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Biosecurity Reference: CFR-Listed Agent and Toxin Summaries

Compiled and Edited by Natalie Barnett

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Abstract

This reference document provides summary information on the animal, plant, zoonotic, and human pathogens and toxins regulated and categorized by 9 CFR 331 and 7 CFR 121, “Agricultural Bioterrorism Protection Act of 2002; Possession, Use and Transfer of Biological Agents and Toxins,” and 42 CFR 73, “Possession, Use, and Transfer of Select Agents and Toxins.” Summary information includes, at a minimum, a description of the agent and its associated symptoms; often additional information is provided on the diagnosis, treatment, geographic distribution, transmission, control and eradication, and impacts on public health.

PREFACE

This summary guide was compiled verbatim from the referenced sources. Minor differences among the sources in style, approach, diction, and organization do not detract from the accuracy or value of the provided reference material.

Contents

1. ANIMAL PATHOGENS

This section introduces the 23 animal pathogens regulated by 9 CFR 331. Summary reference information is provided. Citations and internet addresses for each pathogen are provided in footnotes. This section includes summary information for the following pathogens:

- African horse sickness virus
- African swine fever virus
- Akabane virus
- Avian influenza virus (highly pathogenic)
- Bluetongue virus (exotic)
- Bovine spongiform encephalopathy agent
- Camel pox virus
- Classical swine fever virus
- *Cowdria ruminantium* (Heartwater)
- Foot-and-mouth disease virus
- Goat pox virus
- Japanese encephalitis virus
- Lumpy skin disease virus
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- Menangle virus
- *Mycoplasma capricolum/M. F38/M. mycoides capri* (contagious caprine pleuropneumonia)
- *Mycoplasma mycoides mycoides* (contagious bovine pleuropneumonia)
- Newcastle disease virus (VVND)
- Peste des petits ruminants virus
- Rinderpest virus
- Sheep pox virus
- Swine vesicular disease virus
- Vesicular stomatitis virus (exotic)

1.1 African horse sickness virus[1]

(Perdesiekte, Pestis Equorum, La Peste Equina)

Definition: African horsesickness (AHS) is a highly fatal, viscerotropic, insect-borne viral disease of horses and mules and generally a subclinical disease in other Equidae. The clinical signs and lesions result from selective increased vascular permeability and are characterized by an impairment of the respiratory and circulatory systems.

Etiology: The etiological agent of AHS is a typical orbivirus measuring 68-70 nm in diameter, and the virion is composed of a double-layered protein shell.

The virus is present in the blood and certain organs such as spleen, lung, and lymph nodes in reasonably high concentration, whereas only traces are found in serum, tissue fluids, excretions, and secretions. Viremia generally lasts for about 4-8 days and roughly parallels the febrile reaction. In exceptional cases, viremia may last as long as 17 days in the horse and 28 days in zebra and donkeys.

[1]Committee on Foreign Animal Diseases of the United States Animal Health Association, revised 1998. Foreign Animal Diseases, "The Gray Book," Part IV, Library of Congress Catalog Card Number 17-12842, Pat Campbell & Associates and Carter Printing Company, Richmond, VA. http://www.vet.uga.edu/vpp/gray_book/FAD/ahs.htm

The AHS virus is relatively heat stable, particularly in the presence of protein. It can be stored for at least 6 months at 4° C in saline containing 10 percent serum. Blood in OCG preservative (500 ml glycerin, 500 ml distilled water, 5 g sodium oxalate, and 5 g carbolic acid) can remain infective for more than 20 years; lyophilization may preserve infectivity for as long as 40 years. The virus is readily inactivated at pH values lower than 6.3, but it is relatively stable between values ranging from 6.5 to 8.5.

Nine distinct serotypes of AHS virus are known, the last of which was isolated in 1960. This suggests that, despite its segmented genome, the virus can be regarded as genetically stable and that new serotypes do not readily develop. The present nine serotypes probably evolved over many centuries.

Host Range: Horses, mules, and donkeys have historically been known as hosts for AHS virus, as reflected in the name of the disease. In view of the high mortality rate suffered by horses and mules, these species should be regarded as accidental or indicator hosts. That AHS failed to establish itself outside the tropical regions of Africa tends to indicate that neither horses nor mules or donkeys remain long-term carriers of AHS virus and are therefore not essential for the permanent persistence of the infection in a particular region. Zebra may fulfill this role, but no irrevocable proof has been found to substantiate this view.

The dog has long been known to be susceptible to experimental infection. Infection of dogs also readily occurs following ingestion of infected horse meat. However, it is extremely unlikely that this species becomes infected by insect bites, and it is generally accepted that dogs play no role in the spread or maintenance of AHS.

Camels can ostensibly become inapparently infected with AHS virus, but few details are available as to the level and duration of viremia in this species and its role, if any, in the epizootiology of the disease. A high percentage of African elephant serum samples reacted positively against AHS virus in complement fixation tests, but no neutralizing antibodies could be demonstrated in such samples. No evidence of virus replication could be found in elephants artificially infected with AHS virus. It can therefore be concluded that the African elephant is not susceptible to infection and that the putative serological evidence resulted from abnormal reactions of elephant sera in a complement fixation test.

Geographic Distribution: African horse sickness appears to be endemic in tropical regions of central Africa from where it regularly spreads southwards to southern Africa. The Sahara Desert forms a formidable barrier against northward spread. Occasionally the infection does reach northern African countries, either by spread along the Nile valley or along the West Coast of Africa. The disease has also occurred outside Africa on a few occasions, the most notable of which was the major outbreak in the Near and Middle East from 1959 through 63 and in Spain (1966 and 1987-1990).

In temperate regions such as South Africa, AHS has a definite seasonal occurrence. The first cases are usually noticed towards midsummer, and the disease disappears abruptly after the onset of cold weather in autumn. The disease is most prevalent in warm, low-lying moist areas such as valleys and marshes.

Transmission: African horse sickness is a noncontagious disease, and the virus was the first shown to be transmitted by midges (*Culicoides* spp.). The most significant vector seems to be *Culicoides imicola*, but other species, such as *C. variipennis*, which is common in many parts of the United States, should also be considered as potential vectors.

The virus is transmitted biologically by midges, and these insects are most active just after sunset and at about sunrise.

Although other insects such as mosquitoes have been implicated as biological vectors, and large biting flies (e.g., *Stomoxys*, *Tabanus*) may transmit AHS virus mechanically, the role of these insects in the epizootiology of the disease is regarded as absolutely minimal compared with that played by the *Culicoides* species.

Generally, midges disperse only a few kilometers from their breeding sites, but it has been postulated that they can be borne for longer distances on air currents. Analysis of field observations on the progression of

outbreaks indicates that wind-borne spread of midges may assist the short-distance spread of the disease but that long-distance jumps of the infection are invariably the result of movement of infected Equidae.

Incubation Period: In experimentally induced cases the incubation period usually varies between 5 and 7 days, but it may be as short as 2 days and rarely as long as 14 days. Circumstantial evidence indicates that, following natural infection, the incubation period varies from 7 to 14 days.

Clinical Signs: Four clinical forms of AHS can be distinguished.

The Peracute or Pulmonary Form

This form is characterized by very marked and rapidly progressive respiratory involvement. An acute febrile reaction may be the only clinical sign for a day or two, reaching a maximum of about 104-106° F (40-41° C). This is followed by various degrees of respiratory distress. The breathing may increase to 60 or even 75 respirations per minute, and the animal tends to stand with its forelegs spread apart, its head extended, and the nostrils fully dilated. Expiration is frequently forced with the abdomen showing heave lines. Profuse sweating is common, and spasmodic coughing may be observed terminally with frothy, serofibrinous fluid exuding from the nostrils. The onset of dyspnea is usually very sudden, and death often occurs within 30 minutes to a few hours after its appearance.

The Subacute Edematous or Cardiac Form

The incubation period of this form varies between 7 and 14 days, and the onset of the clinical disease is marked by a febrile reaction of 102-106° F (39-41° C) that lasts for 3-6 days. Shortly before the decline of the fever, characteristic edematous swellings appear. These initially involve the supraorbital fossae and the eyelids and later extend to the lips, cheeks, tongue, intermandibular space, and laryngeal region. Subcutaneous edema sometimes extends a variable distance down the neck towards the chest, often obliterating the jugular groove. Interestingly, no edema of the lower limbs is observed. Terminally, petechial hemorrhages develop in the conjunctivae and under the ventral surface of the tongue. The animal becomes very depressed and may lie down frequently but for very short periods only. Occasionally, signs of colic may develop. Finally, the animal remains prostrate and dies from cardiac failure about 4-8 days after the onset of the febrile reaction. In cases that recover, swellings gradually subside within a period of 3-8 days.

The Acute or Mixed Form

This form represents a mixture of the pulmonary and cardiac forms. Although seldom diagnosed clinically, it is seen at necropsy in the majority of fatal cases of AHS in horses and mules. The disease manifests itself in various ways. Initial pulmonary signs of a mild nature that do not progress are followed by edematous swellings and effusions, and death results from cardiac failure. In the majority of cases, however, the subclinical cardiac form is suddenly followed by marked dyspnea and other signs typical of the pulmonary form.

Horsesickness Fever

This is the mildest form and is frequently overlooked in natural outbreaks. The febrile reaction is usually of the remittent type, with morning remissions and afternoon exacerbation, and lasts for 3-8 days but rarely exceeds 104° F (40° C). Apart from the febrile reaction, other clinical signs are rare and inconspicuous. The conjunctivae may be slightly congested, the pulse rate may be increased, and a certain degree of anorexia and depression may be present. This form of the disease is usually observed in donkeys and zebra or in immune horses infected with a heterologous serotype of AHS virus.

Gross Lesions: The lesions observed at necropsy examination depend largely on the clinical form of disease manifested by the animal before death. In the peracute form the most characteristic changes are edema of the lungs or hydrothorax. In very peracute cases, extensive alveolar edema and mottled hyperemia of the lungs are seen, whereas in cases with a somewhat more protracted course extensive interstitial and subpleural edema is also present, but hyperemia is less evident. Occasionally the lungs may appear reasonably normal, but the thoracic cavity may contain as much as 8 L of fluid. Other less commonly observed lesions are periaortic and peritracheal edematous infiltration, diffuse or patchy

hyperemia of the glandular fundus of the stomach, hyperemia and petechial hemorrhages in the mucosa and serosa of the small and large intestines, subcapsular hemorrhages in the spleen, and congestion of the renal cortex. Most of the lymph nodes are enlarged and edematous, especially those in the thoracic and abdominal cavities. Cardiac lesions are usually not conspicuous, but epicardial and endocardial petechial hemorrhages are sometimes evident.

In the cardiac form the prominent lesion is a yellow gelatinous infiltration in the subcutaneous and intermuscular fascia primarily of the head, neck, and shoulders. Occasionally the lesion may also involve the brisket, ventral abdomen and rump. Hydropericardium is a common feature, and there are extensive petechial and ecchymotic hemorrhages on the epicardium and endocardium, particularly of the left ventricle. The lungs are usually normal or only slightly engorged, and the thoracic cavity rarely contains excess fluid. The lesions in the gastrointestinal tract are generally similar to those found in the pulmonary form, except that submucosal edema of the cecum, large colon, and rectum tends to be far more pronounced.

In the mixed form the lesions seem to represent a combination of those found in the pulmonary and cardiac forms.

Morbidity and Mortality: In susceptible horse populations, the fatalities range between 70 and 95 percent, and the prognosis is extremely poor. In mules, the mortality rate is about 50 percent and in the European and Asian donkey about 5-10 percent. No mortality is observed among African donkeys and zebra.

In enzootic regions, the mortality rate is modified in proportion to the immunity acquired by the equine population as a result of previous vaccination or exposure to natural infection.

Diagnosis

Field Diagnosis

During the early febrile phase of AHS, a field diagnosis may be virtually impossible. However, a presumptive diagnosis should be possible once the characteristic clinical signs have developed and, more particularly, at necropsy.

Specimens for the Laboratory

Confirmation of a presumptive diagnosis is based on virus isolation and identification. This is of particular importance whenever outbreaks occur outside the enzootic regions. The AHS virus can be isolated quite readily from blood collected during the early febrile phase (preferably in heparin or else in other anticoagulants) as well as from spleen, lung, and lymph nodes collected at necropsy.

Specimens for virus isolation should be shipped to the laboratory refrigerated, NOT FROZEN.

Horses that survive infection develop specific antibodies within 10-14 days after infection that reach a peak about 10 days later. It is always advisable to use paired (acute and convalescent phase) serum samples. Serological tests can demonstrate AHS antibodies for 1 to 4 years after infection.

Differential Diagnosis

The clinical signs of AHS, particularly when not fully developed, may be confused with other infections, notably equine encephalosis and equine viral arteritis (EVA). The former disease occurs under the same epizootiological conditions as AHS, and in South Africa the two diseases frequently occur simultaneously. Horses suffering from equine encephalosis usually do not have characteristic lung edema or subcutaneous edema, and the mortality rate is considerably lower than in AHS.

Severe cases of EVA may readily be confused with AHS. The presence of ventral edema (in EVA), particularly of the lower limbs, and the much lower mortality rate should allow differentiation. In countries where piroplasmosis occurs, the early stage of this disease, before blood parasites can be demonstrated and anemia develops, may be confused with AHS.

The necropsy lesions of AHS can be confused with those found in cases of purpura hemorrhagica. In the latter condition, the hemorrhages and edema seem to be more severe and widely distributed than in AHS

and usually involve the limbs and lower abdomen. The highly sporadic occurrence of purpura also aids in differentiation.

Vaccination: The work of Alexander and de Toit has resulted in the development of a live attenuated vaccine that has been used successfully for several decades. However, the adaptation of the virus to the brains of adult mice resulted in a neurotropic vaccine that occasionally caused encephalitis in horses mules and particularly in donkeys. This necessitated an alternate and safer method of attenuation achieved by plaque selection in Vero cell cultures. The vaccine currently used in South Africa consists of two quadrivalent vaccines that are administered 3 weeks apart. Strategic reserves of monovalent vaccines are also maintained.

Extensive work is presently under way to develop potent inactivated and recombinant vaccines that should widen the choice in the near future.

Control and Eradication

Preventive Measures

The most important means of introducing AHS into a hitherto disease free country is by the introduction of equid animals incubating the disease. Zebra and African donkeys that do not develop any clinical sign of disease are particularly dangerous. Equid animals imported from infected countries should be quarantined in insect-proof facilities at the point of entry. At present, there is a minimum 60-day quarantine period for horses brought into the United States from Asia, Africa, and the Mediterranean countries.

Once the disease is introduced into a country, several preventive measures should be taken to prevent further spread and eventually to eradicate the scourge in the shortest possible time. It is essential to isolate and identify the causal virus, but it is imperative that control measures be implemented even before the final diagnosis has been made.

Officials should delineate area of control, taking into consideration geographical borders such as mountains and rivers. The movement of all equid animals within, into, and out of the control zone should be stopped and this restriction rigidly enforced. Furthermore, all equid animals should be stabled, at least from dusk to dawn, and sprayed with insect repellents to reduce the risk of insects feeding on the animals. If sufficient stabling facilities are not available, barns could be used. Even if not insect-proof, such housing will reduce the risk of infection. Additionally, the rectal temperatures of all equid animals in the zone should be taken regularly (preferably twice daily) to detect infected animals as early as possible because overt disease is generally preceded by viremia for about 3 days. Animals with fever should be killed or housed in insect-free stables until the cause of the fever has been established.

Once the diagnosis has been finalized, vaccination of all susceptible animals with the relevant monovalent AHS vaccine should be considered. This decision will be guided largely by the success of measures already taken.

Natural Immunity

Animals that recover from the disease develop a solid life-long immunity against the infecting virus and a partial immunity against heterologous serotypes. Foals from immune dams have a passive immunity that may protect them for up to 6 months.

Public Health: There is no evidence that man can become infected with field strains of AHS virus, either through contact with infected animals or from working in laboratories. However, it has been shown that certain neurotropic vaccine strains may cause encephalitis and retinitis in humans following transnasal infection.

1.2 African swine fever virus[2]

(Peste porcine Africaine, fiebre porcina Africana, maladie de Montgomery)

Definition: African swine fever (ASF) is a tickborne and contagious, febrile, systemic viral disease of swine.

Etiology: The ASF virus is a large (about 200 nm) lipoprotein-enveloped, icosahedral, double-stranded DNA virus. For many years the agent was classified as an iridovirus, but in recent years it was found to have many characteristics of poxvirus; thus, researchers have suggested establishment of a new family for ASF virus (ASFV).

This virus is quite stable and will survive over a wide range of pH. In serum-free medium, ASFV is inactivated at pH 3.9 or lower and at pH 11.5 or higher. In the presence of 25 percent serum, ASFV will remain viable for 7 days at pH 13.4. The virus will survive for 15 weeks in putrefied blood, 3 hours at 50° C, 70 days in blood on wooden boards, 11 days in feces held at room temperature, 18 months in pig blood held at 4° C, 150 days in boned meat held at 39° F, and 140 days in salted dried hams.

Over the years, ASFV isolates with lower virulence have emerged — particularly in the Iberian peninsula. Virulence of isolates varies from highly virulent (essentially 10 percent mortality in 7-10 days after exposure), to moderately virulent (acute illness in which a high percentage of the pigs survive), to low virulence (only seroconversion occures).

Host Range: Initially, domestic and wild pigs (Africa: warthog, bush pig, and giant forest hog; Europe: feral pig) were thought to be the only hosts of ASFV. In 1963, Spanish workers isolated ASFV from the soft tick *Ornithodoros erraticus* collected from ASF-infected farms. Subsequently, researchers showed that ASFV replicates in the tick and that there is transstadial, transovarial, and sexual transmission in *Ornithodoros* ticks. *O. moubata* collected from warthog burrows in Africa were shown to be infected with ASFV. African swine fever in wild pigs in Africa is now believed to cycle between soft ticks living in warthog burrows and newborn warthogs. *Ornithodoros* ticks collected from Haiti, the Dominican Republic, and southern California have been shown to be capable vectors of ASFV, but in contrast to the African ticks, many of the ticks from California died after being infected with ASFV. Many researchers believe that ASFV is really a tick virus and the pig is an accidental host.

Because ASFV-infected ticks can infect pigs, ASFV is the only DNA virus that can qualify as an arbovirus.

Geographic Distribution: African swine fever is present in several African countries and on the island of Sardinia.

Transmission: Even though the soft tick has been shown to be a vector (and in Africa probably the reservoir of ASFV), the primary method of spread from country to country has been through the feeding of uncooked garbage containing ASFV-infected pork scraps to pigs. Once a pig becomes infected, ASFV spreads by direct contact, and contaminated people, equipment, vehicles, and feed. The role of carrier pigs has been difficult to prove experimentally, but circumstantial evidence from the field incriminates carrier pigs. An outbreak of ASF in a contained swine operation in Africa was traced to workers feeding the entrails of guinea fowl to pigs. It was shown that the guinea fowl feed on soft ticks; thus, ASFV was present in the guinea fowl intestines fed to the pigs.

The amount of ASFV needed to infect a pig depends on the route of exposure. Experimentally, a pig can be infected by intramuscular or intravenous inoculation with a 0.13 hemadsorbing dose (HAD_{50}); intranasal-oral inoculation required 18,200 HAD_{50}.

In an ASF endemic area where there are soft ticks, ticks can be the source of infection. However, in these areas in Africa, pigs can be very successfully raised in confinement with double fencing, proper isolation,

[2]Committee on Foreign Animal Diseases of the United States, revised 1998. Animal Health Association, Foreign Animal Diseases, "The Gray Book," Part IV, Library of Congress Catalog Card Number 17-12842, Pat Campbell & Associates and Carter Printing Company, Richmond, VA. http://www.vet.uga.edu/vpp/gray_book/FAD/asf.htm

and sanitary procedures. In Africa, the production system with the highest risk of ASF is the village pig, for these pigs roam. The owners do not practice isolation procedures when the pigs are confined.

In other areas, the disease has to be introduced by infected live pigs or by feeding uncooked garbage containing ASFV-infected pork. Once the disease is introduced into a herd, it spreads by direct and indirect contact with secretions and excretions from infected pigs. Aerosol transmission is not important in the spread of ASF. Because ASFV does not replicate in epithelial cells, the amount of virus shed by an ASF-infected pig is much less than the amount of virus shed by a hog-cholera-infected pig. The blood of a recently infected pig contains a very high ASFV titer: $10^{5.3}$ to $10^{9.3}$ HAD_{50} per milliliter. Therefore, if pigs fight, an infected pig develops bloody diarrhea, or an infected pig is necropsied, blood is shed, and there is massive environmental contamination.

Piglets born of ASF-convalescent dams are free of ASFV and ASF antibody at birth but seroconvert after ingesting colostrum. When piglets from noninfected (control) and ASF-convalescent dams were challenge-inoculated when 7 weeks old, the control piglets developed an average viremia of $10^{7.6}$ and died, whereas the piglets from convalescent gilts developed an average viremia of $10^{4.9}$ and survived. However, because of persistent infection by ASFV, reestablishing a herd using pigs from convalescent animals will not result in an ASFV- free herd. When farmers in Cameroon repopulated their herds using ASF-convalescent animals, the herds experienced recurring periods of high mortality due to ASF.

Incubation Period: After intranasal-oral exposure, pigs usually develop fever and leukopenia in 48 to 72 hours.

Clinical Signs

Highly and Moderately Virulent ASF Isolates

The clinical signs of ASF are influenced by the virulence of the virus and the physiological state (age and pregnancy) of the pig. After inoculation of feeder pigs with either a highly virulent or moderately virulent isolate, the clinical course for both isolates is similar for the first 4-6 days post infection. About 2 DPI, the pigs will develop a fever of 105-107^{o} F (40.5-41.7^{o} C) and white pigs will have a reddened skin, moderate anorexia, and leukopenia. When disturbed the pigs will get up and move about but if left alone will after a short time lie down.

After 4-6 DPI, a difference between the pigs inoculated with the different isolates will become apparent.

Highly Virulent Isolate

The pigs become progressively sicker (eat and move less), and most die between 7 and 10 DPI. It is not unusual to see a pig walking and a short time later to find it dead.

Moderately Virulent Isolate

Pigs infected by moderately virulent ASFV usually have a high fever for 10-12 DPI. Some mortality usually occurs at this time. After 12-14 DPI, temperatures and leukocyte counts start to return to normal levels. It is not unusual to have one or more pigs die as early as 7-8 DPI, but when these pigs are necropsied, the cause of death is frequently hemorrhage into the stomach; the underlying mechanism of death was that ASFV infection caused a thrombocytopenia, resulting in a prolonged bleeding time and hemorrhage from a preexisting gastric ulcer. Very young pigs may have a high mortality and have lesions similar to infection by highly virulent virus.

Pigs affected with either isolate, in addition to the reddened skin, may develop dark red to purple discoloration of the skin on the ears, tail, extremities of the legs, or skin on the hams. This is a nonspecific sign also seen in other diseases. Some groups of pigs will develop diarrhea; this is probably due to disturbed gut physiology and flora rather than a direct effect of the virus because the virus does not replicate in epithelium. In contrast to hog cholera, ASFV-infected pigs do not develop a conjunctivitis or encephalitis, and, despite the high fever, the ASFV-infected pigs stay in good condition, whereas hog cholera-infected pigs quickly lose much weight.

Pregnant animals infected with a high-, moderate-, or low-virulence ASF isolate abort.

Low Virulence Isolates

Nonpregnant animals infected by certain low-virulence ASFV may only seroconvert; pregnant animals will abort.

Other low-virulence ASFV isolates will cause a low fever for 2-3 weeks and then reddened areas 1 cm^2 to many centimeters in size may develop in the skin. These areas then become raised and necrotic. These pigs may also have painless enlargements of joints—particularly the carpal and tarsal joints. This form is referred to as chronic ASF. Many of these pigs will have recurring episodes of a more acute disease and eventually die during an acute episode.

Gross Lesions

Highly Virulent ASFV Infection

Pigs that die peracutely from an infection with a highly virulent ASFV may have poorly developed lesions. Animals that die 7 or more DPI have more classic lesions. Three lesions most consistently found and highly suggestive of ASF infection are as follows:

- Greatly enlarged dark red to black friable spleen
- Very enlarged hemorrhagic gastrohepatic lymph nodes
- Very enlarged hemorrhagic renal lymph nodes

Other lesions described for ASF are more variable and are as follows:

- Dark red to purple areas of skin on ears, feet, and tail
- Petechial hemorrhages on serosal surfaces
- Petechial to ecchymotic hemorrhages in the renal cortex
- Perirenal edema
- Edema of the gall bladder
- Swollen liver
- Edema of the lung.

In pigs infected orally, the submandibular lymph node may be enlarged and have some hemorrhage. Other peripheral lymph nodes may have only edema.

Moderately Virulent Virus

The gross lesions 8-12 DPI in pigs infected with a moderately virulent ASFV are similar to those infected by a highly virulent ASFV. The main difference in the lesions between these two types of isolates is that in infections by a moderately virulent ASFV, the spleen although enlarged, has a more normal color and is not friable.

Low Virulent Virus

The most common lesions in chronic ASF are necrotic skin lesions, consolidated lobules in the lung, generalized lymphadenopathy, swollen joints, and pericarditis.

Aborted fetuses may be anasarcous, and there may be petechial hemorrhages in the placenta, skin, and myocardium, and a mottled liver.

Morbidity and Mortality: The warthog and bush pig develop a viremia but have a very mild or subclinical disease, whereas ASF infection in domestic pigs and European feral pigs can cause a high mortality.

Morbidity in a previously unexposed herd will usually be 100 percent in pigs that have contact with each other. Mortality varies with the virulence of the isolate. Highly virulent isolates will cause about 100 percent mortality. Infection by lesser virulent isolates can cause mortality that varies from a low percentage to 60-70 percent. Factors that can increase mortality in infections by the lesser virulent isolates are concurrent disease, a young age, and pregnancy.

Diagnosis

Field Diagnosis

The highly virulent form of ASF will be easiest to diagnose because essentially 100 percent of the pigs will die. African swine fever caused by the lesser virulent isolates will be more difficult to diagnose but should always be suspected when there are febrile pigs and necropsy findings include the following:

- Greatly enlarged dark red to black spleen
- Very enlarged hemorrhagic gastrohepatic lymph nodes
- Very enlarged hemorrhagic renal lymph nodes.

African swine fever has frequently been misdiagnosed as hog cholera. In contrast to hog cholera, ASFV-infected pigs do not develop a conjunctivitis or encephalitis, and despite the high fever, the ASFV-infected pigs stay in good condition. In contrast, hog cholera-infected pigs are severely depressed and quickly lose much weight; moreover, they usually have a foul smelling diarrhea.

Specimens for Laboratory

The ASFV is present in the blood starting about 2 DPI. In infections by lesser virulent isolates, ASFV can usually be isolated from the blood for 25 or more DPI. Specimens for laboratory diagnosis are as follows:

- Heparinized blood
- Clotted blood or serum
- Submandibular lymph node
- Inguinal lymph node
- Tonsil
- Spleen
- Gastrohepatic lymph node
- Lung
- Liver
- Kidney

Bone marrow should be submitted if there are considerable postmortem changes.

The specimens should be shipped refrigerated or frozen. Pieces of the preceeding tissues, the brain, and any other gross lesion should be submitted in 10 percent buffered formalin.

Aborted fetuses are usually free of virus; therefore, it is necessary to submit a blood sample from the dam.

Laboratory Diagnosis

The initial diagnosis of ASF in a free area requires isolation and identification of the virus. After the initial diagnosis, confirmation of a diagnosis can be made by demonstrating ASF antigen in tissue or ASF antibody.

Differential Diagnosis

Differential diagnoses for ASF should include hog cholera, erysipelas, salmonellosis, and eperythrozoonoisis.

Vaccination: There is no vaccine.

Control and Eradication

Prevention

Introduction of the disease into free areas can be prevented by cooking all garbage fed to pigs (this applies to commercial and backyard pigs and pets [potbellied pigs]) and importing only ASF-disease free pigs.

Eradication

Control and eradication of ASF in developed countries can be accomplished by slaughter and disposal of all acutely infected pigs, widespread testing and elimination of all seropostive animals, and good herd isolation and sanitary practices.

Today (1996), ASF is not as great a threat to the United States as it was several years ago. The major pork-exporting countries have eradicated the disease in domestic pigs.

Public Health: Human beings are not susceptible to ASFV infection.

1.3 Akabane virus[3]

(Congenital arthrogryposis-hydranencephaly syndrome, A-H syndrome, Akabane disease, congenital bovine epizootic A-H syndrome, acorn calves, silly calves, curly lamb disease, curly calf disease, dummy calf disease)

Definition: Congenital arthrogryposis-hydranencephaly (A-H) syndrome is an infectious disease of the bovine, caprine, and ovine fetus caused by intrauterine infection and interference with fetal development after transmission to the dam by biting gnat- or mosquito-transmitted Akabane virus and some other antigenically related members of the Simbu group of arboviruses. Fetal infection may cause abortions, stillbirths, premature births, mummified fetuses, and various dysfunctions or deformities of fetuses or liveborn neonates. Adult animals are not clinically affected while actively infected with virus.

Etiology: The etiologic agents of congenital A-H syndrome are arboviruses of the Simbu group of the family Bunyaviridae. Akabane virus was the first member of the Simbu group to be incriminated in congenital A-H syndrome, but other members (namely Aino, Peaton, and Tinaroo viruses) have the capacity to produce fetal defects. In recent years, Cache Valley virus, a mosquito-borne member of the Bunyaviridae outside the Simbu group, has been found to reproduce a similar syndrome in ruminants within the United States. The Simbu group of viruses is spread only by insect vectors. Spread by contact, infected tissues, exudates, or fomites does not occur.

Host Range: Congenital A-H syndrome associated with Akabane virus and other Simbu group viruses has been reported only in cattle, sheep, and goats. Although antibody against these viruses has been detected in horses, no clinical evidence of fetal infection has been reported. Infections of wild ruminants do occur, and fetal damage must be considered but has not been reported.

Geographic Distribution: In Japan, the periodic outbreaks of AH syndrome have been reported since 1949. Enzootic Akabane virus (and presumably other Simbu group virus) activity has occurred in the northern half of Australia since at least 1931 with occasional temporary epizootic incursions southward dependent on favorable seasons. Reports of A-H syndrome in Israel and other countries in the Middle East, Cyprus, Korea, Zimbabwe, and South Africa have been published in the last decade. Serological surveys indicate that the virus occurs throughout Africa, Asia, and Australia but not Papua New Guinea, the Pacific Islands, or the Americas.

Transmission: The occurrence of A-H syndrome is seasonally and geographically restricted. The location and timing of the infection of the fetus during early pregnancy is consistent with the seasonality of transmission by hematophagous insects. Akabane virus has been isolated from *Aedes vexans* and *Culex triteeniorhynchus* mosquitoes in Japan; *Anopheles funestus* mosquitoes in Kenya; *Culicoides milnei* and *C. imicola* in Africa; *C. oxystoma* in Japan; and *C. brevitarsis* and *C. wadei* gnats in Australia. Confirmation of the biologic transmission by these species is lacking; however, epidemiologic evidence incriminates them. In Australia, *C. brevitarsis* is believed to be the principal vector of Akabane virus. Cache Valley virus has been isolated from at least nine different mosquito species, and antibodies to this virus have been detected in man, as well as wild and domestic animals in the Americas.

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[3]Committee on Foreign Animal Diseases of the United States Animal Health Association, revised 1998. Foreign Animal Diseases, "The Gray Book," Part IV, Library of Congress Catalog Card Number 17-12842, Pat Campbell & Associates and Carter Printing Company, Richmond, VA. http://www.vet.uga.edu/vpp/gray_book/FAD/aka.htm

There is no indication that Akabane virus; other Simbu group viruses or Cache Valley virus is transmitted in any other way than by a vector. Transmission happens months before disease in the fetus is evident.

Incubation Period: Infection of adult animals produces no overt clinical sign, but viremia generally occurs 1-6 days after infection. A natural viremia may last 4 to 6 days before antibodies to Akabane virus are detectable. However, infection of pregnant animals during the first months of gestation may result in fetal infection that is not apparent until much later in pregnancy or at term.

Timing of the infection relative to the stage of gestation is critical to the development of defects in the fetus. In pregnant sheep, the gestational period for the occurrence of fetal abnormalities has been shown to vary from 30-36 days to 30-50 days. This variation in the reported results has been ascribed to (a) differences in the virulence of virus strains used, (b) differences in the passage level of the virus strain used, or (c) differences caused after growth of the virus in the arthropod vectors. Inoculation of pregnant cattle with virus between 62 and 96 days of gestation resulted in fetal lesions; in pregnant goats, the critical period in the gestational cycle was about 40 days.

Clinical Signs: Congenital A-H syndrome is manifested as a seasonally sporadic epizootic of abortions, stillbirths, premature births, and deformed or anomalous bovine, caprine, and ovine fetuses or neonates. The pregnant dam has no clinical manifestation at the time of infection with virus. Sentinel cattle under close observation have no clinical sign during viremia induced by natural infection. If infection develops during the first third of pregnancy, gross fetal damage may occur. At the other end of the disease spectrum, damage to the central nervous system (CNS) may be minor and produce changes in behavior of the new born or young animal. Dystocia at parturition may occur owing to the deformities in the fetus. Badly deformed fetuses are usually dead at birth, and the limbs are locked in the flexed or extended position. Most live neonates have central nervous system degeneration and muscle lesions that prevent the animal from standing or suckling. Torticollis, scoliosis, brachygnathism, and kyphosis may coexist with arthrogryposis. Lesions in the central nervous system are manifested clinically as blindness, nystagmus, deafness, dullness, slow suckling, paralysis, and incoordination.

Mildly affected calves or lambs may improve their mobility with time. However, most eventually die by 6 months as a result of blindness and other neurological defects.

Gross Lesions: An individual fetus or newborn may have arthrogryposis and hydranecephaly or both syndromes. Lesions are associated with damage to the enervation of the musculature and to the central nervous system. Arthrogryposis is the most frequently observed lesion. Affected joints cannot be straightened even by application of force because of ankylosis of the joint in the extended or flexed position. Torticollis, scoliosis, and brachygnathism are observed. There may be shallow erosions about the external nares and muzzle and between the distal digits. Hypoplasia of the lungs and skeletal muscles, fibrinous polyarticular synovitis, fibrinous navel infection, ophthalmia, cataracts, and presternal steatosis occur. Within the CNS, hydranencephaly, hydrocephalus, agenesis of the brain, microencephaly, porencephaly and cerebellar cavitation, fibrinous leptomeningitis, fibrinous ependymitis, and agenesis or hypoplasia of the spinal cord are variously reported. The cerebellum appears intact. Lesions due to Akabane tend to be symmetrical. However, some asymmetry occurs when Aino virus is involved. Akabane virus was isolated from fetuses of naturally infected pregnant cows or ewes by the use of predictive serology. When the mothers seroconverted from negative to positive in Akabane virus neutralization tests, Akabane virus was isolated from the fetus.

Morbidity and Mortality: In endemic areas, animals are exposed and become immune before becoming pregnant; thus, congenital abnormalities are seldom seen in native animals, for antibodies prevent virus from spreading from the site of the bite to the fetus. However, when the infected vector spreads (e.g., during an extended humid summer) to an area where the animals are not immune, A-H syndrome can occur months later in many animals. The disease can also appear when pregnant animals from a disease-free area are moved into an endemic area.

There is no reported damage to the dam in congenital A-H syndrome. Most live-born affected calves, lambs, or kids die shortly after birth or must be slaughtered for humane reasons. Some mildly affected calves do improve gait and learn to follow the herd.

Diagnosis

Field Diagnosis

A field diagnosis of congenital A-H syndrome can be made on the basis of the clinical condition, gross pathologic lesions, and the epidemiology. The sudden onset of aborted, mummified, premature, or stillborn fetuses with arthrogryposis and hydranencephaly should be suggestive. The dam will have had no clinical history of disease. A retrospective study would indicate that the first third of pregnancy occurred during a time of biting insect activity.

Specimens for Laboratory

The following specimens should collected for virus isolation: placenta, fetal muscle, cerebrospinal fluid, and fetal nervous tissue; for serology: fetal or precolostral serum, and serum from the dam. For histopatholgy send pieces of spleen, liver, lung, kidney, heart, lymph nodes, affected muscle, spinal cord and brain in 10 percent buffered formalin.

If the specimens can be delivered to a laboratory within 24 hours, they should be placed on ice. If delivery will take longer, quickfreeze the specimens and do not allow them to thaw during transit.

Laboratory Diagnosis

Virus isolation should be attempted from placenta, fetal muscle, or fetal nervous tissue. The chances of success are very low except with a fetus and placenta aborted before antibodies are generated within an immunocompetent fetus. In the absence of viral isolations, a serologic diagnosis is usually made by demonstrating antibodies in precolostral or fetal serum samples. In adult animals, seroconversion or a demonstrable rise in antibody titer indicates that there was infection. A microtiter neutralization test and an immunofluorescence test are available for detecting and assaying antibodies. Tissues of the dam are free of virus by the time the damage is observed in the fetus or newborn. Low titers (<10) in unpaired serum samples should not be taken as diagnostic because of cross-reaction problems.

Differential Diagnosis

The demonstration that Cache Valley virus, a Bunyavirus that is ubiquitous within the United States, can cause the A-H syndrome means that serological tests are essential to distinguish exotic from enzootic etiologies. It is a reasonable assumption that other Bunyaviridae will be proven to be teratogenic in livestock in the Americas. A variety of nutritional, genetic, toxic, and infectious diseases will produce fetal wastage and deformities. Fetal brain lesions resulting from bluetongue vaccine virus infections of pregnant ewes are similar to those produced within the congenital A-H syndrome. Bluetongue presents the greatest difficulty in the initial differential diagnosis of hydranencephaly. Bovine virus diarrhea infection can cause cerebellar dysplasia in calves. Border disease virus infection can cause undersized, excessively hairy lambs with muscular tremors and skeletal defects. Wesselsbron virus infection can cause congenital porencephaly and cerebral hypoplasia in calves. Serology of the dam and fetus will resolve any confusion.

Vaccination: A formalin-inactivated, aluminum phosphate, gel-absorbed vaccine and an attenuated vaccine have been developed in Japan for Akabane virus. An effective killed vaccine for Akabane virus has been developed but not marketed in Australia. These vaccines induce immunity in the cow or ewe, and the circulating antibodies prevent the virus from reaching the fetus. The vaccines are used prior to exposure to infected vectors. Vaccine is no longer available for economic reasons. Immunizing agents for other Simbu group viruses are not currently available and are not expected to be developed.

Control and Eradication: Techniques for the control of the viral agents that cause congenital A-H syndrome are those typically recommended for other vector-transmitted agents. Control of the vector depends upon disruption of breeding sites, reduction of vector populations with pesticides, and protection

of host animals from feeding by the vectors. In addition to these procedures, animals should be vaccinated before breeding.

Public Health: There is no evidence that humans can be infected by Akabane virus.

1.4 Avian influenza virus[4]

(Fowl Plague)

Definition: Avian influenza (AI) is a disease of viral etiology that ranges from a mild or even asymptomatic infection to an acute, fatal disease of chickens, turkeys, guinea fowls, and other avian species, especially migratory waterfowl.

Etiology: Fowl plague was described in 1878 as a serious disease of chickens in Italy. It was determined in 1955 that fowl plague (FP) virus is actually one of the influenza viruses. The AI viruses, along with the other influenza viruses, make up the virus family Orthomyxoviridae. The virus particle has an envelope with glycoprotein projections with hemagglutinating and neuraminidase activity. These two surface antigens, hemagglutinin (HA) and neuraminidase (NA), are the basis of describing the serologic identity of the influenza viruses using the letters H and N with the appropriate numbers in the virus designation e.g., H7N2. There are now 15 hemagglutinin and 9 neuraminidase antigens described among the Type A influenza viruses. The type designation (A, B, or C) is based upon the antigenic character of the M protein of the virus envelope and the nucleoprotein within the virus particle. All influenza viruses affecting domestic animals (equine, swine, avian) belong to Type A, and Type A influenza virus is the most common type producing serious epidemics in humans. Types B and C do not affect domestic animals.

Classical fowl plague viruses have H7 as one of the surface antigens but can have different N antigens. It was once believed that all H7 viruses are highly pathogenic fowl plague viruses and that no other avian influenza viruses could produce a fowl-plague-like disease. When avirulent AI viruses with the H7 antigens were demonstrated in turkeys in 1971 and highly virulent AI viruses with the H5 antigen were first found in chickens in 1959, the necessity for redefining the term fowl plague or using other terminology became apparent. Because there are highly virulent AI viruses that possess H antigen other than the H7 and H7 AI viruses that do not produce clinical fowl plague, an international assembly of avian influenza specialists proposed that the term fowl plague no longer be used. They suggested that any AI virus, regardless of its HA designation, meeting specified virulence requirements in the laboratory be designated highly pathogenic avian influenza (HPAI). The criteria that serve as the basis for classifying an AI virus as HPAI has more recently been modified to include molecular considerations such as the type of amino acids at the cleavage site of its HA. This chapter will be limited to describing the HPAI and not the AI viruses of less virulence and pathogenicity.

Host Range: Most avian species appear to be susceptible to at least some of the AI viruses. A particular isolate may produce severe disease in turkeys but not in chickens or any other avian species. Therefore, it would be impossible to generalize on the host range for HPAI, for it will likely vary with the isolate. This assumption is supported by reports of farm outbreaks where only a single avian species of several species present on the farm became infected. Swine appear to be important in the epidemiology of infection of turkeys with swine influenza virus when they are in close proximity. Other mammals do not appear to be involved in the epidemiology of HPAI. The infection of humans with an H5 avian influenza virus in Hong Hong in 1997 has resulted in a reconsideration of the role of the avian species in the epidemiology of human influenza.

Geographic Distribution: Highy pathogenic avian influenza viruses have periodically occurred in recent years in Australia (H7), England (H7), South Africa (H5), Scotland (H5), Ireland (H5), Mexico (H5), Pakistan (H7), and the United States (H5). Because laboratory facilities are not readily available in some

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parts of the world to differentiate Newcastle disease and HPAI, the actual incidence of HPAI in the world's poultry flocks is difficult to define. It can occur in any country, regardless of disease control measures, probably because of its prevalence in wild migratory waterfowl, sea birds and shore birds.

Avian influenza has produced losses of variable severity, primarily in turkeys in the United States, since the mid-1960's. The disease outbreaks in turkeys in the United States have been caused by AI viruses with many of the HA designations. It was in the fall of 1983 that a highly virulent H5 virus produced severe clinical disease and high mortality in chickens, turkeys, and guinea fowl in Pennsylvania. This severe disease, clinically indistinguishable from classical fowl plague, occurred after a serologically identical but apparently mild virus had been circulating in poultry in the area for 6 months.

Outbreaks of less virulent AI have frequently been described in domestic ducks in many areas of the world. The AI viruses are often recovered from apparently healthy migratory waterfowl, shore birds, and sea birds worldwide. The epidemiologic significance of these isolations relative to outbreaks in domestic poultry has led to the generally accepted belief that waterfowl serve as the reservoir of influenza viruses.

Transmission: There is a considerable body of circumstantial evidence to support the hypothesis that migratory waterfowl, sea birds, or shore birds are generally responsible for introducing the virus into poultry. Once introduced into a flock, the virus is spread from flock to flock by the usual methods involving the movement of infected birds, contaminated equipment, egg flats, feed trucks, and service crews, to mention a few. Preliminary trapping evidence indicates that garbage flies in the Pennsylvania outbreak were sources of virus on the premises of the diseased flocks. Virus may readily be isolated in large quantities from the feces and respiratory secretions of infected birds. It is logical to assume, therefore, that because virus is present in body secretions, transmission of the disease can take place through shared and contaminated drinking water. Airborne transmission may occur if birds are in close proximity and with appropriate air movement. Birds are readily infected via instillation of virus into the conjunctival sac, nares, or the trachea. Preliminary field and laboratory evidence indicates that virus can be recovered from the yolk and albumen of eggs laid by hens at the height of the disease. The possibility of vertical transmission is unresolved; however, it is unlikely infected embryos could survive and hatch. Attempts to hatch eggs in disease isolation cabinets from a broiler breeder flock at the height of disease failed to result in any AI-infected chickens. This does not mean that broken contaminated eggs could not be the source of virus to infect chicks after they hatch in the same incubator. The hatching of eggs from a diseased flock would likely be associated with considerable risk.

Incubation Period: The incubation period is usually 3 to 7 days, depending upon the isolate, the dose of inoculum, the species, and age of the bird.

Clinical Signs: Infections of HPAI result in marked depression with ruffled feathers, inappetence, excessive thirst, cessation of egg production, and watery diarrhea. Mature chickens frequently have swollen combs, wattles, and edema surrounding the eyes. The combs are often cyanotic at the tips and may have plasma or blood vesicles on the surface with dark areas of ecchymotic hemorrhage and necrotic foci. The last eggs laid, after the onset of illness, are frequently without shells. The diarrhea begins as watery bright green and progresses to almost totally white. Edema of the head, if present, is often accompanied by edema of the neck. The conjunctivae are congested and swollen with occasional hemorrhage. The legs, between the hocks and feet, may have areas of diffuse hemorrhage. Respiratory signs can be a significant feature of the disease, depending on the extent of tracheal involvement. Mucus accumulation can vary. It is not unusual in caged layers for the disease to begin in a localized area of the house and severely affect birds in only a few cages before it spreads to neighboring cages.

Death may occur within 24 hours of first signs of disease, frequently within 48 hours, or be delayed for as long as a week. Some severely affected hens may occasionally recover.

In broilers, the signs of disease are frequently less obvious with severe depression, inappetence, and a marked increase in mortality being the first abnormalities observed. Edema of the face and neck and neurologic signs such as torticollis and ataxia may also be seen.

The disease in turkeys is similar to that seen in layers, but it lasts 2 or 3 days longer and is occasionally accompanied by swollen sinuses.

In domestic ducks and geese the signs of depression, inappetence, and diarrhea are similar to those in layers, though frequently with swollen sinuses. Younger birds may exhibit neurologic signs.

Gross Lesions: Birds that die with the peracute disease and young birds may not have significant gross lesions other than severe congestion of the musculature and dehydration. In the less acute form, and in mature birds, significant gross lesions are frequently observed. They may consist of subcutaneous edema of the head and neck area, which is evident as the skin is reflected. Fluid may exit the nares and oral cavity as the bird is positioned for postmortem examination. The conjunctivae are severely congested—occasionally with petechiation. The trachea may appear relatively normal except that the lumen contains excessive mucous exudate. It may also be severely involved with hemorrhagic tracheitis similar to that seen with infectious laryngotracheitis. When the bird is opened, pinpoint petechial hemorrhages are frequently observed on the inside of the keel as it is bent back. Very small petechia may cover the abdominal fat, serosal surfaces, and peritoneum, which appears as if it were finely splattered with red paint. Kidneys are severely congested and may occasionally be grossly plugged with white urate deposits in the tubules.

In layers, the ovary may be hemorrhagic or degenerated with darkened areas of necrosis. The peritoneal cavity is frequently filled with yolk from ruptured ova, causing severe airsacculitis and peritonitis in birds that survive for 7 to 10 days.

Hemorrhages may be present on the mucosal surface of the proventriculus — particularly at the juncture with the gizzard. The lining of the gizzard peels easily and frequently reveals hemorrhages and erosions underneath. The intestinal muscosa may have hemorrhagic areas — especially in the lymphoid foci such as the cecal tonsils. The gross lesions are not distinctly different from those observed with velogenic viscerotropic Newcastle disease (VVND). The lesions in turkeys and domestic ducks are similar to those in chickens but may not be as marked.

Morbidity and Mortality: The prognosis for flocks infected with HPAI is poor. Morbidity and mortality rates may be near 100 percent within 2 to 12 days after the first signs of illness. Birds that survive are usually in poor condition and resume laying only after a period of several weeks.

Diagnosis

Field Diagnosis

Highly pathogenic avian influenza is suspected with any flock where sudden deaths follow severe depression, inappetence, and a drastic decline in egg production. The presence of facial edema, swollen and cyanotic combs and wattles, and petechial hemorrhages on internal membrane surfaces increases the likelihood that the disease is HPAI. However, an absolute diagnosis is dependent upon the isolation and identification of the causative virus. Commercially available type A influenza antigen-capture enzyme linked immunosorbent assay kits designed for use in human influenza have recently shown promise as a possible rapid diagnositic test for poultry.

Specimens for Laboratory

Specimens sent to the laboratory should be accompanied by a history of clinical and gross lesions, including any information on recent additions to the flock. Diagnosis depends upon the isolation and identification of the virus from tracheal or cloacal swabs, feces, or from internal organs. Specimens should be collected from several birds. It is not unusual for many of the submitted specimens to fail to yield virus. Swabs are the most convenient way to transfer AI virus from tissues or secretions of the suspect bird to brain and heart infusion broth or other cell culture maintenance medium containing high levels of antibiotics. Dry swabs should be inserted deeply to ensure obtaining ample epithelial tissue. Trachea, lung, spleen, cloaca, and brain should be sampled. If large numbers of dead or live birds are to be sampled, cloacal swabs from up to five birds can be pooled in the same tube of broth. An alternative technique is to place 0.5 cm^3 of each tissue into the broth. Blood for serum should be collected from

several birds. If the specimens can be delivered to a laboratory within 24 hours, they should be placed on ice. If delivery will take longer, quickfreeze the specimens and do not allow them to thaw during transit.

Laboratory Diagnosis

Nine to 11-day-old embryonated chicken eggs are inoculated with swab or tissue specimens. Avian influenza virus will usually kill embryos within 48-72 hours. If the virus isolated is identified as a Type A influenza virus, through the AGP or ELISA tests, it is then tested using a battery of specific antigens to identify its serologic identity (HA and NA type).

Sera from infected chickens usually yield positive antibody tests as early as 3 or 4 days after first signs of disease.

Differential Diagnosis

Highly pathogenic avian influenza is easily confused with VVND, because the disease signs and postmortem lesions are similar, and may also be confused with infectious laryngotracheitis and acute bacterial diseases such as fowl cholera and *Escherichia coli.* However, in an area where AI is prevalent, such as during an outbreak, sound presumptive diagnoses can be made by flock history, signs, and gross lesions.

Treatment: Amantadine hydrochloride has been licensed for use in humans to treat influenza since 1966. The medication is effective in reducing the severity of influenza Type A in humans. Experimental evidence indicated possible efficaciousness in poultry when the drug was administered in drinking water to reduce disease losses, but drug-resistant viruses quickly emerged, negating the initial beneficial effects. Thus, the drug is not recommended for use in poultry.

Vaccination: Inactivated oil-emulsion vaccines, although fairly expensive, have been demonstrated to be effective in reducing mortality, preventing disease, or both, in chickens and turkeys. These vaccines may not, however, prevent infection in some individual birds, which go on to shed virulent virus. More economical viable vaccines prepared using naturally avirulent or attenuated strains have the disadvantage of the possible creation of reassortant influenza viruses with unpredictable characteristics. These reassortants could result when a single host bird is simultaneously infected with both the vaccine and another AI virus. Owing to the segmented nature of the influenza virus genome, a reassortment of genetic material can readily occur, creating new influenza viruses. The basic drawback to any vaccine approach for the control of HPAI is the large number of HA subtypes that can cause the disease. Because there is no cross-protection among the 15 known HA subtypes, either a multivalent vaccine will be needed or vaccination postponed until the prevalent disease-causing subtype in the area is identified. A recombinant fowl pox virus vaccine containing the gene that codes for the production of the H5 antigen has recently been licensed. The use of a recombinant insect virus containing the gene for either the H5 or H7 antigen has been used to make these vaccine proteins in insect cell cultures.

Control and Eradication: The practice of accepted sanitation and biosecurity procedures in the rearing of poultry is of utmost importance. In areas where waterfowl, shore birds, or sea birds are prevalent, the rearing of poultry on open range is incompatible with a sound AI prevention program. Appropriate biosecurity practices should be applied, including the control of human traffic and introduction of birds of unknown disease status into the flock. Cleaning and disinfection procedures are the same as those recommended in the chapter on velogenic Newcastle disease.

Public Health: The AI viruses are Type A influenza viruses, and the possibility exists that they could be involved in the development, through genetic reassortment, of new mammalian strains. An influenza virus isolated from harbor seals that died of pneumonia had the HA and NA surface antigens of an influenza virus isolated from turkeys a decade earlier. The infection and deaths of 6 of 18 humans infected with an H5 avian influenza virus in Hong Hong in 1997 has resulted in a reconsideration of the portentous role that the avian species have on the epidemiology of human influezna. Previously there was only one report of a human becoming infected with an H7 AI virus. Is is impossible to predict the importance of AI virus in determining the strains of virus that infect humans. There was no evidence to indicate that humans

coming in contact with large quantities of the H5N2 virus during depopulation efforts in the HPAI outbreak of 1983 in Pennsylvania became infected with the virus.

1.5 Bluetongue virus[5]

(Sore muzzle, pseudo foot-and-mouth disease, muzzle disease)

Definition: Bluetongue (BT) is an insect-borne viral diseases of ruminants characterized by acute or subacute clinical courses in susceptible ruminants. The BT virus (BTV) has also been associated with congenital disease in sheep and cattle.

Etiology: Bluetongue is caused by orbiviruses in the family *Reoviridae*. Other orbiviruses include Ibaraki, Palyam, Eubenangee, and Tilligerry. The viruses are resistant to lipid solvents, which is typical of nonenveloped viruses. The viruses are relatively acid-labile, and slow freezing at -10 to -20 degrees C (14 – 4 degrees F) is deleterious to the virus.

Worldwide, 24 serotypes of BTV have been identified. Five serotypes of BTV have been isolated in the United States. However, only BTV serotypes 10, 11, 13, and 17 are currently active. BTV serotype 2, originally isolated from animals imported into Florida, may not have established itself in the United States; however, epidemiologic studies will be required to resolve this issue.

Acetic acid is an effective disinfectant.

Host Range: The host range of BTV is very broad and includes all ruminant species tested to date. However, the expression of clinical disease varies in different species. Sheep are fully susceptible to BTV.

Geographic Distribution: The geographic distribution of the orbiviruses is extensive, but current knowledge is still incomplete. Virus distribution is based on the presence of certain *Culicoides* species, including *C. variipennis*, *C. imicola*, *C. brevitaris*, and others. Orbivirus infections are common in tropical, subtropical, and temperate climates. Areas with year- round vector activity could easily maintain the virus by a continuous vector-host cycle. Virus persistence in areas with severe winters is not understood. Reintroduction of virus into the area in the warm months by transportation of infected animals or infected *Culicoides* being carried on the wind is probably common. Some research reports suggest that overwintering of the virus in these areas occurs by mechanisms such as (1) prolonged viremias (up to 3 months) in certain animals, (2) transplacental transmission of BTV in the late fall and early winter to the late-term developing fetus with subsequent birth of a viremic calf, and (3) overwintering of the virus in *Culicoides* that may survive through the winter at very low population densities. Virological and serological testing has suggested that BTV exists in North, Central, and South America; Africa; and parts of Asia; Europe; the Middle East; and the South Pacific.

Transmission: Transmission of orbiviruses is primarily by *Culicoides* species, which are biological vectors. Limited experimental studies have also demonstrated that ticks are capable of mechanically or biologically transmitting BTV; however, their role in the epidemiology of BTV is probably minimal. Virus can also be transferred from viremic dams (sheep and cattle) to the developing fetus. Although BTV can be found in the semen of certain rams and bull, it is only isolated at the time of peak viremia; this presence of virus would appear to be the result of blood cells in the semen. Extensive field and experimental studies suggest that transmission of BTV via semen is of not importance in the epidemiology of BTV. The potential for BTV transmission also exists owing to poor management practices such as using the same needle or infected surgical equipment on several animals (mechanical transmission).

[5]Committee on Foreign Animal Diseases of the United States Animal Health Association, revised 1998. Foreign Animal Diseases, "The Gray Book," Part IV, Library of Congress Catalog Card Number 17-12842, Pat Campbell & Associates and Carter Printing Company, Richmond, VA. http://www.vet.uga.edu/vpp/gray_book/FAD/blt.htm

Incubation Period: The incubation period of BT in sheep is usually 7-10 days; however, viremia may appear as early as 3 to 4 days after infection. In cattle, viremia occurs as early as 4 days postinfection, but clinical signs are uncommon. Development of clinical BT in cattle may be the result of hypersensitization. Under laboratory conditions such animals develop clinical signs 10 to 12 days following reexposure to the virus. The incubation of BTV infection in deer is 7 to 12 days.

Clinical Signs of Bluetongue

BT in Sheep

The classic signs of BT in sheep are those of an acute to subacute infection by a virulent strain of virus in fully susceptible animals belonging to the fine wool or mutton breeds. However, the signs of BT are variable. Not all strains of BTV that infect sheep cause clinical disease. In some flocks, no clinical sign is apparent, whereas in other flocks infected by the same virus up to 30 percent may develop signs of disease.

The first sign of illness which begins 7 to 8 days after infection, is a rise in temperature to 106-107^{o} F (41.6-41.7^{o} C). Temperatures may be elevated for 4 to 12 days following the initial rise. Within 24 hours of the initial rise in temperature, excessive salivation and frothing at the mouth develop and are associated with hyperemia and swelling of the buccal and nasal mucosa. If forced to move, sheep may pant like a dog. During the next 2 to 3 days, erosions and ulcerations may develop in the buccal mucosa. By 4 to 7 days in severe cases, extensive ulcerations may be covered by gray necrotic tissue on the dental pad and dorsal surface of the tongue. In addition, affected animals being fed rough feed (stemmy alfalfa) may have more severe lesions of the oral mucosa.

Hyperemia is often observed around the coronary bands of the hooves. Often the hooves are tender and varying degrees of lameness are apparent. In more severe cases, the animals stand with an arched back. If the animals are driven during this time, they may slough their hooves. Animals that recover may have a dark line in the wall of the hoof.

The lesions in the mouth, the reluctance to move, and the necrosis of striated musculature lead to weakness, depression, and rapid weight loss. These can result in prostration and eventual death in severely affected animals. Sheep that recover from severe infections may have a break in the wool 3 to 4 weeks after the fever has subsided. This can lead to partial or complete shedding of wool.

Bluetongue virus infection of pregnant ewes in the first trimester can cause fetal death and resorption, abortion, or birth of "dummy" lambs. Attenuated BTV vaccines can also cause reproductive failure.

BT in Cattle

Bluetongue virus infection in cattle usually does not cause any clinical sign of disease. Subclincal disease is only evidenced by changes in the leukocyte and lymphocyte subpopulation counts in the peripheral blood and a mild acute eosinophilic dermatitis. A consistent fluctuation in rectal temperature is indicative of viremia and a mild disease. Occasionally, field outbreaks of BT disease occur in which as many as 30 percent of the cattle have clinical signs. Experimental data support the contention that clinical BT in cattle occurs as result of prior sensitization to a related orbivirus followed by a later second exposure. After secondary exposure, clinical signs become apparent in 10-12 days. Clinical signs consist of mild hyperemia in the buccal cavity and around the coronary band; vesicular lesions, which lead to ulcerations, in the buccal mucosa; erect hair over the cervical and dorsal thoracic areas; and a definite hyperesthesia. In addition, the dermis becomes thickened with prominent folds apparent in the cervical areas, and a dry crusty exudate leads to matting of hair in affected areas. These lesions may persist for 10 to 20 days. Similar lesions have been reported on teats of cattle with clinical BT. Hoof lesions may be associated with lameness. In some instances severe breaks in the hooves occur 40 to 60 days after infection and are usually followed by foot rot.

Bluetongue virus infection can cause reproductive failure in cattle. Some infected bulls will become temporarily sterile following acute infections. After recovery, production of sperm resumes, and the bulls are capable of settling cows.

Certain strains of BTV are capable of causing fetal death, resorption and or abortion; cell-culture-adapted live virus may be more effective than field virus in establishing fetal infection. Teratogenic effects of BTV in the bovine fetus include hydranencephaly and cerebral cysts that result in "dummy" calves.

Critical factors for fetal infection include the stage of embryonic or fetal development when infection occurs, the immune status of the dam, and the strain(s) of BTV causing infection. The most susceptible period for fetal infections occurs between 60 and 140 days gestation in nonimmune dams. Experimental studies suggest 15 to 20 percent of viremic dams will transmit virus to their fetuses. In areas where strains of BTV are endemic, there is little evidence that BTV has adverse affects on reproduction.

BT in Goats

Bluetongue infection of goats is typically an inapparent infection similar to that described for cattle.

Gross Lesions

BT in Sheep

The lesions of BT in sheep vary greatly depending on (1) the strain of virus, (2) individual animal and breed susceptibility and (3) environmental (stress) factors. Prominent lesions include facial edema, edematous ears, and dry, crusty exudate over the nostrils. Lesions in the oral cavity include focal petechial (pinhead-size) hemorrhages that progress to gray necrotized debris over erosions and ulcerations on the lips; on the dorsal, lateral, and ventral surfaces of the tongue; and on the dental pad. The buccal mucosa may be cyanotic. Hyperemia and occasional erosions can occur on papillae and laminae in the reticulum and omasum.

Lesions in the respiratory system include cyanosis and edema of the nasal mucosa and pharynx, and there may be tracheal hyperemia and congestion. Froth in the trachea is present only when there is pulmonary congestion and edema.

Lesions in the vascular system cause hyperemia, edema, and hemorrhages. A characteristic lesion is hemorrhage at the base of the pulmonary artery. Petechial and ecchymotic (larger than pinhead-size) hemorrhages may be observed at times in the endocardium. Focal gray-white areas of necrosis are often found in the papillary muscles and less frequently in other areas of the myocardium.

The most prominent changes in the skin include dermal and subcutaneous edema of the head and ears. Sometimes an irregular rash (exanthematous eruptions) may progress into serous and crusty exudates on the skin. Hyperemia is prominent at the coronet of the hoof. Often, this reddening is accompanied by petechial or ecchymotic hemorrhages that extend down the horn.

A yellow gelatinous exudate is common in the fascia (connective tissue) along and between skeletal muscles. On the cut surface of the heavy muscle there may be focal hemorrhages and areas that appear dry and gray-white.

Newborn lambs with congenital BT have hydranencephaly or porencephaly. These lesions are characterized by fluid-filled cavities, either occupying the whole of the cranial vault or as cystic cavities in the gray and white matter of the cerebral cortex. Cerebellar dysplasia (abnormal development) with rudimentary medial and lateral lobes may be present. The spinal cord may be dysplastic (abnormal development) and lack white matter. Skeletal deformities may consist of scoliosis (lateral curvature of the spine) and torticollis (twisted neck).

BT in Cattle

Gross lesions in cattle differ in some respects from those observed in sheep. The most prominent lesions involve the skin, mouth, and hooves. Skin lesions are characterized by marked edema that leads to thick folds — particularly in the cervical areas. Lesions may form in the folds as serous exudate accumulates and dries. Dry, crusty exudate is present on the skin over the cervical and thoracic areas. The crusty material results from vesicular eruptions and ulcerations.

The external nares may have erosions covered by crusty exudate that sloughs. Lesions in the mouth start as vesicles and proceed to ulcers covered with grayish necrotic debris. These lesions are more common on

the buccal mucosa and dental pad and rarely the tongue. Hyperemia occurs at the coronary band. In some instances, fissures occur 6 to 8 weeks following infection.

In utero BTV infection may lead to fetal death and resorption, abortion, hydranencephaly, or cerebral cysts.

BT in Deer

Bluetongue in susceptible deer causes widespread hemorrhages throughout the body. These lesions are associated with intravascular thromboses and hemorrhages varying in size from petechial to ecchymotic. In chronic BT, deer may develop severe fissures and even sloughing of hooves. Ulcers covered with gray necrotic debris are found in the buccal mucosa, dental pad, and tongue.

Morbidity and Mortality: In sheep, BT can range from inapparent to severe, depending on the breed, strain of virus, and environmental stress on the animals. Morbidity can reach 100 percent; mortality can vary from 0 to 50 percent. Many animals will recover within a few days to 2 weeks.

In cattle, BTV infection is usually subclinical. Although morbidity can approach 5 percent, cattle typically recover within a few weeks. However, lameness and unthriftiness may persist for prolonged period.

The morbidity and mortality for BTV infection in other species are as follows:

- Goats - - minimal clinical signs
- White-tailed deer - - morbidity approaching 100 percent and a mortality of 80-90 pecent
- Pronghorn antelope - - morbidity approaching 100 percent and a mortality of 80-90 percent
- Bighorn sheep - - morbidity approaching 100 percent and a mortality of 0 to 50 percent
- North American elk - - similar to cattle; the disease is usually subclinical.

Diagnosis

Field Diagnosis

Tentative diagnosis of BT can be made when (1) clinical signs appear in populations known to be susceptible, (2) the occurrence of disease coincides with a prevalence of insect vectors, (3) necropsy of sheep reveals characteristic gross lesions, and (4) a flock history of recent wasting (loss of weight) and pododermatitis (foot rot).

Specimens for Laboratory

Preferred samples for confirmatory diagnosis include sterile heparinized blood samples from animals with clinical signs or spleen or bone marrow, or both, from dead animals. Samples from aborted and congenitally infected newborn animals should include heparinized blood and, if possible, spleen, lung, brain, and serum. If possible, the heparinized whole blood (erythrocytes and white cells) should be washed in saline containing antibiotics and resuspended in saline prior to shipping. This procedure will reduce the antibody that may neutralize the virus if blood-cell lysis occurs.

Specimens should be shipped refrigerated, not frozen. Freezing makes virus isolation difficult.

Laboratory Diagnosis

Confirmatory diagnosis is based on isolation and identification of virus from blood or tissues. Diagnosis for lambs and calves infected in utero is based on serology (if no colostrum has been ingested) or virus isolation, or both.

Differential Diagnosis

Differential diagnoses include plant photosensitization, foot-and-mouth disease, vesicular stomatitis, bovine virus diarrhea, malignant catarrhal fever, infectious bovine rhinotracheitis, parainfluenza-3, contagious ecthyma, and actinobacillosis.

Vaccination: Vaccination has been the primary means of controlling BT disease in sheep. To date, only modified-live (attenuated) virus vaccines have been used. Because of the multiplicity of BTV serotypes

and variable cross-protection between serotypes, vaccination has resulted in varying degrees of success. The serotypes incorporated into the vaccine must be the same as those causing infection in the field. The practice of administering multiple virus serotypes in a single vaccination is argued against by some scientists because (1) an immune response (virus neutralizing antibody) is typically only induced to one, or at best two of the serotypes incorporated in the vaccine and (2) reassortment between the genome segments of the multiple vaccine viruses may occur in the host of a vector feeding on such a vaccinated animal. Although simultaneous infection of sheep, cattle, or *Culicoides* can result in creation of reassortant viruses, there is no evidence that this process has resulted in generation of new serotypes. However, such reassortant events may result in altered virulence and biological transmissibility.

No inactivated or subunit vaccines are currently available, though several experimental vaccine preparations have been studied, including inactivated virus vaccines, subunit vaccines prepared by purification of natural VP2 (viral protein responsible for inducing virus neutralizing antibody), and recombinant VP2 expressed in a baculovirus system.

Control and Eradication: Vaccination can be used in endemic areas.

Vector control measures to impede the spread of BTV infection are not commonly used. However, certain measures have potential effectiveness such as water management (reduction of *Culicoides* breeding sites), use of insecticides and larvacides (spraying breeding areas), and insect repellents in which animals are dipped.

The only applicable treatment available is to minimize animal stress and administer broad-spectrum antibiotics to combat secondary infection.

Public Health: There is only one documented human infection, and that was in a laboratory worker

1.6 Bovine spongiform encephalopathy[6]

BSE is caused by a prion. The term prion derives from 'proteinaceous infectious particle'; it refers to the initially heretical hypothesis that the infectious agent causing those diseases consists only of protein, with no nucleic acid genome. (All previously known pathogens, such as bacteria and viruses, contain nucleic acids, which enable them to reproduce.) The prion hypothesis explained why the mysterious infectious agent is resistant to ultraviolet radiation, which breaks down nucleic acids, but is susceptible to substances that disrupt proteins. [7]

Definition: Bovine spongiform encephalopathy (BSE), widely known as "mad cow disease," is a chronic, afebrile, degenerative disease affecting the central nervous system (CNS) of cattle.

Bovine spongiform encephalopathy belongs to the family of diseases known as the transmissible spongiform encephalopathies (TSE's). These diseases are caused by a transmissible agent that is yet to be fully characterized. They share the following common characteristics:

a. A prolonged incubation period of months or years;

b. A progressive debilitating neurological illness that is always fatal;

c. When examined by electron microscopy, detergent-treated extracts of brain tissue from animals or humans affected by these diseases reveal the presence of scrapie-associated fibrils (SAF's);

d. Pathological changes appear to be confined to the CNS and include vacuolation and astrocytosis;

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[6]Committee on Foreign Animal Diseases of the United States Animal Health Association, revised 1998. Foreign Animal Diseases, "The Gray Book," Part IV, Library of Congress Catalog Card Number 17-12842, Pat Campbell & Associates and Carter Printing Company, Richmond, VA. http://www.vet.uga.edu/vpp/gray_book/FAD/Search/search.htm

[7]Scientific American.com, Ask the Experts: Medicine: What is a prion? http://www.sciam.com/askexpert_question.cfm?articleID=000B256E-B042-1C71-9EB7809EC588F2D7&catID=3

e. The transmissible agent elicits no detectable specific immune response in the host.

Specific types of TSE's include scrapie, which affects sheep and goats; transmissible mink encephalopathy; feline spongiform encephalopathy; chronic wasting disease of deer and elk; and five rare diseases in humans: kuru, Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler-Scheinker syndrome, fatal familial insomnia (FFI), and new variant Creutzfeldt-Jakob disease (nvCJD).

Etiology: The clinical, pathological, and molecular genetic features of BSE, as well as other transmissible spongiform encephalopathies, have led to speculation on the nature of the etiologic agent and the pathogenic mechanisms of the disease. There are three main theories on the nature of the scrapie agent:

1. The virus theory, in which the virus would have to have unusual biochemical and biophysical characteristics that would help explain the remarkable physicochemical properties.

2. The prion theory, in which the agent is conceived of being composed exclusively of a host-coded normal cellular protein (PrPc) that becomes partially protease resistant (PrPBSE) — most likely through a post-translational conformation change after infection. In this theory there is no nonhost component of the agent. That is, a specific informational molecule (nucleic acid e.g., RNA or DNA) is not present.

3. The virino theory, which states that the agent consists of a host-derived protein coat, (PrP being one of the candidates for this protective protein) and a small noncoding regulatory nucleic acid.

All of the proposed theories have some degree of validity. Proponents of the virus and virino theories have concluded that the existence of different scrapie strains unequivocally proves the presence of a nucleic acid component of the infectious agent which, as in conventional viruses, may undergo mutations responsible for phenotypic variations. The problem with these theories is that no agent-specific nucleic acid has been convincingly identified to copurify with infectivity. Moreover, chemical, enzymatic, or physical treatments that usually inactivate or degrade nucleic acids have no effect on the transmissible properties of the infectious agent. Possible reasons for this are that the amount of nucleic acid of the putative agent is too small to be detected with available techniques and that its tight bond to the protein protects it from chemical or physical inactivation. Also weakening the virus and virino theories is the inability to identify any virus particles under the electron microscope, and the failure of an infected host to generate an immune response. Recently small particles resembling virus structures have been observed by electron microscopy.

The prion model involves propagation of a protein-only agent (PrP^{BSE}) whereby PrP^{c} can assume various tertiary structures caused by a combination of host genetics and the introduction of altered (infectious) PrP (PrP^{BSE}). More simply stated, the structure of the infecting PrP^{BSE} imprints upon the normal cellular precursor (PrP^{c}) and results in a conformation change to the protease-resistant form. It is suspected that "strain" differences result from mutations in the PrP gene that may cause proteins "flip" and change shape. Several explanations for scrapie strain genetics in the context of the prion theory have been suggested but none have been proven.

It should be pointed out that the prion theory fails to explain a) how the PrP of the infecting agent originally assumed the aberrant structure associated with infectivity, and b) how the different structures originated as a function of the different strains. Although numerous scrapie strains can be differentiated in a single host (i.e., sheep), the PrP agents associated with these strains have not shown any biochemical and molecular differences; thus, BSE seems to be caused by a single strain type. This BSE strain is different from historical or contemporary isolates from sheep or goats with natural scrapie, as determined by study of incubation periods and brain "lesion profiles" in mice.

Regardless of whether the prion (PrP^{BSE}) is or is not the etiologic agent, the partially protease-resistant form of the prion protein is a marker of infection.

Host Range: Bovine spongiform encephalopathy has been experimentally transmitted to the following species via intracerebral (IC) inoculation: cattle, sheep, and goats, mink, pigs, marmosets, macaques, and

mice. Intracerebral transmission was attempted in hamsters but was not successful. Via the oral route, BSE has been successfully transmitted to cattle, sheep, and goats; mice; and mink. Oral transmission has not been successful in swine. Parenteral and oral transmission has also been attempted in chickens with no evidence of disease thus far.

A transmissible spongiform encephalopathy has been diagnosed in eight species of captive wild ruminants as well as exotic (cheetahs, pumas, a tiger, and an ocelot) and domestic cats. There have been about 81 domestic cat cases of feline spongiform encephalopathy (FSE) in Great Britain and in 1 domestic cat each in Norway, Northern Ireland, and Liechtenstein. The agent isolated from several of these cases using strain typing in mice is indistinguishable from BSE in cattle, which suggests that FSE is actually BSE in exotic and domestic cats. This also appears to be true for the other ruminants. Epidemiological evidence suggests BSE-contaminated feed to be the primary source of infection in these species.

Other cases of spongiform encephalopathy have been reported in kudu, eland, nyala, gemsbok, and a few exotic cats. These too are thought to be linked to contaminated feed.

It has also been suggested that 23 cases (as of January 31, 1998) of a variant form of CJD (nvCJD) (a human disease) in Great Britain (U.K. Department of Health, March 2, 1998) and 1 case in France may be linked to exposure to BSE before the introduction of a specified bovine offal (SBO) ban at slaughter in 1989. The SBO ban excludes from human consumption brain, spinal cord, and other tissues with potential BSE infectivity.

Geographic Distribution: Worldwide there have been more than 170,000 cases since the disease was first diagnosed in 1986 in Great Britain. Over 95 percent of these cases have occurred in the United Kingdom. The disease has also been confirmed in native-born cattle in Belgium, France, Ireland, Luxembourg, the Netherlands, Northern Ireland, Portugal, and Switzerland but is not known to exist in the United States.

Transmission: Different scientific hypotheses have been advanced concerning the origins of BSE. The epidemiologic data suggest that BSE in Great Britain is an extended common source epidemic involving feed containing TSE-contaminated meat and bone meal as a protein source. The causative agent is suspected to be from either scrapie-affected sheep or cattle with a previously unidentified TSE.

Changes in rendering operations in the early 1980's — particularly the removal of a solvent-extraction process that included a steam-heat treatment — may have played a part in the appearance of the disease and the subsequent amplification of the agent in the food chain. A ban on feeding animal protein of ruminant origin to ruminants was enacted in Great Britain in July 1988.

In Great Britain the epidemic peaked in 1992-93, when approximately 1,000 cases were being reported per week. In 1998 it remains on the decline with approximately 100 cases reported per week. Cases that have been detected in other countries appear be a result of importations of live cattle or, more significantly, contaminated feed from Great Britain.

There is no evidence that BSE spreads horizontally; that is, by contact between unrelated adult cattle or from cattle to other species.

New evidence suggests that maternal transmission may occur at an extremely low level. Results of British research show low levels of transmission of BSE from affected cows to their offspring. These results demonstrated that there is approximately a 9 percent increase in the occurrence of BSE in offspring of BSE-affected dams as compared with calves born to dams where BSE was not detected. The study did not ascertain if this was the result of genetic factors or true transmission. The research did, however, point out that, at this level, if maternal transmission does occur, it alone will not sustain the epidemic.

In the naturally infected animals, the agent has been identified by mouse bioassay in the brain, spinal cord, and retina. The route of inoculation into the mice was intracranial. The naturally infected animals were adult cattle exhibiting clinical signs of disease.

Mice fed milk, mammary gland, placenta, lymph nodes, or spleen have failed to develop the disease or to establish subclinical infection of the lymphoreticular system within their natural lifespan.

Another study was conducted to examine the pathogenesis of BSE in cattle; that is the replication (tissue distribution) of the agent during the incubation period. This study, which has not yet been completed, has identified the agent via mouse bioassay in the distal ileum of the experimentally infected calves. It is thought that the agent may be associated with the lymphoid tissue of the intestines. The calves were 4 months of age at the time of oral dosing. First isolation of the agent in the distal ileum was made at 6 months after oral dosing. Subsequent isolations from the distal ileum were made at 10, 14, and 18 months after dosing. Recently this study has also identified infectivity in bone marrow, trigeminal ganglion, dorsal root ganglion, brain, and spinal cord.

No infectivity has been found by parenteral or oral challenge, or both, in over 40 other tissues from clinically ill cattle using the mouse bioassay. It appears as if the distribution of the BSE agent is not as diverse as the scrapie agent in sheep. However, there is a possibility that the agent is present but is at such low levels that the bioassay is not sensitive enough to detect it.

Incubation Period: The incubation period usually ranges from 2 to 8 years. Following the onset of clinical signs, the animal's condition gradually deteriorates until the animal becomes recumbent, dies, or is destroyed. This usually takes from 2 weeks to 6 months. Most cases in Great Britain have occurred in dairy cows (Friesians) between 3 and 6 years of age. The youngest confirmed case occurred in a 20-month-old heifer, and the oldest case was found in a cow 18 years of age.

Clincial Signs: Cattle affected by BSE develop a progressive degeneration of the nervous system. Affected animals may display changes in temperament, abnormalities of posture and movement, and changes in sensation. More specifically, the signs include apprehension, nervousness or aggression, incoordination, especially hind-limb ataxia, tremor, difficulty in rising, and hyperaesthesia to sound and touch. In addition, many animals have decreased milk production or loss of body condition, or both, despite continued appetite.

Gross Lesions: There is no gross lesion associated with BSE.

Morbidity and Mortality: In Great Britain, 19 percent of the dairy herds and 1.6 percent of the beef herds have had one or more cases of BSE. This difference is believed to result from the fact that dairy calves were fed a higher level of protein supplement. The average incidence in herds in Great Britian has been 1.75 cases. However, there have been a few herds with over 30 cases. Affected animals die.

Diagnosis

Field Diagnosis

A field diagnosis of BSE is based on the occurrence of clinical signs of the disease. A bovine animal that has signs of a CNS disturbance should be observed over time (at least 2 weeks) to determine whether the signs become progressively more severe. If, after this interval, improvement or recovery has not taken place, BSE should be suspected and the animal humanely euthanized.

Specimens for Laboratory

Because the BSE agent is considered a human pathogen, protective clothing, gloves, and face protection should be worn when performing the necropsy. The entire brain should be removed intact with a portion of the cranial cervical spinal cord attached. Portions should be placed in a plastic bag and submitted unfixed. The remainder of the brain should be fixed in 10 percent buffered formalin solution. One cerebral hemisphere is removed by cutting the brain stem through the space between the cerebellum and cerebrum with a longitudinal cut between the cerebral hemispheres.

Laboratory Diagnosis

Bovine spongiform encephalopathy currently must be confirmed by histopathological examination of brain tissue. Bilaterally symmetrical degenerative changes are usually seen in the gray matter of the brain stem. These changes are characterized by vacuolation or microcavitation of nerve cells in the brain stem nuclei. The neural perikarya and axons of certain brain stem nuclei contain intracytoplasmic vacuoles of

various sizes that give the impression of a spongy brain. Hypertrophy of astrocytes often accompanies the vacuolation. A diagnosis may also be made by the detection of SAF's using electron microscopy.

Two supplemental tests are available to enhance the diagnostic capabilities for BSE. These are immunohistochemistry and the Western blot technique. In the past, if the brain tissue was not harvested shortly after the animal's death, autolysis often made it very difficult to confirm a diagnosis by histopathology. These tests allow for the possibility of confirming a diagnosis of BSE by detecting PrP^{BSE} even if the brain has been frozen or autolytozed.

Differential Diagnosis

Differentials for BSE include rabies, listeriosis, nervous ketosis, milk fever, grass tetany, lead poisoning, and other toxicities or etiological agents affecting the nervous or musculoskeletal system of adult cattle.

Treatment: There is no known treatment for BSE or any of the TSE's.

Vaccination: There is no preventative vaccine.

Control and Eradication: Bovine spongiform encephalopathy from foreign sources may be prevented by the implementation of import regulations prohibiting live ruminants and ruminant products (especially meat, bone meal, and offal) from countries where BSE may exist. Because the origin of BSE remains unknown, preventing an epidemic of BSE would involve, at a minimum, the prohibition of feeding ruminant proteins to ruminants. The prevention program of any country should also include an active surveillance effort focused on high-risk cattle for the early detection of BSE. Most countries of the world have prohibited the importation of cattle and bovine products from countries known to have BSE. In addition many countries have taken steps to enact regulations prohibiting the feeding of ruminant proteins to ruminants. This is true even in countries such as Australia and New Zealand with no known animal TSE's.

Agricultural officials in countries known to have BSE have taken a series of actions to control and, it is to be hoped, eradicate BSE. These include making BSE a notifiable disease, prohibiting the inclusion of certain animal proteins in ruminants' rations (the feed bans vary depending on the amount of BSE detected), and depopulating certain populations of cattle thought to be of higher risk because of epidemiological findings.

To prevent human exposure to the BSE agent numerous countries have established prohibitions on the inclusion of high risk material in foods, pharmaceuticals, cosmetics, and so forth.

U.S. Actions

With an active surveillance program in place for 8 years, BSE has not been detected in the United States. The United States Department of Agriculture (USDA), Food and Drug Administration (FDA), and industry groups are actively working to maintain this status. The measures USDA, Animal and Plant Health Inspection Service (APHIS), has taken in this regard include prohibitions or restrictions, or both, on certain animal and product imports, ongoing surveillance for the disease in the United States, preparation of an emergency response plan in the unlikely event an introduction were to occur, and continuing educational efforts. The Animal and Plant Health Inspection Service actively shares information and coordinates closely with other Federal agencies, as well as the States, livestock and affiliated industries, veterinary and research communities, and consumer groups, to ensure that the United States has a uniform approach to transmissible spongiform encephalopathies based on sound scientific information.

A comprehensive surveillance program has been implemented by APHIS in the United States to ensure timely detection and swift response in the unlikely event that an introduction of BSE were to occur. This surveillance program entails the location of imports from countries known to have BSE and targeted active and passive surveillance for either BSE or any other TSE in cattle.

To locate each of the 496 British cattle that were imported into this country between January 1, 1981, and July 1989, APHIS has conducted a traceback effort. In July 1989, the United States prohibited the importation of ruminants from countries affected with BSE. As of March 1998, only 17 of these animals are known to be alive in the United States, and these are being carefully monitored by APHIS personnel

on an ongoing basis. In addition, five head of cattle imported from Belgium in 1996 are now under quarantine. In cooperation with the states and industry, APHIS continues to purchase these animals for diagnostic purposes. No evidence of BSE has been found in any of these imported animals.

The United States has had an aggressive, active surveillance program for BSE since May 1990. Bovine spongiform encephalopathy is a notifiable disease, and there are more than 250 Federal and State regulatory veterinarians specially trained to diagnose foreign animal diseases, including BSE. The Animal and Plant Health Inspection Service leads an interagency surveillance program, which includes the Food Safety Inspection Service (FSIS) and the Centers for Disease Control (CDC). The surveillance samples include field cases of cattle exhibiting signs of neurological disease, cattle condemned at slaughter for neurological reasons, rabies-negative cattle submitted to public health laboratories, neurological cases submitted to veterinary diagnostic laboratories and teaching hospitals, and random sampling of cattle that are nonambulatory at slaughter. As of February 21, 1998, over 6,600 brains had been examined for BSE or another form of a transmissible spongiform encephalopathy in cattle. No evidence of either condition has been detected by histopathology or immunohistochemistry.

As of December 12, 1997, APHIS has prohibited the importation of live ruminants and most ruminant products from all of Europe until a thorough assessment of the risks can be made. The new restrictions apply to Albania, Austria, Bosnia-Herzegovina, Bulgaria, Croatia, Czech Republic, Denmark, Federal Republic of Yugoslavia, Finland, Germany, Greece, Hungary, Italy, Former Yugoslav Republic of Macedonia, Norway, Poland, Romania, Slovak Republic, Slovenia, Spain, and Sweden.

This action was taken because, in the past year, the Netherlands, Belgium, and Luxembourg have reported their first cases of BSE in native-born cattle. There is evidence that European countries may have had high BSE risk factors for several years and less than adequate surveillance. Additionally, Belgium reported that the cow diagnosed with BSE was processed into the animal food chain.

The Food and Drug Administration (FDA) has recently established regulations that prohibit the feeding of most mammalian proteins to ruminants. The effective date of this regulation was August 4, 1997.

Public Health

BSE and CJD — Human Health Concerns

On March 20, 1996, the U.K.'s Spongiform Encephalopathy Advisory Committee (SEAC) announced the identification of 10 cases of a new variant form of CJD (nvCJD). All of the patients developed onset of illness in 1994 or 1995. The following features describe how these 10 cases differed from the sporadic form of CJD:

- The affected individuals were much younger than the sporadic CJD patient. Typically, sporadic CJD patients are over 63 years old. The average patient age for the variant form of CJD is 27.5 (range of 16 to 42) years.
- The course of the disease in the nvCJD averaged 13 months. Sporadic CJD cases average a 6-month duration.
- In the variant cases, electroencephalographic (EEG) electrical activity was not typical of sporadic CJD.
- Although brain pathology was recognizable as CJD, the pattern was different from normal CJD, and evidenced large aggregates of prion protein plaques.

Epidemiologic and case studies have not revealed a common risk factor among the cases of nvCJD. According to the SEAC, all victims were reported to have eaten beef or beef products in the last 10 years, but none had knowingly eaten brain material. One of the affected individuals had been a vegetarian since 1991.

The SEAC concluded that, although there was no direct scientific evidence of a link between BSE and nvCJD, on the basis of current data and in the absence of any credible alternative, the most likely

explanation was that the cases were linked to exposure to BSE before the introduction of control measures; namely, the specified bovine offal (SBO) ban in 1989.

Research reported in later 1996 and 1997 has presented further evidence to support a causal association between nvCJD and BSE. Two significant studies published in the October 2, 1997, edition of Nature led the SEAC to conclude that the BSE agent is very likely to be the cause of nvCJD. Dr. Moira Bruce and colleagues at the Institute for Animal Health in Edinburgh, Scotland, inoculated three panels of inbred mice and one panel of crossbred mice with BSE, nvCJD, and sporadic CJD. Interim results indicate that mice inoculated with BSE show the same pattern of incubation time, clinical signs, and brain lesions as mice inoculated with tissues from patients with nvCJD. This provides evidence that BSE and nvCJD have the same signature or are the same "strain." In addition classical CJD and known scrapie strains were not similar to nvCJD or BSE.

Results from another study published by Dr. John Collinge and colleagues of Imperial College School of Medicine, London, United Kingdom, strongly support Bruce's results. Collinge's paper reports experimental transmission of BSE to transgenic mice expressing only human PrP.

The Health and Safety Executive in the United Kingdom now advises that BSE must be considered a biological agent (human pathogen) within the meaning of the Control of Substances Hazardous to Health Regulations 1994.

1.7 Camel pox virus[8]

Camelpox Virus Orthopoxvirus (see description above under Vaccinia)

- generalized pustular disease
- limited to Middle East, parts of Africa & Asia

Vaccinia Virus

- Orthopoxvirus
  - vesicular (blister) lesions (start as rash or papule)
  - may be localized or generalized
  - transmission: direct contact, air, fomites
  - diagnosis: electron microscopy, virus isolation

Vaccinia:

- infects cattle via handling (milking) by infected personnel
- lesions are on teats
- was used for vaccination of people against smallpox

1.8 Classical swine fever virus[9]

Hog Cholera (Note: The preferred term for this disease is now classical swine fever.)

(Classical swine fever, peste du porc, colera porcina, Virusschweinepest)

Definition: Hog cholera (HC) is a highly contagious viral disease of swine that occurs in an acute, a subacute, a chronic, or a persistent form. In the acute form, the disease is characterized by high fever, severe depression, multiple superficial and internal hemorrhages, and high morbidity and mortality. In the

[8]Texas A&M University, Department of Veterinary Pathobiology, College of Veterinary Medicine, Poxviridae. http://vtpb-www.cvm.tamu.edu/vtpb/vet_micro/descriptions_viruses/pox.html#5

[9]Committee on Foreign Animal Diseases of the United States Animal Health Association, revised 1998. Foreign Animal Diseases, "The Gray Book," Part IV, Library of Congress Catalog Card Number 17-12842, Pat Campbell & Associates and Carter Printing Company, Richmond, VA. http://www.vet.uga.edu/vpp/gray_book/FAD/HOC.htm

chronic form, the signs of depression, anorexia, and fever are less severe than in the acute form, and recovery is occasionally seen in mature animals. Transplacental infection with viral strains of low virulence often results in persistently infected piglets, which constitute a major cause of virus dissemination to noninfected farms.

Etiology: Although minor antigenic variants of hog cholera virus (HCV) have been reported, there is only one serotype. Hog cholera virus is a lipid-enveloped pathogen belonging to the family Flaviviridae, genus *Pestivirus*. The organism has a close antigenic relationship with the bovine viral diarrhea virus (BVDV) and the border disease virus (BDV), as demonstrated in the immunodiffusion and immunofluorescence tests. The serum neutralization test can, however, differentiate between HCV and BVDV. In a protein-rich environment, HCV is very stable and can survive for months in refrigerated meat and for years in frozen meat. The virus is sensitive to drying (desiccation) and is rapidly inactivated by a pH of less than 3 and greater than 11.

Host Range: The hosts of HCV are the pig and wild boar.

Geographic Distribution: According to the FAO—WHO—OIE Animal Health Yearbook 1989, HC is recognized in 36 countries and is suspected of being present in another 2. The disease has been eradicated in Australia, Canada, and the United States. Constant progress toward eradication has been made in the countries of the European Economic Community since the guidelines for HC control in individual member states were accepted in 1980.

Transmission: The pig is the only natural reservoir of HCV. Blood, tissues, secretions and excretions from an infected animal contain HCV. Transmission occurs mostly by the oral route; though infection can occur through the conjunctiva, mucous membrane, skin abrasion, insemination, and percutaneous blood transfer (e.g., common needle, contaminated instruments). Airborne transmission is not thought to be important in the epizootiology of HC, but such transmission could occur between mechanically ventilated units within close proximity to each other.

Introduction of infected pigs is the principal source of infection in HC-free herds. Farming activities such as auction sales, livestock shows, visits by feed dealers, and rendering trucks are also potential sources of contagion. Feeding of raw or insufficiently cooked garbage is a potent source of HCV. During the warm season, HCV may be carried mechanically by insect vectors that are common to the farm environment. There is no evidence, however, that HCV replicates in invertebrate vectors. Husbandry methods also play an important role in HC transmission. Large breeding units (100 sows) have a higher risk of recycling infection than small herds. In large breeding units where continuous farrowing is practiced, strains of low virulence may be perpetuated indefinitely until the cycle is interrupted by stamping-out procedures and a thorough cleaning and disinfection are carried out.

Incubation Period: The incubation period is usually 3 to 4 days but can range from 2 to 14 days.

Clinical Signs: The clinical signs of HC are determined by the virulence of the strain and the susceptibility of the host pigs. Virulent strains cause the acute form of the disease, whereas strains of low virulence induce a relatively high proportion of chronic infections that may be inapparent or atypical. These strains are also responsible for the "carrier-sow" syndrome from which persistently infected piglets are produced.

Acute Hog Cholera

In acute HC, the pigs look and act sick. Their disease progresses to death within 10 to 15 days, and remissions are rare. In an affected herd, some pigs will become drowsy and inactive and will stand with arched backs. Other pigs will stand with drooping heads and straight tails. Some pigs may vomit a yellow fluid containing bile. The sick pigs will huddle and pile up on each other in the warmest corner of the enclosure and will rise only if prompted vigorously. Anorexia and constipation will accompany a high fever that may reach 108° F (42.2° C) with an average of 106° F (41.1° C). Pigs may continue to drink and may have diarrhea toward the end of the disease process. Conjunctivitis is frequent and is manifested by encrustation of the eyelids and the presence of dirty streaks below the eyes caused by the accumulation of dust and feed particles. Sick pigs become gaunt and have a weak, staggering gait related to posterior

weakness. In terminal stages, pigs will become recumbent, and convulsions may occur shortly before death. In the terminal stage, a purplish discoloration of the skin may be seen; if present, the lesions are most numerous on the abdomen and the inner aspects of the thighs.

Chronic Hog Cholera

Chronic HC is characterized by prolonged and intermittent disease periods with anorexia, fever, alternating diarrhea and constipation, and alopecia. A chronically infected pig may have a disproportionately large head relative to the small trunk. These runt pigs may stand with arched backs and their hind legs placed under the body. Eventually, all chronically infected pigs will die.

Congenital Hog Cholera

Congenital HCV infection by virulent strains will likely result in abortions or in the birth of diseased pigs that will die shortly after birth. Transplacental transmission with low-virulence strains may result in mummification, stillbirth, or the birth of weak and "shaker" pigs. Malformation of the visceral organs and of the central nervous system occurs frequently. Some pigs may be born virtually healthy but persistently infected with HCV. Such infection usually follows exposure of fetuses to HCV of low virulence in the first trimester of fetal life. Pigs thus infected do not produce neutralizing antibodies to HVC and have a lifelong viremia. The pigs may be virtually free of disease for several months before developing mild anorexia, depression, conjunctivitis, dermatitis, diarrhea, runting, and locomotive disturbance leading to paresis and death. In breeding herds affected with lowvirulence strains of HCV, poor reproductive performance may be the only sign of disease.

Gross Lesions

Acute Hog Cholera

The most common lesion observed in pigs dying of acute HC is hemorrhage. Externally, a purplish discoloration of the skin is the first observation. There may be necrotic foci in the tonsils. Internally, the submandibular and pharyngeal lymph nodes are the first to be affected and become swollen owing to edema and hemorrhage. Because of the structure of the pig lymph node, hemorrhages are located at the periphery of the node. As the disease progresses, the hemorrhage and edema will spread to other lymph nodes. The surface of the spleen, and particularly the edge of the organ, may have raised, dark wedge-shaped areas. These are called splenic infarcts. Infarcts are frequently observed in pigs infected experimentally with older strains of HCV but are less commonly seen with the contemporary strains.

Pinpoint to ecchymotic hemorrhages on the surface of the kidney are very common in HC. Such lesions are easier to see in the decapsulated kidney. Hemorrhages are also found on the surface of the small and large intestine, the larynx, the heart, the epiglottis, and the fascia lata of the back muscles. All serous and mucosal surfaces may have petechial or ecchymotic hemorrhages.

Accumulation of straw-colored fluids in the peritoneal and thoracic cavities and in the pericardial sac may be present.

The lungs are congested and hemorrhagic and have zones of bronchopneumonia.

Chronic Hog Cholera

In chronic HC, the lesions are less severe and are often complicated by secondary bacterial infections. In the large intestine, button ulcers are an expression of such a secondary bacterial infection. In growing pigs surviving for more than 30 days, lesions may be seen at the costochondral junction of the ribs and at the growth plates of long bones.

Congenital Hog Cholera

In pigs infected transplacentally with HCV strains of low virulence, the most commonly seen lesions are hypoplasia of the cerebellum, thymus atrophy, ascites, and deformities of the head and of the limbs. Edema and petechial hemorrhages of the skin and of the internal organs are seen at the terminal stage of the disease.

Morbidity and Mortality: In acute HC, the morbidity and mortality are high.

Diagnosis

Field Diagnosis

Septicemic conditions in which pigs have high fever should be investigated carefully. A thorough history from the herd owner should be obtained to determine if raw garbage was fed, if unusual biological products were used, or if recent additions were made to the herd. Careful observation of the clinical signs and of the necropsy lesions should be recorded. In acute HC, it is helpful to necropsy four or five pigs to increase the probability of observing the representative lesions.

A marked leukopenia is detectable at the time of initial rise in body temperature and persists throughout the course of the acute and chronic disease. This feature was once widely used in the field diagnosis of HC. Nowadays, with the development of more specific laboratory diagnostic methods, which are aimed at demonstrating the virus or its structural antigens in tissues or at detecting specific antibodies in the serum, the white blood count is not as widely used. In endemic areas it could be helpful.

Specimens for Laboratory

For virus isolation and antigen detection, the tonsils are considered essential. In addition, submandibular and mesenteric lymph nodes, spleen, kidneys, and the distal part of the ileum should be collected. In live pigs, tonsil biopsies and whole blood collected with anticoagulants are useful to diagnose HC. Sample collection should be targeted to pigs having fever or showing other signs of the disease. Each sample of tissue should be placed in a separate plastic bag and identified. The samples should not be frozen (interference with fluorescent antibody tissue section test) but kept at refrigeration temperature. The material should be transported and stored in leak-proof containers in accordance with national regulations for transportation of diagnostic biologic samples.

Serum samples for antibody detection should be collected from animals that have recovered from suspected infection or from sows known to have been in contact with infected or suspected cases. A sufficient number of samples should be collected to ensure a high probability of detecting infection.

A complete set of tissues, including the whole brain, should be submitted in 10 percent buffered formalin.

Laboratory Diagnosis

Any clinical diagnosis of HC must be confirmed by the submission of specimens to a specialized diagnostic laboratory that should also have the capability to distinguish between HC and African swine fever.

The laboratory diagnostic procedures for HC have evolved in parallel with the emergence of new technologies. Until the 1960's, laboratory diagnosis was restricted to recognition of gross lesions and confirmation by histopathology. Inoculation of susceptible pigs was often used as final confirmatory test and to determine the virulence of the viruses. Numerous laboratory techniques have been described to diagnose HC, but only a few have gained international acceptance and have been integrated into national HC control programs. Only these will be discussed in this presentation.

In the fluorescent antibody tissue section test (FATST), direct fluorescent antibody technique is applied to detect HC viral antigens in frozen tissues of organs from dead pigs, in biopsy material, or in impression smears. Theoretically, a diagnosis can be confirmed within hours from the reception of the specimen. In countries where the disease has been eradicated, the diagnosis of the "index case" by the FATST alone may be difficult, and confirmation in cell culture may be needed. The FATST may not differentiate HC from BVDV infection; an accurate distinction between the two viruses has to be made before releasing a final diagnosis. Differentiation between HCV and BVDV can readily be made with the immunoperoxidase test using monoclonal antibodies or the serum neutralization test.

The isolation of HCV in cell culture and the identification using fluorescein-labeled hog cholera antibody (fluorescent antibody cell culture test) can provide confirmation in cases where the results of investigation of frozen tissue sections are inconclusive.

As control measures for HC are implemented in a country, virulent strains of HCV will be reduced, and there will be a relative increase of low-virulence strains. As the proportion of subclinical cases in a

national herd increases, it will become increasingly difficult to recognize the disease. The antigen detection systems previously described become less effective; thus, serological tests are essential for a successful control and eventual eradication program.

Approximately 75 percent of pigs infected with acute HC have microscopic lesions of an encephalitis characterized by perivascular cuffing, endothelial proliferation, and microgliosis. This feature is easily recognized in a nonspecialized diagnostic laboratory and may constitute the most important single factor that will cause the pathologist to suspect HC.

Differential Diagnosis

Differential diagnosis of HC should include African swine fever, erysipelas, salmonellosis, eperythrozoonosis, and salt poisoning.

Vaccination: Over the years, numerous regimens of vaccination have been advocated with a variable degree of success. In the past two decades, modified live vaccines (MLV) with no residual virulence for pigs have become available. The lapinized Chinese (C) strain, the Japanese guinea pig cell culture-adapted strain, and the French Thiverval strain have been widely used. All three strains are considered innocuous for pregnant sows and piglets over 2 weeks old.

Control and Eradication: In countries where HC is enzootic, a systematic vaccination program is effective in preventing losses. Experience in the United States and in some countries of the European Union has proven that a strict regimen of vaccination will reduce the number of outbreaks to a level at which complete eradication by sanitary measure alone will be feasible. At that point, vaccination must be stopped. A successful eradication program requires a massive input of funds from a central government and cooperation from the government, the swine industry, and the veterinary profession. Eradication measures will be assisted by strictly enforcing the garbage cooking laws, having an effective swine identification system, and using serological surveys targeted primarily to breeding sows to detect subclinical infections.

In countries where HC has been eradicated and in which the threat of reintroduction is significant, it is essential to initiate an effective serological monitoring system. Sampling may be limited to strategic locations such as the border of an infected neighbor country or be intensified to target populations such as the garbage-fed herds. Such a system has been in effect in the United States since successful eradication in 1976; several thousand samples have been accessed annually.

Public Health: Human beings are not susceptible to HCV infection.

1.9 *Cowdria ruminantium* (Heartwater)[10]

(Cowdriosis)

Definition: Heartwater (HW) is an acute noncontagious infectious disease of ruminants affecting cattle, sheep, goats, and antelope and is caused by the rickettsial organism *Cowdria ruminantium*, which is transmitted by ticks of the genus *Amblyomma*. The disease is characterized by fever, nervous signs, hydropericardium, hydrothorax, ascites, edema of the lungs, and high mortality. In some wild ruminants the agent causes subclinical infection. The name "heartwater" is derived from the hydropericardium, which is commonly seen with this disease.

Etiology: The disease is caused by *Cowdria ruminantium*, a rickettsial agent. It is the only species of the genus *Cowdria*, in the tribe Ehrlichia, family Rickettsiaceae, order Rickettsiales. The organism multiplies in vascular endothelial cells throughout the body and in the reticulum cells of the lymph nodes. The agent is pleomorphic, usually coccoid, occasionally ring-formed, and measures from 400 to over 1,000 nm in diameter. It usually occurs in clumps of from less than five to several thousand organisms within the

[10]Committee on Foreign Animal Diseases of the United States Animal Health Association, revised 1998. Foreign Animal Diseases, "The Gray Book," Part IV, Library of Congress Catalog Card Number 17-12842, Pat Campbell & Associates and Carter Printing Company, Richmond, VA. http://www.vet.uga.edu/vpp/gray_book/FAD/HEA.htm

cytoplasm of infected capillary endothelial cells, especially in the brain. The HW organism is extremely fragile and cannot persist outside of a host for more than a few hours. Because of its fragility, the organism must be stored in dry ice or liquid nitrogen to preserve its infectivity.

Heartwater strains vary in virulence, and although all are apparently pathogenic for sheep and goats, at least one strain is nonpathogenic for cattle.

Host Range: Heartwater causes severe disease in cattle, sheep, goats, and water buffalo; mild disease in some indigenous African breeds of sheep and goats; and inapparent disease in several species of antelope indigenous to Africa. The blesbok (*Damaliscus albifrons*), the black wildebeest (*Connochaetes gnu*), the eland (*Taurotragus oryx oryx*) and the springbok (*Antidorcas marsupialis*) have experimentally been shown to be susceptible to HW, and although the natural disease in these animals is usually mild, deaths in springbok have been attributed to HW. The blesbok and wildebeest are known carriers of *C. ruminantium* and are believed to play a role in the maintenance of the disease in nature.

Nonruminant hosts of *C. ruminantium*, such as guinea-fowl, leopard tortoises, and scrub hare, may also be important in the maintenance of the organism in nature because they are all known carriers of the agent. Although the striped mouse and the multimammate mouse have been shown to be susceptible to *C. ruminantium*, they are not hosts of the vector ticks and are not believed to play a role in the epizootiology of HW.

In the United States the most common deer species, *Odocoileus virginianus* (the white-tailed deer), has been shown by experimental inoculation to be susceptible to *C. ruminantium*. Severe clinical signs were noted along with typical postmortem lesions. The mortality rate was high. *Amblyomma maculatum*, an experimentally proven vector of HW, is a common parasite of white-tailed deer in the southern United States.

The ferret and the albino mouse are susceptible to *C. ruminantium* under experimental conditions, and a mouse agent resembling C. ruminantium has been isolated in South Africa.

Geographic Distribution: Heartwater occurs only where vector ticks of the genus *Amblyomma* are active. For decades, the disease has been known to occur in most countries of Africa south of the Sahara Desert and on the island of Madagascar. The disease has also been reported from Tunisia and the former Yugoslavia; however, the Yugoslavian report is probably erroneous. For the last half-century or more, the disease has been considered one of the most important livestock diseases in Africa and has been surpassed only by trypanosomiasis and East Coast fever.

Heartwater is now known to occur in the Caribbean, where the vector tick *A. variegatum* (tropical bont tick) has been recognized for many years. This tick, now known to occur on numerous Caribbean islands (e.g., Puerto Rico, Antigua, Guadeloupe, Martinique, St. Lucia, Nevis, St. Kitts) was probably introduced to the French Antilles with a shipment of cattle from Senegal in the 1830's. A fatal disease of cattle with neurologic and hemorrhagic signs, which in retrospect could have been HW, was described from Guadeloupe in 1932. Rapid spread of the tropical bont tick in the West Indies is believed to have occurred only after the introduction of the cattle egret from Africa in the early 1960's. Egrets are now known to be efficient porters of the tick. Heartwater has been diagnosed on the islands of Antigua, Guadeloupe, and Marie Galante. A disease closely resembling HW has also been reported from other Caribbean islands (e.g., Cuba and Antigua), but the diagnoses have not been confirmed. *Cowdria ruminantium* is far more widespread in the Caribbean than was formerly believed. Recent serological surveys have demonstrated HW antibodies in cattle from 10 Caribbean islands (Antigua, Dominica, Granada, Guadeloupe, Martinique, Montserrat, St. Kitts, St. Lucia, St. Martin, and St. Vincent).

Transmission: Heartwater is transmitted only by ticks of the genus *Amblyomma*. Of the 12 species known to transmit the disease, *A. variegatum* (tropical bont tick) is by far the most important, for it is widely distributed in Africa and has extended its range to include Yemen, Reunion, the Cape Verde islands, and several islands of the West Indies (26). Other major vector species are the bont tick *A. hebraeum* (in southern Africa), and *A. lepidum* (in East Africa and the Sudan). *Amblyomma astrion* (mainly on buffalo) and *A. pomposum* are also natural vectors of the disease, and five other African ticks

— *A. sparsum, A. gemma*, *A. cohaerans*, *A. marmoreum* and *A. tholloni* (the elephant tick) — have experimentally been shown to be capable of transmitting HW.

Two North American species of *Amblyomma* ticks have been shown by Uilenberg (1982) to be capable of transmitting the disease. They are *A. maculatum* (the Gulf Coast tick) and *A. cajennense*, but neither of these ticks has been incriminated as natural vectors of HW. The former tick is widely distributed in the eastern, southern, and western United States, and was shown by Uilenberg to be as good a vector as one of the principal African vectors, *A. hebraeum*.

Amblyomma ticks are three-host ticks whose life cycles may take from 5 months to 4 years to complete. Because the ticks may pick up the infection as larvae or nymphs and transmit it as nymphs or as adults, the infection can persist in the tick for a very long time. The infection does not pass transovarially. *Amblyomma* ticks are multihost and will feed on a wide variety of livestock, wild ungulates, ground birds, small mammals, reptiles, and amphibians.

Incubation Period: The incubation period is generally shorter in sheep and goats than in cattle. Experimental intravenous inoculation usually results in a febrile response between the 7th and 10th day after inoculation in sheep and goats, and between the 10th and 16th day after inoculation in cattle. Under field conditions, susceptible animals can be expected to show signs of the disease 14 to 28 days after introduction into an HW-infected area.

Clincial Signs: Heartwater occurs in four different clinical forms determined by variations in susceptibility of the hosts and the virulence of various strains of the HW agent.

The relatively rare peracute form of the disease is usually seen in Africa in nonnative breeds of cattle, sheep, and goats introduced to an HW enzootic area. Heavily pregnant cows are especially prone to develop the peracute disease. Sudden death occurs, usually preceded only by a fever, severe respiratory distress, and terminal convulsions. Severe diarrhea may be seen in some breeds of cattle (e.g., Jersey, Guernsey).

The acute form of the disease, by far the most commonly observed syndrome, is seen in nonnative and indigenous domestic ruminants. A sudden fever of up to 107° F (42° C) is followed by inappetance, depression, listlessness, and rapid breathing. Nervous signs then develop, the most prominent being chewing movements, twitching of the eyelids, protrusion of the tongue and circling, often with highstepping gait. The animal may stand with its legs apart and head lowered. The nervous signs increase in severity, and the animal goes down in convulsions. Galloping movements and opisthotonos are commonly seen before death. Hyperesthesia is often observed in the terminal stages of the disease, as is nystagmus and frothing at the mouth. Diarrhea is occasionally seen, especially in younger animals. The acute disease is usually fatal within a week of the onset of signs.

Rarely, the disease may run a subacute course characterized by prolonged fever, coughing (a result of lung edema), and mild incoordination; recovery or death occurs in 1 to 2 weeks. A mild or subclinical form of the disease, known as "heartwater fever," is seen in partially immune cattle or sheep, in calves less than 3 weeks old, in antelope, and in some indigenous breeds of sheep and cattle with high natural resistance to the disease. The only clinical sign in this form of the disease is a transient febrile response.

Gross Lesions: The gross lesions in cattle, sheep, and goats are very similar. Heartwater derives its name from one of the prominent lesions observed in the disease, namely pronounced hydropericardium. The accumulation of straw-colored to reddish fluid in the pericardium is more consistently observed in sheep and goats than in cattle. Ascites, hydrothorax, mediastinal edema, and edema of the lungs, all resulting from increased vascular permeability with consequent transudation, are frequently encountered. Subendocardial petechial hemorrhages are usually seen, and submucosal and subserosal hemorrhages may occur elsewhere in the body. Degeneration of the myocardium and liver parenchyma, splenomegaly, edema of lymph nodes, nephrosis, and catarrhal and hemorrhagic abomasitis and enteritis are all commonly encountered. Meningeal congestion and edema are often present. Brain congestion may occur, but brain lesions can be remarkably few when one considers the severity of the nervous signs observed in this disease.

Morbidity and Mortality: Once signs of the disease have developed, the prognosis is poor for nonnative sheep, goats, and cattle infected with the more virulent strains of the HW organism. The mortality rate in merino sheep may be 80 percent in contrast to 6 percent mortality observed in Persian or Afrikander sheep. Angora goats are extremely susceptible to HW. In cattle, mortality of about 60 percent is not uncommon.

Diagnosis

Field Diagnosis

The presence of *Amblyomma* ticks plus the rather characteristic signs and lesions of heartwater allows tentative field diagnosis of the disease, which must then be confirmed by demonstration of the causative organism, its antigens, or its DNA.

Specimens for Laboratory

From live animals, collect 10 ml of blood using heparin as an anticoagulant and add sufficent DSMO to make a 10 percent concentration; freeze on dry ice. Collect an additional 50 ml of heparinized blood and 10 ml of serum. From a dead animal, submit smears of cerebral cortex or half of the brain unpreserved and a set of tissues in 10 percent buffered formalin.

Laboratory Diagnosis

Demonstration of the Organism: The HW organism stains purplish-blue with Giemsa stain and can be seen by microscopic examination of brain smears prepared as follows: A small piece of cerebrum, cerebellum, hippocampus, or other well-vascularized portion of the brain is macerated between two microscope slides. The resultant pulp is then drawn across a slide with varying pressure, which results in "ridges and valleys" on the slide. The slide is then air-dried, fixed with methanol, and stained with Giemsa. Under low magnification, the capillaries will be found extending from the "thick" areas of the slide. Examination of the capillary endothelial cells under oil immersion will reveal the blue to reddish-purple clumps of organisms. A rapid method for obtaining brain tissue for examination is to drive a large nail through the unopened skull and make a smear from the tissue adhering to the nail. The HW organisms can also be observed in smears prepared from the intima of large blood vessels or in stained sections of kidney glomeruli and lymph nodes.

Although microscopic examination of Giemsa-stained brain smears is still widely employed in HW diagnosis, newer and more sensitive techniques such as the use of DNA probes have been applied to detect *Cowdria* nucleic acids in tissues of infected livestock and ticks. These newer techniques should supplant the older methods of diagnosis as facilities and equipment become more available in HW-enzootic areas.

Antibody Detection: The indirect fluorescent antibody (IFA) test has extensively been used for HW antibody detection, and the newer competitive enzyme-linked immunosorbent assay (CELISA) promises to be a useful addition to the meager array of tests available for the detection of HW antibodies. The cross-reactions described with several *Ehrlichia* spp. Can now be eliminated with the use of more specific antigens and monoclonal antibodies.

Differential Diagnosis

The peracute form of HW can be confused with anthrax. The acute nervous form of HW can be confused with rabies, tetanus, chlamydiosis, bacterial meningitis or encephalitis, cerebral trypanosomiasis, piroplasmosis or theileriosis, and various intoxications such as with strychnine, lead, organophosphates, or chlorinated hydrocarbons. Heavy helminth infestations may produce accumulations of fluid similar to those seen in HW. Arsenical poisoning may resemble the enteric form of the disease, and certain poisonous plants (e.g., Cestrum laevigatum, Pachystigma spp., Pavetta spp.) may produce signs and lesions similar to those seen in HW.

Treatment: Tetracycline antibiotics (especially oxytetracycline) are very effective in the treatment of HW, especially when animals are treated early in the course of the disease. Tetracycline antibiotics

administered before signs appear will suppress the disease entirely, but will allow immunity to develop. Doxycycline and rifamycin are both very effective, and a wide variety of sulfonamides have successfully been used in HW treatment. Treatment for ruminal atony, a commonly observed sequel to this disease, may be indicated, and diuretics may be useful to control fluid accumulations in body cavities.

Vaccination: Animals recovering from the natural disease or from artificial exposure to the organism are solidly immune for a variable period ranging from 6 months to 18 months. Animals exposed to reinfection during this period of resistance will have their immunity reinforced and will remain immune as long as they are periodically reinfected. There is now conclusive evidence that immunity to HW is T-cell mediated and that circulating antibodies play a minor role in immunity.

Calves and lambs are very resistant to *C. ruminantium* in the first 4 weeks of life. This resistance seems to be a true age resistance and has successfully been used in the immunization of cattle and sheep. Calves of less than 4 weeks of age, and lambs in the first week of life can be immunized by intravenous inoculation of HW-infected blood. The infection that follows is usually mild, and upon recovery animals are immune to reinfection because immunity is continuously stimulated by natural exposure to the organism. Older animals or very valuable calves should be examined daily after immunization and should be treated with antibiotics as soon as the febrile response commences. A subcutaneous implant of doxycycline at the time of immunization will eliminate the labor-intensive tetracycline treatment method. The immunity will not be affected by the antibiotic treatment. Flock immunization of sheep and goats can be accomplished by inoculation followed by mass treatment at the end of the incubation period.

Immunologically different strains of the organism do exist, but present evidence indicates that there is considerable cross-protection between different strains, thus allowing successful immunization. However, there are some stains between which there is little cross-protection.

A strain of *C. ruminantium*, attenuated by serial passage in bovine umbilical endothelium cells has been shown to confer solid HW immunity to sheep and goats. This finding suggests that a live-attenuated vaccine to HW may soon be available, but because other strains of the organism have not become attenuated by cell-culture passage, the degree of cross-protection between strains still needs clarification. A universally effective vaccine is probably not imminent.

Control and Eradication

Tick Control

The HW organism is extremely fragile and cannot persist outside of a host for more than a few hours. The principal mode of bringing the disease into an area is thus through introduction of infected ticks or carrier animals. It is not known for how long wild or domestic ruminants can be a source of infection for ticks in nature, but Andrew and Norval (1989) have shown that experimentally infected sheep, cattle, and African buffalo can be a source of infection for nymphs of the bont tick (*A. hebraeum*) for 223, 246, and 161 days, respectively. After molting to adults, the ticks transmit the disease to susceptible sheep. This prolonged carrier state needs to be considered when animals are moved from HW-enzootic to HW-free areas. It is also not known for how long a tick can remain a carrier of the organism. Careful dipping and hand-dressing followed by inspection to ensure the absence of ticks is recommended for animals in transit to HW free areas.

Vector control measures aimed at eradication of *Amblyomma* ticks by dipping of cattle have failed principally because the vector is a multihost tick with a high rate of reproduction. The development of acaricide resistance has further complicated attempts at tick control. In enzootic areas, tick levels are now allowed to remain at levels high enough to permit reinfection of immune animals to booster the immunity.

Chemoprophylaxis

Cattle, sheep, and goats moving into an HW-enzootic area can be protected from HW by prophylactic treatment with tetracycline (short or long-acting) either by feeding or by inoculation. However, they should be kept under surveillance and individually treated if clinical signs are seen.

Public Health: Humans are not known to be susceptible to *Cowdria ruminantium.*

1.10 Foot-and-mouth disease[11]

(Afta epizootica, Bek-en-klouseer, Fiebra aftosa, Fievre aphteuse, Maul-und-Klauenseuche)

Definition: Foot-and-mouth disease (FMD) is a highly contagious viral infection primarily of cloven-hoofed domestic animals (cattle, pigs, sheep, goats, and water buffalo) and cloven-hoofed wild animals. The disease is characterized by fever and vesicles with subsequent erosions in the mouth, nares, muzzle, feet, or teats.

Etiology:

The FMD virus (FMDV) is a member of the genus *Aphthovirus* in the family Picornaviridae. There are seven serotypes of FMDV: A, O, C, Asia 1, and Southern African Territories (SAT) 1, 2 and 3. Within these serotypes, over 60 subtypes have been described, and new subtypes occasionally arise spontaneously. However, at a specific time, there are only a few subtypes causing disease throughout FMD endemic areas. The importance of subtypes is that a vaccine may have to be tailored to the subtype present in the area in which the vaccine is being used.

The FMD virus is pH sensitive; the virion is inactivated when exposed to pH below 6.5 or above 11. However, in milk and milk products, the virion is protected and can survive at 70^{o} C for 15 seconds and pH 4.6. Between pH 6.7 and 9, stability increases with decreasing temperature; the virus in cell culture medium will remain viable for a year at 4^{o} C. The virus in serum or other organic material will survive drying and can be carried on inanimate objects. In meat, the virus can survive for long periods in chilled or frozen bone marrow and lymph nodes.

Host Range: Cloven-footed domestic and wild animals are primarily affected. Examples of other susceptible species are hedgehogs, armadillos, nutrias, elephants, capybaras, rats, and mice.

Geographic Distribution: Foot-and-mouth disease, after World War II, was widely distributed throughout the world. In 1996, endemic areas were Asia, Africa, and parts of South America. In South America, Chile is free, and Uruguay and Argentina have not had an outbreak since April 1994. Most European countries have been recognized as free. Countries belonging to the European Union have stopped FMD vaccination. North and Central America, Australia, New Zealand, Japan, and the British Isles have been free of FMD for many years.

Geographic Serotype Prevalence of FMD: It is interesting how certain serotypes tend to be restricted to certain areas of the world.

Some examples are as follows:

Europe (historically)	A (5) O (1) C (1)
Asia	
Near East	A (22) O (1)
Middle East	A (22) O (1) C Asia (1)
Far East	A O (1) C Asia (1)

[11]Committee on Foreign Animal Diseases of the United States Animal Health Association, revised 1998. Foreign Animal Diseases, "The Gray Book," Part IV, Library of Congress Catalog Card Number 17-12842, Pat Campbell & Associates and Carter Printing Company, Richmond, VA. http://www.vet.uga.edu/vpp/gray_book/FAD/FMD.htm

Africa	Serotype C is uncommon in Africa
Central East to West	A O
Northeast Central and South	SAT 1 and 2
South	SAT 3
South America	A (24), (27) O (1) C (3)

Transmission

The FMD virus can be introduced into a free area by the following means:

1. Direct or indirect contact with infected animals.

2. Spread of aerosol from infected animals (requires proper humidity and temperature). Aerosol from bulk milk trucks spread FMD in England. A person in contact with infected animals can have sufficient FMDV in his or her respiratory tract for 24 hours to serve as a source of infection for susceptible animals.

3. Feeding contaminated garbage (meat, milk, blood, glands, bones, cheese, etc.)

4. Contact with contaminated objects (hands, footwear, clothing).

5. Artificial insemination.

6. Contaminated biologicals such as hormones (extraction procedure may not inactivate the virus).

After an animal becomes infected by any means, the primary mode of spread is then via respiratory aerosols. Other important means of spread are direct and indirect contact. In an outbreak of FMD, the roles of the three primary hosts in transmission are as follows:

- Sheep act as maintenance hosts,
- Pigs act as amplifiers,
- Cattle act as indicators.

When sheep or goats become infected with FMDV, the disease may not be diagnosed for a considerable time because signs and lesions can be very mild. However, during this time, the animals will be producing infectious aerosols, contaminating fomites, and spreading the virus by contact.

Foot-and-mouth disease in pigs spreads very rapidly, for they produce 30 to 100 times more virus in aerosols than sheep or cattle. An infected pig can produce a hundred million infectious doses per day.

When cattle are infected with FMDV, signs and lesions usually develop more rapidly and are more severe than in pigs, sheep, or goats. If cattle, sheep, and pigs are exposed together, cattle will usually get sick first. This may result from increased exposure due to a greater pulmonary tidal volume.

Some animals can be carriers of FMDV. Most ruminant species can harbor the virus in their pharyngeal tissues for a long period. Recovered cattle or vaccinated cattle exposed to diseased animals can become healthy carriers for 6-24 months. Sheep can be carriers for 4-6 months. Although under experimental conditions it has been difficult to demonstrate transmission of FMD from carriers to susceptible livestock, there is strong circumstantial field evidence that carriers may have been the occasional cause of

outbreaks. Also it has been shown that the virus was maintained for many years in a relatively small, isolated group of African buffaloes without the appearance of clinical signs.

Some strains of FMDV seem to have a predilection for certain species. There have been strains that affect pigs but not cattle. In South America, mature cattle have had clinical signs of FMD, when sheep in an adjacent pasture were normal.

Incubation Period: After experimental exposure, signs may develop as early as 12 hours. The usual interval is 24 to 48 hours.

When susceptible animals are in contact with clinically infected animals (peak time of transmission is generally when vesicles rupture), clinical signs usually develop in 3 to 5 days.

Pigs fed infected garbage usually develop signs in 1 to 3 days. Intact oral epithelium is resistant to infection, but during the process of ingesting food there may be injury, and the virus may also enter through the tonsils.

Clinical Signs

Cattle

Initial signs are fever of 103-105^{o} F (39.4-40.6^{o} C), dullness, anorexia, and fall in milk production. These signs are followed by excessive salivation; drooling, serous nasal discharge; shaking, kicking of the feet or lameness; and vesicle (blister) formation. Sites of predilection for vesicles are the tongue, dental pad, gums, soft palate, nostrils, muzzle, interdigital space, coronary band, and teats. Vesicles may be difficult to see. The animal may need to be tranquilized to facilitate a thorough examination.

After vesicle formation, drooling may be more marked, and nasal discharge, lameness or both may increase. Pregnant cows may abort, and young calves may die without developing any vesicle.

The course of an FMD infection is 2 to 3 weeks. Secondary infection may delay recovery. A lactating animal may not recover to preinfection production because of damage to the secretory tissue.

Sequelae to FMD in Cattle

Secondary infection — mouth, nose, feet

Hoof deformation

Low milk production

Mastitis

Unthriftiness — failure to gain weight

Breeding problems

Panting — associated with pituitary gland damage

Diabetes mellitus

Swine

Initial signs are fever of 104-105^{o} F (40-40.6^{o} C), anorexia, reluctance to move, and squeal when forced to move. These signs are followed by vesicles on the coronary band, vesicles on the heals, vesicles in the interdigital space (foot involvement is usually severe), and vesicles on the snout. Mouth lesions are not too common and when they occur are smaller and of shorter duration than in cattle and tend to be a "dry"-type lesion. There is no drooling. Sows may abort. Piglets may die without showing any clinical sign.

Sheep and Goats

Clinical signs, if they occur, tend to be very mild, and may include dullness; fever; and small vesicles or erosions on the dental pad, lips, gums, and tongue. Mild lameness may be the only sign. In lame animals there may be vesicles or erosion on the coronary band or in the interdigital space. Infected animals may abort. Nursing lambs may die without showing any clinical sign.

Gross Lesions

Cattle

The diagnostic lesions are single or multiple vesicles ranging from 2 mm to 10 cm. These can occur at all sites of predilection. Gross lesions on the tongue usually progress in the following manner:

1. A small blanched whitish area develops in the epithelium.
2. Fluid fills the area, and a vesicle (blister) is formed.
3. Vesicle enlarges and may coalesce with adjacent ones.
4. Vesicle ruptures.
5. Vesicular covering sloughs leaving an eroded (red) area.
6. Gray fibrinous coating forms over the eroded area.
7. Coating becomes yellow, brown or green.
8. Epithelium is restored, but line of demarcation remains; line then gradually fades.

Occasionally "dry" FMD lesions develop. Instead of forming a vesicle, the fluid is apparently lost as it forms and the upper layers of the epithelium become necrotic and discolored. The lesion therefore appears necrotic rather than vesicular.

Gross Lesions on the Feet:

The vesicle in the interdigital space is usually large because of the stress on the epithelium caused by movement and weight. The lesion at the coronary band at first appears blanched; then there is separation of the skin and horn. When healing occurs, new horn is formed, but a line resulting from the coronitis is seen on the wall of the hoof.

Gross Cardiac and Skeletal Lesions:

Animals that die may have grayish or yellowish streaking in the myocardium - degeneration and necrosis. These findings are known as "tiger heart". Skeletal muscle lesions occur but are rare.

Swine

Vesicles on the snout can be large and filled with clear or bloody fluid. Mouth lesions are usually the "dry" type and appear as necrotic epithelium. Feet lesions are usually severe, and the hoof can become detached. Animals that die may have grayish or yellowish streaking in the myocardium with degeneration and necrosis ("tiger heart").

Sheep

Lesions in the mouth and vesicles on the coronary band may be few, small, and difficult to find. Animals that die may have grayish or yellowish streaking in the myocardium with degeneration and necrosis ("tiger heart").

Morbidity and Mortality: The morbidity rate is essentially 100 percent in a susceptible population of domestic animals. Mortality is usually less than 1 percent, but in young animals and with certain isolates mortality can be high. In an FMD outbreak in Israel, there was a high mortality (at least 50 percent) in wild mountain gazelles. The same virus caused typical low mortality in cattle. In the gazelles, there was a severe viral pancreatitis that accounted for the high mortality.

Diagnosis

Field Diagnosis

In cattle, FMD should be considered whenever salivation and lameness occur simultaneously and a vesicular lesion is seen or suspected. Fever often precedes other clinical signs; therefore, febrile animals should be carefully examined. Early diagnostic lesions may be found before animals start to salivate, have a nasal discharge, or become lame. To avoid missing a diagnosis, examine the mouth of a lame animal and the feet of any animal with signs or lesions involving the mouth or nostrils. Typically, FMD spreads

rapidly and there is a high clinical attack rate; however, this cannot be counted upon, for a relatively avirulent strain could appear, or more resistant animals (sheep) could be affected.

In pigs, sheep, and goats, FMD should be considered when animals have sore feet, vesicular lesion is suspected, or both.

Specimens for Laboratory Diagnosis

Because the various vesicular diseases have similar clinical signs, a laboratory diagnosis is mandatory. Oral, nasal, foot, or mammary lesions are good sources of specimens. The following should be collected from each of two or three animals:

1. Vesicular fluid (as much as possible).

2. Epithelium covering a vesicle.

3. Flaps of epithelial tissue still attached.

(For 2 and 3 above, try to collect about 0.5 gm.)

Old necrotic or fibrinous material that is difficult to remove is undesirable and often is highly contaminated with bacteria.

4. About 5 ml of blood with anticoagulant (viremia ends about 5 days after the onset of disease).

5. Esophageal—pharyngeal (OP) fluid from convalescent cattle, sheep, or goats.

This should immediately be diluted with an equal volume of cell culture fluid (e.g., Hanks balanced salt solution with lactalbumin hydolysate) and shaken vigorously for about 1 minute. If the solution turns yellow, the pH is low and the virus could be inactivated; discard and collect another sample.

6. Blood for serum (10 ml of serum).

7. From dead animals, collect samples of epithelial lesions, lymph nodes, thyroid, adrenal gland, kidney, and heart (about 10 gm).

8. Full set of tissues in formalin.

If the specimens can be delivered to a laboratory within 24 hours, they should be placed on ice. If delivery will take longer, quickfreeze the specimens, and do not allow them to thaw during transit. If dry ice is used, be sure that the vials are tightly sealed with stopper and tape so that no carbon dioxide enters the vial. The carbon dioxide will lower the pH and inactivate FMDV. Epithelium can also be placed in buffered glycerin and kept at 39^{o} F (4^{o} C) or -4^{o} F (-20^{o} C). Ratio of epithelium to glycerin should not exceed 1:10.

Laboratory Diagnosis

To confirm the initial case of FMD, the virus has to be isolated and identified. After confirmation of the initial case, diagnosis can be made by antigen or nucleic acid detection, or both.

Serological tests are available to detect antibody and differentiate infected and vaccinated animals.

Differential Diagnosis

Differential diagnosis for FMD should include vesicular stomatitis, swine vesicular disease, vesicular exanthema of swine, foot rot, and chemical and thermal burns. In cattle, oral lesions caused by rinderpest, infectious bovine rhinopneumonitis, bovine virus diarrhea, malignant catarrhal fever, and bluetongue can be similar to the later lesions in FMD. In sheep, lesions caused by bluetongue, contagious ecthyma, and lip and leg ulceration can be similar to the later lesions of FMD.

Vaccination: Starting about 1951, FMD vaccine was produced by the Frenkel method. Normal tongue epithelium was removed, minced, placed in a nutrient broth, and inoculated with FMDV. After replication of FMDV, the virus was inactivated with formalin, and aluminum hydroxide was added as an adjuvant. This method as well as virus propagation in cell culture is being used today to produce FMD vaccine.

Outbreaks of FMD have been traced to use of formalin-inactivated vaccine. Apparently, in some cases, vaccine contained viable virus. Today (1996) the classical FMD vaccines are prepared using binary-ethyleneimine (BEI) inactivated virus and aluminum hydroxide-saponin or oil as an adjuvant. Double

emulsion oil vaccines have been shown to produce an immunity of longer duration than aluminum hydroxide-saponin vaccine.

To date, molecular-engineered vaccines have not been as effective or as economical as the cell culture vaccines.

When vaccinating animals, it is important that the vaccine contain the same subtype of virus as is in the area. This necessitates frequent checking of the serotype and subtype during an outbreak because FMD virus frequently changes during natural passage through various species.

Protection induced by a good aluminum hydroxide vaccine decreases rapidly in 4-6 months. A double emulsion oil vaccine can protect for up to 1 year.

Vaccinated animals that are not completely protected can be a source of infection. The virus may replicate and be shed, but the animals may not show any clinical sign of infection.

Control and Eradication: The official attitude of a country regarding control of a disease depends on how seriously the disease affects the country, the financial and technical ability of the country, and what its neighbors are doing. The degree of control of FMD varies as follows:

1. Virtually no control in some Asian and African countries where FMD is enzootic.

2. Protection of valuable or accessible animals or vaccination along a border to provide a buffer zone. (May vaccinate cattle because of severity of the disease but not sheep and goats.)

3. Large-scale vaccination and quarantine with or without slaughter of infected animals.

4. Regulatory measures to prevent entry of FMD virus and quarantine and implementation of an eradication program.

A country where FMD is endemic should be as concerned about introduction of FMD virus as a country that is free of FMD because the introduced virus may be a serotype to which the native animals have no immunity.

The following are the essential features of a control and eradication program:

1. Stop movement of animals and animal products in the area affected.

2. Slaughter infected animals (and known contact animals).

3. Destroy carcasses.

4. Disinfect vehicles leaving the infected area.

5. Perform vaccination.

If eradication by slaughter fails, vaccination may be used to control the outbreak. There are experimental results indicating that potent vaccine may induce significant immunity in 4 days to protect exposed cattle to FMD.

6. Inform and educate the community.

Most developed countries have detailed plans to deal with an outbreak of FMD.

Public Health: In a review of the zoonotic aspects of FMD by K. Bauer in 1997, he reported that, since 1921, FMD virus has been isolated and typed from slightly over 40 human cases. The cases occurred on three continents: Europe, Africa, and South America. Type O predominated, followed by C, and rarely A. Because infection is uncommon, FMD is not considered to be a public health problem.

1.11 Goat pox virus[12]

Sheep and Goat Pox

[12]Committee on Foreign Animal Diseases of the United States Animal Health Association, revised 1998. Foreign Animal Diseases, "The Gray Book," Part IV, Library of Congress Catalog Card Number 17-12842, Pat Campbell & Associates and Carter Printing Company, Richmond, VA. http://www.vet.uga.edu/vpp/gray_book/FAD/SGP.htm

***Definition*:** Sheep and goat pox (SGP) is an acute to chronic disease of sheep and goats characterized by generalized pox lesions throughout the skin and mucous membranes, a persistent fever, lymphadenitis, and often a focal viral pneumonia with lesions distributed uniformly throughout the lungs. Subclinical cases may occur.

Etiology: The virus that causes SGP is a capripoxvirus, one of the largest viruses (170-260 nm by 300-450 nm). It is closely related to the virus that causes lumpy skin disease; SGP virus and lumpy skin disease virus cannot be distinguished serologically. There is only one serotype of SGP virus (SGPV). Various strains of SGPV cause disease only in sheep, others only in goats, and some in both sheep and goats.

The SGPV is very resistant to physical and chemical agents.

Host Range: Sheep and goat pox virus causes clinical disease in sheep and goats. The virus replicates in cattle but does not cause clinical disease. The disease has not been detected in wild ungulate populations.

Geographic Distribution: The disease is endemic in Africa, the Middle East, the Indian subcontinent, and much of Asia.

A goat-pox-like disease was reported in the western United States, but no attempt was made to indentify the agent with a reference serum against SGPV. Serum samples from animals representing the affected group of goats were submitted to the Foreign Animal Disease Diagnostic Laboratory (FADDL) at Plum Island, NY, and tested for antibody to SGPV; no antibodies were found against SGPV. The serums were not tested for antibodies to bovid herpesvirus 2 or contagious ecthyma at the FADDL. Unfortunately, the viral isolate was not available for study. It is conclusive that what was reported in the literature was not goat pox.

***Transmission*:** Contact is the main means of transmission of SGPV. Inhalation of aerosols from acutely affected animals, aerosols generated from dust contaminated from pox scabs in barns and night holding areas, and contact through skin abrasions either by fomites or by direct contact are the natural means of transmitting SGPV. Insect transmission is possible. The virus can cause infection experimentally by intravenous, intradermal, intranasal, or subcutaneous inoculation.

Incubation Period: Under field conditions, the incubation of SGP is between 4 and 8 days. Experimentally, the first sign (fever) may appear within 3 to 5 days after inoculation. The course of the disease is 4 to 6 weeks with various stages of pox lesions present at the same time. Full recovery may take up to 3 months.

Clinical Signs: Sheep and goat pox virus may cause subclinical infection; clinical cases vary from mild to severe. The course of the disease in sheep and goats is similar. The first signs may include fever, depression, conjunctivitis, lacrimation, and rhinitis. Within a few days of the prodromal signs, pox lesions develop in the skin. These are more easily observed on the wool-free or hair-free parts of the body such as the perineum, inguinal area, scrotum, udder, axilla, and muzzle. Lesions do occur in wooled or haired skin. Generally, more severe (extensive) skin lesions correlate with more severe illness. The skin lesion first appears as an erythematous area (macula). This lesion progress to a raised, slightly blanched lesion that presents erythema with edema in the central part of the lesion (papule). Pox lesions with a transudate, representing the vesicular stage of the lesion, may be noted, but rarely is there any gross vesicle in the skin. The center of the lesion then becomes depressed and gray (necrotic) and is surrounded by an area of hyperemia. Late in the course of the disease (2 to 4 weeks after the first signs), the lesions become dry, and a scab forms. A characteristic feature of a pox lesion is that lesions involve the entire epidermis and dermis and penetrate into the subcutaneous tissue; it feels like a nodule. Depending on the severity of the skin lesion, there may be a scar, an area devoid of wool or hair, after the lesion heals. Secondary bacterial infection may complicate the healing process. The muzzle may be swollen, and the nares and oral mucosa may have extensive lesions. In many cases, pneumonia may occur with labored breathing and a respiratory rate approaching 90 per minute. Depression, anorexia, and emaciation are common and may persist. Nervous signs may occur, but how these are related to the SGPV infection is not clear.

Lambs and kids under 1 month of age may suffer a very severe generalized form of SGP. The signs described above for older animals are exaggerated, and there is an increased mortality.

Gross Lesions: At necropsy, skin lesions have congestion, hemorrhage, edema, vasculitis, and necrosis and will be seen to involve all layers of the epidermis, dermis, and, in severe cases, extend into the adjacent musculature. Lymph nodes draining affected areas are enlarged up to eight times their normal size owing to extensive lymphoid proliferation, edema, congestion, and hemorrhage.

Mucous membranes of the eye, mouth, and nose have pox lesions that, in severe cases, may coalesce. In severe cases of SGP, the eyelids may be so seriously affected that the proliferative lesions and inflammation cause the eyes to close. Lesions on the muzzle and nares may coalesce, and proliferative changes and inflammation may be extensive. Pox lesions may occur in the pharynx, epiglottis, and trachea. These usually appear as rounded blanched areas surrounded by an area of hyperemia. Occasionally there may be lesions in the epithelium of the rumen and omasum.

Pox lesions in the lungs may be severe and extensive; the lesions are focal and uniformly distributed throughout the lungs as the result of hematogenous infection. Early lesions are congested areas; these then progress to discrete areas of congestion and edema and finally to white nodules. Areas distal to the pox lesions have lobular atelectasis. Mediastinal lymph nodes are often enlarged up to five times their normal size and may be congested, hemorrhagic, and edematous.

Pox lesions also may be present on the vulva, prepuce, testicles, udder, and teats.

Morbidity and Mortality: The severity of SGP varies depending on the strain of the virus and the age and breed of the animals affected. In adult sheep and goats, morbidity may range to 80 percent with some subclinical infections. Mortality can approach 50 percent. In susceptible lambs and kids under 1 month of age, morbidity may approach 100 percent, and mortality may be as high as 95 perent. Factors that may complicate the course of the disease and increase the mortality are poor nutrition, heavy parasitism, and severe climatic conditions.

Diagnosis

Field Diagnosis

A tentative diagnosis of SGP can be made on the basis of clinical signs consisting of skin lesions, which on, palpation involve the whole thickness of the skin, a persistent fever, lymphadenitis, and often pneumonia; mortality may approach 50 percent in adults and 95 percent in lambs and kids under 1 month of age.

Specimens for Laboratory

For laboratory diagnosis of SGP, skin biopsies of early lesions can be used for virus isolation and histopathologic and electron microscopic studies. Samples aspirated from enlarged lymph nodes can be used for virus isolation. Necropsy samples should include a full set of tissues, but samples of the lungs, trachea, and rumen containing gross lesions are especially valuable for histopathology. Samples for virus isolation should be shipped to the laboratory under wet ice if they will arrive in 2 days and shipped under dry ice if delivery will take longer (send in screw-capped vials with the caps secured with electrical tape). Samples for histopathology should be preserved in 10 percent buffered formalin (DO NOT FREEZE). Serum samples should be taken from acute and chronic cases. Followup serum samples from acute cases may be taken 2 to 3 weeks after the first sample.

Laboratory Diagnosis

The laboratory procedures for the diagnosis of SGP include virus isolation; observation of the virus by electron microscopy; detection of antibody by virus neutralization, the indirect fluorescent antibody test, or both; and characteristic histopathologic lesions.

Differential Diagnosis

Following are several diseases to consider in the differential diagnosis for SGP:

Bluetongue — Animals are depressed and have a nonpurulent conjunctivitis. The muzzle is swollen, congested, and edematous, and there may be a coronitis. Deformed aborted fetuses and deformed newborn sheep and goats may be encountered.

Peste des Petits Ruminants — Conjunctivitis, rhinitis, and oral lesions that are white, raised, and necrotic are common. Pneumonia, diarrhea, and mortality approaching 90 percent in lambs and kids under 1 month of age are characteristic signs.

Contagious Ecthyma (contagious pustular dermatitis, ORF) — This disease is most severe in lambs and kids. The proliferative pox lesions are common on the muzzle and eyes of affected neonates; mortality may approach 50 percent. Nursing females may have proliferative pox lesions on the teats and muzzle. This is a zoonotic disease; lesions in attendants are not uncommon.

Photosensitization — Dry, flaky, inflamed areas are confined to the nonpigmented parts of the skin.

Insect bites — The trauma from insect bites may cause local inflammation, edema, and pruritus. Insects seldom bite mucous membranes.

Parasitic pneumonia — Severe signs of respiratory distress may occur with extensive parasitic lesions; in these cases, there is no pox lesion in the skin.

Caseous lymphadenitis — Focal, raised lesions in the skin represent caseous abscesses; abscesses are not seen in SGP.

Streptothricosis (*Dermatophilus congolensis* infection) — Lesions are superficial and often moist. Lesions are common in the skin of the neck, axillary region, inguinal region, and perineum. The organism may be demonstrated by Giemsa staining.

Mange - Scab-like skin lesions are seen in psoroptic mange. Itching and scratching are not seen in SGP.

***Vaccination*:** In endemic areas, vaccination is an effective means of controlling losses from SGP. Killed vaccines have not proven to be practical under field conditions because they do not provide solid lasting immunity. Several modified live virus vaccines have been used for protection against SGP. The most widely employed vaccine is probably the Romanian strain that has been used effectively for many years. The Kenya O 180 strain is possibly the vaccine with the best safety and efficacy.

Control and Eradication

Prevention

The most likely manner for SGP to enter a new area is by introduction of infected animals. Restrictions on the movement of animals and animal products (meat, hair, wool, and hides) are essential to prevent introduction of SGP. Wool, hair, and hides must be subjected to suitable decontamination procedures before entry into nonendemic areas.

Control

If a new case is confirmed in a new area before extensive spread occurs, the area should be quarantined, infected and exposed animals should be slaughtered, and the premises cleaned and disinfected. Vaccination of susceptible animals on premises surrounding the infected flock(s) should be considered.

If the disease has spread over a large area, the most effective means of controlling losses from SGP is vaccination; however, consideration should be given to eliminating infected and exposed flocks by slaughter; properly disposing of animals and contaminated material; and cleaning and disinfecting contaminated premises, equipment, and facilities.

Eradication

A carrier state has not been shown for SGPV. However, the virus may persist for many months on contaminated premises. The imposition of quarantines on areas and premises containing infected or exposed animals is required to prevent disease spread. Depopulation of infected and exposed flocks should be used if limited spread has occurred. If the disease has spread extensively, massive vaccination followed by cessation of vaccination and control of animal movements from the area represent a strong strategy to control and then eradicate SGP.

Public Health: There is no conclusive evidence that SGPV infects humans. A report from India that implied that goat pox caused human infection was merely based on clinical signs. There was no attempt to isolate the causative virus or perform serology on the convalescent serums of the three patients to differentiate the infection from contagious ecthyma, which is a known zoonotic agent that occurs worldwide. A report from Sweden indicated that human infection occurred during an outbreak of goat pox. Although serological studies seemed to indicate that the apparent causative agent of the outbreak was not vaccinia or contagious ecthyma, no virus was isolated. Therefore, it cannot be said that goat pox virus caused human infection.

1.12 Japanese encephalitis virus[13]

(Japanese B encephalitis)

Definition: Japanese encephalitis (JE) is an arthropod-borne virus disease affecting the central nervous system (CNS) of human beings and, less frequently, horses. The infection also results in the birth of litters of pigs with a high percentage of stillbirths or pigs affected with encephalitis.

Etiology: The JE virus is a member of the family Flaviviridae and is in the genus *Flavivirus*. Host range and other characteristics are described in detail in the International Catalogue of Arboviruses.

Host Range: People and horses are victims of the JE virus infection but appear to be dead-end hosts from an epidemiologic standpoint. Viremia levels in infected human beings and equine species are generally too low to provide potential mosquito vectors with an infective blood meal. Under experimental conditions, however, Gould et al. demonstrated horse to horse transmission by *Culex tritaeniorhynchus*. Cattle are frequently infected in enzootic areas but do not develop sickness or viremia.

Swine in Japan and Taiwan are both victims of disease as well as amplifiers of infection in nature. This is particularly true when swine are bred to farrow at a time when infected mosquitoes make their first appearance. This type of breeding program is practiced in Japan where, because of immunity or natural seasonal lows in transmission, gilts resist infection during pregnancy, and thus losses due to abnormal litters resulting from JE infection are reduced. However, normal newborn piglets soon lose maternally acquired antibody and are fully susceptible to infection from arthropod vectors.

Although JE infection in shoats is subclinical, viremias are sufficiently high to provide emerging broods of *Cu. tritaeniorhynchus*, which feed readily on swine, with a plentiful source of virus-containing blood. Following a period of extrinsic incubation of virus, the mosquitoes are able to transmit the infection to susceptible vertebrate hosts.

In Japan, herons and egrets play a role in the spread of infection to man and other vertebrates and may be responsible for carrying the virus from rural to urban areas. *Cu. tritaeniorhynchus* feeds readily on herons and egrets and ranges sufficiently high off the ground to feed on the young nesting birds.

Geographic Distribution: Human encephalitis in Japan was recognized as early as 1871, and Japanese encephalitis in epidemic form has been known since 1924 when 4,000 human deaths were recorded in Japan. The epidemiology of the disease was studied extensively after World War II in Japan by scientists of the U.S. Army's 406th Medical General Laboratory. Concurrent with vaccination of people and extensive use of agricultural pesticides in the last three decades, the disease has practically disappeared from Japan.

Japanese encephalitis virus infection is widespread throughout temperate and tropical Asia; increasing numbers of human and equine cases have appeared in India, Nepal, China, Philippines, Sri Lanka, and northern Thailand. The disease in humans is sporadic in Indonesia and northern Australia but is not known in the rest of the world.

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[13]Committee on Foreign Animal Diseases of the United States Animal Health Association, revised 1998. Foreign Animal Diseases, "The Gray Book," Part IV, Library of Congress Catalog Card Number 17-12842, Pat Campbell & Associates and Carter Printing Company, Richmond, VA. http://www.vet.uga.edu/vpp/gray_book/FAD/JEN.htm

Transmission: The virus is maintained in nature in a cycle involving *Culex mosquitoes* of the genera *tritaeniorhynchus*, *annulus*, *fuscocephala*, *gelidus*, and *vishnui* complex. Mosquitoes transmit the virus to many species of birds and to swine.

The sequence of events in temperate Asia is initiated by appearance of virus in mosquitoes in late spring followed by the infection and disease in susceptible horses and swine. This is followed by the appearance of disease in man in August and September. In tropical and semitropical areas of Asia, the seasonal nature of the disease is less marked.

Basically, however, it appears the *Culex* mosquitoes and birds are common factors in the epidemiology of JE, regardless of the region of occurrence, and that swine are involved where they are numerous in Asia.

The mechanism of maintaining the virus over the winter in temperate areas has not been elucidated. Overwintering in mosquitoes is a possibility either in infected hibernating mosquitoes or by transovarial passage. It is also possible that bats may carry the virus for prolonged periods.

Incubation Period: In horses, the incubation period is 8 to 10 days. The time between exposure of pregnant swine to an infectious dose of JE virus and delivery of abnormal litters does not seem to be clearly established, although exposure early in gestation appears more likely to result in abnormal litters than later exposure.

Clinical Signs: In horses, initial signs are fever, impaired locomotion, stupor, and grinding of teeth. Blindness, coma, and death follow in more severe cases. Although the clinical signs resemble those seen in horses with Western equine encephalomyelitis and Eastern equine encephalomyelitis, mortality is relatively low. Inapparent or subclinical infections in horses are far more common than cases of recognizable encephalitis.

The principal manifestation of disease in swine is the expulsion of litters of stillborn or mummified fetuses, usually at term. Viable piglets frequently die shortly after birth and exhibit tremor and convulsions before expiring. Experimental infection of boars leads to diminished sperm count and decreased mobility of sperm. Virus has been transmitted to gilts by way of infected semen.

Gross Lesions: In horses, gross lesions are similar to those observed in animals dying from Eastern equine encephalomyelitis and Western equine encephalomyelitis virus infections and are not specific enough to establish an etiologic diagnosis. Litters from infected pigs contain fetuses that are mummified and dark in appearance. Hydrocephalus, cerebellar hypoplasia, and spinal hypomyelinogenesis have been noted.

Morbidity and Mortality: The equine mortality caused by JE has been reported at about 5 percent in Japan and may actually be less than this in Southeast Asia. Mortality in adult pigs is close to zero. Litters of pigs from infected sows may be dead at delivery or, if living, may be quite weak and apt to succumb to encephalitis shortly after birth.

Diagnosis

Field Diagnosis

Presumptive diagnosis can be made in horses that manifest CNS disease accompanied by fever, particularly in an epizootic period. It has been observed that illness in horses at race tracks in Malaysia is frequently due to JE infection. The infection is manifested only by fever and a short period of lethargy. In temperate zones, the disease appears during late summer and early fall.

A presumptive diagnosis in swine is based on the birth of litters with a high percentage of stillborn or weak piglets.

Specimens for Laboratory

One half of a brain from animals having signs of encephalitis should be submitted unfixed and the other half fixed in 10 percent formalin. Paired serum samples collected at least 14 days apart should be submitted from animals that survive. Cerebrospinal fluid from horses with CNS signs should be submitted for detection of JE-specific IgM.

Laboratory Diagnosis

Confirmation of JE can be accomplished by demonstrating seroconversion in animals that survive long enough to yield properly spaced blood samples. Neutralization, complement fixation, hemagglutination inhibition, immunofluorescence, and enzyme-linked immonosorbent assay tests are used to show a rise in titer from the acute stage to death or recovery. Reliance on seroconversion or IgM as a means of diagnosis in horses is not definitive because seroconversion may have resulted from exposure to another nonpathogenic *Flavivirus*.

Demonstration of JE-specific IgM in serum of an encephalitic equine is presumptive evidence of the diagnosis.

Further confirmation of JE in horses can be obtained by examination of the cerebrospinal fluid and the brain. Specific IgM in the spinal fluid is excellent evidence of CNS infection. Although microscopic lesions of the brain are of value, definitive confirmation is based on isolation and identification of the virus from the brain. Virus isolations are more likely to be successful from brains of animals that died after a short course of the disease.

Confirmation of JE in diseased litters of pigs is accomplished by isolation of the virus from fetal brains or brains of piglets that die after manifesting signs of encephalitis. Demonstration of antibody increase in dams bearing affected litters is probably not a reliable measure because seroconversion in such animals would probably have occurred earlier in infection.

Differential Diagnosis

The disease in horses must be differentiated from other viral encephalitides. In Asia, JE is the only recognized arboviral infection causing encephalitis in horses. Because there are many mild or subclinical infections, laboratory confirmation is essential.

Various forms of toxic encephalitis must be considered in differential diagnosis. In temperate-zone Asia, the midsummer seasonal occurrence of JE in horses aids in differential diagnosis.

Japanese encephalitis in pigs must be differentiated from a hemagglutinating DNA virus infection that appears to be as commonplace in Japan as JE and causes the same pattern of disease. There is evidence that the DNA virus infection is established in gilts in the middle or last trimester of pregnancy. Seasonal patterns of DNA virus infection need more complete study, but the disease does appear concurrently with Japanese encephalitis and therefore requires laboratory tests for differentiation.

Another hemagglutinating virus, myxovirus parainfluenza 1 (Sendai), has been shown capable of producing stillbirth in swine under experimental conditions. Encephalitis in neonatal pigs is also associated with a coronavirus infection. This agent is known to cause encephalitis in piglets in at least North America and Europe.

Vaccination: A live attenuated vaccine produced in hamster kidney tissue culture is in widespread use in horses in China. This vaccine reduced disease by about 85 percent. An inactivated vaccine prepared in mouse brain is licensed in Japan, Korea, Taiwan, India, and Thailand for use in humans. A similar inactivated product made in hamster kidney tissue culture has been used to immunize children annually in China since 1965. Live attenuated vaccines are used to immunize pigs in Japan and Taiwan and humans in China.

Control and Eradication: Options for control include elimination of the vectors, prevention of amplification of the infection cycle in birds and pigs, or immunization of horses, pigs, and people. Although some success in vector control was achieved by modification of irrigation methods to minimize breeding of *Cu. tritaeniorhynchus* in Southeast Asia and coincidentally by the use of agricultural pesticides, vector control has never been more than marginally successful. Reduction of the avian reservoir hosts does not appear feasible.

The most promising approach to reducing livestock losses and at the same time reducing the totality of infection in nature is widespread immunization of swine. Live attenuated vaccines are in use in Japan and Taiwan. Immunization of shoats prevents infection in vaccinees and neutralizes their role as amplifiers of

infection in nature. It is anticipated that those animals retained for breeding will remain immune, and, because of immunity or natural seasonal lows in transmission resist infection during pregnancy and therefore bear normal litters. Although controlling the disease in swine dampens the spread of infection in nature, there is a continued threat to horses and human beings from other sources.

The introduction of JE virus into the United States is always a possibility, but whether the infection, once introduced, would become established in nature is difficult to assess. Animal health authorities must continue to be alert to detecting and identifying agents associated with encephalitis in horses and with abnormal litters of pigs. The means for rapid diagnosis and identification of JE are available, although it is doubtful that control of the disease in Asia will be achieved in the near future.

Public Health: Japanese encephalitis can cause an explosive, highly fatal form of human encephalitis.

1.13 Lumpy skin disease virus[14]

(Pseudo-urticaria, Neethling virus disease, exanthema nodularis bovis, knopvelsiekte)

Definition: Lumpy skin disease (LSD) is an acute to chronic viral disease of cattle characterized by skin nodules that may have inverted conical necrosis (sitfast) with lymphadenitis accompanied by a persistent fever.

Etiology: The causative agent of LSD is a capripoxvirus. The prototype strain of LSD is the Neethling virus. The LSD virus (LSDV) is one of the largest viruses (170-260 by 300-450 nm). There is only one serotype of LSDV. The LSDV is very closely related serologically to the virus of sheep and goat pox (SGP) from which it cannot be distinguished by routine virus neutralization or other serological tests. Restriction endonuclease studies of capripoxviruses indicate that LSDV strains are essentially identical with each other and with a Kenyan strain (O 240/KSGP) of sheep and goat pox virus (SGPV). Other strains of SGPV from Kenya were different from the O 240/KSGP strain but similar to each other and resembled strains of SGPV from the Arabian Peninsula. The Kenyan group of SGPV strains showed differences when compared with ones from India, Iraq, and Nigeria.

The LSDV is very resistant to physical and chemical agents. The virus persists in necrotic skin for at least 33 days and remains viable in lesions in air-dried hides for at least 18 days at ambient temperature.

Host Range: Lumpy skin disease is a disorder of cattle. There is inconclusive evidence regarding the infection of water buffalo (*Bubalus*) with LSDV. The African Cape buffalo (*Synercus caffer*) and other wild ungulates have not been infected during epizootics of LSD in Africa. Experimental infection of some species is possible.

Geographic Distribution: Lumpy skin disease was first described in Northern Rhodesia in 1929. Since then, the disease has spread over most of Africa in a series of epizootics. The most recently affected countries include Kuwait in 1986-88 and Egypt in 1988. An outbreak of LSD occurred in Israel in 1989. For the first time, the disease was eradicated by slaughter and vaccination.

Transmission: Biting insects play the major role in the transmission of LSDV. Epidemics of LSD are associated with rainy seasons. The disease spreads in river basins and areas conducive to insect multiplication. *Stomoxys calcitrans* experimentally transmitted LSDV, but biting lice (*Mallophaga* spp.), sucking lice (*Damalinia* spp.), or *Culicoides nubeculosus* did not. In Kenya, *Culex mirificus* but also *Aedes natronius* were in heavy concentration during an LSD epizootic and were associated with transmission. Direct contact seems to play a minor role in the spread of LSD.

Incubation Period: In the field the incubation period is 2 to 5 weeks. Following experimental infection by intradermal inoculation, a lesion usually develops at the inoculation site within 6 to 20 days.

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[14]Committee on Foreign Animal Diseases of the United States Animal Health Association, revised 1998. Foreign Animal Diseases, "The Gray Book," Part IV, Library of Congress Catalog Card Number 17-12842, Pat Campbell & Associates and Carter Printing Company, Richmond, VA .http://www.vet.uga.edu/vpp/gray_book/FAD/LSD.htm

Clinical Signs: Lumpy skin disease virus causes inapparent to severe disease in cattle. All ages of cattle can be affected, but young calves are usually more severely affected. The severity of the disease depends on the dose of the inoculum as well as the susceptibility of the host (*Bos taura* is more susceptible than B*os indicus*) and the route of exposure. A fever 104 to 107^{o} F (40-41.5^{o} C) can occur and can be transitory or last up to 4 weeks. Generally within 2 days after the appearance of the fever, swellings or nodules 1 to 5 cm in diameter appear in the skin and generalization occurs. Depression, anorexia, excessive salivation, oculonasal discharge, agalactia, and emaciation are presented. Nodules 1 to 7 cm in diameter may occur anywhere on the body but especially in the skin of the muzzle, nares, back, legs, scrotum, perineum, eyelids, lower ear, nasal and oral mucosa, and tail. The hair stands erect over early skin lesions. The nodules are painful and involve the epidermis, dermis, and subcutaneous tissue and may even involve the musculature. As the disease progresses, the nodules become necrotic, and eventually a deep scab forms; this lesion is called a sitfast. Secondary bacterial infection can complicate healing and recovery. Lesions on the teats can result in severe secondary bacterial infection with loss of the quarter owing to mastitis.

Where extensive generalization occurs, animals can become lame and reluctant to move because of edema. Lameness also may result from inflammation of the tendons, tendon sheaths (tendosynovitis), joints (synovitis), and laminae (laminitis). Severe edema in the brisket and legs can occur. If secondary bacterial infection develops in the tendon sheaths and joints, pemmanent lameness may result. Superficial lymph nodes such as the mandibular, parotid, prescapular, and prefemoral nodes, draining affected areas of skin become enlarged 4 to l0 times normal size.

Abortion may occur as the result of prolonged fever. Davies has reported intrauterine infection of late-term fetuses in which calves are born with LSD lesions. Temporary or pemmanent sterility in bulls can result from the fever or lesions of the reproductive organs. Cows may not come into estrus for several months after LSD.

The lesions may persist in various stages over a course of 4 to 6 weeks. Final resolution of lesions may take 2 to 6 months, and nodules can remain visible 1 to 2 years. Permanent damage to the hide is inevitable in clinical cases.

Gross Lesions: The gross lesions of LSD are well described. Skin nodules have congestion, hemorrhage, edema, and vasculitis with consequent necrosis and involve all layers of the epidermis, dermis, subcutaneous tissue, and often adjacent musculature. Lymph nodes draining affected areas are enlarged up to 10 times normal size with extensive lymphoid proliferation, edema, congestion, and hemorrhage.

Mucous membranes of the oral and nasal cavities can have pox lesions that coalesce in severe cases. Pox lesions may occur in the pharynx, epiglottis, and trachea. Pox lesions are not easily visualized in the lungs but appear as focal areas of atelectasis and edema. In severe cases, pleuritis can occur with enlargement of the mediastinal lymph nodes.

Synovitis and tendosynovitis with fibrin in the synovial fluid can occur. Pox lesions can be present in the testicles and urinary bladder.

Morbidity and Mortality: Morbidity for LSD varies from 3 to 85 percent and likely depends on prevalence of the mechanical insect vector and the susceptibility of the cattle. Mortality is generally low (1 to 3 percent). In one outbreak in South Africa, mortality was about 20 percent when an anaplasmosis vaccine was prepared from bovine blood contaminated with LSDV. Unusually high mortality (75 to 85 percent) in other outbreaks of LSD was not explained.

Diagnosis

Field Diagnosis

A tentative diagnosis of LSD can be made based upon clinical signs. A contagious disease with generalized skin nodules having a characteristic inverted conical necrosis of skin nodules (sitfast), persistent fever, emaciation, and low mortality suggests LSD.

Specimens for Laboratory

Skin biopsies of early lesions (ones where necrosis has not occurred) provide samples that can be used for virus isolation, histopathology, and electron microscopy. Samples should be taken from at least three animals. Samples aspirated from enlarged lymph nodes can be used for virus isolation. Samples for virus isolation should be shipped to the laboratory under wet ice if they will arrive in 2 days and be shipped under dry ice if more time will be required. Samples for histopathology should be preserved in 10 percent buffered formalin (DO NOT FREEZE). Serum samples should be taken from acute and chronic cases. Followup serum samples (convalescent samples) should be taken 2 to 3 weeks after the first appearance of skin lesions.

Laboratory Diagnosis

To confirm an initial diagnosis in an LSD-free area, the virus has to be isolated and identified. The laboratory procedures for the diagnosis of LSD include virus isolation in lamb testicle or fetal bovine lung cell cultures or both. Virions may be detected by electron microscopy. Herpesviruses may be present in bovine skin samples and cause confusion in cell culture studies. Serological tests include virus neutralization and indirect fluorescent antibody (IFA). The IFA test may measure group-reactive antibody that may be elicited by other pox viruses.

Differential Diagnosis

Listed below are several diseases that should be considered in the differential diagnosis of LSD:

Bovine herpes mammillitis (also called Allerton virus infection caused by Bovid Herpesvirus-2) — The lesions are superficial (involving only the epidermis) and occur predominantly on the cooler parts of the body such as teats and muzzle. Generalized skin lesions can occur accompanied by a transient fever (1 to 3 days). Resolution of the lesion is rapid and results in focal alopecia but no hide damage.

Streptotrichosis (Dermatophilus congolensis infection) — lesions are superficial (often moist and appear as crusts) scabs or 0.5- to 2-cm diameter accumulations of keratinized material. Lesions are common in the skin of the neck, axillary region, inguinal region, and perineum. The organism can be demonstrated by Giemsa staining.

Ringworm — The lesions of ringworm in cattle are grayish, raised, plaque-like, and often pruritic. The organism can be demonstrated with a silver stain.

Hypoderma bovis infection —The parasitic fly larvae of this parasite have a predilection to migrate to the dorsal skin of the back. They cause a nodule with a small central hole through which the larva exits the body, which results in significant hide damage.

Photosensitization — Dry, flaky, inflamed areas are confined to the nonpigmented parts of the skin.

Bovine papular stomatitis — Pox-like lesions occur in the skin of the muzzle, oral cavity, and esophagus. There is no generalized disease.

Insect bites — The trauma from insect bites causes local inflammation, edema, and pruritus. Insects seldom bite mucous membranes.

Urticaria — Delayed hypersensitivity reactions can be confused with LSD. Such lesions generally resolve within 3 to 5 days. An example of this was described by Shimshony (1989) where allergic reactions occurred after vaccination with a foot-and-mouth disease vaccine.

Besnoitiosis (Globidiosis) — Thick-walled cysts in the skin are caused by sporozoan parasites of the genus *Besnoitia*, which are transmitted mechanically by certain biting flies. Histologic sections will reveal the parasites.

Treatment: Treatment is directed at preventing or controlling secondary infection. Animals infected with LSDV generally recover (mortality is usually less than 3 percent). Complete recovery may take several months and may be prolonged where secondary bacterial infection occurs. Loss of production results from severe emaciation, lowered milk production, extensive damage to hides, and loss of draft from lameness. It may take up to 6 months for animals severely affected by LSDV to recover fully.

Vaccination: In endemic areas, vaccination against LSD has been successfully practiced. In the Union of South Africa, an attenuated LSD vaccine is used. In Kenya, sheep and goat pox virus is used. In Egypt, the Romanian strain of sheep and goat pox vaccine has been used successfully for prophylaxis against LSD.

Control and Eradication: The most likely way for LSD to enter a new area is by introduction of infected animals. Biting insects that have fed on infected cattle may travel and be blown for substantial distances. It is likely that LSD spread to Israel via contaminated insects blown across the Sinai Desert. The movement of contaminated hides represents another potential means for this resistant virus to move.

If LSD is confirmed in a new area before extensive spread occurs, the area should be quarantined, infected and exposed animals slaughtered, and the premises cleaned and disinfected. Vaccination of susceptible animals within the quarantine should be considered.

If the disease has spread over a large area, the most effective means of controlling losses from LSD is vaccination. However, even with vaccination, consideration still should be given to eliminating infected and exposed herds by slaughter, proper disposal of animals and contaminated material, and by cleaning and disinfecting contaminated premises, equipment, and facilities.

In the Union of South Africa, the control of insects was not effective in preventing the spread of LSD, but current insecticides together with repellents aid in the prevention of the spread of LSD.

Public Health: There is no evidence that LSDV infects humans.

1.14 Malignant catarrhal fever virus[15]

(Malignant head catarrh, malignant catarrh, snotsiekte)

Definition: Malignant catarrhal fever (MCF) is a generalized viral disease of domestic cattle and buffaloes and many species of wild ruminants characterized by high fever, profuse nasal discharge, corneal opacity, ophthalmia, generalized lymphadenopathy, leukopenia, and severe inflammation of the conjunctival, oral, and nasal mucosas with necrosis in the oral and nasal cavities sometimes extending into the esophagus and trachea. Occasionally central nervous system (CNS) signs, diarrhea, skin lesions, and nonsuppurative arthritis are observed.

Etiology: The etiologic agent of MCF in Africa is a highly cell-associated lymphotropic herpesvirus of the subfamily *Gamma herpesvirinae*. Two viral strains have recently been designated: alcelaphine herpesvirus-1 (AHV-1) and alcelaphine herpesvirus-2 (AHV-2), although some continue to designate this agent as bovid herpesvirus-3. This agent is carried as a latent infection by African antelope of the family Bovidae, subfamily Alcelaphinae which includes wildebeest (*Connochaetes* sp.), hartebeest (*Alcelaphus* sp.), and topi (*Damaliscus* sp.). The wildebeest herpesvirus of MCF (AHV-1) was first isolated by Plowright from a blue wildebeest (*Connochaetes taurinus taurinus*) in 1960.

Epidemiologic evidence suggests that domestic and wild sheep and goats may be additional major reservoirs of a virus causing MCF. Serologic evidence also suggests this virus may be related but not identical to the alcelaphine herpesvirus-l.

Sheep-associated MCF herpesviruses were isolated from domestic cattle in Minnesota in 1977 and from domestic cattle in Austria in 1990. On the basis of morphology and molecular DNA mapping, both isolates appear similar to AHV-1.

Viruses identical or closely related to AHV-1 and AHV-2 have been isolated from several captive wild ruminant species in two U.S. zoos located in Oklahoma City and San Diego. Animals infected with AHV-

[15]Committee on Foreign Animal Diseases of the United States Animal Health Association, revised 1998. Foreign Animal Diseases, "The Gray Book," Part IV, Library of Congress Catalog Card Number 17-12842, Pat Campbell & Associates and Carter Printing Company, Richmond, VA. http://www.vet.uga.edu/vpp/gray_book/FAD/ahs.htm

1 were white-tailed gnu, white-bearded gnu, gaur, greater kudu, Formosan sika deer, axis deer, and nilgai. The AHV-2 was isolated from a topi and a hartebeest at the San Diego Wild Animal Park.

The agent of MCF on deer farms in Scotland and New Zealand has not yet been demonstrated by electron microscopy or isolation of cell-free virus in a cell culture system. However, it has been passaged in lymphocyte cultures, rabbits, and deer.

Host Range: All species of wildebeest, hartebeest, and topi are considered carriers of alcelaphine MCF virus. There is serologic evidence that several other African wild ruminants, such as various species of oryx and addax, may also be reservoir hosts, although MCF virus has not been isolated from these species.

Domestic and wild sheep and goats are also considered reservoir hosts for MCF virus.

Many exotic ruminant species in zoos have been reported affected with MCF, including several wild bovines such as bison, water buffalo, gaur and banteng, and several deer (including white-tailed deer) and antelope species. Interestingly, no cases of MCF have been reported from antelope species that normally cohabitate wildebeest grazing areas in Africa.

In cattle and susceptible wild ruminants, MCF affects all ages, breeds, and sexes.

Geographic Distribution: Sheep-associated MCF occurs worldwide. The alcelaphine antelope-associated form in cattle occurs chiefly in Africa in the natural habitat of wildebeest, hartebeest, and topi. This form of MCF has, however, occurred in zoos and wild animal parks that also kept wildebeest. The increasing popularity in North America and other areas of the world of wild game animal ranches, often in association with domestic cattle raising, increases the possibility that MCF will become a more prevalent disease in cattle and ranched exotic ruminants. There is increasing serologic evidence that cattle may develop low levels of neutralizing antibodies following exposure to MCF, especially of sheep or goat origin, without manifesting clinical disease. There is evidence that stress or some other immunosuppressive effector may be necessary as a precursor of clinical sheep-associated MCF.

Transmission: The MCF virus in wildebeest, hartebeest, and topi is largely cell-associated in adult animals and hence rarely transmissible. However, neonatal wildebeests have been found to shed cell-free MCF virus in nasal and ocular secretions and in feces. Cell-free MCFV has also been demonstrated in nasal secretions of captive adult wildebeests after stress or administration of corticosteroids. Transmission to cattle or other susceptible species may occur by inhalation of cell-free virus in infectious aerosol droplets, ingestion of feed or water contaminated with infectious secretions or feces, or possibly mechanically by arthropods. Masai herdsmen believed cattle acquire MCF by contact with wildebeest placentas or birth hair of neonates. Recent studies have failed to demonstrate infective MCFV in fetal fluids or placentas of wildebeest probably owing to the rapid inactivation of virus by sunlight. The mode of transmission of sheep-associated MCF remains unknown, although relatively close contact between cattle and sheep, especially lambing ewes, is believed necessary. MCF-affected cattle appear to shed only cell-associated virus, and thus cattle-to-cattle transmission is thought to be rare or nonexistent, although there are documented instances where this has occurred.

Incubation Period: The incubation period in natural cases is not known, but epidemiologic evidence indicates it may be as long as 200 days. Experimentally, the incubation period has varied from 9 to 77 days.

Clinical Signs: Clinical MCF in cattle has arbitrarily been divided into four forms as follows:

1. Peracute form: Fever, severe inflammation of the oral and nasal mucosas and hemorrhagic gastroenteritis with a course of 1 to 3 days.

2. Intestinal form: Fever, diarrhea, hyperemia of oral and nasal mucosas with accompanying discharges, and lymphadenopathy with a course of 4 to 9 days.

3. Head and eye form: This is the typical syndrome of MCF with fever, nasal, and ocular discharges progressing from serous to mucopurulent and purulent. Encrustation of the muzzle and nares occurs in later stages, causing obstruction to the nostrils and dyspnea, open-mouthed breathing, and drooling. There

is intense hyperemia and multifocal or diffuse necrosis of the oral mucosa (usually on the lips, gums, and hard and soft palate) and buccal mucosa. Erosion of the tips of buccal papillae, leaving them reddened and blunted, is often encountered.

Ocular signs referable to ophthalmia include lacrimation progressing to purulent exudation, photophobia, hyperemia, and edema of the palpebral conjunctiva and injection of scleral vessels. Corneal opacity, starting peripherally and progressing centripetally, results in partial to complete blindness. Hypopyon may also be seen. Corneal opacity is usually bilateral but occasionally is unilateral. Fever is common and usually high (104-107° F [40-41.6° C]) until the animal becomes moribund, at which time it is hypothermic. Clinical features at early onset have included reddening of the skin of the udder, the coronary bands and interdigital spaces, and marked hyperemia of the oral cavity. Increased thirst accompanies the fever, and anorexia is seen in late stages. Constipation is common in this form of MCF, but terminal diarrhea is sometimes observed.

Nervous signs are not frequently seen but may be manifested by trembling or shivering, uncoordinated gait, and terminal nystagmus.

Necrotic skin lesions occasionally are seen, and horn and hoof coverings may be loosened or sloughed in some cases. The course of the head and eye form, which is invariably fatal, is usually 7 to 18 days.

4. Mild forms: These are syndromes caused by experimental infection of cattle with attenuated viruses and are usually nonfatal.

There is considerable variation and overlap among these artificial categories, and their use has little value.

Although the manifestations of the "head and eye" form of MCF are considered the typical syndrome in cattle, clinical signs in exotic ruminants are often less dramatic and not usually specifically diagnostic, except in members of the subfamily *Bovinae* (i.e., wild cattle). In deer and antelope species MCF tends to be more subtle clinically and usually is manifested by conjunctivitis, photophobia, moderate corneal clouding (often unilateral), fever, depression, variable lymphadenopathy, occasionally diarrhea, and usually a mild serous nasal discharge. Death may be sudden following a brief course of hemorrhagic diarrhea. Inflammation of the oral and nasal cavity is usually less severe than in cattle and only occasionally progresses to mucosal erosions.

There is some suggestion from studying cases of MCF among exotic ruminants of a host-dependent modification with respect to the clinical and pathologic manifestations of MCF virus infection.

Gross Lesions: Gross lesions vary considerably, depending on the form or severity and course of the disease. Animals that die of the peracute disease may have few lesions other than a hemorrhagic enterocolitis.

In the more protracted acute to subacute disease (intestinal and head and eye forms), the carcass may be normal, dehydrated, or emaciated. The muzzle is often encrusted and raw. Cutaneous lesions sometimes occur as a generalized exanthema with exudation of lymph causing crusting and matting of the hair. Where skin is unpigmented, hyperemia is apparent. These lesions are frequently seen in the ventral thorax and abdomen, inguinal region, perineum and loins, and sometimes on the head.

Enlarged lymph nodes are characteristic findings in MCF. All nodes may be involved, but those in the head and neck and periphery are the most consistently prominent. Affected nodes are grossly enlarged and edematous and sometimes have patchy reddened or beige-brown areas on cut surfaces. Hemolymph nodes are also enlarged and prominent. The spleen is slightly enlarged, and Malpighian corpuscles are prominent. Pale areas may be seen in the heart muscle.

Lesions in the respiratory system range from mild to severe. When the clinical course is short, there is slight serous nasal discharge and hyperemia of the nasal mucosa. Later the discharge becomes more copious and mucopurulent to purulent and is accompanied by intense nasal mucosal hyperemia, edema, and small focal erosions.

Occasionally a croupous pseudomembrane formation is seen. Lesions in the nasal passages and turbinates may extend to the frontal sinuses. The pharyngeal and laryngeal mucosas are hyperemic and edematous

and later develop multiple erosions, often covered with gray-yellow pseudomembranes. Inflammation and sometimes petechiation and ulceration are seen in the tracheobronchial mucosa. The lungs are often edematous and sometimes emphysematous but in some cases may appear normal. A bronchopneumonia may complicate chronic cases.

The alimentary tract mucosa may have no gross lesion in peracute cases. When the course is longer, alimentary lesions are commensurately more severe and include mild to severe mucosal inflammation (hyperemia and edema), erosions, and ulcerations— especially on the dental pad and gingival surfaces, the palate, tongue, and buccal papillae. Mucosal inflammation, hemorrhage, and erosions may also be found in the rest of the digestive tract including the esophagus, rumen, omasum, abomasum, small intestines, colon, and rectum. Petechiation may be seen. Feces are usually scant, dry, pasty, or blood stained.

Urinary tract lesions include hyperemia and sometimes marked distention and prominence of bladder mucosal vessels and mucosal edema, perhaps with petechial to severe hemorrhage and occasionally epithelial erosion and ulceration. Kidneys may appear normal or mottled with patches of beige, discolored raised areas. Petechiae or ecchymoses may occur in the renal pelvis and ureters.

The liver is usually slightly enlarged, and, upon close examination, has a prominent reticular pattern. There may be hemorrhages and erosions in the gallbladder mucosa.

In most cases, small arterioles are very prominent and tortuous and have thickened walls. This is usually seen in subcutaneous vessels and those in the thorax, abdomen, and CNS.

Fibrinous polyarthritis is seen in many cases of MCF.

Morbidity and Mortality: Clinical MCF in cattle in the United States is usually sporadic. However, in an outbreak in a Colorado feedlot, morbidity was 37 percent. Morbidity in nonalcelaphine MCF outbreaks in Malaysia ranged from 28 percent to 45 percent. The prognosis in MCF is poor. Once clinical signs are observed, mortality is usually greater than 95 percent (90-100 percent). In some parts of New Zealand, MCF is, along with tuberculosis, the most important cause of mortality in the deer-farming industry.

Diagnosis

Field Diagnosis

A history indicating contact with sheep, goats, or alcelaphine antelope, especially around the period of parturition, associated with typical clinical features of MCF, provides grounds for a tentative diagnosis of MCF.

Gross necropsy lesions consisting of corneal opacity; enlarged lymph nodes; inflammation and erosions in nasal passages, alimentary tract mucosa, and urinary bladder; and prominent tortuous small arteries in the subcutaneous tissue, thorax, and abdomen, provide further evidence for a presumptive diagnosis of MCF.

Specimens for Laboratory

1. For animal transmission and inoculation at least 300 to 500 ml blood in EDTA (1 mg/ml blood), heparin, or ACD solution should be collected and carried or shipped iced, not frozen. For virus isolation in cell culture, 10 to 20 ml of blood in EDTA is preferred. This should also be shipped cold but not frozen.

2. Tissues for virus isolation, FA, or immunoperoxidase examination should also be refrigerated (iced) but NOT FROZEN and should include pieces of spleen, lung, lymph nodes, adrenals, and thyroids as well as unclotted blood. These should be collected as soon after death as possible, for the virus becomes inactivated rapidly in an animal dead more than 1 hour. The most useful specimens for animal inoculation or virus isolation attempts are those collected from a moribund animal immediately after euthanasia.

3. Tissues for histopathology, fixed as thin pieces in 10 percent neutral buffered formalin, should include lung, kidney, liver, adrenals, lymph nodes, eyes, oral epithelium, esophagus, Peyer's patches, urinary bladder, carotid rete, thyroid, heart muscle, skin (if lesions are present), and whole brain.

4. Serum for serology should consist of paired samples taken 3 to 4 weeks apart (i.e., the first during the acute phase of disease and the second during convalescence or at death). Serologic methods currently preferred include virus neutralization and competitive inhibition enzyme-linked immuosorbent assay for MCF antibody.

Laboratory Diagnosis

Microscopic lesions of an extensive fibrinoid necrotizing vasculitis, perivasculitis, and lymphoreticular proliferation in lymphoid organs with mononuclear infiltrations in kidney, liver, adrenals, CNS, etc., are pathognomonic for MCF and are a sound, practical basis for a confirmed diagnosis.

Virologic and serologic examinations provide additional information that may also ultimately lead to a better understanding of the epizootiology and differences between viral strains and the clinical manifestations. Methods used consist of virus isolation, identification of viral isolates, demonstration of the appearance, or rising titers of MCF antibodies and molecular techniques using viral DNA probes, or target DNA amplifying methods such as the polymerase chain reaction (PCR). Because of the presence of MCF antibodies in asymptomatic U.S. cattle, a single antibody positive serologic sample is of limited value in establishing an etiologic diagnosis. The PCR method for demonstrating MCF DNA segments is proving to be useful for identifying MCF carriers as well as diagnosing overtly diseased animals.

Differential Diagnosis

Clinical MCF must be distinguished from other diseases and factors that produce inflammation and erosions and ulcerations of the nasal and alimentary tract mucosas such as BVD mucosal disease, bluetongue, rinderpest, vesicular diseases (FMD, VS), ingested caustics, and some poisonous plants and mycotoxins. The inability to differentiate the alcelaphine clearly from the sheep-associated MCF by clinical observations, lesions, or laboratory means presents an enigma in evaluating the possibility of a foreign animal disease. With our current knowledge, history of association with sheep, goats, or with alcelaphine antelope remains the only practical means of differentiating one form from the other.

Vaccination: Cattle and experimentally infected rabbits recovered from MCF have a solid immunity against all strains of MCF virus.

An effective vaccine is not available for MCF. Some viral strains have undergone limited attenuation after serial passage in cell cultures and offer hope for a future modified live virus vaccine. Experimental killed virus vaccines have been inconsistent in inducing protection against virulent virus challenge, although some have induced significant titers of serum virus neutralizing antibodies

Control and Eradication: Cattle should be kept separated from potential reservoir hosts such as sheep, goats, and wildebeest — especially during lambing, kidding, or calving seasons, respectively.

The stocking of cattle ranches with alcelaphine antelope, wild sheep, or goats should be discouraged or should require a negative MCF serologic test, preferably by the serum-virus neutralization method, or a negative PCR test for any wild ruminants destined for such a facility. Similar testing of such wild ruminants before being placed in, or transferred between, zoos is also recommended as a means to prevent the introduction of potential carriers of MCF virus.

Containment of an outbreak usually means the immediate separation of cattle or the susceptible host from sheep and goats in the case of the domestic disease and the susceptible host from alcelaphine or wild ruminants in the case of alcelaphine MCF.

Public Health: There is no evidence that MCF is infectious for humans.

1.15 Menangle virus[16]

Summary: An outbreak in New South Wales, Australia of a new paramyxovirus (proposed name is Menangle virus) associated with reproductive failure in swine has been reported.

[16]APHIS, USDA, Center for Emerging Issues, November 1998. Menangle Virus, Australia, Emerging Disease Notice. http://www.aphis.usda.gov/vs/ceah/cei/menangle.htm

Fruit bats have been identified as a probable reservoir of infection.

Serologic evidence and possible clinical signs of infection have been reported in two humans with intense occupational exposure to infected pigs.

Australia is a minor player in the international market for swine and swine products and the proportion of all U.S. imports of swine products originating from Australia is less than 0.05%.

To date, there is no evidence that the outbreak has spread beyond the three associated premises where it was originally identified, and therefore, has had little effect on overall swine production in Australia.

At present, the risk to U.S. swine from this new paramyxovirus appears low. This risk could change however, if for example, other reservoirs are identified, new cases are reported in Australia or elsewhere, U.S. imports of live swine, swine products or the reservoir species from Australia increase, or research into this new virus reveals information that would impact transmission risk.

Background: A farrow to weaning swine operation in New South Wales, Australia experienced a decrease in the pregnancy rate and average litter size and an increase in the proportion of mummified fetuses and stillborn piglets from mid-April to early September 1997. An apparently new virus in the family Paramyxoviridae was isolated from brain, lung and heart of stillborn piglets. The virus isolation was conducted at the Elizabeth Macarthur Agricultural Institute in Menangle, New South Wales, Australia and the proposed name for the new virus is Menangle virus. An assay for neutralizing antibodies to the virus was also developed at the Elizabeth Macarthur Agricultural Institute. The infection occurred in all four breeding units at a commercial operation with 2,600 sows, and in two associated grow to finish operations located several hundred kilometers away from the breeding facility. These associated grow to finish operations received batches of pigs at varying intervals from the farrow to weaning operation. Porcine sera (n=1,114) from other swine operations throughout Australia were tested for antibodies and the results indicate that infection appears to be confined to the affected farrow to weaning operation and the two associated grow to finish operations. There are currently no reports of this new virus anywhere else in the world.

As of October 1998, the infection was still circulating in the farrow to weaning operation and in one grow to finish operation. Most pigs become infected at 12 to 16 weeks of age, after colostral immunity wanes. Among replacement gilts raised on the affected premises, approximately two-thirds are seropositive at entry into the breeding herd. Infection is no longer present in the other grow to finish operation. Eradication of infection in this operation is thought to be due to herd immunity achieved when no shipments of 8 week old pigs were received at the operation for a period of 10 weeks. Eradication plans for the other two premises are being developed. Quarantine measures are in effect. Pigs are only allowed to move off the affected premises directly to slaughter. All of the affected premises are quite geographically isolated from other commercial swine operations.

The pathologic lesions of affected stillborn piglets included the following: severe degeneration of the brain and spinal cord, arthrogryposis (rigid fixation of limbs, usually in flexion), brachygnathia (short lower jaw), occasional fibrinous body cavity effusions, pulmonary hypoplasia, and some had nonsuppurative myocarditis. No postnatal animals of any age manifested clinical disease, however greater than 90% of serum samples collected from pigs of all age categories at the affected operations (n=88) contained high titers (¥256) of neutralizing antibodies against the virus. The farrowing rate decreased to approximately 40% and litter size was also decreased.

Workers at the affected swine operations, slaughter workers, and others who had contact with potentially infected pigs were tested for antibodies to this new virus. Of 33 workers at the farrow to weaning operation, one was seropositive. Five workers were tested from the weaning to finish operations and one was seropositive. Another 218 people who had potential exposure to infected pigs (slaughter workers, researchers, animal handlers, veterinarians, pathology laboratory workers, others) were tested and all were seronegative. The two seropositive swine operation workers had onset of an illness in early June lasting 10-14 days, characterized by fever, chills, rigors, drenching sweats, malaise, headache and spotty red rash. It is suspected that the illness in these two workers was caused by the new virus. One of the

seropositive workers had frequent prolonged contact with birthing pigs and the other seropositive worker performed autopsies on pigs without wearing gloves or protective eye wear. Seroprevalence was 5.26% (2/38) for individuals with high occupational exposure (workers at the swine operations), and if all individuals at risk are included, the seroprevalence was 0.78% (2/256). This low prevalence of seroconversion seems to indicate that the virus is not highly infectious to humans.

Investigators hypothesize that a large colony of fruit bats near the infected farrow to weaning operation were the source of the infection. Serum samples from fruit bats of several species collected both before and after the outbreak, were virus neutralization test positive. However, virus has not been isolated from bats. Samples from rodents, birds, cattle, sheep, cats and a dog were all seronegative.

Characteristics of Paramyxovirus Diseases: The family Paramyxoviridae includes three genera which contain many important viruses of both animals and humans. In the genus Morbillivirus are canine distemper virus, rinderpest virus, peste des petits ruminants virus, equine morbillivirus and the human measles virus. The genus Paramyxovirus includes Newcastle disease virus, four serotypes of parainfluenza viruses (including Sendai virus which affects swine), several serotypes of avian paramyxoviruses, and human mumps virus. The third genus, Pneumovirus, includes the respiratory syncytial viruses (RSV) of humans and cattle and the pneumonia virus of mice.

Many paramyxoviruses produce a serious systemic disease (rinderpest, Newcastle disease, canine distemper) while others are primarily respiratory (parainfluenza, RSV). Many diseases caused by paramyxoviruses have a neurological component (canine distemper, Newcastle disease). Intestinal signs are also common. The incubation periods range from 24 hours (parainfluenza 3 in cattle) to 11 days (equine morbillivirus). Transmission is generally by direct contact as virus is usually shed in all secretions. For some paramyxoviruses transmission can also occur by airborne aerosols and dust particles (Newcastle, canine distemper, RSV) and by contaminated feed and water (Newcastle, rinderpest).

Virus stability varies from stable (Newcastle) to very labile (rinderpest, canine distemper, RSV). Morbidity and mortality vary also; parainfluenza viruses are associated with both low morbidity and low mortality whereas rinderpest, canine distemper and equine morbillivirus are associated with high mortality. A carrier state is present in Newcastle disease with virus shedding for at least four weeks post infection.

The gross pathologic lesions and histopathology described in reports of the Australian outbreak of this new virus, are consistent with a neurotropic paramyxovirus. Morbidity in adult sows is extremely low, with reproductive failure the primary clinical effect. La Piedad Michoacan Virus (LPMV) or "blue eye disease", first isolated in Mexico in 1980, is the only other known paramyxovirus which is associated with reproductive failure in pigs. Newcastle disease virus and equine morbillivirus are other paramyxoviruses reported to cause zoonotic illness.

Probable mode of transmission: Respiratory transmission was suspected as the mode of spread in pigs because of the almost 100% prevalence of virus neutralizing titers in the pig population. Additional studies, however, using sentinel pigs in the affected operations, indicate fecal-oral transmission may be more likely. Transmission from fruit bats to pigs is also hypothesized to have occurred by the fecal-oral route. Fruit bats fly over the farrow to weaning operation buildings when departing their roost at dusk and when returning to the roost at dawn. Paths around the buildings which house the pigs are contaminated with fruit bat feces and pigs are moved on these paths.

Lesions in the affected swine fetuses as mentioned above, indicate viremia probably occurs with the virus crossing the placenta. Virus may be present therefore, in meat, secretions, and excretions of affected animals. Research is needed to determine however, if transmission via meat to animals or humans is possible.

Suspected reservoir and its distribution in the U.S.: The suspected reservoir for this apparently new virus is fruit bats. Serum samples from several species of fruit bat in the family Pteropodidiae were found to be positive for the new virus in the virus neutralization test. These species were Pteropus poliocephalus (grey-headed flying fox), P. alecto (black fruit bats), and P. conspicillatus (spectacled fruit bats).

Pteropodids can be found in tropical and subtropical regions of southern and central Asia, Africa, Australia, the Phillipines and other Pacific islands. Fruit bats in Australia have recently been identified as the reservoir of two other new viruses, equine morbillivirus and a lyssavirus, which cause disease in humans and domestic animals.

The following species in the vicinity of the affected premises were tested for neutralizing antibodies to Menangle virus, and were seronegative: rodents (n=19), birds (n=13), cattle (n=60), sheep (n=70), cats (n=25), and dog (n=1).

Pteropodids are not naturally present in the U.S., though they may be imported and exist in U.S. zoos, private collections, or research facilities. The U.S. Fish and Wildlife Service maintains data files on wildlife imports in LEMIS (Law Enforcement Management Information System). The LEMIS system includes only a portion of total wildlife imports. Other imports are recorded in a U.S. Customs database which CEI did not have access to. According to the LEMIS database, there were no imports of the three bat species which tested positive for the new virus (Pteropus poliocephalus, Pteropus alecto or Pteropus conspicillatus), into the U.S. during 1995, 1996 or 1997. There was however, a shipment in 1995 of 65 live bats of the Pteropus genus, of unidentified species, imported into the U.S. from Jamaica. During 1995, 1996 and 1997 there were 4,250 live bats, of genera other than Pteropus, imported into the U.S. from Canada, the Solomon Islands and Jamaica.

CEI's interpretation(s): At present, the risk to the U.S. from this apparently new paramyxovirus appears low. The U.S. imported a small number of live swine from Australia in 1997 and 1998. Imports of swine meat and products from Australia to the U.S. are minimal. The outbreak was limited to three premises and does not appear to have spread. The suspected animal reservoir does not occur naturally in the U.S.

The risk to the U.S. could change, however. The following circumstances would warrant a reevaluation of risk: other reservoirs are identified, new cases are reported in Australia or elsewhere, U.S. imports of live swine, swine products or the reservoir species from Australia increase, or research into this new virus reveals information that would impact transmission risk.

Though at present the risk of introduction to the U.S. of this new virus appears low, should introduction occur, the impact on the U.S. pork industry is potentially great.

1.16 *Mycoplasma capricolum/M. F38/M. mycoides capri*[17]

Contagious Caprine Pleuropneumonia

***Definition*:** Contagious caprine pleuropneumonia (CCPP) is an acute highly contagious disease of goats caused by a mycoplasma and characterized by fever, coughing, severe respiratory distress, and high mortality. The principal lesion at necropsy is fibrinous pleuropneumonia.

***Etiology*:** For many years the causative agent of CCPP was considered to be *M. mycoides capri* (type strain PG-3) because this was the agent most commonly isolated from goats with CCPP. In 1976, however, MacOwan and Minette reported isolating a new mycoplasma (designated F-38) from a CCPP outbreak in Kenya and demonstrated it to be the cause of a highly contagious form of pneumonia resembling the original description of CCPP by Hutcheon in 1881. McMartin et al. presented very convincing arguments supporting this agent as the cause of the classical disease, at least in Africa. Both of these mycoplasmas are now considered to cause CCPP, although the infrequency with which *M. mycoides capri* has been isolated from CCPP in recent years suggests that it may be a minor cause of the disease. Neither of these agents occurs in North America. The name *M. capricolum capripneumoniae* proposed for mycoplasma F-38 by Leach et al. is not in common usage. *Mycoplasma mycoides capri* is easily

[17]Committee on Foreign Animal Diseases of the United States Animal Health Association, revised 1998. Foreign Animal Diseases, "The Gray Book," Part IV, Library of Congress Catalog Card Number 17-12842, Pat Campbell & Associates and Carter Printing Company, Richmond, VA. http://www.vet.uga.edu/vpp/gray_book/FAD/CCP.htm

propagated on standard mycoplasma media, but F-38 is much more fastidious and can easily be missed at diagnosis, which may explain its late recognition as the major cause of CCPP.

M. mycoides mycoides has also been isolated from goats with pneumonia. This agent (the so-called large colony or LC variant of *M. mycoides mycoides*) usually produces septicemia, polyarthritis, mastitis, encephalitis, conjunctivitis, hepatitis, or pneumonia in goats. Some strains of this agent will cause pneumonia closely resembling CCPP, but the agent is not highly contagious and is not considered to cause CCPP. It does occur in North America. *M. capricolum capricolum*, a goat pathogen commonly associated with mastitis and polyarthritis in goats, can also produce pneumonia resembling CCPP, but it usually causes severe septicemia and polyarthritis. This agent (which does occur in the United States) is closely related to mycoplasma F-38 but can be differentiated from it using monoclonal antibodies.

Host Range: Contagious caprine pleuropneumonia is a disease of goats, and where the classical disease has been described, only goats were involved in spite of the presence of sheep and cattle. Mycoplasma F-38, the probable cause of the classic disease, does not cause disease in sheep or cattle.

M. mycoides capri, the other agent considered a cause of CCPP, will result in a fatal disease in experimentally inoculated sheep and can spread from goats to sheep. It is however, not recognized as a cause of natural disease in sheep.

Geographic Distribution: Contagious caprine pleuropneumonia has been described in many countries of Africa, the Middle East, Eastern Europe, the former Soviet Union, and the Far East. It is a major scourge in many of the most important goat-producing countries in the world and is considered by many to be the world's most devastating goat disease.

The classical disease, as caused by mycoplasma F-38, has not been described in North America. The reports of CCPP occurring in the United States and in Mexico were erroneous in that, although similar syndromes were described, the agents isolated were misidentified as *M. mycoides capri* and were subsequently shown to be *M. mycoides mycoides* (LC type). Neither mycoplasma F-38 nor *M. mycoides capri* has been isolated in North America

***Transmission*:** Contagious caprine pleuropneumonia is transmitted by direct contact through inhalation of infective aerosols. Of the two known causative agents, F-38 is far more contagious. Outbreaks of the disease often occur after heavy rains (e.g., after the monsoons in India) and after cold spells. This is probably because recovered carrier animals start shedding the mycoplasmas after the stress of sudden climatic change. It is believed that a long-term carrier state may exist.

Incubation Period: The incubation period can be as short as 6 to 10 days but may be very prolonged (3-4 weeks) under natural conditions

Clinical Signs: The clinical signs described for CCPP from different parts of the world have varied enormously. This is not surprising because at least two different mycoplasmas have been regarded as causative agents of the disease. In many field outbreaks, the clinical picture has probably been further complicated by the presence of viruses and other bacteria (e.g., pasteurella) as part of the etiologic picture.

The classical disease as caused by mycoplasma F-38 is a purely respiratory illness. It is characterized by a fever- of 106^{o} F (41^{o} C), coughing, and a distinct loss of vigor. Affected goats have labored breathing; later they may grunt or bleat in obvious pain. Frothy nasal discharges and stringy salivation are often seen shortly before death. In the acute disease, which occurs in fully susceptible populations of goats, death occurs within 7 to 10 days of the onset of clinical signs. A more chronic form of the disease is often seen in endemic areas and may lead to recovery of a higher percentage of infected animals, many of them carriers of the mycoplasmas.

M. mycoides capri tends to cause a more generalized infection in which septicemia is frequently seen. An acute or peracute septicemic form of the disease involving the reproductive, respiratory and alimentary tracts has been described. In addition, thoracic and reproductive formes of the disease have been

attributed to this agent. The disease is considerably less contagious than F-38-induced disease, and the mortality and morbidity rates are also lower.

Gross Lesions: The gross lesions in classical CCPP are confined to the thoracic cavity. Pea-sized yellowish nodules are seen in the lungs in early cases, whereas in more established cases there is marked congestion around the nodules. The lesions may be confined to one lung or involve both, and an entire lobe may become solidified. The pulmonary pleura becomes thickened, and there may be adhesions to the chest wall. Hutcheon emphasized that the lesions of CCPP do not resemble those of contagious bovine pleuropneumonia (CBPP) in that "no thickening of the interlobular tissue" occurrs, a classical lesion of CBPP. He described a CCPP-diseased lung as resembling a "somewhat granular looking liver", which is his description of the massive hepatization seen in CCPP lungs.

In sharp contrast, *M. mycoides capri* has been reported to cause lesions in a wide variety of organ systems and to produce lung lesions closely resembling those seen in CBPP. The generalized lesions described include encephalitis, meningitis, lymphadenitis, splenitis, genitourinary tract inflammations, and intestinal lesions, none of which are a feature of classical CCPP. The lung lesions, which resemble those seen in CBPP, are usually confined to one lung and reflect various stages of fibrinous pneumonia. Extensive pleuritis is usually present, and various stages of hepatization and marked dilation of interlobular septa is commonly seen. The cardiac and diaphragmatic lobes are the ones most commonly involved. Some describe this as a mild form of CCPP; others argue that it is not CCPP.

Morbidity and Mortality: Morbidity can be 100 percent and mortality may be in the range of 70 percent to 100 percent. Gathering or increased confinement of animals facilitates the spread of the disease.

Diagnosis

Field Diagnosis

A highly contagious disease occurring in goats and characterized by severe respiratory distress, high mortality, and postmortem lesions of fibrinous pleuropneumonia with pronounced hepatization and pleural adhesions warrants a field diagnosis of CCPP.

Specimens for Laboratory

From a dead animal that has had severe clinical disease, the best specimens to submit are affected lung, swabs of major bronchi, and tracheobronchial or mediastinal lymph nodes. All samples should be collected aseptically and if possible, placed in transport medium (heart infusion broth, 20 percent serum, 10 percent yeast extract, benzylpenicillin at 250 to 1000 IU/ ml). Samples should be kept cool and shipped on wet ice as soon as possible. If transport to the laboratory is delayed (more than a few days), samples may be frozen. Blood should be collected for serum.

Laboratory Diagnosis

Diagnosis must be confirmed by isolation of the agent (F-38). The causative agent, once isolated, can be identified by immunofluorescence or by growth or metabolic inhibition tests. Several serological tests can be used in the laboratory for the detection of antibodies to mycoplasma F-38. These include complement fixation (CF), passive hemagglutination (PH), and enzyme-inked immunosorbent assay (ELISA). The latex agglutination test is a very convenient field test for detecting antibodies in whole blood or in serum.

Differential Diagnosis

Clinically, CCPP may be confused with other pneumonic conditions such as pasteurellosis and peste des petits ruminants.

Treatment: The mycoplasmas are sensitive to several broad-spectrum antibiotics (notably the tetracyclines, tylosin, and tiamulin). Although early treatment can be effective, chemotherapy and chemoprophylaxis have not played important roles in CCPP control programs.

Vaccination: A crude vaccine prepared from goat lung was used to vaccinate goats in South Africa after the original outbreak of CCPP in the late 1800's. A combination of this vaccine and other control methods eliminated the disease from the country.

Vaccines to *M. mycoides capri* have been used with little success. This is probably because the disease is usually caused by mycoplasma F-38, first recognized in 1976. Since that time both live attenuated and inactivated F-38 vaccines have been tested with varying degrees of success. The most promising of the experimental vaccines is the lyophilized saponin-inactivated F-38 vaccine shown in field tests to confer 100 percent protection to contact exposure. This vaccine could be of inestimable value in many countries of Africa.

Control and Eradication: Sufficient regulatory restrictions should be maintained to prevent introduction of CCPP into apparently healthy animals. Serologic testing of susceptible animals for importation is a recommended safeguard.

Successful control of the spread of CCPP rests on removing susceptible animals from any possible contact with CCPP-infected animals, whether they are clinically affected or subclinical carriers only. On-farm quarantine of suspicious and contact animals would be very advantageous in stemming the spread of the disease. In an outbreak situation, testing, slaughter, and quarantine would be the methods of choice.

Public Health: Human infection with these mycoplasmas has not been reported.

1.17 *Mycoplasma mycoides mycoides*[18]

Contagious Bovine Pleuropneumonia

***Definition*:** Contagious bovine pleuropneumonia (CBPP) is a highly infectious acute, subacute, or chronic disease, primarily of cattle, affecting the lungs and occasionally the joints, and caused by *Mycoplasma mycoides mycoides*.

***Etiology*:** Contagious bovine pleuropneumonia is caused by *M. mycoides mycoides* small-colony type (SC type). *M. mycoides mycoides* large-colony type is pathogenic for sheep and goats but not for cattle. *M. mycoides mycoides* (SC type) survives well only in vivo and is quickly inactivated when exposed to normal external environmental conditions. *M. mycoides mycoides* does not survive in meat or meat products and does not survive outside the animal in nature for more than a few days. Many of the routinely used disinfectants will effectively inactivate the organism.

Host Range: Contagious bovine pleuropneumonia is predominantly a disease of the genus *Bos*; both bovine and zebu cattle are naturally infected. There are many reported breed differences with respect to susceptibility. In general, European breeds tend to be more susceptible than indigenous African breeds. There does seem to be some age resistance, for animals less than 3 years of age are less resistant to experimental challenge. In zoos the infection has been recorded in bison and yak. Although it has been reported that the domestic buffalo (*Bubalus bubalis*) is susceptible, the disease is difficult to produce experimentally in this species.

Geographic Distribution: Contagious bovine pleuropneumonia is endemic in most of Africa. It is a problem in parts of Asia, especially India and China. Periodically, CBPP occurs in Europe, and outbreaks within the last decade have occurred in Spain, Portugal, and Italy. Contagious bovine pleuropneumonia was eradicated from the United States in the nineteenth century. It is of historical interest that the Bureau of Animal Industries, which is the forerunner of the USDA's Animal and Plant Health Inspection Service, was formed in 1884 specifically to eradicate CBPP. The United States was declared free of CBPP only 9 years later in 1893. Currently, CBPP is not present in the Western hemisphere.

***Transmission*:** Contagious bovine pleuropneumonia is spread by inhalation of droplets from an infected, coughing animal. Consequently, relatively close contact is required for transmission to occur. Outbreaks usually begin as the result of movement of an infected animal into a naive herd. It is widely believed that recovered animals harboring infectious organisms within a pulmonary sequestrum, may become active

[18]Committee on Foreign Animal Diseases of the United States Animal Health Association, revised 1998. Foreign Animal Diseases, "The Gray Book," Part IV, Library of Congress Catalog Card Number 17-12842, Pat Campbell & Associates and Carter Printing Company, Richmond, VA. http://www.vet.uga.edu/vpp/gray_book/FAD/CBP.htm

shedders when stressed. Although this may be a factor in some outbreaks, it has not been substantiated experimentally. There are limited anecdotal reports of fomite transmission, but this means of transmission is not generally thought to be a problem.

Incubation Period: The time from natural exposure to overt signs of disease is variable but generally quite long. It has been shown that healthy animals placed in a CBPP-infected herd may begin showing signs of disease 20 to 123 days later. Experimentally, subsequent to instillation of large quantities of infective material at the tracheal bifurcation, the incubation period is 2 to 3 weeks.

Clinical Signs: Usually the first abnormality noticed is a depressed, inappetent animal with fever. Coughing may be the next sign followed by evidence of thoracic pain and an increased respiratory rate. As pneumonia progresses and animals become increasingly dyspneic, animals are inclined to stand with elbows abducted in an attempt to decrease thoracic pain and increase chest capacity. Auscultation of the lungs reveals any of a wide variety of sounds, depending on how severely the subjacent pulmonary parenchyma is affected.

Crepitations, rales, and pleuritic friction rubs are all possible. Percussion over affected areas reveals dullness. When pulmonary involvement is extensive and severe, there will be very labored respiration and, sometimes, open-mouthed breathing. Occasionally in calves, pneumonia may be accompanied by a polyarthritis. Animals affected in this manner may be very reluctant to move and stand stiffly with a distinctly arched back. Getting up and down may cause obvious discomfort. Large joints may be distended and warm on palpation. If joint pain is severe, animals may be so reluctant to bend the joints that they lie in lateral recumbency with legs outstretched. Contagious bovine pleuropneumonia often evolves into a chronic disease. This form, characterized by ill thrift and recurrent low-grade fever, may be difficult to recognize as pneumonia. Forced exercise may precipitate coughing.

Gross Lesions: The gross pathologic features of CBPP are quite characteristic. If the animal dies, there is usually extensive and marked inflammation of the lung and associated pleurae. In severe cases there can be abundant fluid in the thoracic cavity. The inflammation is not uncommonly unilateral. The initial focus can be in any part of the lung and, in fatal cases, usually has spread locally and extensively to include a sizable segment. The affected pulmonary parenchyma is odorless and often has all stages of lesions with both acute and chronic inflammatory changes adjacent to one another. The predominant gross change is consolidation, or thickening, of individual lobules, which become encased in markedly widened interlobular septa, resulting in the very characteristic marbled appearance. Interlobular septa become distended first by edema, then by fibrin, and finally by fibrosis. The overlying pleura may be very thickened by an irregular layering of yellow fibrin which, with time, becomes fibrosed, often resulting in adhesions between parietal and visceral pleurae. Not uncommonly, within an affected lung will be found a sequestrum - a focus that has undergone coagulative necrosis and is effectively sealed off from the rest of the lung. Such sequestra may even be found in recovered animals. It has been shown that *M. mycoides mycoides* (SC-type) can survive within these sequestra for months or possibly longer.

Morbidity and Mortality: The attack rate with CBPP is variable. It is not thought to be a highly contagious disease. With increased confinement of animals, morbidity rises. The mortality rate with CBPP is quite varied and ranges from 10 to 70 percent in various outbreaks. As with many subacute and chronic infectious diseases, mortality may depend on other intercurrent factors such as plane of nutrition, level of parasitism, and general body condition

Diagnosis

Field Diagnosis

Clinical diagnosis of CBPP is difficult. At postmortem the gross lesions of CBPP are somewhat distinct. Often there is an extensive deposition of fibrin and a large quantity of straw-colored fluid in the thoracic cavity with a prominent marbling of pulmonary parenchyma. Generally, all stages of pathologic changes, from acute through to chronic, are present in one animal. In some chronic cases the nodules of inflammation may not be readily apparent from the pleural surface but can be palpated within the parenchyma. Unlike many other pneumonias, CBPP is often unilateral.

Specimens for Laboratory

From a live animal, nasal swabs, transtracheal washes, or pleural fluid obtained by thoracic puncture all provide good samples for isolation attempts. From a dead animal that has had severe clinical disease, the best specimens to submit are affected lung, swabs of major bronchi, tracheo-bronchial or mediastinal lymph nodes, and joint fluid from those animals with arthritis. All samples should be collected aseptically and, if possible, placed in transport medium (heart infusion broth, 20 percent serum, 10 percent yeast extract, benzylpenicillin at 250 to 1000 IU/ml). Samples should be kept cool and shipped on wet ice as soon as possible. If transport to the laboratory is delayed (more than a few days), samples may be frozen. Blood should be collected for serum.

Laboratory Diagnosis

A definitive diagnosis is made by isolating and identifying the organism. Serology is helpful in the diagnosis of CBPP. Because CBPP is a subacute to chronic disease, most animals will have developed antibodies by the time of clinical disease.

Differential Diagnosis

Clinically, CBPP may be confused with other pneumonic conditions, most especially bovine pasteurellosis. However, bovine pasteurellosis would likely spread much more rapidly and consequently the epidemiologic picture may be distinct.

Treatment*: Mycoplasma mycoides mycoides* (SC-type) is susceptible to a variety of antimicrobials, including streptomycin, oxytetracycline, and chloramphenicol. However, antimicrobial therapy may only serve to slow the progression of the disease or may even in some cases favor the formation of sequestra. In the case of chronically affected animals or subclinically affected carriers, the organisms may be in an inaccessible location within an area of coagulative necrosis, which by definition is not served by a blood supply.

Vaccination: A modified live vaccine is available for use in enzootic areas. A major drawback of this vaccine is that it generates an unpredictable local reaction. For this reason it is often given in the tail tip, which may become necrotic and slough. Immunity subsequent to vaccination is generally good and lasts at least 12 months. The CBPP vaccine is often given in combination with the vaccine for rinderpest.

Control and Eradication

Prevention

Because CBPP is a chronic disease that may exist subclinically in carrier animals, it is important to maintain sufficient regulatory restrictions to prevent its introduction in apparently healthy animals. Serologic testing of susceptible animals for importation is a recommended safeguard.

Control and Eradication

Successful control of the spread of CBPP rests on removing susceptible animals from any possible contact with CBPP-infected animals, whether they are clinically affected or subclinical carriers only. On-farm quarantine of suspicious and contact animals would be very advantageous in stemming the spread of the disease. In an outbreak situation, testing, slaughter, and quarantine would be the methods of choice.

Public Health: There is no evidence to indicate that humans are susceptible to this disease.

1.18 Newcastle disease virus[19]

Velogenic Newcastle Disease

(Exotic Newcastle disease, Asiatic Newcastle disease)

[19]Committee on Foreign Animal Diseases of the United States Animal Health Association, revised 1998. Foreign Animal Diseases, "The Gray Book," Part IV, Library of Congress Catalog Card Number 17-12842, Pat Campbell & Associates and Carter Printing Company, Richmond, VA. http://www.vet.uga.edu/vpp/gray_book/FAD/VND.htm

***Definition*:** Velogenic Newcastle disease (VND) is the most severe form of Newcastle disease and is likely the most serious disease of poultry throughout the world. In chickens it is characterized by lesions in the brain or gastrointestinal tract, morbidity rates near 100 percent, and mortality rates as high as 90 percent in susceptible chickens. Neurologic signs or severe depression are the most obvious clinical sign, and some nonvaccinated birds may be found dead with no detected sign of prior illness.

***Etiology*:** Newcastle disease viruses (NDV's) occur as three pathotypes: lentogenic, mesogenic, and velogenic, reflecting increasing levels of virulence. The most virulent (velogenic) isolates are further subdivided into neurotropic and viscerotropic types. The velogenic isolates are considered exotic to the United States, and the disease caused by these VND isolates is the subject of this chapter.

The Newcastle disease viruses belong to the *Paramyxoviridae* virus family and, like other members of this group, possess two surface proteins that are important to the identification and behavior of the virus. The first, hemagglutinin/neuraminidase (HN) is important in the attachment and release of the virus from the host cells in addition to its serologic identification. The other very important surface protein is the fusion (F) protein, which has a critical role in the pathogenesis of the disease. There are at least nine known types of avian paramyxoviruses based on the antigenic makeup of the hemagglutinin. NDV is the prototype virus for Type 1 avian paramyxoviruses.

Host Range: Inapparently infected carriers that are the most likely source for introduction of VND include numerous species of exotic pet and exposition birds, waterfowl, and domestic poultry. A persistent carrier state has been demonstrated in psittacine and in certain other wild birds whereas virus can be recovered from chickens for shorter periods of time, usually 14 days or less.

Geographic Distribution: Velogenic Newcastle disease is endemic in many countries of Asia, the Middle East, Africa, and Central and South America. Some European countries are considered free of VND. VND has caused high mortality in wild cormorants in Canada and the United States.

***Transmission*:** In many parts of the tropics VND is recurrent in the poultry populations. One possibility is that they are infected from a wild bird reservoir. Additional studies will be required before it can be established which species, if any, are true carriers and which are only transiently infected. It is not known whether the occurrence of VND in wild birds moving in international trade can be reduced by avoiding the capture of certain species or their collection at certain time periods or places. Once introduced into poultry, the virus spreads farm-to-farm by the movement of inapparently infected poultry species; on contaminated objects such as boots, sacks, egg trays, and crates; or by flies or mice. Reports from England that the virus can be wind-borne under certain conditions should be considered even though there was no evidence of airborne transmission between premises with the virus that caused the 1971 outbreak in California. Free-flying wild birds apparently had no role in the spread of VND during that outbreak.

Incubation Period: The incubation period for Newcastle disease after natural exposure varies from 2 to 15 days. For VND in chickens, an incubation period of 2 to 6 days is common. The incubation period in other species of birds may be longer.

Clinical Signs: Velogenic Newcastle disease is a devastating malady in unvaccinated chickens of any age. The first sign in laying chickens is usually a marked drop in egg production followed within 24 to 43 hours by high death losses. At the onset, 10-15 percent of a flock may be lost in 24 hours. After 7 to 10 days, deaths usually subside, and birds surviving 12 to 14 days generally do not die but may display permanent paralysis and other neurologic signs. The reproductive system may be permanently impaired, and thus egg production does not return to previous levels. In vaccinated chickens, or chicks protected by parental antibodies, the clinical signs are less severe and are proportional to the level of protective antibodies.

With viscerotropic strains (VVND), edema of the head, especially around the eyes may become apparent after birds have been sick for 2 or 3 days. This edema usually does not involve the comb and wattle to the extent of highly pathogenic avian influenza (HPAI). A dark ring sometimes forms around the eye, probably due to cyanosis and poor blood circulation in the edematous tissue. This "black eye" appearance is especially visible in white chickens.

Bile-stained, greenish-dark diarrhea may be noted 2 to 3 days after onset of illness. Some birds in an affected flock usually have diarrhea throughout the course of the disease.

The most noteworthy clinical sign in unvaccinated flocks is sudden death without prior indications of illness. The peracute onset often causes the owner to suspect poisoning.

Respiratory distress and signs of neurological disturbances, such as drooping wings, torticollis, and ataxia. may not be as marked as they are with the neurotropic forms of the disease. However, these neurologic signs are frequently observed in chickens that survive infection with the viscerotopic strains for 2 or 3 weeks. Because of lack of experience with viscerotropic strains, poultry owners throughout the United States and Canada may not consider Newcastle disease as a possible diagnosis unless they see the neurologic signs they have seen with the domestic neurotropic viruses.

Neurotropic strains cause respiratory signs soon followed by neurologic signs, including muscular tremors, paralysis of legs or wings, torticollis, and opisthotonos. There is a marked decline in egg production but ususally no diarrhea. Disease signs may differ markedly, depending on the host species. Psittacines or pigeons infected with the viscerotropic strains of virus may display neurologic signs typical of the disease caused by the strains of neurotropic ND in chickens. These same viscerotropic viruses may cause typical signs and lesions of VVND when inoculated into chickens. In some species, such as finches and canaries, clinical disease may not be observed.

Gross Lesions: No gross lesion may be observed in many of the first birds dying in a commercial poultry operation. Peracute deaths are generally due to collapse or dysfunction of the reticuloendothelial system before discernible gross lesions have developed. There is no pathognomonic gross lesion for VVND, but, generally, sufficient lesions can be found to make a tentative diagnosis if enough birds are examined. Because of the marked similarities between the gross lesions of VVND and higly pathogenic avian influenza, a final diagnosis in the first flocks must await virus isolation and identification. In a continuing outbreak where numerous flocks are involved, gross observations may eventually be all that is necessary when typical lesions are present.

Edema of the interstitial tissue of the neck, especially near the thoracic inlet, may be marked. After the trachea and esophagus are exposed during necropsy examination, straw colored fluid may drip from these tissues. Congestion and occasionally hemorrhage may be seen in the trachea generally corresponding to the rings of cartilage.

Proventriculus

Petechial and small ecchymotic hemorrhages may be present on the mucosa of the proventriculus. These small hemorrhagic foci tend to be found near the base of the papillae and concentrated around the posterior and anterior orifices.

Intestine

Peyer's patches, cecal tonsils, and other focal aggregations of lymphoid tissue in the gut wall usually are markedly involved and are responsible for the term viscerotropic applied to this form of Newcastle disease. These areas progressively become edematous, hemorrhagic, necrotic, and ulcerative. In chickens that have died from VVND, these involved lymphoid areas can often be observed without opening the gut.

Reproductive System

Ovaries may be edematous, hemorrhagic, or degenerated. Yolk peritonitis can frequently be observed in layers as a result of VVND, and rough, misshapen eggs are frequently laid by recovering hens.

Neurotropic strains of VND may cause few gross lesions other than in the trachea and lungs. There will be no gross lesion in the brain of diseased birds. Gross lesion patterns usually differ markedly between the disease caused by the viscerotropic and neurotropic velogenic viruses.

Morbidity and Mortality: Clinical VND is most severe in chickens, peafowl, guineas, pheasant, quail and pigeons. Turkeys may develop a milder form of the disease. Severity of disease in psittacine and passerine birds is variable. In susceptible chickens, the morbidity and mortality rates can be as high as 100 percent

and 90 percent, respectively. In some species such as finches and canaries, clinical disease may not be observed.

Diagnosis

Field Diagnosis

A tentative diagnosis of VND may be made on the basis of history, clinical signs, and gross lesions, but because of similarities to other diseases such as fowl cholera and highly pathogenic avian influenza, confirmation requires virus isolation and identification.

Specimens for Laboratory

Virus can readily be recovered from sick or recently dead birds. Swabs are the most convenient way to transfer VND virus from tissues or secretions of the suspect bird to brain and heart infusion broth or other cell culture maintenance medium containing high levels of antibiotics. Trachea, lung, spleen, cloaca, and brain should be sampled. Swabs should be inserted deeply to ensure obtaining ample epithelial tissue. If large numbers of dead or live birds are to be sampled, cloacal swabs from up to five birds can be pooled in the same tube of broth. An alternate technique is to place 0.5 cm^3 of each tissue into the broth. If the specimens can be delivered to a laboratory within 24 hours, they should be placed on ice. If delivery will take longer, quick-freeze the specimens and do not allow them to thaw during transit.

Laboratory Diagnosis

In the laboratory, virus isolation is attempted by inoculating 9- to 11-day-old embryonating chicken eggs. Chorioallantoic fluid (CAF) is collected from all embryos dying after 24 hours postinoculation and tested for hemagglutination (HA) activity. If positive, the hemagglutination-inhibition (Hl) test is used with known NDV-positive serum to confirm the presence of NDV in the CAF. If NDV is found, it is characterized by inoculating 4- to 6-week-old chickens free of ND antibodies with the suspect CAF by swabbing the cloaca, instilling into the nares or conjuctival sac, or injecting into the thoracic air sac. If VVND virus is present, the inoculated chicks usually die in 3 to 7 days, revealing typical visceral lesions on postmortem examination. Neurotroph ic VVD viruses will cause severe neurologic and respiratory signs in inoculated chickens but no visceral lesions. If no bird dies in 10 days, the NDV is not considered to be the velogenic, viscerotropic type but is either a lentogen or mesogen.

Differential Diagnosis

The viscerotropic, velogenic Newcastle disease in poultry can be confused with highly pathogenic avian influenza, infectious laryngotracheitis, fowl cholera, and coryza.

Vaccination: Vaccination with viable or inactivated oil emulsion vaccines, or both, can markedly reduce the losses from VND in poultry flocks. If eradication of the virus is not the goal of the control program, vaccines can be used to lessen the impact of the disease. Their use, however, can make the complete eradication of the virus much more problematic by increasing the difficulty of identifying infected flocks. There is little doubt, however, that vaccination makes the flock more refractive to infection when exposed and reduces the quantity of virus shed by infected flocks.

Control and Eradication: Before 1972, VND was introduced into the United States on several occasions by unrestricted introduction of exotic pet birds, especially psittacine birds. Because pet birds are not usually associated with domestic poultry, VND outbreaks were rare. Since 1973, restrictions on the importation of exotic birds requiring the quarantining and testing of imported birds in approved quarantine facilities have reduced but not eliminated the threat of VND in the United States. Illegally imported exotic bird species remain the source of frequent outbreaks of VND in private or commercial aviaries.

The establishment of a strict quarantine and destruction of all infected and exposed birds with financial indemnification for losses followed by thorough cleaning and disinfection of premises were the main features necessary for eradication of VND virus from the poultry producing area of southern California. Flocks may be safely and humanely destroyed using carbon dioxide in air-tight chambers and the carcasses disposed of by burying, composting, or rendering, depending upon the geographic area and the

numbers involved. The VND virus has been recovered from effluent water for as long as 21 days and from carcasses for 7 days when the daytime temperatures were over 90 degrees F. It is recommended that premises be kept free of domestic poultry for an additional 30 days after cleaning and disinfection are completed.

Insects and mice associated with the poultry should be destroyed before depopulation of a flock begins. Usually 48 hours is sufficient to control these vectors. As soon as all birds are killed and the manure and feed removed, all equipment and structural surfaces should be thoroughly cleaned using high-pressure spray equipment. The entire premises should then be sprayed with an approved residual disinfectant such as the cresylics or phenolics. Preliminary disinfection will probably inactivate most of the viruses on the surface of floors, equipment, cages, etc., but no disinfectant is effective unless it is applied to scrupulously cleaned surfaces free of all organic material.

Cleaning and disinfecting commercial poultry premises are time-consuming and expensive operations. All manure must be removed down to a bare concrete floor. If the floor is earthen, at least the top inch of soil should be removed with the manure. Manure can be safely disposed of by burying it at least 5 feet deep or by composting. If composting is used, the manure piles should be tightly covered with black polyethylene sheets in a manner to prevent access by birds, insects, and rodents during composting. These piles of manure should remain tightly covered and undisturbed at least 90 days during warm weather and for longer periods during cold weather. Recent studies indicate that proper composting can decompose carcasses and manure, and thus inactivate viruses in only a few weeks.

Feathers, usually numerous around commercial poultry premises, can be burned outside the buildings, and in some cases inside, with the careful use of a flame thrower, or they can be removed and the area wet down with disinfectant. The hot sun and high daytime temperatures will assist in destroying the virus in the area of the houses. Extremely cold temperatures will make the cleaning and decontamination process much more difficult, and the results more uncertain.

In 1997, because neither the neurotropic or viscertropic strain of velogenic Newcastle disease was known to exist in the United States, USDA-APHIS declared both types to be exotic and therefore indistinguishable as to the response of disease control officials should they occur in the United States.

Surveillance

The most difficult part of the VND eradication program is locating inapparently infected and exposed birds.

Repeated vaccination at 30 to 50 day intervals protects most chickens against clinical manifestation of VND. However, vaccine does not prevent all chickens in a flock from becoming infected, showing no disease sign, or shedding virulent virus. As individual chickens become susceptible and get exposed to the virus, they become infected and also shed the virus for a time. Thus, the virulent virus continues to be present in apparently healthy, vaccinated flocks. The advantages of using vaccines as part of a VND eradication program must be weighed against the difficulty created in finding asymptomatic but infected and virus-shedding flocks. In such instances owners should be encouraged to observe strict biosecurity measures to reduce the chances of their flocks being exposed to VND virus.

Infected carriers in vaccinated flocks can be detected using one of two systems. In the first, all birds dying during a 24-hour period are collected twice a week, and cloacal swabs and brains are collected and cultured for the presence of VND virus using the diagnostic sampling procedures described earlier. Birds in VND-infected flocks that die from Marek's disease, leukosis, gout, and numerous other disease conditions may yield VND virus—especially if their immune system was impaired by those diseases before death. In the second virus detection system, susceptible sentinel birds are placed in vaccinated flocks. The sentinel birds must be unvaccinated and obtained from a specific pathogen-free source to be certain that they do not inadvertently serve as a source of diseases for the suspect flock. In most instances the sentinel birds die from VND within a week or so after placement if there is VND virus present in the flock; however, in some cases it is sometimes difficult to place sentinel birds so they are adequately exposed to any VND virus that may be in the flock — especially in caged-layer flocks.

Public Health: Although people may become infected with VND virus, the resulting disease is typically limited to a conjunctivitis. Recovery is usually rapid, and the virus is no longer present in eye fluids after 4 to 7 days. Infections have occurred mostly in laboratory workers and vaccinating crews with rare cases in poultry handlers. No instance of transmission to humans through handling or consuming of poultry products is known. Individuals with conjunctivitis from VND virus should not enter poultry premises or come in contact with live avian species.

1.19 Peste des petits ruminants virus[20]

(Pest of Small Ruminants, stomatitis-pneumoenteritis complex or syndrome pseudorinderpest of small ruminants and kata [Pidgin English for catarrh])

Definition: Peste des petits ruminants (PPR) is an acute or subacute viral disease of goats and sheep characterized by fever, erosive stomatitis, conjunctivitis, gastroenteritis, and pneumonia. Goats are usually more severely affected than sheep.

Etiology: Peste des petits ruminants is caused by a paramyxovirus of the *Morbillivirus* genus. Other members of the genus include rinderpest virus (RPV), measles virus (MV), canine distemper virus (CDV), and phocid distemper virus (PDV) of sea mammals (seals). For many years, PPR virus was considered a variant of RPV, specifically adapted for goats and sheep, that had lost its virulence for cattle. It is now known that the two viruses are distinct though closely related antigenically.

Host Range: Peste des petits ruminants is primarily a disease of goats and sheep. However, there is one report of naturally occurring PPR in captive wild ungulates from three families: *Gazellinae* (*dorcas gazelle*), *Caprinae* (Nubian ibex and Laristan sheep), and *Hippotraginae* (gemsbok). Experimentally, the American white-tailed deer (*Odocoileus virginianus*) is fully susceptible. The role of wildlife on the epizootiology of PPR in Africa remains to be investigated. Cattle and pigs are susceptible to infection with PPRV, but they do not exhibit clinical signs. Such subclinical infections result in seroconversion, and cattle are protected from challenge with virulent RPV. Cattle and pigs do not, however, play a role in the epizootidogy of PPR because they are apparently unable to transmit the disease to other animals.

Geographic Distribution: Presently, PPR occurs in most African countries situated in a wide belt between the Sahara and Equator, the Middle East (Arabian Peninsula, Israel, Syria, Iraq, Jordan), and the Indian subcontinent.

Transmission: Peste des petits ruminants is not very contagious and transmission requires close contact. Ocular, nasal, and oral secretions and feces are the sources of virus. Contact infection occurs mainly through inhalation of aerosols produced by sneezing and coughing. Fomites such as bedding may also contribute to the onset of an outbreak. As in rinderpest (RP), there is no known carrier state. Infected animals may transmit the disease during the incubation period.

Incubation Period: Peste des petits ruminants has an incubation period of 4 to 5 days.

Clinical Signs: The disease usually appears in the acute form, with an incubation period of 4 to 5 days followed by a sudden rise in body temperature to 104-106° F (40-41° C). The temperature usually remains high for about 5 to 8 days before slowly returning to normal preceeding recovery or dropping below normal before death. Affected animals appear ill and restless and have a dull coat, dry muzzle, and depressed appetite. Accompanying these nonspecific signs are a series of changes that make up a highly characteristic syndrome. From the onset of fever, most animals have a serous nasal discharge, which progressively becomes mucopurulent. The discharge may remain slight or may progress, resulting in a profuse catarrhal exudate, which crusts over and occludes the nostrils. At this stage, animals have respiratory distress, and there is much sneezing in an attempt to clear the nose. Small areas of necrosis

[20]Committee on Foreign Animal Diseases of the United States Animal Health Association, revised 1998. Foreign Animal Diseases, "The Gray Book," Part IV, Library of Congress Catalog Card Number 17-12842, Pat Campbell & Associates and Carter Printing Company, Richmond, VA. http://www.vet.uga.edu/vpp/gray_book/FAD/PDP.htm

may be seen on the visible nasal mucous membranes. The conjunctiva usually becomes congested, and the medial canthus may have some crusting. As with the nose, there may be profuse catarrhal conjunctivitis resulting in matting of the eyelids.

Necrotic stomatitis is common. It starts as small, roughened, red, superficial necrotic foci on the gum below the incisor teeth. These areas may resolve within 48 hours or progressively increase to involve the dental pad, the hard palate, cheeks and their papillae, and the dorsum of the anterior part of the tongue. Necrosis may result in shallow irregular nonhemorrhagic erosions in the affected areas of the mouth and deep fissures on the tongue. Necrotic debris may collect at the oral commissures, and scabs may form along the mucocutaneous junction of the lips. There may be excessive salivation but not to the extent of drooling.

At the height of development of oral lesions, most animals manifest severe diarrhea, often profuse but not hemorrhagic. As it progresses, there is severe dehydration, emaciation, and dyspnea followed by hypothermia, and death usually occurs after a course of 5 to 10 days. Bronchopneumonia, evidenced by coughing, is a common feature in the later stages of PPR. Pregnant animals may abort.

Secondary latent infections may be activated and complicate the clinical picture.

Gross Lesions: The pathology caused by PPR is dominated by inflammatory and necrotic lesions in the mouth and the gastrointestinal tract. Unlike RP, there is also a definite, albeit inconstant, respiratory system component; hence, the synonym stomatitis-pneumoenteritis complex.

Emaciation, conjunctivitis, erosive stomatitis involving the inside of the lower lip and adjacent gum, cheeks near the commissures, and the free portion of the tongue are frequent lesions. In severe cases, lesions may also be found on the hard palate, pharynx, and upper third of the esophagus. The necrotic lesions do not evolve into ulcers because the basal layer of the squamous epithelium is rarely penetrated.

The rumen, reticulum, and omasum rarely have lesions. Sometimes, there may be erosions on the pillars of the rumen. The abomasum is a common site of regularly outlined erosions and often oozes blood.

Lesions in the small intestine are generally moderate, being limited to small streaks of hemorrhages and, sometimes, erosions in the first portion of the duodenum and the terminal ileum. Peyer's patches are the site of extensive necrosis, which may result in severe ulceration. The large intestine is usually more severely affected with congestion around the ileocecal valve, at the ceco-colic junction, and in the rectum. In the posterior part of the colon and the rectum, discontinuous streaks of congestion ("zebra stripes") form on the crests of the mucosal folds.

In the respiratory system, small erosions and petechiae may be visible on the nasal mucosa, turbinates, larynx, and trachea. Bronchopneumonia may be present, usually confined to the anteroventral areas and is characterized by consolidation and atelectasis. There may be pleuritis, which may become exudative and results in hydrothorax.

The spleen may be slightly enlarged and congested. Most lymph nodes throughout the body are enlarged, congested, and edematous. Erosive vulvovaginitis similar to the lesions in the oral mucocutaneous junction may be present.

Morbidity and Mortality: The incidence of PPR in an enzootic area may be similar to that of rinderpest (RP) in that a low rate of infection exists continuously. When the susceptible population builds up, periodic epizootics (outbreaks) occur, that receive more attention than usual. Such epizootics may be characterized by almost 100 percent mortality among affected goat and sheep populations.

The prognosis of acute PPR is usually poor. The severity of the disease and outcome in the individual is correlated with the extent of mouth lesions. Prognosis is good in cases where the lesions resolve within 2 to 3 days. It is poor when extensive necrosis and secondary bacterial infections result in an unpleasant, fetid odor from the animal's breath. Respiratory involvement is also a poor prognostic sign. A morbidity rate of 80-90 percent and a casefatality rate of 50-80 percent are not uncommon — particularly in goats.

Young animals (4 to 8 months) have more severe disease, and morbidity and mortality are higher. Both field and laboratory observations indicate that PPR is less severe in sheep than in goats. Nevertheless,

field outbreaks have been reported in the humid zones of west Africa in which no distinction could be made between the mortality rates in sheep and in goats. Poor nutritional status, stress of movement, and concurrent parasitic and bacterial infections enhance the severity of clinical signs.

Diagnosis

Field Diagnosis

In the field, a presumptive diagnosis can be made on the basis of clinical, pathological, and epizootiological findings.

Laboratory confirmation is an absolute requirement — particularly in areas or countries where PPR has not previously been reported.

Specimens for Laboratory

Specimens to submit include blood in EDTA anticoagulant, clotted blood or serum (if possible, paired sera), mesenteric lymph nodes, spleen, lung, tonsils, and sections of the ileum and large intestine.

Swabs of serous nasal and lachrymal discharges may also be useful. All samples should be shipped fresh (not frozen) on ice within 12 hours after collection.

Laboratory Diagnosis

A wide range of laboratory procedures have been described for detecting virus or viral antigen, viral nucleic acid, and antibody.

Differential Diagnosis

Rinderpest. Clinical RP is rare in goats and sheep in Africa. In India, these species are quite often involved in RP outbreaks. Clinically, RP and PPR are similar, but the former should be the prime suspect if the disease involves both cattle and small ruminants.

Confirmation requires virus isolation and cross-neutralization.

Pasteurellosis. Enzootic pneumonia or the septicemic form of pasteurellosis is characterized by obvious respiratory signs, infrequent diarrhea, and a fatality rate rarely exceeding 10 percent.

Contagious caprine pleuropneumonia. There is no digestive system involvement, and the clinical signs and lesions are confined to the respiratory system and pericardium.

Bluetongue. Swelling of the lips, muzzle, and oral mucosa, together with edema of the head region, should serve to differentiate bluetongue from PPR. Coronitis, common in bluetongue, is not a feature of PPR. Also, sheep are more affected than goats.

Heartwater. There is often central nervous system involvement, including convulsions. There is no diarrhea.

Contagious ecthyma (contagious pustular dermatitis, orf). The orf virus causes proliferative, not necrotic lesions, that involve the lips rather than the whole oral cavity. The absence of nasal discharges and diarrhea also distinguish orf from PPR.

Foot-and-mouth disease. This condition is comparatively mild, and the most characteristic clinical sign, lameness, is not a feature of PPR.

Nairobi sheep disease. Sheep are more severely affected than goats. It is limited geographically to parts of east and central Africa (Kenya, Uganda, Tanzania, Ethiopia, Somalia and Congo [formerly Zaire]). Diagnosis requires isolation and serologic identification of the virus.

Coccidiosis. There is no upper digestive tract and respiratory system involvement.

Plant or mineral poisoning. Several plants and minerals may cause severe intestinal lesions. Case history and absence of fever should distinguish poisoning from PPR.

Treatment: There is no specific treatment for PPR. However, drugs that control bacterial and parasitic complications may decrease mortality.

Vaccination: The tissue culture rinderpest vaccine at a dose of 102.5 $TCID_{50}$ protects goats for at least 12 months against PPR. The vaccine is currently used in many African countries for vaccination against PPR. The efficacy notwithstanding, its wide use is disadvantageous for the ongoing Pan-African rinderpest campaign (PARC) because it is impossible to determine if seropositive small ruminants have been vaccinated or naturally infected with RPV. A homologous attenuated PPR vaccine is being tested and may soon be commercially available.

Control and Eradication: Eradication is recommended when PPR appears in new areas. Methods that have been successfully applied for RP eradication in many areas would be appropriate for PPR. These should include quarantine, slaughter, and proper disposal of carcasses and contact fomites, decontamination, and restrictions on importation of sheep and goats from affected areas.

Public Health: Peste des petits ruminants is not infectious for humans.

1.20 Rinderpest virus[21]

Definition: Rinderpest (RP) is a contagious viral disease of cattle, domestic buffalo, and some species of wildlife. It is characterized by fever, oral erosions, diarrhea, lymphoid necrosis, and high mortality.

Etiology: Rinderpest virus (RPV) is a single-stranded RNA virus in the family Paramyxoviridae, genus Morbillivirus. It is immunologically related to canine distemper virus, human measles virus, peste des petits ruminants virus, and marine mammal morbilliviruses. There is only one serotype of rinderpest virus, but field strains vary widely in virulence, ease of transmission, and host affinity.

Rinderpest virus is a relatively fragile virus. Sunlight is lethal, and the vaccine must therefore be kept in a brown bottle and protected from light; virus in a thin layer of blood is inactivated in 2 hours. Moderate relative humidity inactivates the virus more quickly than either high or low humidity. The virus is very sensitive to heat, and both lyophilized and reconstituted virus should therefore be kept cold; lyophilized virus stored at -20^{o} C is viable for years. Vaccine reconstituted in pure water quickly loses potency. Vaccine is more stable in a saline solution; reconstitution in a molar concentration of sulfate ions greatly increases resistance to heat.

Rinderpest virus is rapidly inactivated at pH 2 and 12 (10 minutes); optimal for survival is a pH of 6.5-7. The virus is inactivated by glycerol and lipid solvents.

Transmission: Rinderpest was established as an infectious disease in 1754 when susceptible animals were infected by placing bits of material previously dipped in morbid discharge into an incision made in the dewlap. In 1899, cattle were infected with a bacteria-free filtrate.

Secretions and excretions, particularly nasal-ocular discharges and feces, 1 to 2 days before clinical signs to 8 to 9 days after onset of clinical signs contain large quantities of virus. Spread of RP is by direct and indirect (contaminated ground, waters, equipment, clothing) contact with infected animals; aerosol transmission is not a significant means of transmission (it occurs only in a confined area and over a short distance). A major reason RP spreads in Africa is that the herds are nomadic. Cattle follow the grass and thus move great distances, and during the dry season, many herds will use the same well or watering area, and thus there is ample opportunity for cross-infection. It is said that a good fence will control RP.

There is only one serotype of RPV; recovered or properly vaccinated animals are immune for life, and there is no vertical transmission, arthropod vector, or carrier state. For these reasons, RPV is an ideal virus to be targeted for eradication.

Highly virulent strains of RPV are responsible for epizootics in susceptible animals and tend to die out. Milder strains tend to persist in an area, and the disease is not recognized as RP unless serology is performed.

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[21]Committee on Foreign Animal Diseases of the United States Animal Health Association, revised 1998. Foreign Animal Diseases, "The Gray Book," Part IV, Library of Congress Catalog Card Number 17-12842, Pat Campbell & Associates and Carter Printing Company, Richmond, VA. http://www.vet.uga.edu/vpp/gray_book/FAD/RIN.htm

The roles the various hosts can play in the disease are as follows:

- Cattle and domestic buffalo — highly susceptible
- Sheep and goats in Africa — subclinical infection and seroconversion, but there is no transmission to other animals.
- Sheep and goats in India — when infected by low-passage goat RP vaccine will transmit to domestic buffalo.
- Pigs — Swayback pigs in Thailand and the Malay peninsula can be naturally infected and may die. European pigs can be infected by ingestion of RPV-infected meat and will transmit to cattle and other pigs.

Host Range: Most wild and domestic cloven-footed animals can be infected.

Geographic Distribution: Rinderpest is present in the Indian subcontinent, Near East, and sub-Saharan Africa.

Wild ungulates:

Highly susceptible — African buffalo, wildebeest, kudu, eland, giraffe, warthog

Fairly susceptible — Thompson gazelle, hippopotamus

Wild ungulates are infected by contact with cattle and can transmit to cattle. In the absence of RP in cattle, the disease dies out in wildlife.

Incubation Period: The incubation period varies with the strain of virus, dosage, and route of exposure. Following natural exposure, the incubation period ranges from 3 to 15 days but is usually 4 to 5 days.

Clinical Signs: Depending on the strain of virus, resistance of the animal affected, and concurrent infection, RP can appear as a peracute, acute, or mild infection.

Peracute Form

This form is seen in highly susceptible and young animals. The only signs of illness are a fever of 104-107^{o} F (40-41.7^{o} C), congested mucous membranes, and death within 2 to 3 days after the onset of fever.

Acute or Classic Form

This form of the disease progresses as follows:

Small amounts of virus may be in nasal and ocular secretions before the onset of fever

Fever of 104-106^{o} F (40-41.1^{o} C)

Serous to mucopurulent ocular discharge

Serous to mucopurulent nasal discharge.

Leukopenia

Depression

Anorexia

Constipation

Oral erosions — Salivation may be abundant and frothy.

Fever decreases and viral titer drops.

Diarrhea — May be very watery or hemorrhagic, or both.

Dehydration, emaciation

Prostration and death 6 to 12 days after onset of illness.

Gross Lesions: Oral lesions are variable; some isolates cause good oral lesions and with others there is no oral lesion. Oral lesions start as small grey foci that may coalesce. The grey (necrotic) epithelium then sloughs off and leaves a red erosion.

Mouth — Lesions occur on the gums, lips, hard and soft palate, cheeks, and base of the tongue. Early lesions are grey, necrotic, pinhead-sized areas that later coalesce and erode and leave red areas.

Esophagus — Brownish necrotic or eroded areas.

Rumen and reticulum — Lesions are rare.

Omasum — Erosions and hemorrhage are rare.

Abomasum — Congestion and edema.

Small intestine — Necrosis or erosion of Peyer's patches in the jejunum; necrosis or erosions over the lymphoid area in the ileum (ingesta adhering to the intestinal mucosa indicates areas of necrotic epithelium).

Cecum and colon — The wall may be edematous, and there may be blood in the lumen and blood clots on the mucosa. Lesions are usually more severe in the upper colon (edema of the wall, erosions in the mucosa, and congestion). The lesions may be accentuated at the cecocolic junction. Further down the colon, the colonic ridges may be congested; this is referred to as "tiger striping". Tiger striping can occur in other diarrheas and probably results from tenesmus.

Severity of intestinal lesions varies between isolates.

Lymph nodes — Generally swollen and edematous.

Liver — There may be petechial to ecchymotic hemorrhages in the gall bladder.

Lung — There may be emphysema, congestion, and areas of pneumonia.

Diagnosis

Field Diagnosis

Rinderpest should be considered in all ages of cattle whenever there is a rapidly spreading acute febrile disease accompanied by the preceeding clinical signs and lesions of RP. The all ages stipulation is important because this will be one of the major differences between bovine virus diarrhea-mucosal disease, which predominately affects animals between 4 and 24 months of age.

Specimens for Laboratory

Because the viral titer drops when the fever falls and diarrhea starts, specimens should preferably be collected from animals with a high fever and oral lesions. The following samples should be collected from live animals:

- Blood in EDTA or heparin
- Blood for serum
- Swabs containing lacrimal fluid
- Necrotic tissue from the oral cavity
- Aspiration biopsies of superficial lymph nodes

For the best specimens, a febrile animal should be slaughtered and specimens collected. If this cannot be done, then collect specimens from moribund animals. Collect the blood samples listed above and sections of:

- Spleen
- Lymph nodes
- Tonsil

The preceeding samples should be transported to the laboratory on wet ice — NOT FROZEN.

A complete set of tissues, including sections of all lesions, should be collected in 10 percent formalin.

Laboratory Diagnosis

To confirm the initial diagnosis in a free area, the virus has to isolated and identified.

Differential Diagnosis

The differential diagnosis for RP should include bovine virus diarrhea (mucosal disease), infectious bovine rhinotracheitis, malignant catarrhal fever, foot-and-mouth disease, vesicular stomatitis, salmonellosis, paratuberculosis, and arsenic poisoning.

Vaccination: The following types of RP vaccine have been used:

- Lapinized in China and Korea
- Avianized-lapinized in Korea
- Goat-adapted in India
- cell-culture-adapted in Africa, Middle East, and India.

An experimental vaccinia-vectored vaccine containing the F and H genes of RPV has protected against challenge inoculation of virulent virus

The two most commonly used vaccines today (1996) are the goat-adapted and cell-culture-adapted vaccines. The goat-adapted vaccine is only partially attenuated; it will cause disease in animals with low innate resistance or concurrent latent disease and kills sheep and goats. The cell-culture-attenuated vaccine was developed by Plowright in Kenya in the 1960's. This is a safe vaccine for many species and produces life-long immunity in cattle (animals challenge-inoculated 7 years after vaccination were protected). In endemic areas where cattle have been vaccinated, colostral immunity will interfere with the vaccination of calves up to 11 to 12 months of age. Because the duration of colostral immunity is variable, the recommendation is to vaccinate calves annually for 3 years.

One of the biggest problems with the cell-culture-adapted vaccine has been stability. The lyophilized virus has to be kept cold (cold chain) until used. The combination of maintenance of the cold chain and remoteness of vaccination sites made RP vaccination very expensive. Because of the uncertainty that the vaccine being used was viable, in areas of Africa it is and was the policy to vaccinate animals every year in the hope that one of the vaccinations would immunize the animal. Researchers at Plum Island in the early 1990's greatly increased the stability of the lyophilized vaccine by modifying the stabilizers and lyophilization process. This change in production is now being used in some production facilities in Africa.

Experimentally, the vaccinia-vectored RP vaccine protected cattle against challenge inoculation with RPV. This vaccine is undergoing field testing. This vaccine could be particularly useful in an eradication program because vaccinia-vectored-RP-vaccine immunized animals can be differentiated serologically from animals having antibody induced by live virus. The vaccinia-vectored vaccine would enable a country toward the end of an eradication program to maintain herd immunity to RP without using a live RP virus.

Control and Eradication: Countries and areas free of RP should prohibit unrestricted movement of RP-susceptible animals and uncooked meat products from areas infected by RP or practicing RP vaccination. Because recovered animals are not carriers, and there are good serological techniques, zoological ruminants and swine can be imported with proper quarantine and testing. If an outbreak occurs, the area should be quarantined, infected and exposed animals slaughtered and buried or burned, and ring vaccination considered.

Experimentally it has been shown that RPV will not be transmitted by bovine embryo transfer if the embryos have been processed by the technique recommended by the International Embryo Transfer Society and the OIE.

High-risk countries (those trading with, or geographically close to, infected countries) can protect themselves by having all susceptible animals vaccinated before they enter the country or vaccinating the national herd, or both. If an outbreak occurs, the area should be quarantined and ring vaccinated.

Endemic countries should vaccinate the national herd. Owing to the uncertainty of vaccine potency, the recommendation is to vaccinate annually for at least 4 years, followed by annual vaccination of calves.

Foci of infection should be quarantined and stamped out. Wildlife, sheep, and goats should be monitored serologically. Serological monitoring of sheep and goats could be complicated by using RP vaccine to protect against peste des petits ruminants.

Public Health: There is no report of RPV infection in a human.

1.21 Sheep pox virus

See Goat Pox

1.22 Swine vesicular disease[22]

Definition: Swine vesicular disease (SVD) is an acute, contagious viral disease of swine caused by an enterovirus and characterized by fever and vesicles with subsequent erosions in the mouth and on the snout, feet, and teats.

Etiology: Swine vesicular disease virus is in the enterovirus group of picornaviruses and is closely related to the human enterovirus Coxsackie B-5 and unrelated to known porcine enteroviruses. Some researchers believe this is a case where a human pathogen transferred to pigs through the eating of human feces. The virion is a roughly spherical 28 nm single-stranded RNA virus. This pathogen is resistant over a wide pH range (2.5-12), relatively resistant to heat (inactivated at 157^{o} F [69^{o} C]), and persists for a long time (up to 2 years) in salted, dried, and smoked meat products.

Host Range: Pigs are the only natural host. Baby mice can be experimentally infected, and there has been accidental laboratory infection of humans.

Geographic Distribution: Swine vesicular disease first occurred in Italy and was subsequently recognized in Hong Kong, England, Scotland, Wales, Japan, Malta, Austria, Belgium, France, the Netherlands, Germany, Poland, Switzerland, Greece, and Spain. Outbreaks in the 1990's were reported in Italy, Spain, and Portugal.

Transmission: The disease can be introduced into a herd by feeding garbage containing infected meat scraps, by introducing infected animals, or by contacting infected feces (e.g., an improperly cleaned truck).

Recent outbreaks in Europe appeared after the introduction of animals that had no clinical sign of SVD, which indicates that there is a subclinical form of the disease. After the initial infection, the disease spreads through contact of susceptible pigs with infected pigs and infected feces.

Incubation Period: Signs of SVD develop in 2 to 3 days after eating contaminated feed and in 2 to 7 days after contact with infected pigs.

Clinical Signs: Clinical signs are very similar to those of foot-and-mouth disease and other vesicular diseases. There is a fever, vesicles in the mouth and on the snout and feet, and lameness, all of which are grossly indistinguishable from FMD. More suggestive of SVD is an unsteady gait, shivering, and chorea (jerking) type leg movements due to an encephalitis.

Gross Lesions: Vesicles are indistinguishable from those of foot-and-mouth disease, vesicular stomatitis, and vesicular exanthema of swine. See the foot-and-mouth disease chapter.

Morbidity and Mortality: Morbidity in SVD is lower, and lesions are less severe, than in foot-and-mouth disease. There is essentially no mortality in SVD.

Diagnosis

See Foot-and-mouth disease.

[22]Committee on Foreign Animal Diseases of the United States Animal Health Association, revised 1998. Foreign Animal Diseases, "The Gray Book," Part IV, Library of Congress Catalog Card Number 17-12842, Pat Campbell & Associates and Carter Printing Company, Richmond, VA. http://www.vet.uga.edu/vpp/gray_book/FAD/SVD.htm

Serology is complicated by cross reactions with other undefined porcine enteroviruses.

Differential Diagnosis

Differential diagnosis for SVD should include foot-and-mouth disease, vesicular stomatitis, vesicular exanthema of swine, and chemical and thermal burns.

Vaccination: There is no vaccine.

Control and Eradication: Prevention measures are similar to those for FMD: control of animals imported from infected areas, and sanitary disposal of garbage from international aircraft and ships

Eradication measures consist of quarantining infected farms and areas, slaughtering and disposing of infected and contact pigs, and cleaning and disinfecting infected premises.

Public Health: Human infection has been reported in laboratory personnel working with the virus. Caution should be taken when working with infected material.

1.23 Vesicular stomatitis virus (exotic)[23]

Definition: Vesicular stomatitis (VS) is a viral disease characterized by fever, vesicles, and subsequent erosions in the mouth and epithelium on the teats and feet. Horses, cattle, and pigs are naturally susceptible; sheep and goats are rarely affected.

Etiology: The vescular stomatitis virus is a Vesiculovirus in the family Rhaboviridae. The virion is a large bullet-shaped (65-185 nm) RNA virus. There are two serotypes of VSV: New Jersey and Indiana 1. In the serotype Indiana 1, there are two subtypes: Indiana 2 (Cocal) and Indiana 3 (Alagoas). In addition to these two serotypes of VSV, there are other viruses within the genus Vesiculovirus that can experimentally cause vesicular lesions in domestic animals and infect humans; these are as follows:

Piry — first isolated from an opossum in Brazil.

Chandipura — first isolated from a person in India.

Isfahan — isolated from sandflies and humans in Iran.

Effective disinfectants are 2 percent, sodium carbonate - 4 percent, sodium hydroxide - 2 percent, iodophore disinfectants and chlorine dioxide disinfectants.

Host Range: The host range in decreasing order of severity of infection are horses, donkeys, mules, cattle, swine, and man.

South American cameilids develop clinical infection.

Sheep and goats are quite resistant and rarely become affected.

Vesicular stomatitis virus has also been shown experimentally to infect a wide host range, including deer, raccoons, bobcats, and monkeys.

Geographic Distribution: Classical VS occurs only in North and Central America and the northern part of South America. Serotypes New Jersey and Indiana I occur in the United States and Central America. Serotypes New Jersey and Indiana 1, 2, and 3 occur in South America.

Transmission: The vesicular stomatitis virus has been shown to be transmitted by the sand fly (Lutzomyia shannoni) and the black fly (Simuliidae). Transovarial transmission has been shown to occur in both flies. The VS-NJ serotype was isolated from a variety of field-collected hematophagous insects such as Culicoides (biting midges), Simuliidae (black flies), Aedes (mosquitoes) and nonbiting insects such as Chloropidae (eye gnats), Anthomyiidae, and Musca (house flies) during the 1982 epizootic in the southwestern United States. Except for Lutzomyia and Simulidae, the role of these other insects in the transmission of VSV is unknown. Before the 1982 outbreak in the United States, people, on the basis of

[23]Committee on Foreign Animal Diseases of the United States Animal Health Association, revised 1998. Foreign Animal Diseases, "The Gray Book," Part IV, Library of Congress Catalog Card Number 17-12842, Pat Campbell & Associates and Carter Printing Company, Richmond, VA. http://www.vet.uga.edu/vpp/gray_book/FAD/VST.htm

past experience, expected an outbreak to stop about 2 weeks after a killing frost. In the 1982 outbreak, cases and spread occurred through the winter. The winter spread of the disease is believed to have resulted from movement of infected animals and the resulting exposure of uninfected animals to contaminated waterers and feed bunks as well as contact with infected animals. It is known that VSV can be spread by a contaminated milking machine. Overwintering did not occur in the 1995 outbreak in the United States.

Humans may be infected by contact and by aerosol.

Epidemology: The disease occurs throughout the year in subtropical and tropical areas of the Americas. The disease occurs sporadically during the warm months in southern and western United States. Epidemics have occurred irregularly at 10 to 15 year intervals. The virus is spread by insect vectors, movement of infected animals, and contaminated objects. Researchers have shown transovarial transmission in the sand fly and black fly; this may be a way the virus can overwinter.

Incubation Period: A vesicle appears in about 24 hours after intradermal lingual inoculation of VSV. This similar to the incubation period for foot-and-mouth disease.

In humans, the incubation period is 24 to 48 hours.

Clincial Signs: Animals develop a fever ranging to 104-106^{o} F (40-41^{o} C).

Horse

Vesicles in the mouth may cause the animal to chomp its jaws, drool, and rub its mouth on the manger or other objects. Lesions on the coronary band can cause lameness.

Cattle and pigs

See the clinical signs section in the FMD chapter. The signs are very similar.

Humans

In humans, VSV causes an influenza-like illness; there is fever, headache, muscular aches, and blisters in the mouth similar to those caused by herpesvirus. The disease course is 4 to 7 days.

Morbidity and Mortality

Interesting data on the economic effect of VS in cattle were collected by Alderink during the 1982 outbreak of VS in Colorado. In 13 of the dairy herds studied there were 2,404 cows and 378 cases of VS. Lesion distribution in these 378 was as follows:

Oral lesions only	263 animals (69.3%)
Teat lesions only	87 animals (23%)
Oral and teat lesions	22 animals (5.8%)
Foot lesions only	7 animals (1.9%)

Herds experiencing primarily oral lesions had an attack rate of 19.8 percent. The attack rate in two of four herds with teat lesions was 55.8 percent and in the other two herds 1.6 percent. The clinical course in cases with oral lesions was 23.8 days. Mastitis complicated 72% of the cases with teat lesions.

The total cost to the 13 dairymen was $95,752, which came to an average cost of $253 per case. The approximate cost of a case with only oral lesions was $174 in contrast to an average cost of $568 for cases with teat lesions. Of the total $95,752 loss, 46 percent was for cows culled; 30 percent was for decreased production; 11 percent for deaths; and 11 percent for drugs, labor, weight loss, and veterinary charges.

Differences Between VS and FMD

The characteristics of VS are as follows:

- Horses affected.
- Sporadic incidence in the herd (see preceeding).
- Distribution of lesions in an animal (small percentage of animals have lesions at more than one site of predilection; see preceeding).
- No rumen lesions observed at necropsy.
- No heart lesions observed at necropsy.
- Vesicular stomatitis is less severe in young animals.
- Stabled animals usually not affected.

In spite of these differences, do not attempt to make a final differential diagnosis in the field; get laboratory confirmation of the diagnosis.

Diagnosis

See Foot-and-mouth disease.

Differential Diagnosis

Differential diagnosis for VS in cattle should include foot-and-mouth disease, foot rot, and chemical and thermal burns. In cattle, oral lesions caused by rinderpest, infectious bovine rhinopneumonitis, bovine virus diarrhea, malignant catarrhal fever, and bluetongue can be similar to the later lesions in FMD. In pigs, the differential diagnosis for VS should include foot-and-mouth disease, swine vesicular disease, vesicular exanthema of swine, foot rot, and chemical and thermal burns. In sheep, the differential diagnosis for VS lesions should include bluetongue, contagious ecthyma, lip and leg ulceration, and footrot.

Control and Eradication

- Control movement of animals — no movement from an infected premise, except for slaughter, for 30 days after last lesion has healed.
- Separate infected and healthy animals.
- Stable animals if possible.
- Disinfect milking machines between cows.
- Milk infected cows last.
- Control insects.
- Commercial vaccines are available, but efficacy has not been field-tested.

Public Health: Vesicular stomatitis (New Jersey and Indiana) infection frequently occurs in man and causes influenza-like symptoms but rarely results in vesicles. Other vesicular stomatitis viruses (Piry, Isfahan, and Chandipura) are much more infectious for man.

2. PLANT PATHOGENS

This section introduces the 10 plant pathogens regulated by 7 CFR 121. Summary reference information is provided. Citations and internet addresses for each pathogen are provided in footnotes. This section includes summary information for the following pathogens:

- *Liberobacter africanus*
- *Liberobacter asiaticus*
- *Peronosclerospora philippinensis*
- *Phakopsora pachyrhizi*
- Plum pox potyvirus
- *Ralstonia solanacearum*, race 3, biovar
- *Sclerophthora rayssiae var. zeae*
- *Synchytrium endobioticum*
- *Xanthomonas oryzae pv. oryzicola*
- *Xylella fastidiosa* (citrus variegated chlorosis strain)

2.1 *Liberobacter africanus*[24]

Candidatus Liberibacter

Disease name: Citrus greening disease

Other Names:

- Greening
- Blotchy mottle
- Decline (likubin)
- Dieback
- Leaf mottle
- Vein phloem degeneration
- Yellow branch
- Yellow shoot (huang lung bin)

Causative Agent: An uncharacterized Gram-negative bacterium known as citrus greening disease bacterium for which the name *Liberibacter*, also seen as *Candidatus Liberibacter*, has been proposed Synonyms None Crops Affected Citrus crops Description of the Agent

The organism is poorly characterized. It is a filamentous Gram-negative organism that has not yet been cultured under controlled in the laboratory. It is transmitted by insects including:

- *Diaporina citri*
- *Trioza erytreae*
- and by the plant dodder (*Cuscuta spp.*)

Symptoms: Citrus greening can be a systemic disease, especially in younger trees. The first signs are seen in the leaves where yellowing along the veins of leaves or a blotchy mottle of leaves. There is sometimes a chlorotic mottle that resembles zinc or iron deficiencies. Fruit is misshapen and bitter. It often falls prematurely and fruit that stays on the tree does not color and seeds do not develop. The root systems do not develop properly.

[24]CBWInfo.com, September 2002. Factsheets on Chemical and Biological Warfare Agents, Version 2.1, http://www.cbwinfo.com/Biological/PlantPath/CGD.html

There are strain differences in symptoms with the Asian strains showing more severe symptoms than the African strains.

Prevention and Treatment: There are relatively few treatments. One of the most effective is heat treatment. Graftwood heated to 48-58°C (119-134°F) for several (conditions somewhat similar to pasteurization) eleiminate the disaease-causing agent.

The most effective method of preventing the disease is to control the insect vectors. This can be done by use of insecticides or (preferably) with predatory wasps that lay their eggs in the larvae of the vectors.

Agricultural practices including the careful selection of vector-free production areas, good crop husbandry and careful selection of trees.

Other Comments: Citrus greening disease is capable of being extremely destructive and causing catastrophic losses of an important crop. Infection rates of up to 95% have been seen in Thailand and crop losses of 100% have been seen in some areas of South Africa and it has eliminated citrus production in some parts of the country.

2.2 *Liberobacter asiaticus*

See *Liberobacter africanus*.

2.3 *Peronosclerospora philippinensis*[25]

Description: The Peronosporaceae family of microorganisms causes downy mildew diseases. Downy mildew primarily attacks young, tender leaves, twigs, and fruits. While a film of water is needed on plant tissue for spore germination, and a wet, humid environment and mild temperatures are necessary for infection and growth, when conditions are right downy mildew can spread rapidly through fields. Symptoms of downy mildew infection include small, pale yellow spots with indefinite borders on the upper leaf surface. There may also be purplish discoloration of the upper leaf surface on some hosts. A downy growth of fruiting structures and hyphae on the underside of the leaf may be visible directly under the spots, or on fruits or stems.

Exotic target pathogens include: *Peronosclerospora philippinensis*, which causes downy mildew disease of sugarcane and maize

2.4 *Phakopsora pachyrhizi*[26]

Synonym: Australasian Soybean Rust

Description: Soybean rust is a disease caused by the fungus *Phakopsora pachyrhizia*. It is most destructive in the tropical and subtropical regions of Asia and Australia. It has recently been found in Africa and South America. It is not known to occur in the United States.

The rust fungus causes lesions on the leaves, stem and pods. The main effects on the soybean plant is the destruction of photosynthetic tissue, premature defoliation and reduction of the number of days to maturity. The effect on yield is lower seed weight and fewer pods and seeds.

The rust pathogen has a wide host range of over 30 legume species. It is an obligate pathogen, which means it needs living host cells to survive.

Disease Cycle and Symptoms: The most common symptom is brown to reddish brown lesions on the leaves. If you look closely at the lesions you will often see tiny bumps within the lesion. The bumps are spore structures called uredinia, which give the leaf the typical "rusty" look. They are found most abundantly on the underside of the leaf, with fewer and smaller uredinia forming on the top of the leaf. Masses of clear to yellow-brown microscopic urediniospores are released from the uredinia. They are transported by air currents to other soybean plants and can be transported over long distances.

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[25]Cooperative Research Centre for Tropical Plant Protection - http://www.tpp.uq.edu.au/disease/downy.htm
[26]Plant Health Initiative, a North Central Soybean Research Program - http://www.planthealth.info/rust/rust.htm

Soybean rust can be diagnosed by microscopic examination of infected leaves at a plant disease clinic.

Environmental factors are critical to the incidence and severity of soybean rust. Long periods of leaf wetness are needed for urediospore germination, as well as temperatures between 15 and 30 degrees C and a high relative humidity of 75-80%. Urediospores are able to penetrate the plant cells directly, rather than through stomatal openings or wounds in the leaf tissue. Thus infection is relatively quick, about 9-10 days. The fungus eventually forms another type of spore called a teliospore. Their role in the epidemiology of this rust disease is not clear.

Rust Management: The most effective and efficient control measure for rust diseases is the use of resistant soybean varieties. Soybean pathologists and breeders at North Central universities and the USDA are working to identify the resistance of soybean lines under field conditions at international locations where the disease is endemic.

2.5 Plum pox potyvirus[27]

Description: Plum pox is the most serious disease of plums, apricots and peaches in Europe. It is caused by the plum pox virus (PPV). Different strains of the virus occur with variable host susceptibility and symptoms. Yield and overall economic losses have been catastrophic to plum and peach growers in many countries.

Disease distribution: First identified in Bulgaria, plum pox is now reported in most European countries, in parts of Asia and northern Africa, and in South America (Chile). In October 1999, the D-strain of PPV was discovered on peaches grown in Adams County, Pennsylvania.

Host plants: PPV infects virtually all cultivated fruit tree species of the genus Prunus. Severe symptoms occur on apricot, plum, and peach trees. Almond trees may be infected occasionally but show few symptoms. Sweet and sour cherries are affected by only some strains of PPV. The strain reported in the United States does not affect cherries. The disease also affects some wild Prunus species, especially blackthorn (Prunus spinosa) which has been a natural source of infection in many countries.

Symptoms: Plum pox symptoms can vary greatly, making the disease difficult to recognize. Symptoms may appear on the leaves or fruits of infected trees. In some cases, only part of the infected tree or even only a single branch will show symptoms.

Symptoms are easiest to see in the spring when leaves show chlorotic spots, bands, or rings, vein clearing and even leaf deformation. Infected fruits show chlorotic spots or rings. Diseased plums and apricots may be deformed with internal browning of the flesh and pale rings or spots on the stones.

Visual symptoms are not always a reliable indicator of disease. The disease may not be visible until several months or years after the tree has been infected. However, PPV can also be detected by laboratory analysis of tissue samples or by grafting test material to highly susceptible hosts and watching for symptoms to develop.

Biology: The main source of plum pox is infected trees or budwood. The virus is spread in the field by several species of aphids. The aphids acquire the virus during feeding and then transmit it to nearby healthy plants. The aphids can only transmit PPV for a short period of time after acquiring it.

The virus can also survive in roots and may spread by natural root grafting. The suckers produced from the remaining roots of rogued infected trees often carry the virus and must be removed.

Long distance spread of PPV occurs through the movement and use of infected propagative materials (grafting and budding of infected material). Some strains of PPV can be transmitted through seed and pollen. PPV cannot be spread by mechanical means such as pruning.

[27]Canadian Food Inspection Agency, Plant Products Directorate, Plant Health and Production Division, Horticulture Section. http://www.inspection.gc.ca/english/plaveg/hort/ppv/infoe.shtml

Control: Plum pox is a difficult disease to control. Once introduced it can quickly spread and become established in the native vegetation. Control of the virus requires regular surveys, removal of infected trees, and replacement with resistant varieties or non-host crops. There is no anti-virus treatment that can be applied to infected trees or orchards.

The use of disease-free propagative material at all times is crucial in preventing introduction to new areas.

2.6 *Ralstonia solanacearum*, race 3, biovar 2[28]

Background: There are three races of Bacterial wilt, *Ralstonia solanacearum*, which have been distinguished on the basis of pathogenicity. Race 3 of the pathogen mainly affects potatoes and tomatoes. Race 3 is also referred to as upland wilt or Biovar 2 as it has adapted to cooler temperatures and its importance in Europe has increased in recent years. The adaptability of the bacterium means that it also poses an increased threat to Western Australian potato growers.

Distribution: Bacterial wilt of potato occurs throughout Asia, Africa, Europe, Mexico, the Caribbean and South America. It has also been recorded in all states of Australia except Tasmania and Western Australia. The most serious reports have come from Queensland, New South Wales and Victoria. In these states there are areas where the disease is considered endemic. Bacterial wilt was introduced on one or more occasions through the planting of uncertified seed.

Potential impact: Bacterial wilt can cause losses of up to 75% in potato crops. Disease severity increases if *R. solanacearum* is found in association with root nematodes. Nematode infestation changes the physiology of the plants, increasing susceptibility to Bacterial wilt. For further information on state quarantine regulations refer to the Potato Industry Protection Plan and Reference Manual.

Plants affected: The main host of Bacterial wilt, *Ralstonia solanacearum* - race 3, is potatoes. Other hosts include solanaceous crops such as tomatoes, the garden plant *Pelargonium x hortorum* and the weed *Solanum nigrum* (blackberry nightshade).

Season of occurrence: Bacterial wilt persists in the soil all year round. It is also spread efficiently in infected soil as well as in water runoff from irrigation. In general, race 3 of the bacterium survives better under conditions of high soil moisture and low temperature. Race 3 will usually decrease in virulence when temperatures exceed 35°C. The disease is most severe at 24-35°C and is seldom found in temperate climates where the mean temperature for any winter month falls below 10°C. The main pathway for spread is by latently infected seed potatoes and other vegetative propagating materials. The greatest risk to WA is illegally imported seed potatoes that may be infected with the bacteria.

Symptoms

Foliage: The first visible symptom of Bacterial wilt is a wilting of the leaves at the ends of the branches during the heat of the day with recovery at night. This symptom can be mistaken for water stress. Plants eventually fail to recover and die. Plants may also look stunted and begin to yellow.

Stems: As the disease develops, a streaky brown discolouration of the stem may be observed on stems and the leaves have a bronze tint. A white, slimy mass of bacteria exudes from vascular bundles when broken or cut.

Tubers: External symptoms may be visible depending on the stage of development of the disease. Symptoms may be confused with those of ring rot. Bacterial wilt can be distinguished by the ooze that often emerges from the eyes and stem-end of infected tubers ('sore eye'). Cutting the diseased tuber will reveal a discolouration of the tissue about 4-5 mm below the skin, browning and necrosis of the vascular ring and immediately surrounding tissues up to 0.5 cm each side of the ring.

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[28] Edited and published by Information Services, Agriculture Western Australia, Locked Bag No. 4, Bentley Delivery Centre, WA 6983 By Chris Stansbury, Simon McKirdy, Alison Mackie and Greg Power, HortguardTM Initiative AGWEST.
http://www.agric.wa.gov.au/agency/pubns/factsheets/2001/fs00701.pdf

2.7 *Sclerophthora rayssiae* var. *zeae*[29]

Synonym: Brown stripe downy mildew

Causal organism: *Sclerophthora rayssiae* var. *zeae*, Mastigomycotina (Fungi)

Description: Fungal disease which is an only downy mildew disease occurring on corn in Japan. The disease begins to occur in the rainy season. The lesion is yellow to yellowish brown, delimited by the leaf vein, rectangular to stripe, 0.5-2 cm in length and 3-7 mm in width. The lesion elongates long, and becomes purple red, and the entire leaf withers. The damage is limited to lower leaves and not so severe now in Japan. About 30 degrees in temperature of the soil is suitable for the occurrence. The pathogen is considered to infect fingergrass, too.

2.8 *Synchytrium endobioticum*[30]

Synchytrium endobioticum (Schilberzky) Percival (Fungi)

Synonyms: Potato Wart or Potato Canker

Hosts**:** *Synchytrium endobioticum* is an obligate parasite of certain members of the order Solanaceae with potato (Solanum tuberosum) being the only cultivated host. In Mexico some wild Solanum species are also known as hosts.

Distribution

Asia: Bhutan, China, India, Lebanon, Nepal

Africa: Algeria, South Africa, Tunisia

Europe: All countries, except Portugal where it was eradicated.

North America:Canada (NFLD), USA (eradicated), Mexico.

Pacific:New Zealand's south island

South America: Bolivia, Chile, Falkland Is., Peru, Uruguay

Biology**:** S. endobioticum is a soil borne fungal parasite which does not produce hyphae, but sporangia containing anywhere from 200-300 motile zoospores. In the spring, at temperatures above 8·C and given sufficient moisture, the overwintering sporangium found in decaying warts in the soil germinate and release uninucleate zoospores. The zoospores possess a single flagellum (tail) which enables them to move in soil water to reach the host. Once a suitable host cell is encountered, the zoospore will shed it's tail and penetrate the cell. The infected cell swells as the enclosed fungus forms a short-lived but quickly reproducing structure, the summer sporangium, from which numerous zoospores are released to infect neighbouring cells. This cycle of infection and release may be repeated for as long as conditions are suitable, resulting in the host tissue becoming thoroughly infected. The cells surrounding the infected ones also swell and the tissue proliferates, producing the characteristic cauliflower-like appearance.

Under certain conditions of stress, such as water shortage, the zoospores may fuse in pairs to form zygotes. In this case, the host cell wall remains closely attached, forming an outer layer to the resistant, thick-walled structure called the winter sporangium. Mature winter sporangium are released into the soil from rotting warts. They can remain viable for up to 30 years and can be found at depths of up to 50 cm. The disease can be spread by infected seed tubers which may have incipient warts, or in infested soil attached to tubers. The sporangia can survive digestion by animals, and therefore can be spread by fecal material.

[29]National Institute of Livestock and Grassland Sciences, Japanese site. http://ss.ngri.affrc.go.jp/diseases/de1.htm

[30]Wallace, S., and R. Favrin, Canadian Food Inspection Agency, Plant Pest Information, Plant Health Survey Unit. http://www.inspection.gc.ca/english/ppc/science/pps/datasheets/synende.shtml

Detection & Identification

Symptoms: The disease appears on all underground parts except the roots. Buds on stems, stolons, and tubers are the centres of infection and abnormal growth activity leading to wart formation. Above ground symptoms are not usually apparent although there maybe a reduction in plant vigour.

Identification: The warts vary from very small protuberances to large intricately branched systems. A typical wart is initially white in colour, roughly spherical, but it is usually not a solid structure. It is soft and pulpy and can be cut more easily than a tuber. Morphologically it consists of distorted, proliferated branches and leaves grown together into a mass of hyperplastic tissue resembling the head of a cauliflower. Often a developing wart may become exposed at or above the soil line, in which case it will turn green. As the warts become older, whether above or below ground, they darken and decay. It is not uncommon for the entire tuber to be replaced by the warty proliferation. Warts which develop on potatoes in storage may be the same colour as the tuber.

2.9 *Xanthomonas oryzae pv. oryzicola*[31]

Common name: Bacterial leaf streak

Diagnostic summary: Damage to plants - browning and drying of leaves, reduced 1000-grain weight under severe condition.

Signs: Initial symptoms are dark-green and water-soaked streaks on interveins from tillering to booting stage streaks later enlarge to become yellowish gray and translucent bacterial exudates on surface of lesions - lesions turn brown to grayish white then dry - browning and drying of entire leaves

Symptoms: Initially, small, dark-green and water-soaked streaks on interveins from tillering to booting stage. Streaks dark-green at first and later enlarge to become yellowish gray and translucent. Numerous small yellow beads of bacterial exudates on surface of lesions on humid conditions. Very small yellow beads instead of bacterial exudates during dry season. Lesions turn brown to grayish white then dry when disease is severe. Yellow halo around lesions on susceptible cultivars. Browning and dying of entire leaves. Bleached and grayish white leaves

Confirmation: The linear streaks or narrow transparent streak can be seen against the sunlight. When the advancing part of the streaks are cut and placed in a glass with water, mass of bacterial cells would be seen coming out from the leaf making the water turbid after 5 minutes.

Problems with similar symptoms: BLS is the only leaf spot disease with transparent narrow streaks as compared with other leaf diseases like brown spot, narrow brown spot, and bacterial blight.

At an early stage, the symptom looks similar to that of the narrow brown leaf spot. At a later stage, when the streaks coalesced, the symptoms of bacterial leaf streak look the same as those of bacterial blight.

Bacterial leaf streak can be distinguished from bacterial blight by its thinner translucent lesions with the yellow bacterial ooze.

Why and where it occurs: The disease is transmitted through seeds to the next planting season. Planting of infected seeds, which are collected from diseased fields produce diseased seedlings. The bacteria, which is present in the water or those surviving in the debris left after harvest, are also sources of inoculum in the next planting season.

The bacterial cells in beads on leaves when moistened by dew or rain disperse and spread by wind cause new infection or damage on the same leaves or other leaves. High temperature and high humidity also favor new infection and development of lesions.

The disease usually occurs during the early stage of planting from maximum tillering to panicle initiation. Older plants are more resistant to the disease.

[31] Suparyono, JLA Catindig, and IP Oña, International Rice Research Institute. http://www.knowledgebank.irri.org/ricedoctor_mx/Fact_Sheets/Diseases/Bacterial_Leaf_Streak.htm

Causal agent or factor: The bacteria causing the disease *X. oryzae* pv. oryzicola occur as rods. They are 1.2 x 0.3-0.5 µm in dimension. They are single, occasionally in pairs but not in chains. The bacteria have no spores and no capsules. They move with the aid of a single polar flagellum. They are Gram-negative and aerobic and can grow favorably at 28 °C.

The bacterial colonies on nutrient agar are pale yellow, circular, smooth, convex, and viscid and have an entire margin. Their growth on slant is filiform. Growth in nutrient broth is moderate with a surface ring growth without a definite pellicle.

Factors affecting development of the pest: Presence of the bacteria on leaves and in the water or those surviving in the debris left after harvest - high temperature and high humidity - early stage of planting from maximum tillering to panicle initiation

Host range: Species of wild rice such as Oryza spontanea, O. perennis balunga, O. nivara, O. breviligulata, O. glaberrima, and Leersia hexandra Sw. (southern cutgrass) are alternate hosts of the disease.

Mechanism of damage: The bacterium penetrates the leaf through natural openings (such as stomata and hydatodes), leaf injuries or artificial wounds due to wind, insect bites, or others. Initial infection is observed only on the parenchyma cells in between the veins of the leaf. Highest infection occurs at midday, during which the leaf stomata are fully opened. After gaining entrance, the bacterium multiplied in the substomatal cavity and progressed intercellularly in the parenchyma. Soon after lesions develop, bacterial exudates form on the surface of the lesions under moist conditions during the night. Under dry conditions, these exudates become small, yellow beads that eventually fall into the irrigation water. When the leaves are wet from dew or rain, with the aid of wind, the bacteria is carried from field to field by irrigation water.

Economic importance: Bacterial leaf streak is widely distributed in Taiwan, southern China, Southeast Asian countries, India, and West Africa. The disease is not reported to occur in temperate countries including Japan. Losses as high as 32.3% in 1000-grain weight due to BLS were reported.

At three disease intensities, the estimated yield losses were 8.3%, 13.5%, and 17.1% in the wet season and 1.5%, 5.9%, and 2.5% during the dry season.

Management principles: The disease can be controlled by proper application of fertilizers and proper planting spacing, the use of resistant varieties, and hot water treated seeds.

Practicing field sanitation is important. Ratoons, straws and volunteer seedlings left after harvest can be destroyed to minimize the initial inoculum at the beginning of the season. Providing good drainage system especially in seedbeds can also manage this disease.

Planting of resistant varieties, which are available at IRRI and at National Research Institute, is the most effective method of controlling bacterial leaf streak. Fallow field and allowing to dry thoroughly is also recommended.

2.10 *Xylella fastidiosa* (citrus variegated chlorosis strain)[32]

Disease Name: Almond leaf scorch, Citrus variegated chlorosis, Phony peach disease, Pierce's disease, Plum leaf scald

Agent Type: Bacterium

Target: Vines,fruit and timber trees

Related Agents: None

Listed by the Australia Group: Yes

[32]CBWInfo.com, September 2002. Factsheets on Chemical and Biological Warfare Agents, Version 2.1. http://www.cbwinfo.com/Biological/Pathogens/SecondaryBW/SecTY.html#TY8

Comments: *Xylella fastidiosa* is a bacterium that lives in the xylem of plants that is crucial for the movement of liquids and nutrients. As they block transmission of water the plant begins to die. This starts at the edges of leaves and progresses into the central tissues of the plant with the roots also being affected. The disease is transmitted rapidly by insects known as sharpshooter leafhoppers and also inadvertently by grafting.

3. OVERLAP PATHOGENS AND TOXINS (ANIMAL AND HUMAN/ZOONOTIC)

This section introduces the 20 zoonotic pathogens and toxins regulated by 9 CFR 331 and 42 CFR 73. Summary reference information is provided. Citations and internet addresses for each pathogen or toxin are provided in footnotes. This section includes summary information for the following pathogens and toxins:

- *Bacillus anthracis*
- *Botulinum neurotoxins* (more than 0.5 mg)
- *Botulinum neurotoxin producing species of Clostridium*
- *Brucella abortus*
- *Brucella melitensis*
- *Brucella suis*
- *Burkholderia mallei (*formerly *Pseudomonas mallei)*
- *Burkholderia pseudomallei (*formerly *Pseudomonas pseudomallei)*
- *Clostridium perfringens epsilon toxin* (more than 100 mg)
- *Coccidioides immitis*
- *Coxiella burnetii*
- Eastern equine encephalitis virus
- Francisella tularensis
- Hendra virus
- Nipah virus
- Rift Valley fever virus (non-vaccine strain (MP-12))
- Shigatoxin (more than 100 mg)
- Staphylococcal enterotoxins (more than 5 mg)
- T-2 toxin (more than 1,000 mg)
- Venezuelan equine encephalitis virus (non-vaccine strain (TC-83))

3.1 *Bacillus anthracis*[33]

Clinical Manifestations: Anthrax is caused by *Bacillus anthracis*. Humans acquire the disease directly from contact with infected herbivores or indirectly via their products. The clinical forms include (1) cutaneous anthrax (eschar with edema), from handling infected material (this accounts for more than 95 percent of cases); (2) intestinal anthrax, from eating infected meat; and (3) pulmonary anthrax, from inhaling spore-laden dust. Several other Bacillus spp, in particular *B cereus* and to a lesser extent *B subtilis* and *B licheniformis*, are periodically associated with bacteremia/septicemia, endocarditis, meningitis, and infections of wounds, the ears, eyes, respiratory tract, urinary tract, and gastrointestinal tract. *Bacillus cereus* causes two distinct food poisoning syndromes: a rapid-onset emetic syndrome characterized by nausea and vomiting, and a slower-onset diarrheal syndrome.

Structure and Classification: Bacillus species are rod-shaped, endospore-forming aerobic or facultatively anaerobic, Gram-positive bacteria; in some species cultures may turn Gram-negative with age. The many species of the genus exhibit a wide range of physiologic abilities that allow them to live in every natural environment. Only one endospore is formed per cell. The spores are resistant to heat, cold,

[33]Turnbull, Peter C. B. Medical Microbiology, Fourth Edition, Bacillus chapter. http://gsbs.utmb.edu/microbook/ch015.htm

radiation, desiccation, and disinfectants. *Bacillus anthracis* needs oxygen to sporulate; this constraint has important consequences for epidemiology and control. In vivo, *B anthracis* produces a polypeptide (polyglutamic acid) capsule that protects it from phagocytosis. The genera Bacillus and *Clostridium* constitute the family Bacillaceae. Species are identified by using morphologic and biochemical criteria.

Pathogenesis: The virulence factors of *B anthracis* are its capsule and three-component toxin, both encoded on plasmids. Bacillus cereus produces numerous enzymes and aggressins. The principal virulence factors are a necrotizing enterotoxin and a potent hemolysin (cereolysin). Emetic food poisoning probably results from the release of emetic factors from specific foods by bacterial enzymes.

Host Defenses: The reasons for marked differences in susceptibility to anthrax among different animal species are not known. The protective actions of the live-spore animal vaccine or the human chemical vaccines are based on induction of humoral and cell-mediated immunity to the protective antigen component of anthrax toxin.

Epidemiology: Individuals at risk for anthrax include those in contact with infected animals or animal products. Episodes of *B cereus* food poisoning occur sporadically worldwide and result from ingestion of contaminated food in which the bacteria have multiplied to high levels under conditions of improper storage after cooking.

Diagnosis: Cutaneous anthrax is diagnosed on the basis of the characteristic papule (early) or eschar (later) with extensive surrounding edema, backed by a history of exposure to animals or their products. Diagnosis is confirmed by observation of characteristic encapsulated bacilli in polychrome methylene blue-stained smears of blood, exudate, lymph, cerebrospinal fluid, etc., and/or by culture. Other Bacillus infections are diagnosed by culture of the bacteria.

Control: Control in animals is essential for control in humans. In endemic areas, animals that die suddenly should be handled cautiously and livestock should be vaccinated annually. A human vaccine is available for individuals in high-risk occupations. Anthrax is readily treated with antibiotics (e.g., penicillin, tetracycline, chloramphenicol, gentamicin, or erythromycin).

Pharmaceutical, Agricultural, and Industrial Importance: Many of the physiologic properties and specialized metabolites of Bacillus species are used in the pharmaceutical, agricultural, and food industries. On the other hand, the resistance of the spores to sterilization and disinfection makes them problem contaminants in foods, medical supplies, surgical procedures, etc.

3.2 Botulinum neurotoxins[34]

Introduction: Botulinum toxin is very strong poison produced by the microorganism *Clostridium botulinum. C. botulinum* is classified as a single species but consists of at least three genetically distinguishable groups of organisms. These are alike in their abilities to produce neurotoxins with similar pharmacological activities but diverse serologic properties (toxin types A, B, C, D, E, F, and G). These types are defined by the International Standards for *Clostridium botulinum* Antitoxin. Botulinum toxins are the causative agents of botulism, a potentially fatal condition of neuromuscular paralysis. Botulism is characterized by symmetric, descending, flaccid paralysis of motor and autonomic nerves, usually beginning with the cranial nerves. Blurred vision, dysphagia, and dysarthria are common initial complaints. The diagnosis of botulism is based on compatible clinical findings; history of exposure to suspect foods; and supportive ancillary testing to rule out other causes of neurological dysfunction that mimic botulism, such as stroke, Guillain-Barré syndrome, and myasthenia gravis. Treatment includes supportive care and trivalent equine antitoxin, which reduces mortality if administered early.

[34]Price, Colonel Richard, ed. Applied Science and Analysis, Inc., The ASA Newsletter, ISSN 1057-9419, Botulinum Toxin: From Poison to Medicinal Agent by Jiri Patocka (Department of Toxicology, Military Medical Academy, Hradec Kralove and Faculty of Health and Social Care University of South Bohemia, Ceské Budejovice) and Miroslav Splino (Department of Epidemiology, Military Medical Academy, Hradec Kralove, Czech Republic). http://www.asanltr.com/newsletter/02-1/articles/Botulinum.htm

Clinicians are the first to treat patients in any type of botulism outbreak. It is important that they know how to recognize, diagnose, and treat this rare but potentially lethal disease. Recently, the potential terrorist use of botulinum toxin has become an important concern. Human botulism is primarily caused by the strains of *C. botulinum* that produce toxin types A, B, and E. The strains of *C. baratii*, which produce type F toxin and *C. butyricum*, which produces type E toxin, also have been implicated in human botulism. Strains of *C. botulinum* that produce type C or type D toxin for the most part cause botulism only in nonhuman species. All these neurotoxigenic organisms are anaerobic, gram-positive, spore-forming bacilli and are commonly found in soils throughout the world. *C. botulinum* organisms cause food poisoning because the heat-resistant spores survive food preservation methods that kill non-sporulating organisms; they subsequently produce a potent neurotoxin under anaerobic, low-acid (pH > 4.6), and low solute conditions. The toxins affect a broad range of vertebrate species, but the evolutionary utility of toxin production to the bacterial host organisms is unclear.

Epidemiology: Four clinical forms of botulism occur in humans: foodborne botulism; wound botulism; infant botulism; and, rarely, adult infectious botulism. Foodborne botulism is a public health emergency because the contaminated food may still be available to other people besides the patient. Studies in monkeys indicate that, if aerosolized, botulinum toxin also can be absorbed through the lungs. The persistence of botulinum toxin is very high: it remains in nonmoving water and food for weeks. Important changes in the epidemiology of botulism have emerged in the past few decades. Recently identified vehicles for foodborne botulism include homemade salsa and traditionally prepared salted or fermented fish. In recent years, restaurant-associated outbreaks accounted for a large proportion of botulism cases. Botulism is not spread from one person to another. Foodborne botulism can occur in all age groups. Infant botulism occurs in a small number of susceptible infants each year who harbor *C. botulinum* in their intestinal tract. It usually affects children in the first year of life (mortality 2 %), with the onset of obstipation, lethargy, ptosis, swallowing discomfort, hypotonia, general weakness, and distressed breathing. It accounts for 5 - 10 % of the Sudden Infant Death Syndrome (SIDS).

Clinical Features of Botulism: Foodborne botulism is caused by ingestion of preformed toxin produced in food by *C. botulinum*. The most frequent source is home-canned foods, in which spores that survive an inadequate cooking and canning process germinate and produce toxin in the anaerobic environment of the canned food. In the event of intentional foodborne poisoning with botulinum toxin, the signs and symptoms developing after ingestion would probably resemble those of naturally occurring foodborne botulism. If aerosolized toxin was inhaled, the incubation period might be slightly longer and gastrointestinal symptoms might not occur. The clinical syndrome of foodborne botulism is dominated by neurologic symptoms resulting from a toxin-induced block of the voluntary motor and autonomic cholinergic junctions. With foodborne botulism, symptoms begin within 6 hours to 2 weeks (most commonly between 12 and 36 hours) after eating toxin-containing food. Although the syndrome is similar for each toxin type, type A toxin has been associated with more severe disease and a higher fatality rate than type B or type E toxin. Symptoms from any toxin type may range from subtle motor weakness or cranial nerve palsies to rapid respiratory arrest. The initial symptoms of foodborne botulism may be gastrointestinal and can include nausea, vomiting, abdominal cramps, or diarrhea; after the onset of neurologic symptoms, constipation is more typical. Dry mouth, blurred vision, and diplopia are usually the earliest neurologic symptoms. These initial symptoms may be followed by dysphonia, dysarthria, dysphagia, and peripheral muscle weakness. Symmetric descending paralysis is characteristic of botulism. Paralysis begins with the cranial nerves, the upper extremities, the respiratory muscles, and, finally, the lower extremities in a proximal-to-distal pattern. Onset usually occurs 18 to 36 hours after exposure. In severe cases, extensive respiratory muscle paralysis leads to ventilatory failure and death unless supportive care is provided. Patients have required ventilatory support for up to 7 months before the return of muscular function, but ventilatory support is most commonly needed for 2 to 8 weeks. Clinical recovery generally occurs over weeks to months; electron microscopic evidence suggests that clinical recovery correlates with the formation of new presynaptic end plates and neuromuscular junctions. Before mechanical ventilation and intensive supportive care, up to 60% of patients died. Death now occurs in 5%

to 10% of cases of foodborne botulism; early deaths result from a failure to recognize the severity of disease, whereas deaths after 2 weeks result from complications of long-term mechanical ventilatory management. Administration of trivalent or heptavalent botulinum antitoxin is recommended as the treatment for Botulinum toxin poisoning (in USA, CDC - Center for Disease Control, Atlanta, Georgia).

Editor's Note:

1. *Botulinum toxins are very potent toxins with real potential as a biological agent for both warfare and terrorism. They also have great utility in medicine to treat spasmodic muscle disorders. Ironically, the more common they become in medical treatment, the more of a potential threat they become because they are produced commercially in relatively large amounts. But to put this in perspective, BotoxA is sold in vials of 100 U (20 U = 1 ng) as stated on the package insert. Even though it may only take 90 g to contaminate a typical large reservoir, it will take over a billion of these vials to contaminate the reservoir to 1 ng/L.*
2. *The toxicity of botulinum toxin is usually expressed in mouse unit (U), where one unit (U) of Botulinum toxin A is the median intraperitoneal lethal dose (LD_{50}) in mice and is approximately 20 units/nanogram (1U=about 0.05 ng). The lethal dose in humans is not known, but extrapolating monkey data to a 70 kg human implies a parenteral lethal dose of nearly 3000 U, i.e., 4.3 µg. According to JAMA, 285, 28 Feb 2001, "Botulinum Toxin as a Biological Weapon" by Stephen S. Amon, et. al., the lethal dose for humans, extrapolated from monkey data is 0.09-0.15 µg of toxin by iv and im., 0.70-0.90 µg by inhalation and 70 µg orally.*

3.3 Botulinum neurotoxin producing species of *Clostridium*[35]

Clostridium botulinum is an anaerobic, Gram-positive, spore-forming rod that produces a potent neurotoxin. The spores are heat-resistant and can survive in foods that are incorrectly or minimally processed. Seven types (A, B, C, D, E, F and G) of botulism are recognized, based on the antigenic specificity of the toxin produced by each strain. Types A, B, E and F cause human botulism. Types C and D cause most cases of botulism in animals. Animals most commonly affected are wild fowl and poultry, cattle, horses and some species of fish. Although type G has been isolated from soil in Argentina, no outbreaks involving it have been recognized.

See also – Botulinum neurotoxins

3.4 *Brucella abortus*[36]

Clinical Manifestations: Brucellosis is a severe acute febrile disease caused by bacteria of the genus Brucella. Relapses are not uncommon; focal lesions may occur in bones, joints, genitourinary tract, and other sites. Hypersensitivity reactions can follow occupational exposure. Infection may be subclinical. Chronic infections may occur.

Structure: Brucellae are Gram-negative coccobacilli; non-spore-forming and non-motile; aerobic, but may need added CO2.

Classification and Antigenic Types: Three species (*B melitensis, B abortus, B suis*) are important human pathogens; *B canis* is of lesser importance. Species are differentiated by production of urease and H2S, dye sensitivity, cell wall antigens and phage sensitivity. The major species are divided into multiple biovars.

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[35]U.S. Food & Drug Administration, Center for Food Safety & Applied Nutrition, Foodborne Pathogenic Microorganisms and Natural Toxins Handbook - Clostridium botulinum. http://vm.cfsan.fda.gov/~mow/chap2.html

[36]Alton, G. G., and J.R.L. Forsyth. Medical Microbiology, Fourth Edition, Brucella chapter. http://gsbs.utmb.edu/microbook/ch028.htm

Pathogenesis: Portals of entry are the mouth, conjunctivae, respiratory tract and abraded skin. Organisms spread, possibly in mononuclear pha gocytes, to reticuloendothelial sites. Small granulomas reveal a mononuclear response; hypersensitivity is a major factor.

Host Defenses: Effective host defense depends mainly upon cell-mediated immunity.

Epidemiology: Brucellosis is a zoonosis, acquired from handling of infected animals or consuming contaminated milk or milk products. Exposure is frequently occupational. The disease is now uncommon in the United States and Britain but common in the Mediterranean and Arabian Gulf regions, Latin America, Africa, and parts of Asia.

Diagnosis: Diagnosis can be made clinically if there is a history of exposure. Blood cultures may be positive in early disease but serology is mainstay of diagnosis. Interpretation is complicated by subclinical infections and persistent levels of antibody.

Control: Brucellosis is prevented by pasteurizing milk, eradicating infection from herds and flocks, and observing safety precautions (protective clothing and laboratory containment). The disease is treated with doxycycline, streptomycin and rifampin.

3.5 *Brucella melitensis*

See *Brucella abortus*

3.6 *Brucella suis*

See *Brucella abortus*

3.7 *Burkholderia mallei* (formerly *Pseudomonas mallei*)

Only two species, *P mallei* and *P pseudomallei*, produce specific human diseases: glanders and melioidosis. Unlike most pseudomonads, *P mallei* and *P pseudomallei* can cause disease in otherwise healthy individuals. *Pseudomonas mallei* is the agent of glanders, a disease primarily of equines. Humans generally become infected by inhalation or by direct contract through abraded skin. These infections are frequently fatal within 2 weeks of onset, although chronic infections also have been reported. Today, *P mallei* infections of equines are controlled and are rarely encountered in the western world. Similarly, melioidosis, an endemic glanderslike disease of animals and a human pulmonary infection caused by *P pseudomallei*, is rare in the western hemisphere. Melioidosis is still found in Southeast Asia, and travelers returning from that area are sometimes infected.[37]

Glanders[38]

(Droes, Farcy, Malleus)

Definition: Glanders is a highly contagious disease of solipeds caused by *Pseudomonas mallei* and characterized by nodular lesions of the lungs and other organs as well as ulcerative lesions of the skin and mucous membranes of the nasal cavity and respiratory passages. The disease typically has a progressive course and poses a significant human health risk.

Etiology: Glanders is caused by the bacteria *Pseudomonas mallei*. Former names of this pathogen include *Loefflerella mallei*, *Pfeifferella mallei*, *Malleomyces mallei*, *Actinobacillus mallei*, *Corynebacterium mallei*, *Mycobacterium mallei*, and *Bacillus mallei*. In experimental infection of guinea pigs *Ps. mallei* produces a tenacious capsule that may serve to protect it from phagocytosis. The organism

[37]Iglewski, Barbara H. Medical Microbiology, Fourth Edition, Pseudomonas chapter. http://gsbs.utmb.edu/microbook/ch027.htm

[38]Committee on Foreign Animal Diseases of the United States Animal Health Association, revised 1998. Foreign Animal Diseases, "The Gray Book," Part IV, Animal Health Association, Library of Congress Catalog Card Number 17-12842, Pat Campbell & Associates and Carter Printing Company, Richmond, VA. http://www.vet.uga.edu/vpp/gray_book/FAD/GLA.htm

is closely related to *Ps. pseudomallei*, the cause of melioidosis, and is serologically indistinguishable in some cases. Genetic homology between *Ps. mallei* and *Ps. pseudomallei* approaches 70 percent. Because of this many consider them to be biotypes or isotypes.

The organism is destroyed by direct sunlight and is sensitive to desiccation. It is readily killed by common disinfectants. It may survive for up to 6 weeks in infected stables.

Host Range: Glanders is primarily a disease of solipeds — particularly horses, donkeys, and mules. Traditionally, donkeys have been regarded as most likely to experience the acute form of the disease and horses a more chronic form, with mules intermediate in susceptibility. Recent reports suggest that chronic and even latent infections are equally likely in mules. Carnivores are susceptible to disease if they consume glandered meat; felids appear to more susceptible than canids, and outbreaks of glanders in captive wild felids have been reported. Several laboratory animals are susceptible to infection including, hamsters and guinea pigs. The susceptibility of the latter species formed the basis of the Strauss reaction in the diagnosis of the disease. Humans also are susceptible to infection with glanders, which is an important occupational disease of veterinarians, farriers, and other animal workers. Swine and cattle are resistant to infection with Ps. mallei, but goats can be infected.

Geographic Distribution: Glanders is currently limited to parts of Asia, Africa, the Middle East, and Asia (specifically Turkey, Syria, Iraq, Iran, Pakistan, India, Burma, Indonesia, the Philippines, China, and Mongolia) and possibly the Balkan states, former Soviet republics, Mexico, and South America. Cross-reactions with serological tests for *Ps. pseudomallei* may confound estimates of worldwide distribution. Although glanders was once widespread throughout the world, it has been eradicated from many countries by diligent test and slaughter programs.

Transmission: The disease is introduced into horse populations by diseased or latently infected animals. Ingestion of the pathogen, present in secretions from infected animals, constitutes the major route of infection in glanders. Experimental evidence suggests that inhalation of the organism is less likely to result in typical cases of the disease. Although invasion by way of skin lesions is possible, it is regarded as being of minor importance in the natural spread of the disease. Close proximity alone does not usually result in transmission of glanders; transmission is facilitated if the animals share feeding troughs or watering facilities or if they nuzzle each other.

Incubation Period: After artificial infection, a fever, 106 degrees F (41 degrees C), develops in about 3 days and clinical signs within a week. After natural infection, weeks or months may elapse before manifestations of the disease are apparent. Such latent infections are a feature of the epidemiology of glanders.

Clincial Signs: Classical descriptions of glanders distinguish between cutaneous, nasal, and pulmonary forms of the disease, but in most outbreaks these forms are not clearly distinct and may occur simultaneously in an animal. Chronic infections with slow progression of an insidious disease are more common than the acute form of glanders. The acute form (more common in donkeys and mules than in horses) typically progresses to death within about a week.

The nasal form of glanders is characterized by unilateral or bilateral nasal discharge. The yellowish-green exudate is highly infectious. The nasal mucosa has nodules and ulcers. These ulcers may coalesce to form large ulcerated areas, or they may heal as stellate scars of the mucosa. In some cases the septum may even be perforated. Nasal lesions are accompanied by enlargement and induration, or sometimes rupture and suppuration, of regional lymph nodes.

In the cutaneous form of glanders, multiple nodules may develop in the skin of the legs or other parts of the body. These nodules may rupture, leaving ulcers that discharge a yellow exudate to the skin surface and heal slowly. Cutaneous lymphatic vessels in the region become involved. They become distended and firm by being filled with a tenacious, purulent exudate (These may be referred to as "Farcy pipes.") In the pulmonary form of glanders, lesions in the lungs develop in concert with nasal and cutaneous lesions or may be the sole manifestation of the disease (typical of latent cases). The lung lesions begin as firm nodules or as a diffuse pneumonic process. The nodules are gray or white and firm, surrounded by a

hemorrhagic zone, and may become caseous or calcified. Clinical signs in animals with lung lesions may only range from inapparent infection to mild dyspnea, or severe coughing and obvious lower respiratory tract involvement.

Lesions may also occur in the liver or spleen and, in male animals, glanderous orchitis is a common lesion.

Gross Lesions: Nodular lesions of glanders are most consistently found beneath the pleura of the lung. In some acute cases, however, a more diffuse form of lobular pneumonia may be present. The nodular lesions, typically about 1 cm in diameter, consist of a gray or white core of necrotic material that may become calcified and are surrounded by a zone of hyperemia and edema. Similar lesions may be found in other viscera. Glanderous orchitis may be seen in intact males.

Nasal lesions consist of submucosal nodules surrounded by a small zone of hyperemia. These nodules may rupture, leaving exudative ulcers. As new lesions develop it is not unusual to find small nodules, ulcers, and scars side by side. Lymphadenitis of associated lymph nodes is a consistent finding. In some cases laryngeal lesions similar to the nasal lesions may be found.

Cutaneous lesions consist of cord-like thickening of subcutaneous lymphatics along which are distributed chains of nodules, some of which are ulcerated.

Morbidity and Mortality: When horses, donkeys, and mules are concentrated, the morbidity can be high.

Diagnosis

Field Diagnosis

Typical nodules, ulcers, scars, and a debilitated condition can be sufficient to diagnose glanders. Unfortunately, many cases of glanders are latent and clinically inapparent. Therefore, systematic testing is essential to identify all infected animals in an outbreak. The mallein test has been the mainstay of field diagnosis. Mallein is a lysate of *Ps. mallei* containing both endotoxins and exotoxins elaborated by the organism. Infected animals are allergic to mallein and exhibit local and systemic hypersensitivity after mallein inoculation similar to that exhibited in tuberculin testing. Inoculation with mallein may trigger a humoral serologic reaction to the complement fixation test. This seroconversion is thought to be transient but may be permanent if the animal undergoes repeated mallein testing. This is extremely important to consider if animals are destined for export to countries that depend on the complement fixation test.

The preferred method of application of mallein is intrapalpebral. The mallein (0.1 ml) is injected into the dermis of the lower eyelid. In positive cases marked edema of the eyelid, purulent conjunctivitis, photophobia, pain, and depression may be observed within 12 to 72 hours. The test is usually read 48 hours after injection.

The ophthalmic mallein test consists of the instillation of mallein into the conjunctival sac. A positive reaction is characterized by development of severe purulent conjunctivitis within 6 to 12 hours. A larger volume of dilute mallein (2.5 MI) may be injected subcutaneously, causing fever, local swelling, and pain in positive animals.

Specimens for Laboratory

A whole or section of a lesion and a serum sample should be collected aseptically. The samples should be kept cool and shipped on wet ice as soon as possible. Sections of lesions in 10 percent buffered formalin and air-dried smears of exudate on glass slides should be submitted for microscopic examination.

Laboratory Diagnosis

The causative organism may be cultured from fresh lesions or lymph nodes. It may also be demonstrated micoscopically in films made from this material.

The Strauss reaction is observed when infectious material from glanders patients is injected intraperitoneally into male guinea pigs. In positive cases, the guinea pig develops localized peritonitis involving the scrotal sac. Glanderous orchitis follows with painful enlargement of the testes. The testis becomes enlarged and painful and ultimately necrotic and is discharged through the scrotal skin.

A variety of serologic tests for glanders have been developed. They are superior to mallein testing in sensitivity and specificity. The complement fixation test is widely used and is reported to have an overall accuracy of 95 pecent. A counter-immunoelectrophoresis test has been described. Recently a dot enzyme-linked immunosorbent assay has been developed and found to be superior to all previously described tests in its sensitivity. This test is inexpensive, rapid, and easy to perform and is not influenced by anticomplement activity. Cross-reactions with Ps. pseudomallei, the cause of melioidosis, are features of all of the serological tests for glanders. Therefore, these tests will result in false positive reactions in animals from areas where melioidosis is endemic.

Differential Diagnosis

Signs of glanders must be distinguished from strangles, epizootic lymphangitis, ulcerative lymphangitis, melioidosis, and other forms of pneumonia. Purulent sinusitis, guttural pouch empyema, and other causes of nasal catarrh should also be considered. Skin lesions may be similar to those of dermatophilosis or dermatomycoses such as sporotrichosis. Knowledge of the progressive debilitating nature of glanders and application of serological or mallein tests will serve to distinguish glanders from other similar diseases.

Strangles is caused by Streptococcus equi. It is characterized by fever, anorexia, and depression with swollen submandibular lymph nodes and mucopurulent nasal discharge. The nasal discharge is usually bilateral, whereas it is most often unilateral in cases of glanders. Skin nodules and typical lung lesions are absent. Animals with strangles will not react to mallein testing or serological tests for glanders. S. equi is readily demonstrable. Strangles does not develop into a chronic, debilitating condition, and most infected horses recover within a few weeks.

Epizootic lymphangitis (caused by Histoplasma farciminosum) is characterized by cutaneous nodules originating from superficial lymph vessels. In epizootic lymphangitis, conjunctivitis is a common lesion. Demonstration of the infectious agent and application of the mallein test and serological testing will help distinguish between these diseases.

Ulcerative lymphangitis (caused by Corynebacterium pseudotuberculosis) is characterized by dermatitis and abscess formation predominantly in the pectoral and ventral abdominal regions. Standard diagnostic tests are again valuable in distinguishing this disease from glanders.

Melioidosis (caused by Ps. pseudomallei) is characterized by multiple abscesses in a variety of tissues and organs. Unlike glanders, it is not specifically a disease of equids and occurs most often in sheep, goats, and swine. It is characterized by dyspnea and lameness, but a wide array of clinical signs may be elicited. Diagnosis is confirmed by isolation of the causative organism. Serological cross-reactions occur with Ps. mallei.

Treatment: Ps. mallei is sensitive to many antimicrobials, but the risk of spreading infection to other equids or to people dictates that infected animals be destroyed. This policy has successfully eradicated glanders from most parts of the world. Sulfonamides have traditionally been used for the treatment of human infection.

Vaccination: Protective vaccines have not been developed.

Control and Eradication: In endemic areas, routine testing and destruction of positive animals have proven successful in the eradication of the disease. Particular care is required where animals are congregated — most often for military purposes. In endemic areas, communal feeding and watering sites should be avoided.

Ps. mallei is quite sensitive to heat, desiccation, and common disinfectants. In warm, moist environments, however, it may remain viable for several months. In outbreaks it is important to bury or burn all contaminated bedding and foodstuffs to prevent infection of susceptible animals. Stalls and harness equipment should be thoroughly disinfected. Removal of susceptible species from contaminated premises for a period of months is advisable.

Public Health: People are susceptible to glanders. The human form of the disease is painful and frequently fatal. Laboratory workers and animal attendants are most at risk. Symptoms of glanders in

people include nodular eruption on the face, legs, and arms; involvement of the nasal mucosa; and later pyemia and metastatic pneumonia. Human glanders may be confused with a variety of other diseases, including typhoid fever, tuberculosis, syphilis, erysipelas, lymphangitis, pyemia, yaws, and melioidosis. The diagnosis can be confirmed by serology and by isolation of the causative organism.

3.8 *Burkholderia pseudomallei* (formerly *Pseudomonas pseudomallei*)[39]

Melioidosis

(*Burkholderia pseudomallei*)

What is melioidosis? Melioidosis, also called Whitmore's disease, is an infectious disease caused by the bacterium *Burkholderia pseudomallei*. Melioidosis is clinically and pathologically similar to glanders disease, but the ecology and epidemiology of melioidosis are different from glanders. Melioidosis is predominately a disease of tropical climates, especially in Southeast Asia where it is endemic. The bacteria causing melioidosis are found in contaminated water and soil and are spread to humans and animals through direct contact with the contaminated source. Glanders is contracted by humans from infected domestic animals.

Why has melioidosis become a current issue? *Burkholderia pseudomallei* is an organism that has been considered as a potential agent for biological warfare and biological terrorism.

How common is melioidosis and where is it found? Melioidosis is endemic in Southeast Asia, with the greatest concentration of cases reported in Vietnam, Cambodia, Laos, Thailand, Malaysia, Myanmar (Burma), and northern Australia. Additionally, it is seen in the South Pacific, Africa, India, and the Middle East. In many of these countries, *Burkholderia pseudomallei* is so prevalent that it is a common contaminate found on laboratory cultures. Moreover, it has been a common pathogen isolated from troops of all nationalities that have served in areas with endemic disease. A few isolated cases of melioidosis have occurred in the Western Hemisphere in Mexico, Panama, Ecuador, Haiti, Brazil, Peru, Guyana, and in the states of Hawaii and Georgia. In the United States, confirmed cases range from none to five each year and occur among travelers and immigrants.

How is melioidosis transmitted and who can get it? Besides humans, many animal species are susceptible to melioidosis. These include sheep, goats, horses, swine, cattle, dogs, and cats. Transmission occurs by direct contact with contaminated soil and surface waters. In Southeast Asia, the organism has been repeatedly isolated from agriculture fields, with infection occurring primarily during the rainy season. Humans and animals are believed to acquire the infection by inhalation of dust, ingestion of contaminated water, and contact with contaminated soil especially through skin abrasions, and for military troops, by contamination of war wounds. Person-to-person transmission can occur. There is one report of transmission to a sister with diabetes who was the caretaker for her brother who had chronic melioidosis. Two cases of sexual transmission have been reported. Transmission in both cases was preceded by a clinical history of chronic prostatitis in the source patient.

What are the symptoms of melioidosis? Illness from melioidosis can be categorized as acute or localized infection, acute pulmonary infection, acute bloodstream infection, and chronic suppurative infection. Inapparent infections are also possible. The incubation period (time between exposure and appearance of clinical symptoms) is not clearly defined, but may range from 2 days to many years.

Acute, localized infection: This form of infection is generally localized as a nodule and results from inoculation through a break in the skin. The acute form of melioidosis can produce fever and general muscle aches, and may progress rapidly to infect the bloodstream.

[39]CDC, Division of Mycotic and Bacterials Disease, Disease Information, Melioidosis. http://www.cdc.gov/ncidod/dbmd/diseaseinfo/melioidosis_g.htm

Pulmonary infection: This form of the disease can produce a clinical picture of mild bronchitis to severe pneumonia. The onset of pulmonary melioidosis is typically accompanied by a high fever, headache, anorexia, and general muscle soreness. Chest pain is common, but a nonproductive or productive cough with normal sputum is the hallmark of this form of melioidosis.

Acute bloodstream infection: Patients with underlying illness such as HIV, renal failure, and diabetes are affected by this type of the disease, which usually results in septic shock. The symptoms of the bloodstream infection vary depending on the site of original infection, but they generally include respiratory distress, severe headache, fever, diarrhea, development of pus-filled lesions on the skin, muscle tenderness, and disorientation. This is typically an infection of short duration, and abscesses will be found throughout the body.

Chronic suppurative infection: Chronic melioidosis is an infection that involves the organs of the body. These typically include the joints, viscera, lymph nodes, skin, brain, liver, lung, bones, and spleen.

How is melioidosis diagnosed? Melioidosis is diagnosed by isolating *Burkholderia pseudomallei* from the blood, urine, sputum, or skin lesions. Detecting and measuring antibodies to the bacteria in the blood is another means of diagnosis.

Can melioidosis be spread from person to person? Melioidosis can spread from person to person by contact with the blood and body fluids of an infected person. Two documented cases of male-to-female sexual transmission involved males with chronic prostatic infection due to melioidosis.

Is there a way to prevent infection? There is no vaccine for melioidosis. Prevention of the infection in endemic-disease areas can be difficult since contact with contaminated soil is so common. Persons with diabetes and skin lesions should avoid contact with soil and standing water in these areas. Wearing boots during agricultural work can prevent infection through the feet and lower legs. In health care settings, using common blood and body fluid precautions can prevent transmission.

Is there a treatment for melioidosis? Most cases of melioidosis can be treated with appropriate antibiotics. Burkholderia psuedomallei, the organism that causes melioidosis, is usually sensitive to imipenem, penicillin, doxycycline, amoxycillin-clavulanic acid, azlocillin, ceftazidime, ticarcillin-vulanic acid, ceftriaxone, and aztreonam. Treatment should be initiated early in the course of the disease. Although bloodstream infection with melioidosis can be fatal, the other types of the disease are nonfatal. The type of infection and the course of treatment can predict any long-term sequelae.

3.9 *Clostridium perfringens* epsilon toxin[40]

Enterotoxemia is one of the very important diseases and in some areas it is the most prevalent disease of goats. Despite the fact that it is also called "Overeating Disease" [or Pulpy Kidney Disease] it is not caused by overeating. Actually, the cause (etiology) of the disease is the toxin (poison) produced by the bacterium *Clostridium perfringens* type C or type D.

The bacteria are normally present in the soil and the intestinal tract in relatively small numbers. Under certain conditions the organisms proliferate in the intestine and produce toxin in lethal quantities.

The Type D infection is probably far more common than Type C. The latter type produces a toxin called "Beta Toxin" which causes intestinal necrosis and severe intestinal hemorrhage. It occurs in adult goats.

Epsilon toxin is produced by the Type D bacteria. It produces vascular damage and increases the permeability (openness) facilitating its own absorption. In the animals that die with neither signs nor tissue changes, an extremely large amount of toxin was absorbed very rapidly. When less toxin is produced, the animal lives longer and there is more time for clinical signs and pathological changes to develop.

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[40]Ayers, J. L., 1992. Extension Goat Handbook, National Agricultural Library, ENTEROTOXEMIA; Los Olivos, S. B. Guss; Pennsylvania State U., University Park. http://www.inform.umd.edu/EdRes/Topic/AgrEnv/ndd/goat/ENTEROTOXEMIA.html

The Acute course of disease lasts 4-26 hours and usually ends in death. Initially the temperature may go to 105F with severe abdominal pain (the kid cries so loudly it is best described as screaming). Profuse slimy or water diarrhea will occur. Depression, wobbly gait, recumbancy (lying down on side often with head down) occur early. Convulsions often occur intermittently and may be accompanied by continuous or intermittent opisthotonos (head thrown straight over back). The animal may slip into a coma before death or die groaning or even crying. These signs occur in kids but can occur in adult milking goats from either Type C or D bacteria.

Tissue Changes: Type D causes mild to moderate (occasionally severe) inflammation and even hemorrhage of the small intestinal mucosa. Petechial hemorrhages may be present anywhere in the body but especially on the epicardium and endocardium. The pericardial sac may contain slight excess of yellow fluid. Microscopic examination of the brain may reveal degeneration of the vascular endothelium with perivascular and intercellular edema with foci of necrosis in several subcortical areas.

The petechial hemorrhages, especially on the epicardium should make one think of enterotoxemia. However, one should look for at least two other signs which together give good presumptive evidence of enterotoxemia; these are, glucosuria and the presence of many short, plump gram positive rods on an intestinal smear.

Prevention and Treatment: Vaccination, with *Cl perfringens* type C and D toxoid by the following schedule along with the good feeding practices of making changes and increases in feed and milk gradually, has provided excellent prevention of the disease.

3.10 *Coccidioides immitis*[41]

Synonym: Coccidioidomycosis, Valley fever, Desert fever

Characteristics: Dimorphic fungus, reproduces by arthroconidia, swelling of arthroconidia in vivo into spherules (which burst and release endospores)

Pathogenicity: Systemic mycosis beginning as a respiratory infection; primary infection asymptomatic or influenza-like; 1/5 clinical cases develop erythema nodosum; rare progression to disseminated disease (more common in pregnant women, blacks and filipinos); progressive, frequently fatal granulomatous disease with lung lesions and abscesses throughout body. Meningitis common, 90% fatal if not treated. Increasingly important in immunocompromised patients.

Epidemiology: Primary infections common in arid and semiarid areas of Western Hemisphere (California to South Texas, northern Argentina, Paraguay, Colombia, Venezuela, Mexico and Central America); dusty fomites from endemic areas can transmit infection elsewhere; affects all ages, both sexes and all races; most frequent in summer after wind and dust storms

Host Range: Humans, domestic animals, cattle and horses, sheep, swine, wild desert rodents and other animals

Infectious Dose: Unknown

Mode of Transmission: Inhalation of infective arthroconidia from soil (arthroconidium is 2-5 µm with high propensity for airborne transmission and retention in deep pulmonary spaces); laboratory accidents involving cultures are common; parasitic spherules not usually infective (larger size 30-60 µm of spherule reduces effectiveness of this form as an airborne pathogen) but accidental inoculation of infected pus or other materials can result in granuloma formation.

Incubation Period: One to 4 weeks in primary infection; disseminated infection may develop insidiously, sometimes without recognized symptoms of primary infection.

Communicability: Not directly transmitted from person to person; parasitic form on dressings or casts and in clinical specimen may however change to the infective form after 7 days.

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[41]Office of Laboratory Security, January 2000. ©Health Canada, Population and Public Health Branch, Material and Safety Data Sheet. http://www.hc-sc.gc.ca/pphb-dgspsp/msds-ftss/msds40e.html

Resevoir: Soil; especially in and around indian middens and rodent burrows, in regions with appropriate temperature, moisture and soil requirements.

Zoonosis: Not directly transmitted from animal to humans.

Vectors: None.

Drug Suseptibility: Sensitive to Amphotericin B, ketoconazole and itraconazole for chronic infections, fluoconazole for meningeal infections.

Suseptibility to Disinfectanta: Susceptible to 1% sodium hypochlorite, phenolics, glutaraldehyde, formaldehyde; susceptibility to 70% ethanol questionable.

Physical Incapacitation: Spores can withstand dry heat; inactivation by moist heat 121°C for at least 15 min.

Survival Outside Host: Arthrospores survive for months to years in soil, dust.

First Aid/Treatment: Amphotericin B for severe or disseminated forms.

Immunization: None

Prophalaxis: None

Containment Requirements: Biosafety level 3 practices, containment equipment and facilities are recommended for all activities with cultures of C. immitis and for processing soil or other materials known or likely to contain infectious arthroconidia

3.11 *Coxiella burnetii*[42]

Q Fever

Overview: Q fever is a zoonotic disease caused by *Coxiella burnetii*, a species of bacteria that is distributed globally. In 1999, Q fever became a notifiable disease in the United States but reporting is not required in many other countries. Because the disease is underreported, scientists cannot reliably assess how many cases of Q fever have actually occurred worldwide. Many human infections are inapparent.

Cattle, sheep, and goats are the primary reservoirs of *C. burnetii*. Infection has been noted in a wide variety of other animals, including other breeds of livestock and in domesticated pets. *Coxiella burnetii* does not usually cause clinical disease in these animals, although abortion in goats and sheep has been linked to *C. burnetii* infection. Organisms are excreted in milk, urine, and feces of infected animals. Most importantly, during birthing the organisms are shed in high numbers within the amniotic fluids and the placenta. The organisms are resistant to heat, drying, and many common disinfectants. These features enable the bacteria to survive for long periods in the environment. Infection of humans usually occurs by inhalation of these organisms from air that contains airborne barnyard dust contaminated by dried placental material, birth fluids, and excreta of infected herd animals. Humans are often very susceptible to the disease, and very few organisms may be required to cause infection.

Ingestion of contaminated milk, followed by regurgitation and inspiration of the contaminated food, is a less common mode of transmission. Other modes of transmission to humans, including tick bites and human to human transmission, are rare.

Signs and Symptoms in Humans: Only about one-half of all people infected with *C. burnetii* show signs of clinical illness. Most acute cases of Q fever begin with sudden onset of one or more of the following: high fevers (up to 104-105° F), severe headache, general malaise, myalgia, confusion, sore throat, chills, sweats, non-productive cough, nausea, vomiting, diarrhea, abdominal pain, and chest pain. Fever usually lasts for 1 to 2 weeks. Weight loss can occur and persist for some time. Thirty to fifty percent of patients with a symptomatic infection will develop pneumonia. Additionally, a majority of patients have abnormal results on liver function tests and some will develop hepatitis. In general, most patients will recover to

[42]CDC, Viral and Rickettsial Zoonoses Branch. http://www.cdc.gov/ncidod/dvrd/qfever/

good health within several months without any treatment. Only 1%-2% of people with acute Q fever die of the disease.

Chronic Q fever, characterized by infection that persists for more than 6 months is uncommon but is a much more serious disease. Patients who have had acute Q fever may develop the chronic form as soon as 1 year or as long as 20 years after initial infection. A serious complication of chronic Q fever is endocarditis, generally involving the aortic heart valves, less commonly the mitral valve. Most patients who develop chronic Q fever have pre-existing valvular heart disease or have a history of vascular graft. Transplant recipients, patients with cancer, and those with chronic kidney disease are also at risk of developing chronic Q fever. As many as 65% of persons with chronic Q fever may die of the disease.

The incubation period for Q fever varies depending on the number of organisms that initially infect the patient. Infection with greater numbers of organisms will result in shorter incubation periods. Most patients become ill within 2-3 weeks after exposure. Those who recover fully from infection may possess lifelong immunity against re-infection.

Diagnosis: Because the signs and symptoms of Q fever are not specific to this disease, it is difficult to make an accurate diagnosis without appropriate laboratory testing. Results from some types of routine laboratory tests in the appropriate clinical and epidemiologic settings may suggest a diagnosis of Q fever. For example, a platelet count may be suggestive because persons with Q fever may show a transient thrombocytopenia. Confirming a diagnosis of Q fever requires serologic testing to detect the presence of antibodies to *Coxiella burnetii* antigens. In most laboratories, the indirect immunofluorescence assay (IFA) is the most dependable and widely used method. *Coxiella burnetii* may also be identified in infected tissues by using immunohistochemical staining and DNA detection methods.

Coxiella burnetii exists in two antigenic phases called phase I and phase II. This antigenic difference is important in diagnosis. In acute cases of Q fever, the antibody level to phase II is usually higher than that to phase I, often by several orders of magnitude, and generally is first detected during the second week of illness. In chronic Q fever, the reverse situation is true. Antibodies to phase I antigens of *C. burnetii* generally require longer to appear and indicate continued exposure to the bacteria. Thus, high levels of antibody to phase I in later specimens in combination with constant or falling levels of phase II antibodies and other signs of inflammatory disease suggest chronic Q fever. Antibodies to phase I and II antigens have been known to persist for months or years after initial infection.

Recent studies have shown that greater accuracy in the diagnosis of Q fever can be achieved by looking at specific levels of classes of antibodies other than IgG, namely IgA and IgM. Combined detection of IgM and IgA in addition to IgG improves the specificity of the assays and provides better accuracy in diagnosis. IgM levels are helpful in the determination of a recent infection. In acute Q fever, patients will have IgG antibodies to phase II and IgM antibodies to phases I and II. Increased IgG and IgA antibodies to phase I are often indicative of Q fever endocarditis.

Treatment: Doxycycline is the treatment of choice for acute Q fever. Antibiotic treatment is most effective when initiated within the first 3 days of illness. A dose of 100 mg of doxycycline taken orally twice daily for 15-21 days is a frequently prescribed therapy. Quinolone antibiotics have demonstrated good in vitro activity against *C. burnetii* and may be considered by the physician. Therapy should be started again if the disease relapses.

Chronic Q fever endocarditis is much more difficult to treat effectively and often requires the use of multiple drugs. Two different treatment protocols have been evaluated: 1) doxycycline in combination with quinolones for at least 4 years and 2) doxycycline in combination with hydroxychloroquine for 1.5 to 3 years. The second therapy leads to fewer relapses, but requires routine eye exams to detect accumulation of chloroquine. Surgery to remove damaged valves may be required for some cases of *C. burnetii* endocarditis.

Prevention: In the United States, Q fever outbreaks have resulted mainly from occupational exposure involving veterinarians, meat processing plant workers, sheep and dairy workers, livestock farmers, and

researchers at facilities housing sheep. Prevention and control efforts should be directed primarily toward these groups and environments.

- The following measures should be used in the prevention and control of Q fever:
- Educate the public on sources of infection.
- Appropriately dispose of placenta, birth products, fetal membranes, and aborted fetuses at facilities housing sheep and goats.
- Restrict access to barns and laboratories used in housing potentially infected animals.
- Use only pasteurized milk and milk products.
- Use appropriate procedures for bagging, autoclaving, and washing of laboratory clothing.
- Vaccinate (where possible) individuals engaged in research with pregnant sheep or live *C. burnetii*.
- Quarantine imported animals.
- Ensure that holding facilities for sheep should be located away from populated areas. Animals should be routinely tested for antibodies to *C. burnetii*, and measures should be implemented to prevent airflow to other occupied areas.
- Counsel persons at highest risk for developing chronic Q fever, especially persons with pre-existing cardiac valvular disease or individuals with vascular grafts.
- A vaccine for Q fever has been developed and has successfully protected humans in occupational settings in Australia. However, this vaccine is not commercially available in the United States. Persons wishing to be vaccinated should first have a skin test to determine a history of previous exposure. Individuals who have previously been exposed to *C. burnetii* should not receive the vaccine because severe reactions, localized to the area of the injected vaccine, may occur. A vaccine for use in animals has also been developed, but it is not available in the United States.

Significance for Bioterrorism: *Coxiella burnetii* is a highly infectious agent that is rather resistant to heat and drying. It can become airborne and inhaled by humans. A single C. burnetii organism may cause disease in a susceptible person. This agent could be developed for use in biological warfare and is considered a potential terrorist threat.

3.12 Eastern equine encephalitis virus[43]

Clinical Features: Symptoms range from mild flu-like illness to frank encephalitis, coma and death.

Etiologic Agent: Eastern equine encephalitis virus, member of the family Togaviridae, genus Alphavirus. Closely related to western and Venezuelan equine encephalitis viruses.

Incidence: 153 confirmed cases in the U.S. since 1964.

Sequelae: Mild to severe neurologic deficits in survivors.

Costs: Total case costs range from $21,000 for transiently infected individuals to $3 million for severely infected individuals. Insecticide applications can cost as much as $1.4 million depending on the size of area treated.

Transmission: Mosquito-borne

Risk Groups: Residents of endemic areas and visitors. Persons with outdoor work and recreational activities.

Surveillance: National Notifiable Diseases Surveillance System

Trends: Risk exposure increases as population expands into endemic areas

[43]CDC, Division of Vector-Borne Infectious Diseases, Arboviral Encephalitides, Fact Sheet http://www.cdc.gov/ncidod/dvbid/arbor/eeefact.htm

Challenges:

- No licensed vaccine for human use
- No effective therapeutic drug
- Unknown overwintering cycle
- Control measures expensive
- Limited financial support of surveillance and prevention

3.13 Francisella tularensis[44]

Tuleramia

Background: Tularemia, a bacterial zoonosis, is caused by *Francisella tularensis*, one of the most infectious pathogenic bacteria known. It requires inoculation or inhalation of as few as 10 organisms to cause disease.

F. tularensis is a small, nonmotile, aerobic, gram-negative coccobacillus. It has a thin lipopolysaccharide-containing envelope and is a hard, non-spore-forming organism that survives for weeks at low temperatures in water, moist soil, hay, straw, and decaying animal carcasses.

Tularemia occurs throughout much of North America and Eurasia. In the U.S., human cases have been reported from every state except Hawaii, with the majority occurring in south-central and western states.

F. tularensis is found in widely diverse animal hosts and habitats and can be recovered from contaminated water, soil, and vegetation. A variety of small mammals, including voles, mice, water rats, squirrels, rabbits, and hares are natural reservoirs of infection. They acquire infection through tick, fly, and mosquito bites and by contact with contaminated environments. Epizootics with sometimes extensive die-offs of animal hosts may herald outbreaks of tularemia in humans.

Humans can become incidentally infected through diverse environmental exposures: bites by infected arthropods; handling infectious animal tissues or fluids; direct contact with or ingestion of contaminated food, water, or soil; and inhalation of infective aerosols. Humans can develop severe and sometimes fatal illness, but do not transmit the disease to others.

Worldwide incidence of naturally occurring tularemia is unknown. It is likely that the disease is greatly under-recognized and under-reported. In the U.S., reported cases have dropped sharply from several thousand/year prior to 1950 to fewer than 200/year in the 1990s. Between 1985 and 1992, 1409 cases and 20 deaths were reported in the U.S., a case fatality rate of 1.4%. Most U.S. cases occur June–September, when arthropod-borne transmission is most common. Cases in winter most commonly occur among hunters and trappers who handle infected animal carcasses.

F. tularensis could be used as a biological weapon in a number of ways, but an aerosol release would likely have the greatest adverse medical and public health consequences.

Airborne *F. tularensis* would be expected to principally cause pleuropneumonitis, but some exposures might contaminate the eye, resulting in ocular tularemia; penetrate broken skin, resulting in ulceroglandular or glandular disease; or cause oropharyngeal disease with cervical lymphadenitis.

[44]Abstracted from Dennis, D.T., T. V. Inglesby, D. A. Henderson et al., Journal of the American Medical Association, June 6, 2001; vol. 285, no. 21:2763-2773. CDC, Abstract: Tularemia as a Biological Weapon: Medical and Public Health Management.
http://www.bt.cdc.gov/Agent/Tularemia/tularemia-biological-weapon-abstract.asp

Release in a densely populated area would be expected to result in an abrupt onset of large numbers of acute, nonspecific febrile illness beginning 3–5 days later (incubation range 1–14 days), with pleuropneumonitis developing in a significant proportion of cases during the ensuing days and weeks.

Pathogenesis and clinical manifestations of tularemia: *F. tularensis* can infect humans through the skin, mucous membranes, gastrointestinal tract, and lungs. It is a facultative intracellular bacterium that multiplies within macrophages. The major target organs are the lymph nodes, lungs and pleura, spleen, liver, and kidney. Untreated, bacilli inoculated into skin or mucous membranes multiply, spread to regional lymph nodes and further multiply, and then may disseminate to organs throughout the body.

Bacteremia may be common in the early phase of infection. The initial tissue reaction to infection is a focal, intensely suppurative necrosis consisting largely of accumulations of polymorphonuclear leukocytes, followed by invasion of macrophages, epithelioid cells, and lymphocytes.

Suppurative lesions become granulomatous, and histopathological examination of the granulomas shows a central necrotic, sometimes caseating, zone surrounded by a layer of epithelioid cells, multinucleated giant cells, and fibroblasts in a radial arrangement, typical of other granulomatous conditions such as tuberculosis and sarcoidosis.

Humans with inhalational exposures also develop hemorrhagic inflammation of the airways early in the course of illness, which may progress to bronchopneumonia. Histopathological examination of the lungs shows alveolar spaces filled with an exudate of mononuclear cells. Pleuritis with adhesions and effusion and hilar lymphadenopathy are common in radiological and pathological findings.

Primary clinical forms vary in severity and presentation according to virulence of the infecting organism, dose, and site of inoculum.

The onset of tularemia is usually abrupt, with fever (38 degrees C–40 degrees C), headache, chills and rigors, generalized body aches (often prominent in the low back), coryza, and sore throat. A pulse-temperature dissociation has been noted in as many as 42% of patients. A dry or slightly productive cough and substernal pain or tightness frequently occur with or without objective signs of pneumonia, such as purulent sputum, dyspnea, tachypnea, pleuritic pain, or hemoptysis. Nausea, vomiting, and diarrhea may occur.

Sweats, fever, chills, progressive weakness, malaise, anorexia, and weight loss characterize the continuing illness.

In general, tularemia would be expected to have a slower progression of illness and a lower case-fatality rate than either inhalational plague or anthrax. Milder forms of inhalational tularemia would be indistinguishable from Q fever; another potential bioterrorism agent; establishing a diagnosis of either would be problematic without reference laboratory testing.

Diagnosis: Rapid diagnostic testing for tularemia is not widely available. Physicians who suspect inhalational tularemia in patients presenting with atypical pneumonia, pleuritis, and hilar lymphadenopathy should promptly collect specimens of respiratory secretions and blood and alert the laboratory to the need for special diagnostic and safety procedures.

F. tularensis may be identified through direct examination of secretions, exudates, or biopsy specimens using Gram stain, direct fluorescent antibody, or immunohistochemical stains. Microscopic demonstration of *F. tularensis* using fluorescent-labeled antibodies is a rapid diagnostic procedure performed in designated reference laboratories in the National Public Health Laboratory Network; test results can be available within several hours of receiving the specimens, if the laboratory is alerted and prepared.

Growth of *F. tularensis* in culture is the definitive means of confirming the diagnosis of tularemia. It can be grown from pharyngeal washings, sputum specimens, and even fasting gastric aspirates in a high proportion of patients with inhalational tularemia. It is only occasionally isolated from blood.

Vaccination: In the United States, a live attenuated vaccine derived from avirulent *F. tularensis* biovar palaearctica (type B) has been used to protect laboratorians routinely working with the bacterium. Until

recently, this vaccine was available as an investigational new drug. It is currently under review by the Food and Drug Administration.

Treatment: In a contained casualty setting, where individual patient management is possible, the working group recommends parenteral antimicrobial therapy. Streptomycin is the drug of choice. Gentamicin, which is more widely available and can be used intravenously, is an acceptable alternative. Treatment with aminoglycosides should be continued for 10 days. Tetracyclines and chloramphenicol are also used, but relapses and primary treatment failures occur at a higher rate with these bacteriostatic agents than with aminogylcosides, and they should be given for at least 14 days to avoid relapse. Both streptomycin and gentamicin are recommended as first-line treatment of tularemia in children.

In a mass casualty setting, doxycycline and ciprofloxacin, administered orally, are the preferred choices for treatment of both adults and children.

Since it is unknown whether drug-resistant organisms might be used in a bioterrorist event, antimicrobial susceptibility testing of isolates should be conducted quickly and treatments altered according to test results and clinical responses.

Antibiotics for treating patients infected with tularemia in a bioterrorist event are included in the national pharmaceutical stockpile maintained by CDC, as are ventilators and other emergency equipment.

Postexposure prophylaxis: Persons beginning treatment with streptomycin, gentamicin, doxycycline, or ciprofloxacin in the incubation period of tularemia and continuing treatment daily for 14 days might be protected against symptomatic infection. Therefore, if an attack is discovered before individuals become ill, exposed persons should be prophylactically treated with 14 days of oral doxycycline or ciprofloxacin.

If an attack is discovered only after individuals become ill, persons potentially exposed should begin a fever watch. Those who develop an otherwise unexplained fever or flu-like illness within 14 days of presumed exposure should begin treatment as outlined above.

Postexposure prophylactic treatment of close contacts of tularemia patients is not recommended because person-to-person transmission is not known to occur.

Infection control and environmental decontamination: Isolation is not recommended for tularemia patients, given the lack of person-to-person transmission. In hospitals, standard precautions are recommended.

Laboratory personnel should be alerted when tularemia is suspected. Routine diagnostic procedures can be performed in biosafety level 2 conditions. Examination of cultures in which *F. tularensis* is suspected should be done in a biological safety cabinet. Manipulation of cultures and other procedures that might produce aerosols or droplets (e.g., grinding, centrifuging, vigorous shaking, animal studies) should be conducted under biosafety level 3 conditions.

Bodies of patients who die of tularemia should be handled using standard precautions. Autopsy procedures likely to produce aerosols or droplets should be avoided.

Clothing or linens contaminated with body fluids of patients with tularemia should be disinfected per standard hospital procedure.

Under natural conditions, *F. tularensis* can survive for extended periods in a cold, moist environment. Information is not available about survivability of an intentionally released aerosol form of *F. tularensis*, but the working group predicts a short half-life due to desiccation, solar radiation, oxidation, and other environmental factors and a very limited risk from secondary dispersal. Following an urban release, the risk to humans of acquiring tularemia from infected animals or arthropods is likely small and can be reduced by educating the public to avoid sick or dead animals and to take precautions to protect against biting arthropods.

3.14 Hendra virus[45]

Equine Morbillivirus Pneumonia

(Note: Equine morbillivirus pneumonia has been renamed "Hendra virus disease")

(Acute respiratory syndrome)

Definition: Equine morbillivirus pneumonia (EMP) is an acute febrile respiratory infection of horses characterized by fever, increased respiratory and heart rates, respiratory distress, and death.

Etiology: The cause of EMP is a newly recognized virus in the genus Morbillivirus and was named equine morbillivirus (EMV). When EMV was tested against antisera to a range of paramyxoviruses, morbilliviruses, and pneumoviruses there was a very weak reaction with only rinderpest virus.

History: Equine morbillivirus pneumonia has been reported only in Australia. The disease was first recognized on one property in Hendra, Australia, in September 1994. In this outbreak, 20 horses were sick and 13 died, and 2 humans were infected and 1 died. A retrospective diagnosis of EMP was made for horses that died in August 1994 on a property in Mackay about 1,100 km from the first reported case of EMP. In this outbreak four horses were infected and two died; the farmer became ill, recovered, had a relapse, and died of EMV infection 13 months later. There was no evidence of epidemiological association between these outbreaks.

Host Range: Equine morbillivirus has naturally caused disease in horses and humans. Experimentally, cats and guinea pigs have been infected. The disease in cats is very similar to the disease in horses. The recent detection of neutralizing antibody to EMV in the native Australian fruit-bats suggests a possible resevoir for EMV.

Geographic Distribution: Equine morbillivirus pneumonia has been reported only in Australia.

Transmission: On the basis of epidemiologic findings and experimental results with cats, EMV is not readily transmissible. In the field, very few horses in contact with infected horses became ill, and even horses placed in uncleaned stalls previously occupied by an infected horse did not become ill. For both animals and humans, EMV seems to require direct contact with respiratory secretions of infected animals. If a fruit-bat is the reservoir, the mechanism for spread to the horse is not known.

Incubation Period: The incubation period in horses is 8 to 14 days.

Clinical Signs: The initial case of EMP in both outbreaks was a pregnant mare on pasture. Clinical signs in field cases at Hendra were fever up to 105.8 degrees F (41 degrees C), severe respiratory distress, and death. Two horses that recovered had mild myoclonic twitching. In the Mackay outbreak, the pregnant mare had "severe respiratory distress, ataxia, and marked swelling of the head — particularly of the infraorbital fossa and cheeks. The second horse, a stallion (horse B), reportedly showed aimless pacing, muscle trembling and a haemorrhagic nasal discharge" and died.

In experimentally infected horses, the first sign of disease was fever that ranged from 102.2 to 106 degrees F (39 to 41.2 degrees C). In some animals, as the disease progressed, heart and respiratory rates increased up to 72 and 60 per minute, respectively, breathing became labored, and the animal's demeanor varied from somnolence to mild agitation.

Gross Lesions: The most distinctive gross lesion in EMP is a bilateral "pulmonary edema characterized by gelatinous distention of subpleural lymphatics." The lungs are heavy and congested; the edema is more prominent in the ventral parts and the lungs vary from a mottled yellowish-brown to dark blue. The major air passages are essentially normal. In one experimental horse, there was also a bilateral dilation of the pulmonary lymphatics and a very enlarged dark spleen.

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[45]Committee on Foreign Animal Diseases of the United States Animal Health Association, revised 1998. Foreign Animal Diseases, "The Gray Book," Part IV, Library of Congress Catalog Card Number 17-12842, Pat Campbell & Associates and Carter Printing Company, Richmond, VA.
http://www.vet.uga.edu/vpp/gray_book/FAD/EMP.htm

Morbidity and Mortality: The number of animals that have been infected naturally and experimentally is small, but using these numbers, mortality is high in animals infected. Owing to the low transmissibility of EMV, morbidity has been low.

Diagnosis

Field Diagnosis

Equine morbillivirus pneumonia should be suspected when a horse that dies has been febrile and the necropsy findings include a pulmonary edema characterized by gelatinous distention of subpleural lymphatics.

Specimens for Laboratory

Specimens sent to the laboratory for culture should consist of pieces of lung, liver, spleen, kidney, lymph nodes, brain, and heparinized blood. For serology, serum samples from acute and convalescent animals should be submitted. For histopathologic examination, send a complete set of tissues in 10 percent formalin.

Laboratory Diagnosis

To confirm a suspected diagnosis of EMP, the virus has to be isolated and identified. The disease can tentatively be diagnosed by histopathology as well as histochemical and molecular biological techniques.

Differential Diagnosis

Because of the pulmonary edema, African horse sickness is a primary consideration in a differential diagnosis.

Other causes of acute death are poisons, intoxications (botulism), and acute bacterial diseases such as anthrax.

Treatment: There is no treatment for the primary disease.

Vaccination: There is no vaccine.

Control and Eradication: In Australia, disease control and eradication procedures consisted of slaughter of known infected horses and extensive serological surveillance. No antibody to EMV was detected in sera from 98 horses remaining on the Hendra property, in sera from horses on adjacent properties, nor in sera from horses on trace-back and trace-forward properties. No EMV antibody was detected in sera collected from the Queensland population of nonracing horses selected using a stratified proportional approach.

Public Health: If one considers that there have been three human infections and two human deaths in only two outbreaks of this disease, maximum precautions should be used if this disease is suspected. The three individuals infected had extensive contact with the infected horses and "assisted in their necropsies without gloves, mask, or protective eyeware". It should be noted, however, that others also had contact with the animals and conducted or participated in necropsies and did not get infected; thus it is suspected that "direct contact with respiratory secretions of infected animals seems to be necessary for transmission".

3.15 Nipah virus[46]

Overview: Nipah virus is a newly recognized zoonotic virus. The virus was ‘discovered’ in 1999. It has caused disease in animals and in humans, through contact with infectious animals. The virus is named after the location where it was first detected in Malaysia. Nipah is closely related to another newly recognized zoonotic virus (1994), called Hendra virus, named after the town where it first appeared in Australia. Both Nipah and Hendra are members of the virus family Paramyxoviridae. Although members of this group of viruses have only caused a few focal outbreaks, the biologic property of these viruses to

[46]World Health Organizaion, Nipah Virus Factsheet. http://www.who.int/inf-fs/en/fact262.html

infect a wide range of hosts and to produce a disease causing significant mortality in humans has made this emerging viral infection a public heath concern.

Natural Host: It is currently believed that certain species of fruit bats are the natural hosts of both Nipah and Hendra viruses. They are distributed across an area encompassing northern, eastern and south-eastern areas of Australia, Indonesia, Malaysia, the Philippines and some of the Pacific Islands. The bats appear to be susceptible to infection with these viruses, but do not themselves become ill. It is not known how the virus is transmitted from bats to animals.

Transmission: The mode of transmission from animal to animal, and from animal to human is uncertain, but appears to require close contact with contaminated tissue or body fluids from infected animals. Nipah antibodies have been detected in pigs, other domestic and wild animals. The role of species other than pigs in transmitting infection to other animals has not yet been determined.

It is unlikely that Nipah virus is easily transmitted to man, although previous outbreak reports suggest that Nipah virus is transmitted from animals to humans more readily than Hendra virus. Despite frequent contact between fruit bats and humans there is no serological evidence of human infection among bat carers. Pigs were the apparent source of infection among most human cases in the Malaysian outbreak of Nipah, but other sources, such as infected dogs and cats, cannot be excluded. Human-to-human transmission of Nipah virus has not been reported.

Clinical features: The incubation period is between 4 and 18 days. In many cases the infection is mild or inapparent (sub-clinical). In symptomatic cases, the onset is usually with "influenza-like" symptoms, with high fever and muscle pains (myalgia). The disease may progress to inflammation of the brain (encephalitis) with drowsiness, disorientation, convulsions and coma. Fifty percent of clinically apparent cases die.

Treatment: No drug therapies have yet been proven to be effective in treating Nipah infection. Treatment relies on providing intensive supportive care. There is some evidence that early treatment with the antiviral drug, ribavirin, can reduce both the duration of feverish illness and the severity of disease. However, the efficacy of this treatment in curing disease or improving survival is still uncertain.

Protection of Health Care Professionals: The risk of transmission of Nipah virus from sick animals to humans is thought to be low, and transmission from person-to-person has not yet been documented, even in the context of a large outbreak. Therefore, the risk of transmission of Nipah virus to health care workers is thought to be low. However, transmission without percutaneous exposure (through a break in the skin barrier) is theoretically possible, as respiratory secretions contain the virus. This is why it has been categorized as a biohazardous agent that should be managed in a high-level biosecurity laboratory. It is recommended that close contact with body fluids and infected tissues be avoided if Nipah infection is suspected.

Outbreaks of Nipah and Hendra Viruses: From September 1998 - April 1999, there was a large outbreak of encephalitis in Malaysia. During the investigation of this outbreak, Nipah virus, a previously unrecognized virus, was identified as the causal agent. A total of 265 people were infected, of whom 105 died. Ninety-three percent of cases had occupational exposure to pigs. An associated outbreak among abattoir workers in Singapore during March 1999 led to 11 cases, with 1 death. These workers had been handling pigs that had been imported from the outbreak areas in Malaysia.

There have been 3 recognized outbreaks of Hendra virus in Australia in 1994, 1995 and1999. Three human cases, leading to 2 deaths were recorded in the 1994 and 1995 outbreaks. In 1995 a horse was infected, with associated human cases. The precise mode of virus transmission to the three Australian patients is not fully understood. All 3 individuals appear to have acquired their infection as a result of close contact with horses which were ill and later died.

3.16 Rift Valley fever virus[47]

(Infectious enzootic hepatitis of sheep and cattle)

Definition: Rift Valley fever (RVF) is an arthropod-borne (primarily mosquito), acute, febrile, viral disease of sheep, cattle, and goats. The disease in these species is characterized by high abortion rates, high mortality in neonates, and hepatic necrosis. Humans are highly susceptible. Symptoms in humans in most cases are those of an acute undifferentiated febrile disease; severe cases (about 1 percent) resemble a dengue-like disease accompanied by hemorrhage, meningoencephalitis, retinopathy, and sometimes death.

Etiology: Rift Valley fever is caused by a three-stranded RNA virus in the Phlebovirus genus of the family Bunyaviridae. All isolates are serologically similar. Detection of differences between isolates requires RNA fingerprinting.

Rift Valley fever virus is inactivated by lipid solvents, detergents, and low pH. At neutral or alkaline pH in the presence of protein such as serum, the virus can remain viable for up to 4 months at 4 degrees C. Specimens stored below 0 degrees C will retain infectivity for 8 years. Rift Valley fever virus in aerosols has a half-life in excess of 77 minutes at 25 degrees C and 30 percent relative humidity. Humans have been infected by aerosols generated during the slaughtering procedure, by handling aborted fetuses, performing necropsies, and conducting laboratory procedures.

Contaminated surfaces should be washed to remove large amounts of organic matter and disinfected using strong solutions of sodium or calcium hypochlorite; residual chorine should exceed 5,000 ppm. Solutions having a pH of 6.2 (acetic acid) or lower are also effective.

Host Range: Rift Valley fever virus infects many species of animals and humans. Neonatal lambs, kids, calves, and puppies are highly susceptible and have a high mortality. Sheep and cattle are the primary species affected and the primary amplifiers of the virus. Humans are highly susceptible to RVF virus infection and are readily infected by mosquitoes and aerosols. Humans develop a sufficient viremia to be a source of infection for mosquitoes and thus could introduce the disease into uninfected areas.

Geographic Distribution: Rift Valley fever has been found to occur in most of Africa.

Transmission: Historically, explosive outbreaks of the disease have occurred simultaneously over a wide area of Africa at 5 to 15 year intervals. The outbreaks have generally occurred in otherwise dry areas following periods of heavy rainfall. The long interval between outbreaks in animals allows for the development of a susceptible population. For many years, the reservoir during the interepidemic periods was unknown. Then researchers found RVF virus to be present in dormant eggs of the mosquito Aedes lineatopinnis located in the soil of grassland depressions known as dambos. When these depressions become full of water, the eggs hatch, and infected mosquitoes develop. These mosquitoes infect an amplifying host (ruminant), which serves as a source of infection for many other genera of mosquitos that rapidly spread the disease. If the area of infected mosquitoes extends into areas of susceptible animals, there are many clinical cases. In contrast, in most areas of Africa the disease is enzootic and best monitored by the use of sentinel animals.

In Africa, many of the species of mosquitoes in the genera Aedes, Anopheles, Culex, Eretmapoites, and Mansonia can transmit RFV. In North America, mosquitoes in the genera Aedes, Anopheles, and Culex experimentally are capable vectors of RFV. Experimentally, Culex pipiens, an important vector in Egypt, was shown to feed preferentially on febrile rather than normal sheep. Experimentally, vector competence of Culex pipiens also increased with increasing holding temperature.

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[47]Committee on Foreign Animal Diseases of the United States Animal Health Association, revised 1998. Foreign Animal Diseases, "The Gray Book," Part IV, Library of Congress Catalog Card Number 17-12842, Pat Campbell & Associates and Carter Printing Company, Richmond, VA. http://www.vet.uga.edu/vpp/gray_book/FAD/RVF.htm

Incubation Period: Experimentally, the incubation period in newborn lambs, kids, calves, and puppies, is about 12 hours. In adult sheep, cattle, goats, and dogs the incubation period may be as long as 3 days. In humans, the incubation period is 4 to 6 days.

Clincial Signs: Clinical signs depend on the species affected and physiologic conditions such as age and pregnancy. Lambs develop a fever of 104-107 degrees F (40-42 degrees C) accompanied by anorexia and become weak and die about 36 hours after inoculation. Mortality in lambs under 1 week of age exceeds 90 percent. Mortality in lambs over a week old is greater than 20 percent. Adult sheep develop a fever of 104-106 degrees F (40-41 degrees C), along with a mucopurulent nasal discharge, and they may vomit. If animals are pregnant, abortion will be the most prominent sign. Mortality, particularly in ewes that abort, may reach 20 to 30 percent. Calves develop a fever of 104-106 degrees F (40-41.1 degrees C) and become depressed. Mortality can range from 10 to 70 percent. Adult cattle develop a fever of 104-106 degrees F (40-41.1 degrees C), have excessive salivation, anorexia, and weakness; some may develop a fetid diarrhea. If animals are pregnant, abortion will be the most prominent sign. Mortality is usually less than 10 percent.

Humans develop influenza-like symptoms with fever of 100-104 degrees F (37.8-40 degrees C), headache, muscular pain, weakness, and nausea plus epigastric discomfort and photophobia. Most people recover in 4 to 7 days; however, a small percentage of infected individuals will develop complications. Some may develop a hemorrhagic syndrome of jaundice, hematemesis, melena, and petechiae 2 to 4 days after becoming febrile and die. Others will develop a meningoencephalitis, and a third group a retinopathy 5 to 15 days after becoming febrile.

Gross Lesions: The primary lesion in RVF is hepatic necrosis. In aborted fetuses and in neonatal animals, particularly lambs and calves, hepatic necrosis can be massive. The liver may be enlarged and yellowish, have petechial hemorrhages, and be friable. Older animals may have a focal hepatic necrosis; this may be visible as small pale foci in the parenchyma or be seen only by histopathologic examination. In both neonatal and older animals that die, there may be widespread cutaneous hemorrhages, petechial to ecchymotic hemorrhages on parietal and visceral serosal membranes, and a hemorrhagic enteritis.

Morbidity and Mortality: Rift Valley fever causes a high mortality in young lambs, calves, and kids. Mortality in adult sheep is about 20 percent and in adult cattle about 10 percent. A high percentage of pregnant animals may abort.

Vaccination: Several vaccines have been used to protect against RVF infection. Rift Valley fever virus was first attenuated by serial intracerebral inoculation of mice (Smithburn strain). One inoculation of this vaccine produced protection in 6 to 7 days and immunity that lasted at least 3 years. However, when administered to pregnant ewes, it caused abortion, and the vaccine was pathogenic for man. Because of these problems with the attenuated vaccine, inactivated vaccines produced from cell-culture-propagated virus were developed. These vaccines protected; however, they had the disadvantages of requiring two inoculations for protection, annual vaccination, and large amounts of antigen. When the epizootic occurred in Egypt, enough inactivated vaccine could be produced to vaccinate only the more valuable breeding stock. Recently a mutagen-attenuated Vero-cell-propagated vaccine has been developed for use in people. The vaccine has also been tested in sheep and cattle. The vaccine causes no adverse effect in neonatal lambs, calves, or pregnant sheep or cattle. Bovine fetuses inoculated with the vaccine via a laparotomy continued a normal development and were seropositive when born. This vaccine also has the advantage that one inoculation induces rapid immunity, and as few as 10 plaque-forming units of the virus induce protection. Thus, many doses of vaccine can be produced quickly.

Attenuated vaccines induce a higher and more persistent serum antibody neutralizing titer than inactivated vaccines. Animals and people vaccinated with inactivated vaccine should have their RVF neutralizing antibody titer determined annually or be revaccinated. A serum neutralization titer of 20 or greater is protective. Lambs and calves that receive colostrum from a convalescent dam or dam vaccinated with an attenuated virus are passively protected for about 3 months.

Control and Eradication: In RVF enzootic areas, vaccination is the only practical method of preventing low-level losses. Movement of animals from an enzootic area to RVF-free areas during the period of virus activity should be discouraged to prevent an epizootic. Mosquito control during an epizootic is logical but not practical for large areas; it could be used to reduce human exposure in limited areas. Slaughter of sick animals is not recommended because of the risk of human infection from aerosols of blood and body fluids. In an epizootic, widespread vaccination of all susceptible animals to prevent infection of amplifying hosts and thus infection of vectors is the only way to prevent infection of animals and man.

Public Health: Humans are highly susceptible to infection. In an enzootic or epizootic area, protective measures should be taken to prevent infection by mosquitoes. Of even more importance, protective measures should be taken to prevent infection by aerosols produced during the handling of infected fetuses and tissues and in laboratory procedures. People who could be exposed to the virus should be vaccinated.

3.17 Shigatoxin[48]

Shiga Toxin-Producing Escherichia coli (STEC)

What is Shiga toxin-producing Escherichia coli? Shiga toxin-producing Escherichia coli is a type of enterohemorrhagic E. coli (EHEC) bacteria that can cause illness ranging from mild intestinal disease to severe kidney complications. Other types of enterohemorrhagic E. coli include the relatively important serotype E. coli O157:H7, and more than 100 other non-O157 strains such as O111 and O26.

What are the symptoms? Typical symptoms include severe abdominal cramping, sudden onset of watery diarrhea, frequently bloody, and sometimes vomiting and a low-grade fever. Most often the illness is mild and self-limited generally lasting 1-3 days. However, serious complications such as hemorrhagic colitis, Hemolytic Uremic Syndrome (HUS), or postdiarrheal thrombotic thrombocytopenic purpura (TTP) can occur in up to 10% of cases.

How soon do the symptoms appear? The incubation period ranges from 1 to 8 days, though typically it is 3 to 5 days.

How do you get Shiga toxin-producing Escherichia coli? Cases and outbreaks of Shiga toxin-producing Escherichia coli have been associated with the consumption of undercooked beef (especially ground beef), raw milk, unpasteurized apple juice, contaminated water, red leaf lettuce, alfalfa sprouts, and venison jerky. The bacteria have also been isolated from poultry, pork and lamb. Person-to- person spread, via fecal->oral transmission, may occur in high-risk settings like day care centers and nursing homes. Further studies are being done to better understand the modes of transmission.

Who gets infected with Shiga toxin-producing Escherichia coli? Although anyone can get infected, the highest infection rates are in children under age 5. Elderly patients also account for a large number of cases. Outbreaks have occurred in child-care facilities and nursing homes.

How is it treated? For mild illness, antibiotics have not been shown to shorten the duration of symptoms and may make the illness more severe in some people. Severe complications, such as Hemolytic Uremic Syndrome, require hospitalization.

How can you keep from getting it?

- COOK meats and poultry thoroughly. Ground beef should reach at least 155 degrees F (until the meat is no longer pink and juices run clear).
- DO NOT consume raw milk or unpasteurized dairy products.
- WASH YOUR HANDS after using the bathroom or changing diapers and before preparing or eating food.

[48]Communicable Disease Epidemiology Program (303 692-2700), March 2001. Colorado Department of Public Health & Environment. http://www.cdphe.state.co.us/dc/Epidemiology/stec_fs.html

3.18 Staphylococcal enterotoxins[49]

Overview: *S aureus* can express two different types of toxin with superantigen activity, enterotoxins, of which there are six serotypes (A, B, C, D, E and G) and toxic shock syndrome toxin (TSST-1). Enterotoxins cause diarrhea and vomiting when ingested and are responsible for staphylococcal food poisoning. When expressed systemically, enterotoxins can cause toxic shock syndrome (TSS) - indeed enterotoxins B and C cause 50% of non-menstrual TSS. TSST-1 is very weakly related to enterotoxins and does not have emetic activity. TSST-1 is responsible for 75% of TSS, including all menstrual cases. TSS can occur as a sequel to any staphylococcal infection if an enterotoxin or TSST-1 is released systemically and the host lacks appropriate neutralizing antibodies. Tampon-associated TSS is not a true infection, being caused by growth of *S aureus* in a tampon and absorption of the toxin into the blood stream. TSS came to prominence with the introduction of super-absorbent tampons; and although the number of such cases has decreased dramatically, they still occur despite withdrawal of certain types of tampons from the market.

Superantigens stimulate T cells non-specifically without normal antigenic recognition. Up to one in five T cells may be activated, whereas only 1 in 10,000 are stimulated during antigen presentation. Cytokines are released in large amounts, causing the symptoms of TSS. Superantigens bind directly to class II major histocompatibility complexes of antigen-presenting cells outside the conventional antigen-binding grove. This complex recognizes only the Vb element of the T cell receptor. Thus any T cell with the appropriate Vb element can be stimulated, whereas normally antigen specificity is also required in binding.[50]

Staphylococcal enterotoxin B[50]

Staphylococcus aureus produces a number of exotoxins, one of which is Staphylococcal enterotoxin B, or SEB. Such toxins are referred to as exotoxins since they are excreted from the organism, and since they normally exert their effects on the intestines they are called enterotoxins. SEB is one of the pyrogenic toxins that commonly causes food poisoning in humans after the toxin is produced in improperly handled foodstuffs and subsequently ingested. SEB has a very broad spectrum of biological activity. This toxin causes a markedly different clinical syndrome when inhaled than it characteristically produces when ingested. Significant morbidity is produced in individuals who are exposed to SEB by either portal of entry to the body.

SEB is the second most common source of outbreaks of food poisoning. Often these outbreaks occur in a setting such as a church picnic or other community event, due to common source exposure in which contaminated food is consumed. Although an aerosolized SEB toxin weapon would not likely produce significant mortality, it could render 80 percent or more of exposed personnel clinically ill and unable to perform their mission for 1-2 weeks. The demand on the medical and logistical systems could be overwhelming. For these reasons, SEB was one of the 7 biological agents stockpiled by the U.S. during its old bioweapons program, which was terminated in 1969.

Mechanism of Toxicity: Staphylococcal enterotoxins belong to a class of potent immune stimulants known as bacterial superantigens. Superantigens bind to monocytes at major histocompatibility complex type II receptors rather than the usual antigen binding receptors. This leads to the direct stimulation of large populations of T-helper cells while bypassing the usual antigen processing and presentation. This induces a brisk cascade of pro-inflammatory cytokines (such as tumor necrosis factor, interferon, interleukin-1 and interleukin-2), with recruitment of other immune effector cells, and relatively deficient activation of counter-regulatory negative feedback loops. This results in an intense inflammatory response that injures host tissues. Released cytokines are thought to mediate many of the toxic effects of SEB.

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[49]Foster, Timothy. Medical Microbiology, Fourth Edition, Staphylococcus chapter. http://gsbs.utmb.edu/microbook/ch012.htm

[50]© The University of Iowa, Virtual Naval Hospital, USAMRIID's Medical Management of Biological Casualties Handbook, Staphylococcal Enterotoxin B, U.S. Army Medical Research, Institute of Infectious Diseases, 1997-2003. http://www.vnh.org/BIOCASU/19.html

Signs and Symptoms: Latent period of 3-12 hours after aerosol exposure is followed by sudden onset of fever, chills, headache, myalgia, and nonproductive cough. Some patients may develop shortness of breath and retrosternal chest pain. Patients tend to plateau rapidly to a fairly stable clinical state. Fever may last 2 to 5 days, and cough may persist for up to 4 weeks. Patients may also present with nausea, vomiting, and diarrhea if they swallow the toxin. Presumably, higher exposure can lead to septic shock and death.

Diagnosis: Diagnosis is clinical. Patients present with a febrile respiratory syndrome without CXR abnormalities. Large numbers of patients presenting in a short period of time with typical symptoms and signs of SEB pulmonary exposure would suggest an intentional attack with this toxin.

Treatment: Treatment is limited to supportive care. Artificial ventilation might be needed for very severe cases, and attention to fluid management is important.

Prophylaxis: Use of protective mask. There is currently no human vaccine available to prevent SEB intoxication.

Isolation and Decontamination: Standard Precautions for healthcare workers. SEB is not dermally active and secondary aerosols are not a hazard from patients. Decon with soap and water. Destroy any food that may have been contaminated.

Clinincal Features: Symptoms of SEB intoxication begin after a latent period of 3-12 hours after inhalation, or 4-10 hours after ingestion. Symptoms include nonspecific flu-like symptoms (fever, chills, headache, myalgias), and specific features dependent on the route of exposure. Oral exposure results in predominantly gastrointestinal symptoms: nausea, vomiting, and diarrhea. Inhalation exposures produce predominantly respiratory symptoms: nonproductive cough, retrosternal chest pain, and dyspnea. GI symptoms may accompany respiratory exposure due to inadvertent swallowing of the toxin after normal mucocilliary clearance.

The fever may last up to five days and range from 103 to 106 degrees F, with variable degrees of chills and prostration. The cough may persist up to four weeks, and patients may not be able to return to duty for two weeks.

Diagnosis: Diagnosis of SEB intoxication is based on clinical and epidemiologic features. Because the symptoms of SEB intoxication may be similar to several respiratory pathogens such as influenza, adenovirus, and mycoplasma, the diagnosis may initially be unclear. All of these might present with fever, nonproductive cough, myalgia, and headache. SEB attack would cause cases to present in large numbers over a very short period of time, probably within a single 24-hour period. Naturally occurring pneumonias or influenza would involve patients presenting over a more prolonged interval of time. Naturally occurring staphylococcal food poisoning cases would not present with pulmonary symptoms. SEB intoxication tends to plateau rapidly to a fairly stable clinical state, whereas inhalational anthrax, tularemia pneumonia, or pneumonic plague would all continue to progress if left untreated. Tularemia and plague, as well as Q fever, would be associated with infiltrates on chest radiographs. Other diseases, including Hantavirus pulmonary syndrome, Chlamydia pneumonia, and CW agent inhalation (mustard, phosgene), should also be considered.

Medical Management: Currently, therapy is limited to supportive care. Close attention to oxygenation and hydration is important, and in severe cases with pulmonary edema, ventilation with positive end expiratory pressure, vasopressors and diuretics might be necessary. Acetaminophen for fever, and cough suppressants may make the patient more comfortable. The value of steroids is unknown. Most patients would be expected to do quite well after the initial acute phase of their illness, but generally would be unfit for duty for one to two weeks. Severe cases risk death from pulmonary edema and respiratory failure.

Prophylaxis: Although there is currently no human vaccine for immunization against SEB intoxication, several vaccine candidates are in development. Preliminary animal studies have been encouraging. A vaccine candidate is nearing transition to advanced development for safety and immunogenicity testing in man. Superantigens: enterotoxins and toxic shock syndrome toxin

3.19 T-2 toxin[51]

Tricothecene Mycotoxicosis

Description of Agent: The tricothecene mycotoxins are a large group of low-molecular-weight toxins produced by several species of filamentous fungi. Most are potent inhibitors of eukaryotic protein synthesis and of mitochondrial respiration. Those toxins most frequently isolated from agricultural products, and likewise mentioned most often in the context of belligerent use, include diacetoxyscirpenol (DAS), Nivalenol, 4-Deoxynivalenol (DON), and especially T-2. T-2 is one of the most stable of these toxins, and thus, perhaps, the most likely to be employed in a terrorist or warfare application. Naturally-occurring mycotoxicosis presents as Alimentary Toxic Aleukia, a lethal condition related to the consumption of moldy grains. Intentional use of T-2 by belligerents might involve aerosolization or the deliberate contamination of foodstuffs. Disease results from inhalation, ingestion, or skin contact, since T-2, unlike other BW agents, possesses significant vesicant properties.

Signs and Symptoms: Dermal exposure leads to symptoms within minutes and manifests as erythema accompanied by pain and a burning sensation. Blisters form and progress to necrosis with a leathery blackening of he skin. Inhalational exposure produces a rapid onset of nose and throat pain, with nasal discharge, cough, dyspnea, wheezing, chest pain, and hemoptysis. Eyes are likewise affected with intense burning and a foreign body sensation. Gastrointestinal exposure leads to anorexia, nausea, abdominal cramping, and hematemesis and hematochaezia. Systemic toxicity may follow exposure by any route and is manifest by weakness and ataxia, followed, in fatal cases, by tachycardia, hypothermia, and hypotension. Survivors of acute illness may manifest hematologic toxicity in the subacute phase.

Diagnosis: Prompt diagnosis is based on clinical and epidemiologic grounds. Blood, tissue, and environmental samples may be assayed for confirmatory evidence using gas-liquid chromatography and mass spectrometry.

Treatment: Therapy is largely supportive. Standard poison management techniques, such as the use of superactivated charcoal, are useful when administered early to casualties with gastrointestinal exposure.

Prophylaxis: Physical means, such as protective masks, are the only available protection. Unlike the situation with most other BW agents, the skin must also be protected against mycotoxin attack.

Decontamination and Isolation: Clothing of T-2 victims should be removed and treated (exposed to 5% hypochlorite for 6-10 hours) or destroyed. Skin may be decontaminated with soap and water. Eye exposure should be managed with copious saline irrigation. Isolation is not required. Instruments and surfaces should be decontaminated by heating to 500(F for 30 minutes or by brief exposure to 1N NaOH. Standard disinfectants effective against most other BW agents are often inadequate to inactivate the very stable mycotoxins.

Outbreak Control: Mycotoxin-induced disease is not contagious, but the stability of the toxins in the presence of heat and ultraviolet light make for the possibility of persistence in the environment following release.

3.20 Venezuelan equine encephalitis virus[52]

Venezuelan Equine Encephalitis

Description of Agent: Venezuelan Equine Encephalitis (VEE) is a mosquito borne alphavirus disease maintained in nature predominantly in a horse-mosquito-horse cycle, although thousands of natural human infections also occur each year. Large equine epizootics typically precede the appearance of

[51]United States Army Institute of Infectious Diseases, Biological Agent Information Papers, Tricothecene Mycotoxicosis.
http://www.nbc-med.org/SiteContent/MedRef/OnlineRef/GovDocs/BioAgents.html#TRICOTHECENE

[52]United States Army Institute of Infectious Diseases,Biological Agent Information Papers.
http://www.nbc-med.org/SiteContent/MedRef/OnlineRef/GovDocs/BioAgents.html#VENEZUELAN

human cases. Use of VEE as a weapon would presumably involve aerosolization, and such usage might be suspected on the basis of a preponderance of human cases and/or the presence of VEE outside of its typically well-localized focus in the Americas.

Signs and Symptoms: VEE may be thought of as an incapacitating agent with a mortality rate (in naturally-occurring disease) of less than 1%. Susceptibility is nearly 100%, and disease is characterized by its sudden onset following a 1-5 day incubation period. Initial symptoms include generalized malaise, severe headache, fever and rigors, photophobia, and myalgias. Cough, sore throat, and vomiting and diarrhea may follow. Only a small percentage of cases actually progress to encephalitis, which is more frequent in young children and is marked by meningismus, convulsions, coma, and paralysis. Route of exposure probably has little effect on the proportion of cases developing neurologic disease. In the majority of cases without neurologic sequelae, full recovery occurs in 1-2 weeks.

Diagnosis: The diagnosis of VEE is largely clinical. Naturally-occurring disease can often be suspected on epidemiologic grounds given its geographic specificity and the likely presence of concomitant disease in equines. Significant leukopenia and lymphopenia is often present. Virus may be cultured from serum and a VEE-specific IgM ELISA is available.

Treatment: Treatment of VEE is largely supportive. Ribavirin has some in vitro activity, but appears of little benefit clinically.

Prophylaxis: Prevention of naturally-occurring VEE is dependent largely upon mosquito control efforts and the immunization of horses. A human vaccine, TC-83, is available as an investigational product through USAMRIID (Ft Detrick MD 21702), and has been given to a large number of laboratory workers as a single 0.5 ml SQ dose. A second investigational vaccine, formalin-inactivated C-84, has been used to boost (0.5 ml SQ) serologic non-responders.

Decontamination and Isolation: Universal precautions should be practiced when dealing with VEE patients. Virus may be destroyed by heat (80 degrees C for 30 minutes) and by ordinary disinfectants (such as 0.5% hypochlorite).

Outbreak Control: Humans are infectious for mosquitoes for at least 72 hours after the onset of symptoms. Efforts at mosquito control thus become paramount to the prevention of secondary VEE cases following intentional or natural VEE outbreaks. In the event of intentional release of VEE virus by belligerents, the potential would be high for the development of an equine epizootic if the proper mosquito vector were present; veterinary vaccination would be useful in such circumstances.

4. HUMAN PATHOGENS AND TOXINS

This section introduces the 29 human pathogens and toxins regulated by 42 CFR 73. Summary reference information is provided. Citations and internet addresses for each pathogen or toxin are provided in footnotes. This section includes summary information for the following pathogens and toxins:

- Abrin (more than 100 mg)
- Cercopithecine herpesvirus 1 (Herpes B virus)
- *Coccidioides posadasii*
- Conotoxins (more than 100 mg)
- Crimean-Congo haemorrhagic fever virus
- Diacetoxyscirpenol (more than 1,000 mg)
- Ebola viruses
- Lassa fever virus
- Marburg virus
- Monkeypox virus
- Ricin (more than 100 mg)
- *Rickettsia prowazekii*
- *Rickettsia rickettsii*
- Saxitoxin (more than 100 mg)
- Shiga-like ribosome inactivating proteins (more than 100 mg)
- South American haemorrhagic fever viruses (Junin (non-vaccine strain (Candid #1)), Machupo, Sabia, Flexal, Guanarito)
- Tetrodotoxin (more than 100 mg)
- Tick-borne encephalitis complex (flavi) viruses (Central European Tick-borne encephalitis, Far Eastern Tick-borne encephalitis, [Russian Spring and Summer encephalitis, Kyasanur Forest disease, Omsk Hemorrhagic Fever])
- Variola major virus (Smallpox virus)
- Variola minor virus (Alastrim)
- *Yersinia pestis*

4.1 Abrin[53]

Abrin is a potent toxin that has been isolated from the seeds of Abrus precatorius (or Rosary pea). Its use as a tool for research was described in 1972 by Sharon and Lis. Abrin exists in two forms, abrin a and abrin b. Both are composed of two chains, an A-chain and a B-chain. A disulfide bond between Cys247 of the A-chain and Cys8 of the B-chain links the A and B chains. The A-chain is 251 residues and is divided into 3 folding domains. The A-chain catalytically inactivates 60S ribosomal subunits by removing adenine from positions 4 and 324 of 28S rRNA therefore inhibiting protein synthesis. The B-chain is a galactose specific lectin that facilitates the binding of abrin to cell membranes. The B-chain of both forms of abrin consist of 268 amino acid residues and share 256 identical residues. Comparison of their sequences with that of the ricin's B-chain shows that 60% of the residues of abrin's B-chain are identical to those of the ricin's B-chain and that two saccharide-binding sites in ricin B-chain identified by a crystallographic study are highly conserved in abrin B-chain.

[53]Patocka, Jiri. Department of Toxicology, Military Medical Academy, Hradec Kralove, Czech Republic, Abrin and Ricin - Two Dangerous Poisonous Proteins. http://www.asanltr.com/newsletter/01-4/articles/Abrin&RicinRev.htm

The mechanism of toxic action of abrin is identical to that of ricin, but the toxicity of abrin in mice is 75 times that of ricin (0.04 µg/kg for abrin compared to3 µg/kg for ricin.) The diagnosis, clinical features, treatment, protection, prophylaxis and so on is also the same for both abrin and ricin intoxications.

Rosary Pea, Precatory beans, crabs eye, jequirity bean [54]

Abrus precatorius, legume or pea family

Toxicity Rating: High. Even one bean can kill.

Animals Affected: All animals may be affected, although the primary risk is to pets.

Dangerous Parts of the Plan: The beans are the primary risk.

Class of Signs: Severe gastrointestinal irritation, vomiting, diarrhea, abdominal pain, collapse, death.

Plant Description: Twisting perennial vine, grows naturally in tropical climates. Rosary pea is established in certain areas of southern Florida. The leaves are alternate and compound, with 8 to 15 leaflets. The flowers are small, and can be any shade of white to red to purple. The seed pod is about 1 and 1/2 inches long, containing several seeds, bright red with a black spot.

Signs: The toxic signs resulting from rosary pea ingestion are very similar to those of castorbean, except rosary pea contains a more powerful toxin. It is reported that one seed if well-chewed can kill an adult human. The toxins are a protein called abrin and a glycoside called abric acid, which cause severe gastrointestinal signs: vomiting (if the species can vomit), diarrhea (which may be bloody) and abdominal pain. This progresses to weakness, shock and death within a short period of time.

The plant does not grow in Indiana, but sometimes seeds are imported to make jewelry and rosaries. If the seed is swallowed without damage to the seed coat, poisoning is unlikely, and the seed will tend to pass without incident. In cases where the seed coat is chewed or opened (as in drilling to make jewelry), toxic signs and death are likely.

First Aid: If rosary pea was ingested, contact a veterinarian immediately. Prevent further exposure and get other animals away from the source. Emergency measures may be used to eliminate the toxin from the stomach and intestines. Once gastrointestinal signs appear, it may be inadvisable to try to evacuate the stomach and intestines for fear of doing even greater damage, but a veterinarian will decide this. Beyond this, care is symptomatic and supportive of the digestive upset, weakness, and shock. Affected animals are likely to die even with care.

Prevention: Rosary pea should never be allowed around the home or pasture for the safety of animals and humans alike. If jewelry or rosaries are made of rosary pea, discard them immediately.

4.2 Cercopithecine herpesvirus 1[55]

(Herpes B virus)

Name: Cercopithecine Herpes Virus 1

Synonym or Cross Reference: B virus, Monkey B virus; Herpesviridae, Alphaherpesvirinae, Herpes simiae, CHV-1, Simian B disease, Genus Simplexvirus:

Characteristics: Herpesvirus, dsDNA, envelope, 100 nm diameter, icosahedral capsid

Pathogenicity: Acute, usually fatal, ascending encephalmyelitis; febrile onset with headache, vesicular skin lesions at site of exposure and variable neurological patterns; involvement of the respiratory centre and death 1 day to 3 weeks after onset of symptoms in over 70% of cases; occasional recoveries with considerable residual disability.

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[54]Cooperative Extension Service, Purdue University. Indiana Plants Poisonous to Livestock and Pets. http://www.vet.purdue.edu/depts/addl/toxic/plant53.htm

[55]Office of Laboratory Security, June 2001. © Health Canada, Population and Public Health Branch, Material and Safety Data Sheet. http://www.hc-sc.gc.ca/pphb-dgspsp/msds-ftss/msds81e.html

Epidemiology: Occurs in veterinarians, laboratory workers and others handling Eastern Hemisphere (Old World) monkeys or tissue cultures derived from monkeys; 31 reported cases with 68% mortality; naturally occurring alpha herpes virus infecting free - living or captive macaques; biological counterpart of herpes simplex virus in humans.

Host Range: Humans, monkeys, rabbits, guinea pigs, mice

Infectious Dose: Unknown

Mode of Transmission: B virus occurs in monkeys as a latent infection and is reactivated spontaneously resulting in shedding of virus in saliva, during periods of stress, higher rates of virus shedding occur; transmission to man occurs after a monkey bite or by direct or indirect contact / exposure of naked skin (broken or mucous membranes) to infected saliva, tissues, tissue fluid or monkey cell cultures; splashes or droplets of infected fluids to eye; aerosols exposure of CHV-1 is likely to be minimal; human-to-human transmission has been documented in one case.

Incubation Period: 3 days up to 4 weeks

Communicability: One reported case where virus was transmitted from person-to-person via direct contact of broken skin with monkey bite of another individual.

Reservoir: Most commonly found in macaque monkeys and other Old World monkeys frequent enzootic infection of captive Macaca mulatta (rhesus) and M. fascicularis (cynomolgus) monkeys.

Zoonosis: Yes

Vectors: None

Drug Susceptibility: Efficacy of acyclovir under investigation and reported to be effective.

Susceptibility to Disinfectants: Susceptible 1% sodium hypochlorite, 70% ethanol, 2% glutaraldehyde, formaldehyde.

Physical Inactivation: Inactivated by heat (50-60° C for at least 30 min), sensitive to lipid solvents, acidic pH and detergent solutions.

Survival Outside Host: Virus can remain viable in monkey saliva, CNS tissue and monkey kidney cell cultures; may survive for up to 7 days at 37° C, weeks at 4° C, very stable at -70° C.

Surveillance: Monitor for symptoms after exposure to bites by a monkey with known or suspicious lesion of B-virus; serological confirmation, PCR.

First Aid/Treatment: Wash and scrub exposed area / wound thoroughly with soap, concentrated solution of detergent, povidone-iodine or chlorhexidine and water, irrigate area with running water for 15-20 minutes; antiviral therapy should be administered.

Immunization: None available.

Prophylaxis: Administration of specific IG, if available may be effective; acyclovir or ganciclovir may be effective.

Laboratory-Acquired Infections: 36 reported cases with 64% deaths (23 died); most cases associated with individuals working directly with living Old World Monkeys; exposure to in vitro monkey tissues (primary rhesus monkey kidney) has been associated with laboratory infection. Other cases occurred following the cleaning of a monkey skull, puncture with contaminated needle or cut by infected glass, researcher died of CHV-1 after having been splashed in eye when moving a monkey cage.

Sources/Specimens: Oral secretions, thoracic and abdominal viscera and CNS tissues of naturally infected macaques.

Primary Hazards: Bites from monkeys with oral herpes lesions; exposures of broken skin or mucous. membranes to oral secretions or to infectious culture fluids; importance of aerosol exposure is not known

Special Hazards: Infected monkey cell cultures are a potential hazard.

Containment Requirements: Biosafety level 4 practices, containment equipment and facilities are recommended for activities involving manipulation of the virus.

Protective Clothing: Street clothing is removed and complete laboratory clothing is used the nature of which depends on the level 4 design (suit laboratory/cabinet line).

Other Precautions: Proper methods of restraint and protective clothing (longed sleeved garments, face shield or surgical mask and goggles or glasses) to help prevent bites and scratches of handlers hands.

Spills: Allow aerosols to settle; wearing protective clothing, gently cover spill with paper towel and apply 1% sodium hypochlorite, starting at perimeter and working towards the centre; allow sufficient contact time before clean up (30 min).

Disposal: Decontaminate all materials removed from the containment laboratory by steam sterilization, chemical disinfection, incineration or by gaseous methods; these include liquid and solid wastes.

Storage: In sealed containers that are appropriately labelled and contained within the level 4 facility.

4.3 *Coccidioides posadasii*[56]

Coccidioidomycosis (Posada's disease, desert rheumatism, desert fever)

Area of endemicity is southwest USA, northern Mexico, South America

Agents: *Coccidioides immitis* and *C. posadasii* (considered by some to be the most virulent fungus). 1992 is the centennial of the first description of the disease.

Epidemiology: Susceptibility to disseminated coccidioidomycosis is race dependent: Filipinos > blacks > whites

Lifecycle: Grows as septated, filamentous fungus in desert soil. Produces aerobuoyant arthroconidia conidia grow to form spherules in tissue (which are somewhat similar to sporangia in appearance). Spherules are sac-like thick-walled structures containing roughly 100-500 endospores. When the spherules lyse, they release the endospores that can then travel to other tissue sites and begin a new round of spherule production.

Disease: Mostly asymptomatic to mild pulmonary disease incubation period is 10-16 days after inhalation. May manifest allergic reactions, patient becomes skin test positive even if asymptomatic.

Desert bumps: erythema nodosum (but may see erythema multiforme)

Desert rheumatism: arthralgias and arthritis

Secondary coccidioidomycosis: ca: 2-8% of symptomatic infections and pulmonary cavity may be seen; see calcifications with resolution of 2° forms of disease.

Disseminated coccidioidomycosis: usually resolves spontaneously; acute meningitis; multiple cutaneous and subcutaneous bone lesions; may follow reactivation due to steroid therapy; chronic pulmonary disease usually shows "coin" lesion on X-ray.

Diagnosis: History of travel in endemic areas; coccidioidin skin test; examine sputum for spherules (biopsy material may be required); comp fix versus coccidiodin (serology of cocci is improving because of spherule antigens available); X-ray of chest may not be helpful.

4.4 Conotoxins[57]

Pathophysiology: Envenomation is associated with 18 species of conus. Venom from a cone shell is delivered via a detachable radula (a dartlike barb) formed in the radular sheath and delivered, after receiving venom in the pharynx, by an extensible proboscis. Cone shells' venom is a neurotoxin.

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[56]University of VA Health System, Medial Microbiology, Mycology 2: Lifecycles and Pathogenesis, Systemic Mycoses. http://hsc.VA.edu/med-ed/micro/myc/myc2.html#vir

[57]Shepherd, Suzanne (MD, MS, DTM&H, FACEP, Program Director, Associate Professor, Department of Emergency Medicine, University of Pennsylvania Medical Center) and James Martin (MD, Fellow, Department of Emergency Medicine, Division of Hyperbaric Medicine, Hospital of the University of Pennsylvania). eMedicine article "Conidae." http://www.emedicine.com/emerg/topic109.htm

Types of conotoxins and their effects:

- W-conotoxin - Hinders the voltage-dependent entry of calcium into the nerve terminal and inhibits acetylcholine release
- M-conotoxin - Modifies muscle sodium channels
- A-conotoxin - Blocks the nicotinic acetylcholine receptor
- Sleeper peptide - Found primarily in Conus geographus, induces a deep sleep state in test animals

A sting most commonly occurs on the hand and/or fingers of an unsuspecting handler, as well as on the feet of swimmers in shallow tropical waters. Local paresthesias and ischemia characterize minor stings. Serious envenomations may result in paralysis and respiratory failure. Death is typically secondary to cardiac failure. C geographus may produce rapid cerebral edema, coma, respiratory arrest, and cardiac failure. Disseminated intravascular coagulation (DIC) also may be evident.

Mortality/Morbidity: A high risk of death is associated with envenomation by certain species of cones, particularly C geographus. Morbidity includes mild symptoms (eg, nausea, weakness, diplopia) lasting several hours. Two to 3 weeks of symptoms may be associated with more severe exposures.

Symptoms: Sharp burning or stinging sensation, local paresthesias, perioral paresthesias, generalized paresthesias, nausea, diplopia, malaise, weakness, dysphagia, areflexia, aphonia, paralysis, apnea, pruritus

No antivenin is available for cone shell envenomation.

4.5 Crimean-Congo haemorrhagic fever virus[58]

Crimean-Congo haemorrhagic fever (CCHF) is a viral haemorrhagic fever of the Nairovirus group. Although primarily a zoonosis, sporadic cases and outbreaks of CCHF affecting humans do occur. The disease is endemic in many countries in Africa, Europe and Asia, and during 2001, cases or outbreaks have been recorded in Kosovo, Albania, Iran, Pakistan, and South Africa.

The disease was first described in the Crimea in 1944 and given the name Crimean haemorrhagic fever. In 1969 it was recognized that the pathogen causing Crimean haemorrhagic fever was the same as that responsible for an illness identified in 1956 in the Congo, and linkage of the 2 place names resulted in the current name for the disease and the virus. CCHF is a severe disease in humans, with a high mortality rate. Fortunately, human illness occurs infrequently, although animal infection may be more common. The geographical distribution of the virus, like that of its tick vector, is widespread. Evidence of CCHF virus has been found in Africa, Asia, the Middle East and Eastern Europe. Healthcare workers in endemic areas should be aware of the illness and the correct infection control procedures to protect themselves and their patients from the risk of nosocomial (hospital-acquired) infection.

CCHF Virus: The virus which causes CCHF is a Nairovirus, a group of related viruses forming one of the five genera in the Bunyaviridae family of viruses. All of the 32 members of the Nairovirus genus are transmitted by argasid or ixodid ticks, but only three have been implicated as causes of human disease: the Dugbe and Nairobi sheep viruses, and CCHF, which is the most important human pathogen amongst them.

CCHF Reservoirs and Vectors: The CCHF virus may infect a wide range of domestic and wild animals. Many birds are resistant to infection, but ostriches are susceptible and may show a high prevalence of infection in endemic areas. Animals become infected with CCHF from the bite of infected ticks.

A number of tick genera are capable of becoming infected with CCHF virus, but the most efficient and common vectors for CCHF appear to be members of the Hyalomma genus. Trans-ovarial (transmission of the virus from infected female ticks to offspring via eggs) and venereal transmission have been

[58]World Health Organization, revised November 2001. Fact Sheet No. 208. http://www.who.int/inf-fs/en/fact208.html

demonstrated amongst some vector species, indicating one mechanism which may contribute to maintaining the circulation of the virus in nature.

However, the most important source for acquisition of the virus by ticks is believed to be infected small vertebrates on which immature Hyalomma ticks feed. Once infected, the tick remains infected through its developmental stages, and the mature tick may transmit the infection to large vertebrates, such as livestock. Domestic ruminant animals, such as cattle, sheep and goats, are viraemic (virus circulating in the bloodstream) for around one week after becoming infected.

Humans who become infected with CCHF acquire the virus from direct contact with blood or other infected tissues from livestock during this time, or they may become infected from a tick bite. The majority of cases have occurred in those involved with the livestock industry, such as agricultural workers, slaughterhouse workers and veterinarians.

Clinical Features: The length of the incubation period for the illness appears to depend on the mode of acquisition of the virus. Following infection via tick bite, the incubation period is usually one to three days, with a maximum of nine days. The incubation period following contact with infected blood or tissues is usually five to six days, with a documented maximum of 13 days.

Onset of symptoms is sudden, with fever, myalgia (aching muscles), dizziness, neck pain and stiffness, backache, headache, sore eyes and photophobia (sensitivity to light). There may be nausea, vomiting and sore throat early on, which may be accompanied by diarrhoea and generalised abdominal pain. Over the next few days, the patient may experience sharp mood swings, and may become confused and aggressive. After two to four days, the agitation may be replaced by sleepiness, depression and lassitude, and the abdominal pain may localize to the right upper quadrant, with detectable hepatomegaly (liver enlargement).

Other clinical signs which emerge include tachycardia (fast heart rate), lymphadenopathy (enlarged lymph nodes), and a petechial rash (a rash caused by bleeding into the skin), both on internal mucosal surfaces, such as in the mouth and throat, and on the skin. The petechiae may give way to ecchymoses (like a petechial rash, but covering larger areas) and other haemorrhagic phenomena such as melaena (bleeding from the upper bowel, passed as altered blood in the faeces), haematuria (blood in the urine), epistaxis (nosebleeds) and bleeding from the gums. There is usually evidence of hepatitis. The severely ill may develop hepatorenal (i.e., liver and kidney) and pulmonary failure after the fifth day of illness.

The mortality rate from CCHF is approximately 30%, with death occurring in the second week of illness. In those patients who recover, improvement generally begins on the ninth or tenth day after the onset of illness.

Diagnosis: Diagnosis of suspected CCHF is performed in specially-equipped, high biosafety level laboratories. IgG and IgM antibodies may be detected in serum by enzyme-linked immunoassay (the "ELISA" or "EIA" methods) from about day six of illness. IgM remains detectable for up to four months, and IgG levels decline but remain detectable for up to five years.

Patients with fatal disease do not usually develop a measurable antibody response and in these individuals, as well as in patients in the first few days of illness, diagnosis is achieved by virus detection in blood or tissue samples. There are several methods for doing this. The virus may be isolated from blood or tissue specimens in the first five days of illness, and grown in cell culture. Viral antigens may sometimes be shown in tissue samples using immunofluorescence or EIA.

More recently, the polymerase chain reaction (PCR), a molecular method for detecting the viral genome, has been successfully applied in diagnosis.

Treatment: General supportive therapy is the mainstay of patient management in CCHF. Intensive monitoring to guide volume and blood component replacement is required.

The antiviral drug ribavirin has been used in treatment of established CCHF infection with apparent benefit. Both oral and intravenous formulations seem to be effective.

The value of immune plasma from recovered patients for therapeutic purposes has not been demonstrated, although it has been employed on several occasions.

Prevention and Control: Although an inactivated, mouse brain-derived vaccine against CCHF has been developed and used on a small scale in Eastern Europe, there is no safe and effective vaccine widely available for human use. The tick vectors are numerous and widespread and tick control with acaricides (chemicals intended to kill ticks) is only a realistic option for well-managed livestock production facilities.

Persons living in endemic areas should use personal protective measures that include avoidance of areas where tick vectors are abundant and when they are active (Spring to Fall); regular examination of clothing and skin for ticks, and their removal; and use of repellents.

Persons who work with livestock or other animals in the endemic areas can take practical measures to protect themselves. These include the use of repellents on the skin (e.g. DEET) and clothing (e.g. permethrin) and wearing gloves or other protective clothing to prevent skin contact with infected tissue or blood.

When patients with CCHF are admitted to hospital, there is a risk of nosocomial spread of infection. In the past, serious outbreaks have occurred in this way and it is imperative that adequate infection control measures be observed to prevent this disastrous outcome.

Patients with suspected or confirmed CCHF should be isolated and cared for using barrier nursing techniques. Specimens of blood or tissues taken for diagnostic purposes should be collected and handled using universal precautions. Sharps (needles and other penetrating surgical instruments) and body wastes should be safely disposed of using appropriate decontamination procedures.

Healthcare workers are at risk of acquiring infection from sharps injuries during surgical procedures and, in the past, infection has been transmitted to surgeons operating on patients to determine the cause of the abdominal symptoms in the early stages of (at that moment undiagnosed) infection. Healthcare workers who have had contact with tissue or blood from patients with suspected or confirmed CCHF should be followed up with daily temperature and symptom monitoring for at least 14 days after the putative exposure.

4.6 Diacetoxyscirpenol[59]

DAS

Trichothecene Mycotoxins (see also T-2 Toxin)

Trichothecene (pronounced “tri-ko-thee-seen”) mycotoxins are a large family of fungal toxins that produce nausea, vomiting, diarrhea, skin irritation, and internal bleeding. Fusarium, Stachybotrys, and other trichothecene-producing molds can infect food grains such as corn, rye, barley, oats, and millet, as well as straw and hay. Historically, trichothecene mycotoxins have been known to cause severe health problems in humans and animals who have eaten contaminated food. In 1944, for example, 30 percent of the population of Orenburg district, near Siberia, was affected by alimentary toxic aleukia (ATA), an often fatal disease caused by the ingestion of trichothecenes.

T-2 toxin and diacetoxyscirpenol (DAS) are the most potent members of the trichothecene family. They have an estimated lethal dose in humans of 3 to 35 milligrams (thousands of a gram), making them moderately toxic compared to chemical nerve agents such as sarin. Nevertheless, microgram doses of T-2 toxin and DAS are sufficient to cause vomiting, sustained nausea, and skin and eye irritation. According to one source, trichothecenes are approximately 10 to 500 times more potent than mustard gas in inducing skin necrosis.

[59]Tucker, Jonathan B. (Ph.D). National Gulf War Resource Center, “Mycotoxins and Gulf War Illness: A Possible Link.” http://www.mold-help.org/war.htm

Harvesting and extraction of infected grain can yield large quantities of trichothecenes for warfare purposes. Crude extracts of Fusarium containing a mixture of trichothecenes are more potent than pure T-2 toxin, and aflatoxin interacts synergistically with trichothecenes to increase their combined toxicity.

Military dissemination of these toxins would probably be in the form of a powder or smoke aerosol or, alternatively, sprayed in large, liquid drops as a ground contaminant. Trichothecenes can enter the body by absorption through the skin or eyes, inhalation (in aerosol form), or ingestion in contaminated food or water. When solubilized in the solvent dimethylsulfoxide (DMSO), T-2 toxin and DAS are readily absorbed through the skin. It is also known that trichothecenes are about 10 times more toxic when inhaled as an aerosol than by oral or subcutaneous administration.

An advantage of the trichothecenes from a military standpoint are that they are extremely stable, particularly in dry powder form. They can be stored for years at room temperature with no loss of activity and retain their full potency even after being boiled in water for an hour. Accordingly, these toxins are difficult to decontaminate from clothing and equipment and are highly persistent in the environment, although they are eventually broken down by soil bacteria.

Symptoms of Trichothecene Poisoning: Trichothecenes exert their toxic effects by inhibiting protein synthesis, and are therefore able to harm multiple organ systems simultaneously. They do the most damage to rapidly dividing cells such as those in the lining of the gastrointestinal tract, the bone marrow, and the lymph nodes, giving rise to symptoms similar to those of radiation poisoning. Because there is a large gap between the incapacitating dose and the lethal dose of trichothecenes, many casualties will become sick but not die. The delay in the appearance of symptoms after exposure depends on the dose and the means of delivery. Initial symptoms may occur within an hour of inhalation or as long as 12-24 hours after skin contact. After a single low-level exposure, the peak effects tend to occur in one to three days.

Initial symptoms of trichothecene poisoning are burning and irritation of the mucous membranes, including conjunctivitis (eye inflammation), nasal irritation (with or without bleeding), and sore throat. If the agent is delivered as an aerosol, coughing and shortness of breath are accompanied by burning of the lungs and secondary pulmonary edema. Nausea and persistent vomiting are common, as are diarrhea (which may be bloody) and dehydration. Damage to exposed areas of skin is often extensive: the symptoms range from redness and inflammation to the formation within several hours of small (1 centimeter diameter), hard, fluid-filled blisters. Other early symptoms are shortness of breath, dizziness, chest pain, low blood pressure and rapid heartbeat.

Delayed symptoms of high-dose trichothecene exposure include hemorrhage and necrosis of the mucous membranes, such as the lining of the gastrointestinal tract, resulting in bloody diarrhea; neurological disorders, and depressed bone-marrow activity leading to immune-system impairment and increased susceptibility to multiple bacterial and viral infections.

Because there are no known antidotes to the trichothecenes, treatment is limited to supportive care. Decontamination is possible if the victim washes extensively with large amounts of soap and water, but must be performed immediately after exposure. Although T-2 toxin and DAS are broken down by the body, some of the resulting metabolic products--such as HT-2 toxin--are also highly toxic. (HT-2 toxin is about two-thirds as toxic as T-2 toxin. Studies have also shown that both T-2 toxin and DAS are lipid-soluble and may persist for weeks in skin and fat tissue. Unfortunately, little research has been done on the chronic effects of sublethal trichothecene exposures.

4.7 Ebola viruses[60]

Ebola Hemorrhagic Fever

[60]CDC, Special Pathogens Branch. http://www.cdc.gov/ncidod/dvrd/spb/mnpages/dispages/ebola.htm

What is Ebola hemorrhagic fever? Ebola hemorrhagic fever (Ebola HF) is a severe, often-fatal disease in humans and nonhuman primates (monkeys, gorillas, and chimpanzees) that has appeared sporadically since its initial recognition in 1976.

The disease is caused by infection with Ebola virus, named after a river in the Democratic Republic of the Congo (formerly Zaire) in Africa, where it was first recognized. The virus is one of two members of a family of RNA viruses called the Filoviridae. There are four identified subtypes of Ebola virus. Three of the four have caused disease in humans: Ebola-Zaire, Ebola-Sudan, and Ebola-Ivory Coast. The fourth, Ebola-Reston, has caused disease in nonhuman primates, but not in humans.

Where is Ebola virus found in nature? The exact origin, locations, and natural habitat (known as the "natural reservoir") of Ebola virus remain unknown. However, on the basis of available evidence and the nature of similar viruses, researchers believe that the virus is zoonotic (animal-borne) and is normally maintained in an animal host that is native to the African continent. A similar host is probably associated with Ebola-Reston which was isolated from infected cynomolgous monkeys that were imported to the United States and Italy from the Philippines. The virus is not known to be native to other continents, such as North America.

Where do cases of Ebola hemorrhagic fever occur? Confirmed cases of Ebola HF have been reported in the Democratic Republic of the Congo, Gabon, Sudan, the Ivory Coast, Uganda, and the Republic of the Congo. An individual with serologic evidence of infection but showing no apparent illness has been reported in Liberia, and a laboratory worker in England became ill as a result of an accidental needle-stick. No case of the disease in humans has ever been reported in the United States. Ebola-Reston virus caused severe illness and death in monkeys imported to research facilities in the United States and Italy from the Philippines; during these outbreaks, several research workers became infected with the virus, but did not become ill.

Ebola HF typically appears in sporadic outbreaks, usually spread within a health-care setting (a situation known as amplification). It is likely that sporadic, isolated cases occur as well, but go unrecognized. A table showing a chronological list of known cases and outbreaks is available.

How is Ebola virus spread? Infections with Ebola virus are acute. There is no carrier state. Because the natural reservoir of the virus is unknown, the manner in which the virus first appears in a human at the start of an outbreak has not been determined. However, researchers have hypothesized that the first patient becomes infected through contact with an infected animal.

Treating patients with Ebola HF during outbreak of the disease in Kikwit, Democratic Republic of the Congo, in 1995.

After the first case-patient in an outbreak setting is infected, the virus can be transmitted in several ways. People can be exposed to Ebola virus from direct contact with the blood and/or secretions of an infected person. Thus, the virus is often spread through families and friends because they come in close contact with such secretions when caring for infected persons. People can also be exposed to Ebola virus through contact with objects, such as needles, that have been contaminated with infected secretions.

Nosocomial transmission refers to the spread of a disease within a health-care setting, such as a clinic or hospital. It occurs frequently during Ebola HF outbreaks. It includes both types of transmission described above. In African health-care facilities, patients are often cared for without the use of a mask, gown, or gloves. Exposure to the virus has occurred when health care workers treated individuals with Ebola HF without wearing these types of protective clothing. In addition, when needles or syringes are used, they may not be of the disposable type, or may not have been sterilized, but only rinsed before reinsertion into multi-use vials of medicine. If needles or syringes become contaminated with virus and are then reused, numerous people can become infected.

Ebola-Reston appeared in a primate research facility in Virginia, where it may have been transmitted from monkey to monkey through the air. While all Ebola virus species have displayed the ability to be spread through airborne particles (aerosols) under research conditions, this type of spread has not been documented among humans in a real-world setting, such as a hospital or household.

What are the symptoms of Ebola hemorrhagic fever? The incubation period for Ebola HF ranges from 2 to 21 days. The onset of illness is abrupt and is characterized by fever, headache, joint and muscle aches, sore throat, and weakness, followed by diarrhea, vomiting, and stomach pain. A rash, red eyes, hiccups and internal and external bleeding may be seen in some patients.

Researchers do not understand why some people are able to recover from Ebola HF and others are not. However, it is known that patients who die usually have not developed a significant immune response to the virus at the time of death.

How is Ebola hemorrhagic fever clinically diagnosed? Diagnosing Ebola HF in an individual who has been infected only a few days is difficult because early symptoms, such as red eyes and a skin rash, are nonspecific to the virus and are seen in other patients with diseases that occur much more frequently. However, if a person has the constellation of symptoms described above, and infection with Ebola virus is suspected, isolate the patient and notify local and state health departments and the CDC.

What laboratory tests are used to diagnose Ebola hemorrhagic fever? Antigen-capture enzyme-linked immunosorbent assay (ELISA) testing, IgM ELISA, polymerase chain reaction (PCR), and virus isolation can be used to diagnose a case of Ebola HF within a few days of the onset of symptoms. Persons tested later in the course of the disease or after recovery can be tested for IgM and IgG antibodies; the disease can also be diagnosed retrospectively in deceased patients by using immunohistochemistry testing, virus isolation, or PCR.

How is Ebola hemorrhagic fever treated? There is no standard treatment for Ebola HF. Patients receive supportive therapy. This consists of balancing the patient's fluids and electrolytes, maintaining their oxygen status and blood pressure, and treating them for any complicating infections.

How is Ebola hemorrhagic fever prevented? The prevention of Ebola HF in Africa presents many challenges. Because the identity and location of the natural reservoir of Ebola virus are unknown, there are few established primary prevention measures.

If cases of the disease do appear, current social and economic conditions often favor the spread of an epidemic within health-care facilities. Therefore, health-care providers must be able to recognize a case of Ebola HF should one appear. They must also have the capability to perform diagnostic tests and be ready to employ practical viral hemorrhagic fever isolation precautions, or barrier nursing techniques. These techniques include the wearing of protective clothing, such as masks, gloves, gowns, and goggles; the use of infection-control measures, including complete equipment sterilization; and the isolation of Ebola HF patients from contact with unprotected persons. The aim of all of these techniques is to avoid any person's contact with the blood or secretions of any patient. If a patient with Ebola HF dies, it is equally important that direct contact with the body of the deceased patient be prevented.

CDC has developed a set of tools to meet health-care facilities' needs. In conjunction with the World Health Organization, CDC has developed practical, hospital-based guidelines, entitled Infection Control for Viral Haemorrhagic Fevers In the African Health Care Setting. The manual describes how to recognize cases of viral hemorrhagic fever, such as Ebola HF, and prevent further nosocomial transmission by using locally available materials and few financial resources. Similarly, a practical diagnostic test that uses tiny samples from patients' skin has been developed to retrospectively diagnose Ebola HF in suspected case-patients who have died.

4.8 Lassa fever virus[61]

Lassa Fever

What is Lassa fever? Lassa fever is an acute viral illness that occurs in West Africa. The illness was discovered in 1969 when two missionary nurses died in Nigeria, West Africa. The cause of the illness was found to be Lassa virus, named after the town in Nigeria where the first cases originated. The virus, a

[61]CDC, Special Pathogens Branch. http://www.cdc.gov/ncidod/dvrd/spb/mnpages/dispages/lassaf.htm

member of the virus family Arenaviridae, is a single-stranded RNA virus and is zoonotic, or animal-borne.

In areas of Africa where the disease is endemic (that is, constantly present), Lassa fever is a significant cause of morbidity and mortality. While Lassa fever is mild or has no observable symptoms in about 80% of people infected with the virus, the remaining 20% have a severe multisystem disease. Lassa fever is also associated with occasional epidemics, during which the case-fatality rate can reach 50%.

Where is Lassa fever found? Lassa fever is an endemic disease in portions of West Africa. It is recognized in Guinea, Liberia, Sierra Leone, as well as Nigeria. However, because the rodent species which carry the virus are found throughout West Africa, the actual geographic range of the disease may extend to other countries in the region.

How many people become infected? The number of Lassa virus infections per year in West Africa is estimated at 100,000 to 300,000, with approximately 5,000 deaths. Unfortunately, such estimates are crude, because surveillance for cases of the disease is not uniformly performed. In some areas of Sierra Leone and Liberia, it is known that 10%-16% of people admitted to hospitals have Lassa fever, which indicates the serious impact of the disease on the population of this region.

In what animal host is Lassa virus maintained? The reservoir, or host, of Lassa virus is a rodent known as the "multimammate rat" of the genus Mastomys. It is not certain which species of Mastomys are associated with Lassa; however, at least two species carry the virus in Sierra Leone. Mastomys rodents breed very frequently, produce large numbers of offspring, and are numerous in the savannas and forests of West, Central, and East Africa. In addition, Mastomys generally readily colonize human homes. All these factors together contribute to the relatively efficient spread of Lassa virus from infected rodents to humans.

How do humans get Lassa fever? There are a number of ways in which the virus may be transmitted, or spread, to humans. The Mastomys rodents shed the virus in urine and droppings. Therefore, the virus can be transmitted through direct contact with these materials, through touching objects or eating food contaminated with these materials, or through cuts or sores. Because Mastomys rodents often live in and around homes and scavenge on human food remains or poorly stored food, transmission of this sort is common. Contact with the virus also may occur when a person inhales tiny particles in the air contaminated with rodent excretions. This is called aerosol or airborne transmission. Finally, because Mastomys rodents are sometimes consumed as a food source, infection may occur via direct contact when they are caught and prepared for food.

Lassa fever may also spread through person-to-person contact. This type of transmission occurs when a person comes into contact with virus in the blood, tissue, secretions, or excretions of an individual infected with the Lassa virus. The virus cannot be spread through casual contact (including skin-to-skin contact without exchange of body fluids). Person-to-person transmission is common in both village and health care settings, where, along with the above-mentioned modes of transmission, the virus also may be spread in contaminated medical equipment, such as reused needles (this is called nosocomial transmission).

What are the symptoms of Lassa fever? Signs and symptoms of Lassa fever typically occur 1-3 weeks after the patient comes into contact with the virus. These include fever, retrosternal pain (pain behind the chest wall), sore throat, back pain, cough, abdominal pain, vomiting, diarrhea, conjunctivitis, facial swelling, proteinuria (protein in the urine), and mucosal bleeding. Neurological problems have also been described, including hearing loss, tremors, and encephalitis. Because the symptoms of Lassa fever are so varied and nonspecific, clinical diagnosis is often difficult.

How is the disease diagnosed in the laboratory? Lassa fever is most often diagnosed by using enzyme-linked immunosorbent serologic assays (ELISA), which detect IgM and IgG antibodies as well as Lassa antigen. The virus itself may be cultured in 7 to 10 days. Immunohistochemistry performed on tissue specimens can be used to make a post-mortem diagnosis. The virus can also be detected by reverse transcription-polymerase chain reaction (RT-PCR); however, this method is primarily a research tool.

Are there complications after recovery? The most common complication of Lassa fever is deafness. Various degrees of deafness occur in approximately one-third of cases, and in many cases hearing loss is permanent. As far as is known, severity of the disease does not affect this complication: deafness may develop in mild as well as in severe cases. Spontaneous abortion is another serious complication.

What proportion of people die from the illness? Approximately 15%-20% of patients hospitalized for Lassa fever die from the illness. However, overall only about 1% of infections with Lassa virus result in death. The death rates are particularly high for women in the third trimester of pregnancy, and for fetuses, about 95% of which die in the uterus of infected pregnant mothers.

How is Lassa fever treated? Ribavirin, an antiviral drug, has been used with success in Lassa fever patients. It has been shown to be most effective when given early in the course of the illness. Patients should also receive supportive care consisting of maintenance of appropriate fluid and electrolyte balance, oxygenation and blood pressure, as well as treatment of any other complicating infections.

What groups are at risk for getting the illness? Individuals at risk are those who live or visit areas with a high population of Mastomys rodents infected with Lassa virus or are exposed to infected humans. Hospital staff are not at great risk for infection as long as protective measures are taken.

How is Lassa fever prevented? Primary transmission of the Lassa virus from its host to humans can be prevented by avoiding contact with Mastomys rodents, especially in the geographic regions where outbreaks occur. Putting food away in rodent-proof containers and keeping the home clean help to discourage rodents from entering homes. Using these rodents as a food source is not recommended. Trapping in and around homes can help reduce rodent populations. However, the wide distribution of Mastomys in Africa makes complete control of this rodent reservoir impractical.

When caring for patients with Lassa fever, further transmission of the disease through person-to-person contact or nosocomial routes can be avoided by taking preventive precautions against contact with patient secretions (together called VHF isolation precautions or barrier nursing methods). Such precautions include wearing protective clothing, such as masks, gloves, gowns, and goggles; using infection control measures, such as complete equipment sterilization; and isolating infected patients from contact with unprotected persons until the disease has run its course.

4.9 Marburg virus[62]

Marburg Hemorrhagic Fever

What is Marburg hemorrhagic fever? Marburg hemorrhagic fever is a rare, severe type of hemorrhagic fever which affects both humans and non-human primates. Caused by a genetically unique zoonotic (that is, animal-borne) RNA virus of the filovirus family, its recognition led to the creation of this virus family. The four species of Ebola virus are the only other known members of the filovirus family.

Marburg virus was first recognized in 1967, when outbreaks of hemorrhagic fever occurred simultaneously in laboratories in Marburg and Frankfurt, Germany and in Belgrade, Yugoslavia (now Serbia). A total of 37 people became ill; they included laboratory workers as well as several medical personnel and family members who had cared for them. The first people infected had been exposed to African green monkeys or their tissues. In Marburg, the monkeys had been imported for research and to prepare polio vaccine.

Where do cases of Marburg hemorrhagic fever occur? Recorded cases of the disease are rare, and have appeared in only a few locations. While the 1967 outbreak occurred in Europe, the disease agent had arrived with imported monkeys from Uganda. No other case was recorded until 1975, when a traveler most likely exposed in Zimbabwe became ill in Johannesburg, South Africa – and passed the virus to his traveling companion and a nurse. 1980 saw two other cases, one in Western Kenya not far from the Ugandan source of the monkeys implicated in the 1967 outbreak. This patient's attending physician in Nairobi became the second case. Another human Marburg infection was recognized in 1987 when a

[62]CDC, Special Pathogens Branch. http://www.cdc.gov/ncidod/dvrd/spb/mnpages/dispages/marburg.htm

young man who had traveled extensively in Kenya, including western Kenya, became ill and later died. In 1998, an outbreak occurred in Durba, Democratic Republic of the Congo. Cases were linked to individuals working in a gold mine. After the outbreak subsided, there were still some sporadic cases that occurred in the region.

Where is Marburg virus found? Marburg virus is indigenous to Africa. While the geographic area to which it is native is unknown, this area appears to include at least parts of Uganda and Western Kenya, and perhaps Zimbabwe. As with Ebola virus, the actual animal host for Marburg virus also remains a mystery. Both of the men infected in 1980 in western Kenya had traveled extensively, including making a visit to a cave, in that region. The cave was investigated by placing sentinels animals inside to see if they would become infected, and by taking samples from numerous animals and arthropods trapped during the investigation. The investigation yielded no virus: The sentinel animals remained healthy and no virus isolations from the samples obtained have been reported.

How do humans get Marburg hemorrhagic fever? Just how the animal host first transmits Marburg virus to humans is unknown. However, as with some other viruses which cause viral hemorrhagic fever, humans who become ill with Marburg hemorrhagic fever may spread the virus to other people. This may happen in several ways. Persons who have handled infected monkeys and have come in direct contact with their fluids or cell cultures, have become infected. Spread of the virus between humans has occurred in a setting of close contact, often in a hospital. Droplets of body fluids, or direct contact with persons, equipment, or other objects contaminated with infectious blood or tissues are all highly suspect as sources of disease.

What are the symptoms of the disease? After an incubation period of 5-10 days, the onset of the disease is sudden and is marked by fever, chills, headache, and myalgia. Around the fifth day after the onset of symptoms, a maculopapular rash, most prominent on the trunk (chest, back, stomach), may occur. Nausea, vomiting, chest pain, a sore throat, abdominal pain, and diarrhea then may appear. Symptoms become increasingly severe and may include jaundice, inflammation of the pancreas, severe weight loss, delirium, shock, liver failure, massive hemorrhaging, and multi-organ dysfunction.

Because many of the signs and symptoms of Marburg hemorrhagic fever are similar to those of other infectious diseases, such as malaria or typhoid fever, diagnosis of the disease can be difficult, especially if only a single case is involved.

Antigen-capture enzyme-linked immunosorbent assay (ELISA) testing, IgM-capture ELISA, polymerase chain reaction (PCR), and virus isolation can be used to confirm a case of Marburg hemorrhagic fever within a few days of the onset of symptoms. The IgG-capture ELISA is appropriate for testing persons later in the course of disease or after recovery. The disease is readily diagnosed by immunohistochemistry, virus isolation, or PCR of blood or tissue specimens from deceased patients.

Are there complications after recovery? Recovery from Marburg hemorrhagic fever may be prolonged and accompanied by orchititis, recurrent hepatitis, transverse myelitis or uvetis. Other possible complications include inflammation of the testis, spinal cord, eye, parotid gland, or by prolonged hepatitis.

Is the disease ever fatal? Yes. The case-fatality rate for Marburg hemorrhagic fever is between 23-25%.

How is Marburg hemorrhagic fever treated? A specific treatment for this disease is unknown. However, supportive hospital therapy should be utilized. This includes balancing the patient's fluids and electrolytes, maintaining their oxygen status and blood pressure, replacing lost blood and clotting factors and treating them for any complicating infections.

Sometimes treatment also has used transfusion of fresh-frozen plasma and other preparations to replace the blood proteins important in clotting. One controversial treatment is the use of heparin (which blocks clotting) to prevent the consumption of clotting factors. Some researchers believe the consumption of clotting factors is part of the disease process.

Who is at risk for the illness? People who have close contact with a human or non-human primate infected with the virus are at risk. Such persons include laboratory or quarantine facility workers who handle non-human primates that have been associated with the disease. In addition, hospital staff and family members who care for patients with the disease are at risk if they do not use proper barrier nursing techniques.

How is Marburg hemorrhagic fever prevented? Due to our limited knowledge of the disease, preventive measures against transmission from the original animal host have not yet been established. Measures for prevention of secondary transmission are similar to those used for other hemorrhagic fevers. If a patient is either suspected or confirmed to have Marburg hemorrhagic fever, barrier nursing techniques should be used to prevent direct physical contact with the patient. These precautions include wearing of protective gowns, gloves, and masks; placing the infected individual in strict isolation; and sterilization or proper disposal of needles, equipment, and patient excretions.

In conjunction with the World Health Organization, CDC has developed practical, hospital-based guidelines, titled Infection Control for Viral Haemorrhagic Fevers In the African Health Care Setting. The manual can help health-care facilities recognize cases and prevent further hospital-based disease transmission using locally available materials and few financial resources.

4.10 Monkeypox virus[63]

Monkeypox is a viral disease with a clinical presentation in humans similar to that seen in the past in smallpox patients. Smallpox no longer occurs, following its worldwide eradication in 1980, whereas monkeypox is still seen as a sporadic disease in parts of Africa.

The virus responsible for monkeypox is related to the virus that used to cause smallpox (both are orthopoxviruses). Vaccination against smallpox (no longer necessary) also gave protection against monkeypox. Before the eradication of smallpox, vaccination was widely practised and protected against both diseases. However, children born after 1980 have not been vaccinated against smallpox and are likely to be more susceptible to monkeypox than older members of the population. The death rate from monkeypox is highest in young children, reaching about 10%.

Most cases occur in remote villages of Central and West Africa close to tropical rainforests where there is frequent contact with infected animals. Monkeypox is usually transmitted to humans from squirrels and primates through contact with the animal's blood or through a bite.

Following reports of ongoing cases of human monkeypox in the Democratic Republic of the Congo ("DRC", formerly Zaire) representing a new pattern of the disease, the Ministry of Health in DRC and the World Health Organization (WHO) organized two investigations in February and October 1997.

In the past, an outbreak of monkeypox in DRC would have been limited to the village and would not last long because it did not spread extensively after the first patients recovered. However, the present study indicates that monkeypox disease is changing its pattern of infection in humans. The outbreak had a much higher rate of person to person transmission than seen previously, and spread through many generations of transmission, thus maintaining the outbreak for more than a year.

Previous studies over a twenty-year period had shown that the rate of transmission of monkeypox within households was low, suggesting that the disease had a low potential for transmission from person to person. Outbreaks were generally self-limiting after one or two sequential transmissions. However, the two recent WHO studies have to-date shown that:

- The outbreak in DRC presents the largest cluster of monkeypox cases - albeit with less severe clinical disease - ever reported: 511 suspect cases were identified with onset of disease between February 1996 and October 1997; some of these suspect cases are thought to be chickenpox and laboratory confirmation studies are underway;

[63] World Health Organization, revised December 1997. Fact Sheet No. 161. http://www.who.int/inf-fs/en/fact161.html

- The proportion of suspect cases who were 16 years of age or older (21%) was higher than previously reported (8%). Young children had mostly been affected in previous outbreaks;
- The percentage of suspect cases from person to person transmission (78%) was higher than previously reported (30%). This was associated with the clustering of cases in household compounds and prolonged chains of transmission from person to person;
- Secondary attack rates among contacts of primary cases (those thought to be infected by animal contact) have been calculated, on a preliminary basis, at 8%, which is similar to secondary attack rates of 4-12% estimated in Zaire in the early 1980s;
- The proportion of deaths (2%) was lower than previously reported (10%); all were aged under eight years and died within three weeks of disease onset.

The ending of vaccination programmes against smallpox in the late 1970's has probably led to an increase in susceptibility to monkeypox and could explain the larger size of the most recent outbreak, the higher proportion of patients aged 15 and over, and the spread through many generations of transmission.

WHO is concerned that monkeypox could pose a public health problem in this region of DRC and therefore vigilance must be maintained by strengthening detection systems for monkeypox and completely investigating future outbreaks.

4.11 Ricin[64]

Ricin is a potent protein cytotoxin derived from the beans of the castor plant (Ricinus communis). Castor beans are ubiquitous worldwide, and the toxin is fairly easy to extract; Therefore, ricin is potentially widely available. When inhaled as a small particle aerosol, this toxin may produce pathologic changes within 8 hours and severe respiratory symptoms followed by acute hypoxic respiratory failure in 36-72 hours. When ingested, ricin causes severe gastrointestinal symptoms followed by vascular collapse and death. This toxin may also cause disseminated intravascular coagulation, microcirculatory failure and multiple organ failure if given intravenously in laboratory animals.

Ricin's significance as a potential biological warfare toxin relates in part to its wide availability. Worldwide, one million tons of castor beans are processed annually in the production of castor oil; the waste mash from this process is 5% ricin by weight. The toxin is also quite stable and extremely toxic by several routes of exposure, including the respiratory route.

Ricin is actually made up of two hemagglutinins and two toxins. The toxins, RCL III and RCL IV, are dimers with molecular weights of about 66,000 daltons. The toxins are made up of two polypeptide chains, an A chain and a B chain, which are joined by a disulfide bond. Ricin can be produced relatively easily and inexpensively in large quantities in a fairly low technology setting. Ricin can be prepared in liquid or crystalline form, or it can be lyophilized to make a dry powder. It could be disseminated as an aerosol, injected into a target, or used to contaminate food or water on a small scale. Ricin is stable under ambient conditions, but is detoxified by heat (80OC for 10 min., or 50OC for about an hour at pH 7.8) and chlorine (>99.4% inactivation by 100 mg/L FAC in 20 min.). Low chlorine concentrations, such as 10 mg/L FAC, as well as iodine at up to 16 mg/L, have no effect on ricin. Ricin's toxicity is marginal when comparing its LD50 to other toxins, such as botulinum and SEB (incapacitating dose). An enemy would need to produce it in large quantities to cover a significant area on the battlefield, thus potentially limiting large-scale use of ricin by an adversary.

Mechanism of Toxicity: Ricin is very toxic to cells. It acts by inhibiting protein synthesis. The B chain binds to cell surface receptors and the toxin-receptor complex is taken into the cell; the A chain has endonuclease activity and extremely low concentrations will inhibit DNA replication and protein synthesis. In rodents, the histopathology of aerosol exposure is characterized by necrosis of upper and

[64]U.S. Army Medical Research Institute of Infectious Diseases. RICIN, A Chapter from Medical Management of Biological Casualities Handbook. http://www.healthri.org/environment/biot/ricin_chapter.htm

lower respiratory epithelium, causing tracheitis, bronchitis, bronchiolitis, and interstitial pneumonia with perivascular and alveolar edema. There is a latent period of 8 hours post-inhalation exposure before histologic lesions are observed in animal models. In rodents, ricin is more toxic by the aerosol route than by other routes of exposure.

Clinical Features: The clinical picture in intoxicated victims would depend on the route of exposure. After aerosol exposure, signs and symptoms would depend on the dose inhaled. Accidental sublethal aerosol exposures which occurred in humans in the 1940's were characterized by acute onset of the following symptoms in 4 to 8 hours: fever, chest tightness, cough, dyspnea, nausea, and arthralgias. The onset of profuse sweating some hours later was commonly the sign of termination of most of the symptoms. Although lethal human aerosol exposures have not been described, the severe pathophysiologic changes seen in the animal respiratory tract, including necrosis and severe alveolar flooding, are probably sufficient to cause death from ARDS and respiratory failure. Time to death in experimental animals is dose dependent, occurring 36-72 hours post inhalation exposure. Humans would be expected to develop severe lung inflammation with progressive cough, dyspnea, cyanosis and pulmonary edema.

By other routes of exposure, ricin is not a direct lung irritant; however, intravascular injection can cause minimal pulmonary perivascular edema due to vascular endothelial injury. Ingestion causes necrosis of the gastrointestinal epithelium, local hemorrhage, and hepatic, splenic, and renal necrosis. Intramuscular injection causes severe local necrosis of muscle and regional lymph nodes with moderate visceral organ involvement.

Signs and Symptoms: Acute onset of fever, chest tightness, cough, dyspnea, nausea, and arthralgias occurs 4 to 8 hours after inhalational exposure. Airway necrosis and pulmonary capillary leak resulting in pulmonary edema would likely occur within 18-24 hours, followed by severe respiratory distress and death from hypoxemia in 36-72 hours.

Diagnosis: An attack with aerosolized ricin would be primarily diagnosed by the clinical and epidemiological setting. Acute lung injury affecting a large number of geographically clustered cases should raise suspicion of an attack with a pulmonary irritant such as ricin, although other pulmonary pathogens could present with similar signs and symptoms. Other biological threats, such as SEB, Q fever, tularemia, plague, and some chemical warfare agents like phosgene, need to be included in the differential diagnosis. Ricin-induced pulmonary edema would be expected to occur much later (1-3 days post exposure) compared to that induced by SEB (about 12 hours post exposure) or phosgene (about 6 hours post exposure). Ricin intoxication would be expected to progress despite treatment with antibiotics, as opposed to an infectious process. There would be no mediastinitis as seen with inhalation anthrax. Ricin patients would not be expected to plateau clinically as occurs with SEB intoxication.

Treatment: Management is supportive and should include treatment for pulmonary edema. Gastric lavage and cathartics are indicated for ingestion, but charcoal is of little value for large molecules such as ricin.

Prophylaxis: There is currently no vaccine or prophylactic antitoxin available for human use, although immunization appears promising in animal models. Use of the protective mask is currently the best protection against inhalation.

Isolation and Decontamination: Standard Precautions for healthcare workers. Ricin is non-vloatile, and secondary aerosols are not expected to be a danger to health care providers. Decontaminate with soap and water. Hypochlorite solutions (0.1% sodium hypochlorite) can inactivate ricin.

4.12 *Rickettsia prowazekii*[65]

Name: *Rickettsia prowazekii*, *Rickettsia canadensis* (Formerly *R. canada*)

[65]Office of Laboratory Security, January 2001. © Health Canada, Population and Public Health Branch, Material and Safety Data Sheet. http://www.hc-sc.gc.ca/pphb-dgspsp/msds-ftss/msds128e.html

Synonym or Cross Reference: Louse-borne typhus fever, Epidemic typhus, Typhus exanthematicus, Classical typhus fever, Brill-zinsser disease.

Characteristics: Pleomorphic, gram-negative bacterium, obligate intracellular

Pathogenicity: Variable onset, often sudden with headache, chills, fever, prostration, and general pains; macular eruption on the 5 and 6th day on upper trunk and spreading to entire body (except face, palms or soles); pronounced toxemia; disease terminates approximately 2 weeks after onset of fever; case fatality rate from 10-40% in absence of treatment; may recrudesce years later as Brill-Zissner disease.

Epidemiology: Occurs in areas of poor hygiene and that are louse-infected; endemic foci in mountainous regions of Central and South America, in Africa and Asia; in USA, last louse-borne outbreak was in 1921 and now exists as a zoonosis of flying squirrels (33 infections between 1976 and 1984).

Host Range: Humans, squirrels

Infectious Dose: <10 organisms

Mode of Transmission: The body louse is infected by feeding on the blood of a patient with acute typhus fever; infected lice excrete rickettsiae in their feces and defecate at time of feeding; man is infected by rubbing feces or crushed lice into the bite or into superficial abrasions; inhalation of infective louse feces from dust; transmission from squirrel may be the bite of squirrel flea.

Incubation Period: From 1 to 2 weeks, commonly 12 days

Communicability: Not directly transmitted from person-to-person; patients are infective for lice during the febrile illness and possibly for 2 to 3 days after temperature returns to normal; louse is infective within 2 to 6 days after infected meal (infective earlier if crushed).

Reservoir: Humans maintain the infection during inter-epidemic periods; importance of flying squirrel has not yet been demonstrated.

Zoonosis: Possibly by flying squirrel

Vectors: Body louse - Pediculus humanus

Drug Susceptibility: Sensitive to tetracyclines, chloramphenicol doxycycline

Susceptibility to Disinfectants: Susceptible to 1% sodium hypochlorite, 70% ethanol, glutaraldehyde, formaldehyde

Physical Inactivation: Susceptible to moist heat (121° C for at least 15 min) and dry heat (160-170° C for at least 1 hour).

Survival Outside Host: Rickettsia may remain viable in louse fecal material and the dead louse for weeks.

Surveillance: All immediate contacts should be observed for 2 weeks for symptoms of illness; *R. prowazekkii* is cross reactive with *R. typhi*.

First Aid/Treatment: For seriously ill patient with possible typhus, antibiotic therapy should begin without waiting for laboratory confirmation; treatment with chloramphenicol or tetracyclines

Immunization: No commercially available vaccine for use (persons entering high endemic areas are immunized eg. military); live vaccine prepared from the attenuated strain E of *R. prowazekii* has shown promise

Prophylaxis: Use of residual insecticide applied to clothing and treatment of hair for louse eggs

Primary Hazards: Accidental parenteral inoculation and exposure to infectious aerosols

Containment Requirements: Biosafety level 2 practices and containment for non-propagative activities (serological procedures, smears); biosafety level 3 for all manipulations of infectious materials, including animal necropsy, and the inoculation, incubation and harvesting of embryonated eggs or tissue cultures; arthropods and flying squirrels should be handled using biosafety level 3 practices and facilities

4.13 *Rickettsia rickettsii*[66]

Name: *Rickettsia rickettsii*

Synonym or Cross Reference: Rocky Mountain Spotted Fever (RMSF), New World spotted fever, Tick-borne typhus fever, Sao Paulo fever

Characteristics: Pleomorphic gram-negative obligate intracellular bacterium 0.3-1.5 µm in size

Pathogenicity: Sudden onset with moderate to high fever persisting 2 to 3 weeks, malaise, deep muscle pain, severe headache, chills and conjunctival injection; maculopapular rash appears on extremities 3rd day and spreads rapidly; hemorrhages are common; 15-20% case fatality rate in absence of therapy but with treatment death is uncommon; clinical syndrome may be confused with atypical measles, ehrlichiosis, meningococcemia and enteroviral infection.

Epidemiology: Occurs through USA during spring, summer and fall; many cases along eastern seaboard and the Rocky Mountain region; adult males infected most frequently in western USA and in East, incidence is higher in children; some infections in Canada, Central and South America.

Host Range: Humans, dogs, rodents, various other small animals

Infectious Dose: < 10 organisms

Mode of Transmission: Ordinarily by bite of an infected tick; several hours of attachment are required before the rickettsiae become reactivated to infect humans; by contamination of skin with crushed tissues or feces of tick.

Incubation Period: From 3 to 14 days

Communicability: Not directly transmitted from person-to-person; tick remains infectious for life, approximately 18 months.

Reservoir: Maintained in nature in ticks by transovarian and transstadial passage; transmitted to humans, dogs, various rodents and animals.

Zoonosis: Yes - bite from ticks from infected animals; contamination of broken skin or mucous membranes with feces or internal contents of crushed tick from infected animal.

Vectors: Ticks:

- East and South USA - dog tick, Dermancentor variabilis
- Northwest USA - wood tick, D.andersoni
- Southwest USA - Lone Star tick, Amyblyomma americanum
- Latin America - A. cajennense

Drug Susceptibility: Sensitive to tetracyclines and chloramphenicol

Susceptibility to Disinfectants: Susceptible to 1% sodium hypochlorite, 70% ethanol, glutaraldehyde, formaldehyde.

Physical Inactivation: Sensitive to moist heat (121° C for at least 15 min) and dry heat (160-170° C for at least 1 hour).

Survival Outside Host: Organism is stable in tick tissues or blood under ambient environmental conditions, surviving up to 1 year; sensitive to drying-feces of infected ticks quickly lose their infectivity on drying.

Surveillance: Monitor for febrile illness and report symptoms immediately; confirm with IF, IgG does not appear until 7 to 10 days after onset.

First Aid/Treatment: Appropriate antibiotic therapy in the early stages of infection should be initiated on clinical and epidemiologic considerations without waiting for laboratory confirmation of the diagnosis.

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[66]Office of Laboratory Security, January 2001. © Health Canada, Population and Public Health Branch, Material and Safety Data Sheet. http://www.hc-sc.gc.ca/pphb-dgspsp/msds-ftss/msds129e.html

Immunization: None licensed; but may be available as killed experimental product from U.S. CDC

Prophylaxis: Early antibiotic treatment lead to delayed onset of illness or relapse (tetracyclines and chloramphenicol do not kill Rickettsia).

Primary Hazards: Accidental parenteral inoculation and exposure to infectious aerosols (successful aerosol transmission documented in non-human primates).

Containment Requirements: Biosafety level 2 practices, containment and facilities for all non-propagative laboratory procedures including serological and fluorescent antibody tests, and staining of impression smears; biosafety level 3 for all other manipulations of known or potentially infectious materials, including necropsy of infected animals and trituration of their tissues, and inoculation, incubation and harvesting of embryonated eggs or tissue culture.

Other Precautions: Animal biosafety level 2 practices and facilities are recommended for holding of experimentally infected rodents, however, all necropsy and manipulation of tissue should be conducted at Biosafety level 3.

4.14 Saxitoxin[67]

Characteristics: Saxitoxin is the parent compound of a family of chemically related neurotoxins. In nature they are predominantly produced by marine dinoflagellates, although they have also been identified in association with such diverse organisms as blue-green algae, crabs, and the blue-ringed octopus. Human intoxications are principally due to ingestion of bivalve molluscs which have accumulated dinoflagellates during filter feeding.

The resulting intoxication, known as paralytic shellfish poisoning (PSP), is known throughout the world as a severe, life-threatening illness requiring immediate medical intervention.

Saxitoxin and its derivatives are water-soluble compounds that bind to the voltage-sensitive sodium channel, blocking propagation of nerve-muscle action potentials. Consistent with this mechanism of action, victims typically present with neurological symptoms and in severe cases, death results from respiratory paralysis.

The natural route of exposure to these toxins is oral. In a BW scenario, the most likely route of delivery is by inhalation or toxic projectile. In addition, saxitoxin could be used in a confined area to contaminate water supplies.

Clinical Features: After oral exposure, absorbtion of toxins from the gastrointestinal tract is rapid. Onset of symptoms typically begins 10-60 minutes after exposure, but may be delayed several hours depending upon the dose and individual idiosyncrasy. Initial symptoms are numbness or tingling of the lips, tongue and fingertips, followed by numbness of the neck and extremities and general muscular incoordination. Nausea and vomiting may be present, but typically occur in a minority of cases. Other symptoms may include a feeling of light headedness, or floating, dizziness, weakness, aphasia, incoherence, visual disturbances, memory loss and headache. Cranial nerves are often involved, especially those responsible for ocular movements, speech, and swallowing. Induced reflexes are normal and the patient remains conscious. Respiratory distress and flaccid muscular paralysis are the terminal stages and can occur 2-12 hours after intoxication. Death results from respiratory paralysis. Clearance of the toxin is rapid and survivors for 12-24 hours will usually recover. Complete recovery may require 7-14 days. There are no known cases of inhalation exposure to saxitoxin in the medical literature, but data from animal experiments suggest the entire syndrome is compressed and death may occur in minutes.

[67] February 1, 1996. Handbook on the Medical Aspects of NBC Defensive Operations FM 8-9, Part II – Biological, Annex B, Clinical Data Sheets for Selected Biological Agents. http://www.mega.nu:8080/nbcmans/8-9-html/part_ii/annexb2.htm

Diagnosis: Routine Laboratory Findings. Routine laboratory evaluation is not particularly helpful. Cardiac conduction defects may develop. Elevation of serum creatine kinase levels in some patients has been reported.

Differential Diagnosis: Exposure to tetrodotoxin or the ciguatera toxins can manifest very similar signs and symptoms. Ciguatoxins (by oral exposure) typically demonstrate a much greater degree of gastrointestinal involvement, and can also be differentiated by a history of eating finfish rather than shellfish. Tetrodotoxin intoxication is nearly identical to that caused by the saxitoxins except that hypotension typically plays a greater role in severe intoxication. Differential diagnosis may require toxin detection. Gas chromatographic analysis of food or stomach contents can rule out pesticide exposure.

Therapy: Management is supportive and standard management of poison ingestion should be employed if intoxication is by the oral route. Toxins are rapidly cleared and excreted in the urine, so diuresis may increase elimination. Charcoal hemoperfusion has been advocated, but remains unproven in its utility. Intubation and mechanical respiratory support may be required in severe intoxication. Timely resuscitation would be imperative, albeit very difficult, after inhalation exposure on the battlefield. Specific antitoxin therapy has been successful in animal models, but is untested in humans.

Prophylaxis: No vaccine against saxitoxin exposure has been developed for human use.

4.15 Shiga-like ribosome inactivating proteins[68]

Name of the Organism: *Shigella* spp. (*Shigella sonnei*, *S. boydii*, *S. flexneri*, and *S. dysenteriae*)

Shigella are Gram-negative, nonmotile, nonsporeforming rod-shaped bacteria. The illness caused by Shigella (shigellosis) accounts for less than 10% of the reported outbreaks of foodborne illness in this country. Shigella rarely occurs in animals; principally a disease of humans except other primates such as monkeys and chimpanzees. The organism is frequently found in water polluted with human feces.

Name of Disease: Shigellosis (bacillary dysentery).

Nature of Disease: Symptoms -- Abdominal pain; cramps; diarrhea; fever; vomiting; blood, pus, or mucus in stools; tenesmus. Onset time -- 12 to 50 hours. Infective dose -- As few as 10 cells depending on age and condition of host. The Shigella spp. are highly infectious agents that are transmitted by the fecal-oral route. The disease is caused when virulent Shigella organisms attach to, and penetrate, epithelial cells of the intestinal mucosa. After invasion, they multiply intracellularly, and spread to contiguous epitheleal cells resulting in tissue destruction. Some strains produce enterotoxin and Shiga toxin (very much like the verotoxin of *E. coli* O157:H7).

Diagnosis of Human Illness: Serological identification of culture isolated from stool.

Associated Foods: Salads (potato, tuna, shrimp, macaroni, and chicken), raw vegetables, milk and dairy products, and poultry. Contamination of these foods is usually through the fecal-oral route. Fecally contaminated water and unsanitary handling by food handlers are the most common causes of contamination.

Relative Frequency of Disease: An estimated 300,000 cases of shigellosis occur annually in the U.S. The number attributable to food is unknown, but given the low infectious dose, it is probably substantial.

Complications: Infections are associated with mucosal ulceration, rectal bleeding, drastic dehydration; fatality may be as high as 10-15% with some strains. Reiter's disease, reactive arthritis, and hemolytic uremic syndrome are possible sequelae that have been reported in the aftermath of shigellosis.

[68] U.S. Food & Drug Administration, Center for Food Safety & Applied Nutrition, Foodborne Pathogenic Microorganisms and Natural Toxins Handbook, Bad Bug Book. http://www-nehc.med.navy.mil/nepmu2/pmttoolbox/NAVMED%20P-5010%5CFDA%20Foodborne%20Pathogenic%20Microorganisms%20and%20Natural%20Toxins%20H~F54%5CPathogenic%20Bacteria%5CShigella%20spp.doc

Target Populations: Infants, the elderly, and the infirm are susceptible to the severest symptoms of disease, but all humans are susceptible to some degree. Shigellosis is a very common malady suffered by individuals with acquired immune deficiency syndrome (AIDS) and AIDS-related complex, as well as non-AIDS homosexual men.

Food Analysis: Organisms are difficult to demonstrate in foods because methods are not developed or are insensitive. A genetic probe to the virulence plasmid has been developed by FDA and is currently under field test. However, the isolation procedures are still poor.

Selected Outbreaks: In 1985, a huge outbreak of foodborne shigellosis occurred in Midland-Odessa, Texas, involving perhaps as many as 5,000 persons. The implicated food was chopped, bagged lettuce, prepared in a central location for a Mexican restaurant chain. FDA research subsequently showed that S. sonnei, the isolate from the lettuce, could survive in chopped lettuce under refrigeration, and the lettuce remained fresh and appeared to be quite edible. In 1985-1986, several outbreaks of shigellosis occurred on college campuses, usually associated with fresh vegetables from the salad bar. Usually an ill food service worker was shown to be the cause. In 1987, several very large outbreaks of shigellosis (S. sonnei) occurred involving thousands of persons, but no specific food vector could be proven. In 1988, numerous individuals contracted shigellosis from food consumed aboard Northwest Airlines flights; food on these flights had been prepared in one central commisary. No specific food item was implicated, but various sandwiches were suspected. **NOTE - Although all Shigella spp. have been implicated in foodborne outbreaks at some time, S. sonnei is clearly the leading cause of shigellosis from food. The other species are more closely associated with contaminated water. One in particular, S. flexneri, is now thought to be in large part sexually transmitted.

4.16 South American haemorrhagic fever viruses[69]

Junin (Candid #1), Machupo, Sabia, Flexal, Guanarito

Arenaviruses

What are the Arenaviridae? Vero E6 tissue culture cell infected with an arenavirus. Image shows extracellular virus particles budding from the cell surface. Click the image to see a high-resolution version. Image courtesy Cynthia Goldsmith, MS, Infectious Disease Pathology Activity, DVRD, NCID, CDC. Go to high-resolution version.

The Arenaviridae are a family of viruses whose members are generally associated with rodent-transmitted disease in humans. Each virus usually is associated with a particular rodent host species in which it is maintained. Arenavirus infections are relatively common in humans in some areas of the world and can cause severe illnesses.

The virus particles are spherical and have an average diameter of 110-130 nanometers. All are enveloped in a lipid (fat) membrane. Viewed in cross-section, they show grainy particles that are ribosomes acquired from their host cells. It is this characteristic that gave them their name, derived from the Latin "arena," which means "sandy." Their genome, or genetic material, is composed of RNA only, and while their replication strategy is not completely understood, we know that new viral particles, called virions, are created by budding from the surface of their hosts' cells.

When were the members of this virus family recognized? The first arenavirus, lymphocytic choriomeningitis virus (LCMV), was isolated in 1933 during a study of an epidemic of St. Louis encephalitis. Although not the cause of the outbreak, LCMV was found to be a cause of aseptic (nonbacterial) meningitis. By the 1960s, several similar viruses had been discovered and they were classified into the new family Arenaviridae. Since Tacaribe virus was found in 1956, new arenaviruses have been discovered on the average of every one to three years. A number of arenaviruses cause hemorrhagic disease. Junin virus, isolated in 1958, was the first of these to be recognized. This virus causes Argentine hemorrhagic fever in a limited agricultural area of the pampas in Argentina. Several

[69]CDC, Special Pathogens Branch. http://www.cdc.gov/ncidod/dvrd/spb/mnpages/dispages/arena.htm

years later, in 1963, in the remote savannas of the Beni province of Bolivia, Machupo virus was isolated. The next member of the virus family to be associated with an outbreak of human illness was Lassa virus in Africa in 1969. Most recently, Guanarito and Sabia viruses were added to this family.

What viruses are included in the virus family? The arenaviruses are divided into two groups: the New World or Tacaribe complex and the Old World or LCM/Lassa complex. Viruses in these groups that cause illness in humans are listed below:

Virus	Disease
Lassa virus	Lassa fever
Junin virus	Argentine hemorrhagic fever
Machupo virus	Bolivian hemorrhagic fever
Guanarito virus	Venezuelan hemorrhagic fever
Sabia	Brazilian hemorrhagic fever

What kinds of animal hosts do these viruses have? These viruses are zoonotic, meaning that, in nature, they are found in animals. Each virus is associated with either one species or a few closely related rodents, which constitute the virus' natural reservoir. Tacaribe complex viruses are generally associated with the New World rats and mice (family Muridae, subfamily Sigmodontinae). The LCM/Lassa complex viruses are associated with the Old World rats and mice (family Muridae, subfamily Murinae). Taken together, these types of rodents are located across the greater proportion of the earth's land mass, including Europe, Asia, Africa, and the Americas. One notable exception is Tacaribe virus, found in Trinidad, which was isolated from a bat.

How are arenaviruses spread? The rodent hosts of arenaviruses are chronically infected with the viruses; however, the viruses do not appear to cause obvious illness in them. Some Old World arenaviruses appear to be passed from mother rodents to their offspring during pregnancy, and thus remain in the rodent population generation after generation. Some New World arenaviruses are transmitted among adult rodents, likely via fighting and inflicting bites. Only a portion of the rodents in each host species is infected at any one time, and in many cases only in a limited portion of the host's geographical range. The viruses are shed into the environment in the urine or droppings of their infected hosts.

Human infection with arenaviruses is incidental to the natural cycle of the viruses and occurs when an individual comes into contact with the excretions or materials contaminated with the excretions of an infected rodent, such as ingestion of contaminated food, or by direct contact of abraded or broken skin with rodent excrement. Infection can also occur by inhalation of tiny particles soiled with rodent urine or saliva (aerosol transmission). The types of incidental contact depend on the habits of both humans and rodents. For example, where the infected rodent species prefers a field habitat, human infection is associated with agricultural work. In areas where the rodent species' habitat includes human homes or other buildings, infection occurs in domestic settings.

Some arenaviruses, such as Lassa and Machupo viruses, are associated with secondary person-to-person and nosocomial (health-care setting) transmission. This occurs when a person infected by exposure to the virus from the rodent host spreads the virus to other humans. This may occur in a variety of ways. Person-to-person transmission is associated with direct contact with the blood or other excretions, containing virus particles, of infected individuals. Airborne transmission has also been reported in connection with certain viruses. Contact with objects contaminated with these materials, such as medical equipment, is also associated with transmission. In these situations, use of protective clothing and disinfection procedures (together called barrier nursing) help prevent further spread of illness.

The other arenaviruses: Ampari, Flexal, Ippy, Mobala, Mopeia, Latino, Parana, Pichinde, Tacaribe, and Tamiamican cause infections in laboratory personnel, especially when high concentrations of virus are being processed.[70]

4.17 Tetrodotoxin[71]

Name of the Organism: Tetrodotoxin (anhydrotetrodotoxin 4-epitetrodotoxin, tetrodonic acid)

Nature of Acute Disease: Pufferfish Poisoning, Tetradon Poisoning, Fugu Poisoning

Nature of Disease: Fish poisoning by consumption of members of the order Tetraodontiformes is one of the most violent intoxications from marine species. The gonads, liver, intestines, and skin of pufferfish can contain levels of tetrodotoxin sufficient to produce rapid and violent death. The flesh of many pufferfish may not usually be dangerously toxic. Tetrodotoxin has also been isolated from widely differing animal species, including the California newt, parrotfish, frogs of the genus Atelopus, the blue-ringed octopus, starfish, angelfish, and xanthid crabs. The metabolic source of tetrodotoxin is uncertain. No algal source has been identified, and until recently tetrodotoxin was assumed to be a metabolic product of the host. However, recent reports of the production of tetrodotoxin/anhydrotetrodotoxin by several bacterial species, including strains of the family Vibrionaceae, Pseudomonas sp., and Photobacterium phosphoreum, point toward a bacterial origin of this family of toxins. These are relatively common marine bacteria that are often associated with marine animals. If confirmed, these findings may have some significance in toxicoses that have been more directly related to these bacterial species.

Diagnosis of Human Illness: The diagnosis of pufferfish poisoning is based on the observed symptomology and recent dietary history.

Associated Foods: Poisonings from tetrodotoxin have been almost exclusively associated with the consumption of pufferfish from waters of the Indo-Pacific ocean regions. Several reported cases of poisonings, including fatalities, involved pufferfish from the Atlantic Ocean, Gulf of Mexico, and Gulf of California. There have been no confirmed cases of poisoning from the Atlantic pufferfish, Spheroides maculatus. However, in one study, extracts from fish of this species were highly toxic in mice. The trumpet shell Charonia sauliae has been implicated in food poisonings, and evidence suggests that it contains a tetrodotoxin derivative. There have been several reported poisonings from mislabelled pufferfish and at least one report of a fatal episode when an individual swallowed a California newt.

Relative Frequency of Disease: From 1974 through 1983 there were 646 reported cases of pufferfish poisoning in Japan, with 179 fatalities. Estimates as high as 200 cases per year with mortality approaching 50% have been reported. Only a few cases have been reported in the United States, and outbreaks in countries outside the Indo-Pacific area are rare.

Course of Disease and Complications: The first symptom of intoxication is a slight numbness of the lips and tongue, appearing between 20 minutes to three hours after eating poisonous pufferfish. The next symptom is increasing paraesthesia in the face and extremities, which may be followed by sensations of lightness or floating. Headache, epigastric pain, nausea, diarrhea, and/or vomiting may occur. Occasionally, some reeling or difficulty in walking may occur. The second stage of the intoxication is increasing paralysis. Many victims are unable to move; even sitting may be difficult. There is increasing respiratory distress. Speech is affected, and the victim usually exhibits dyspnea, cyanosis, and hypotension. Paralysis increases and convulsions, mental impairment, and cardiac arrhythmia may occur. The victim, although completely paralyzed, may be conscious and in some cases completely lucid until shortly before death. Death usually occurs within 4 to 6 hours, with a known range of about 20 minutes to 8 hours.

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[70] Pfau, Charles J. Medical Microbiology, Fourth Edition, Arenaviruses chapter. http://gsbs.utmb.edu/microbook/ch057.htm

[71] U.S. Food & Drug Administration, Center for Food Safety & Applied Nutrition, Foodborne Pathogenic Microorganisms and Natural Toxins Handbook, Bad Bug Book. http://vm.cfsan.fda.gov/~mow/chap39.html

Target Populations: All humans are susceptible to tetrodotoxin poisoning. This toxicosis may be avoided by not consuming pufferfish or other animal species containing tetrodotoxin. Most other animal species known to contain tetrodotoxin are not usually consumed by humans. Poisoning from tetrodotoxin is of major public health concern primarily in Japan, where "fugu" is a traditional delicacy. It is prepared and sold in special restaurants where trained and licensed individuals carefully remove the viscera to reduce the danger of poisoning. Importation of pufferfish into the United States is not generally permitted, although special exceptions may be granted. There is potential for misidentification and/or mislabelling, particularly of prepared, frozen fish products.

4.18 Tick-borne encephalitis complex (flavi) viruses

(Central European Tick-borne encephalitis, Far Eastern Tick-borne encephalitis, [Russian Spring and Summer encephalitis, Kyasanur Forest disease, Omsk Hemorrhagic Fever])

European Tick Borne Encephalitis[72]

European tick-borne encephalitis is the most important recognized arboviral illness in Europe. Widely scattered areas have reported cases of encephalitis following tick bites. These include the east coast of Sweden, rural areas of Poland, Czechoslovakia and Central Europe including Austria, Bavaria, Hungary, and the former Soviet Republics.

Travellers to Austria are particularly concerned to read signs in airports, other public places, and pharmacies asking "Have you been vaccinated against encephalitis?" The following information is, therefore, being made available.

Agent and Epidemiology: This encephalitis is caused by group B arbovirus or flavivirus, whose family also includes the agents of yellow fever, dengue, and St. Louis encephalitis. It is similar to the Powassan virus, a sporadic cause of tick-borne encephalitis in North America. It is tick-borne, with the tick found in forested areas. The virus is excreted in milk, and can be spread via unpasteurized cow, goat or sheep milk products.

Clinical Features: The incubation period is 7-14 days. The disease has a diphasic form in 50% of the cases with a non-specific influenza-like illness lasting about a week, then a 1-3 day asymptomatic phase, followed by a central nervous system phase. There is an abrupt onset of signs of encephalitis such as tremor, dizziness and altered sensorium. Twenty percent of cases have minor neuropsychiatric sequelae and 1-5% die.

Advice to Travellers: The risk from a single tick exposure is very low and would be mainly to those travelling in wooded areas. The most practical advice is to take precautions against being bitten by ticks (e.g., wear long-sleeved clothing, no shorts or sandals, and use insect repellents). As well, unpasteurized cow, goat and sheep milk products should be avoided.

If bitten by a tick, it is prudent to consult a local physician. In Austria, immune globulin is administered for certain types of exposure. To be effective, it must be immune globulin obtained in Austria, as there would be insufficient appropriate antibodies in any North American product. There are no specific gamma globulins in Canada and the United States.

A vaccine, FSME-IMMUN®, is manufactured in Austria and should be considered for long-term travellers, but the time factor would preclude its use for short-term tourists. The recommended regime is 3 doses of vaccine, the second dose usually being given 1-3 months after the first (but this can be shortened to 2 weeks) and the third after 9-12 months. The vaccine is not approved for marketing in Canada, but is available under Health Canada's Special Access Programme (SAP). The mandate of the SAP is to provide practitioners with access to non-marketed drugs for patients facing serious or life-threatening conditions when conventional therapy has failed, is unsuitable or unavailable.

[72]Health Canada, Population and Public Health Branch, July 23, 2002. http://www.hc-sc.gc.ca/pphb-dgspsp/tmp-pmv/travel/tick_e.html

Health Canada's Special Access Program has found that the human serum albumin (HAS), used as a stabilizer in this vaccine, was prepared from plasma collected from European donors. These donors are properly screened according to European guidelines and criteria, and the FSME-IMMUN® is marketed and commonly used in Europe. However, due to the prevalence of Bovine Spongiform Encephalopathy (BSE) in European countries, there is a theoretical possibility that donors living in these countries may be silent carriers of prion agents through their exposure to BSE-infected beef products.

It is well known and recognized that, to date, the risk of transmission of vCJD by blood and blood products, is strictly theoretical, as no case of CJD or vCJD has been shown to be related to the administration of blood or blood products to a patient. The theoretical risk of vCJD to the Canadian population for vaccines manufactured with human albumin has been estimated by the Population and Public Health Branch of Health Canada to be 1 in 100 million per treatment, per person vaccinated.

Tick-borne encephalitis[73]: Tick-borne encephalitis virus circulates in small wild animals, mostly rodents, and is transmitted by Ixodes ticks. Humans may also become infected by drinking goat's milk.

It has a wide area of distribution across Europe and the former USSR, and its seasonal incidence is reflected in one of the many pseudonyms 'Russian spring-summer encephalitis'. Genetic sequencing has allowed Western tick-borne encephalitis virus, which is endemic in Germany, Austria and much of Europe, to be distinguished from Far-Eastern tick-borne encephalitis virus which is found across the former Soviet Union.

After one to two weeks incubation the virus causes a sudden onset of fever, headache nausea and photophobia. In mild cases this resolves after a week, but in more severe cases there is a second phase of illness with meningoencephalitis, or myelitis. The latter tends to cause flaccid paralysis of the upper limb and shoulder girdle. Respiratory muscle and bulbar (brainstem) involvement lead to respiratory failure and death.

Far Eastern tick-borne encephalitis has a higher case fatality rate, but the Western form is often associated with sequelae (after effects).

A formalyn inactivated vaccine given as two doses 4-6 weeks apart, has been recommended for those likely to be exposed in the endemic forested areas of Europe and the former USSR.

4.19 Variola virus[74]

Variola major virus (Smallpox virus)

Variola minor virus (Alastrim)

The Disease: Smallpox is a serious, contagious, and sometimes fatal infectious disease. There is no specific treatment for smallpox disease, and the only prevention is vaccination. The name smallpox is derived from the Latin word for “spotted” and refers to the raised bumps that appear on the face and body of an infected person.

There are two clinical forms of smallpox. Variola major is the severe and most common form of smallpox, with a more extensive rash and higher fever. There are four types of variola major smallpox: ordinary (the most frequent type, accounting for 90% or more of cases); modified (mild and occurring in previously vaccinated persons); flat; and hemorrhagic (both rare and very severe). Historically, variola major has an overall fatality rate of about 30%; however, flat and hemorrhagic smallpox usually are fatal. Variola minor is a less common presentation of smallpox, and a much less severe disease, with death rates historically of 1% or less.

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[73]Solomon, Tom (Lecturer in Neurology and Medical Microbiology University of Liverpool), 2002. The Encephalitis Support Group 2002, Tick-borne encephalitis. http://www.esg.org.uk/ESG/Support/the_illness/Types%20of%20Encephalitis/Tic.htm

[74]CDC, December 9, 2002 Public Health Emergency Preparedness & Response, Smallpox Fact Sheet. http://www.bt.cdc.gov/agent/smallpox/overview/disease-facts.asp

Smallpox outbreaks have occurred from time to time for thousands of years, but the disease is now eradicated after a successful worldwide vaccination program. The last case of smallpox in the United States was in 1949. The last naturally occurring case in the world was in Somalia in 1977. After the disease was eliminated from the world, routine vaccination against smallpox among the general public was stopped because it was no longer necessary for prevention.

Where Smallpox Comes From: Smallpox is caused by the variola virus that emerged in human populations thousands of years ago. Except for laboratory stockpiles, the variola virus has been eliminated. However, in the aftermath of the events of September and October, 2001, there is heightened concern that the variola virus might be used as an agent of bioterrorism. For this reason, the U.S. government is taking precautions for dealing with a smallpox outbreak.

Transmission: Generally, direct and fairly prolonged face-to-face contact is required to spread smallpox from one person to another. Smallpox also can be spread through direct contact with infected bodily fluids or contaminated objects such as bedding or clothing. Rarely, smallpox has been spread by virus carried in the air in enclosed settings such as buildings, buses, and trains. Humans are the only natural hosts of variola. Smallpox is not known to be transmitted by insects or animals.

A person with smallpox is sometimes contagious with onset of fever (prodrome phase), but the person becomes most contagious with the onset of rash. At this stage the infected person is usually very sick and not able to move around in the community. The infected person is contagious until the last smallpox scab falls off.

Smallpox Disease

Incubation Period **(Duration: 7 to 17 days)** ***Not contagious***	**Exposure to the virus** is followed by an incubation period during which people do not have any symptoms and may feel fine. This incubation period averages about 12 to 14 days but can range from 7 to 17 days. During this time, people are not contagious.
Initial Symptoms ***(Prodrome)*** **(Duration: 2 to 4 days)** ***Sometimes contagious****	The **first symptoms** of smallpox include fever, malaise, head and body aches, and sometimes vomiting. The fever is usually high, in the range of 101 to 104 degrees Fahrenheit. At this time, people are usually too sick to carry on their normal activities. This is called the *prodrome* phase and may last for 2 to 4 days.
Early Rash **(Duration: about 4 days)** ***Most contagious***	A **rash emerges** first as small red spots on the tongue and in the mouth.
	These spots develop into sores that break open and spread large amounts of the virus into the mouth and throat. At this time, the person becomes **most contagious**.
Rash distribution:	Around the time the sores in the mouth break down, a rash appears on the skin, starting on the face and spreading to the arms and legs and then to the hands and feet. Usually the rash spreads to all parts of the body within 24 hours. As the rash appears, the fever usually falls and the person may start to feel better.
	By the third day of the rash, the rash becomes raised bumps.
	By the fourth day, the bumps fill with a thick, opaque fluid and often have a depression in the center that looks like a bellybutton. (This is a major distinguishing characteristic of smallpox.)
	Fever often will rise again at this time and remain high until scabs form over the bumps.
Pustular Rash **(Duration: about 5 days)** ***Contagious***	The bumps become **pustules**—sharply raised, usually round and firm to the touch as if there's a small round object under the skin. People often say the bumps feel like BB pellets embedded in the skin.

Pustules and Scabs **(Duration: about 5 days)** ***Contagious***	The pustules begin to form a crust and then **scab**. By the end of the second week after the rash appears, most of the sores have scabbed over.
Resolving Scabs **(Duration: about 6 days)** ***Contagious***	The scabs begin to fall off, leaving marks on the skin that eventually become pitted **scars**. Most scabs will have fallen off three weeks after the rash appears. The person is contagious to others until all of the scabs have fallen off.
Scabs resolved ***Not contagious***	Scabs have fallen off. Person is no longer contagious.

* Smallpox may be contagious during the prodrome phase, but is most infectious during the first 7 to 10 days following rash onset.

4.20 *Yersinia pestis*[75]

Plague

Clinical Features:

- Bubonic plague: enlarged, tender lymph nodes, fever, chills and prostration
- Septicemic plague: fever, chills, prostration, abdominal pain, shock and bleeding into skin and other organs
- Pneumonic plague: fever, chills, cough and difficulty breathing; rapid shock and death if not treated early

Etiologic Agent: *Yersinia pestis* - bacillus

Incidence: In the U.S., 1 to 40 cases reported annually (avg = 13 cases) by western states, 1971-1995; Worldwide, 2861 cases reported by 10 countries to WHO in 1995

Sequelae: Rare, consequences of disseminated intravascular coagulation, lung damage. Mortality 50-90% if untreated; 15% when diagnosed and treated.

Transmission: Flea-borne, from infected rodents to humans; direct contact with infected tissues or fluids from handling sick or dead animals; respiratory droplets from cats and humans with pneumonic plague

Reservoirs: Primarily wild rodents in U.S. (especially rock squirrels, ground squirrels, prairie dogs, other burrowing rodents); commensal rats may be important elsewhere

Risk Groups: In the U.S., persons exposed to rodent fleas, wild rodents, or other susceptible animals in enzootic areas of western states

Most cases occur in southwestern states of NM, AZ, CO, and in CA

Highest rates in Native Americans, especially Navajos; other risk groups: hunters; veterinarians and pet owners handling infected cats; campers or hikers entering areas with outbreaks of animal plague

Surveillance: National Notifiable Disease Surveillance System (NNDSS) for animal plague surveillance, for reports of human cases, and laboratory testing of fleas, animal tissues and serum specimens, and serosurveys of carnivores

CDC, Fort Collins, is a WHO Collaborating Center for Reference and Research on Plague Control, and reports all human plague cases in the U.S. to WHO

[75]CDC, June 22, 2001. Division of Vector-Borne Infectious Diseases, Plague Fact Sheet. http://www.cdc.gov/ncidod/dvbid/plague/facts.htm

5. BW AGENT CHARACTERISTICS (SUBSET)[76]

Disease	Transmit Man to Man	Infective Dose (Aerosol)	ncubation Period	Duration of Illness	Lethality (approx. case fatality rates)	Persistence of Organism	Vaccine Efficacy (aerosol exposure)
Inhalation anthrax	No	8,000-50,000 spores	1-6 days	3-5 days (usually fatal if untreated)	High	Very stable - spores remain viable for > 40 years in soil	2 dose efficacy against up to 1,000 LD_{50} in monkeys
Brucellosis	No	10 -100 organisms	5-60 days (usually 1-2 months)	Weeks to months	<5% untreated	Very stable	No vaccine
Cholera	Rare	10-500 organisms	4 hours - 5 days (usually 2-3 days)	$\geq$ 1 week	Low with treatment, high without	Unstable in aerosols & fresh water; stable in salt water	No data on aerosol
Glanders	Low	Assumed low	10-14 days via aerosol	Death in 7-10 days in septicemic form	> 50%	Very stable	No vaccine
Pneumonic Plague	High	100-500 organisms	2-3 days	1-6 days (usually fatal)	High unless treated within 12-24 hours	For up to 1 year in soil; 270 days in live tissue	3 doses not protective against 118 LD_{50} in monkeys
Tularemia	No	10-50 organisms	2-10 days (average 3-5)	$\geq$ 2 weeks	Moderate if untreated	For months in moist soil or other media	80% protection against 1-10 LD_{50}
Q Fever	Rare	1-10 organisms	10-40 days	2-14 days	Very low	For months on wood and sand	94% protection against 3,500 LD_{50} in guinea pigs
Smallpox	High	Assumed low (10-100 organisms)	7-17 days (average 12)	4 weeks	High to moderate	Very stable	Vaccine protects against large doses in primates
Venezuelan Equine Encephalitis	Low	10-100 organisms	2-6 days	Days to weeks	Low	Relatively unstable	TC 83 protects against 30-500 LD_{50} in hamsters
Viral Hemorrhagic Fevers	Moderate	1-10 organisms	4-21 days	Death between 7-16 days	High for Zaire strain, moderate with Sudan	Relatively unstable - depends on agent	No vaccine
Botulism	No	0.001 mg/kg is LD_{50} for type A	1-5 days	Death in 24-72 hours; lasts months if not lethal	High without respiratory support	For weeks in nonmoving water and food	3 dose efficacy 100% against 25-250 LD_{50} in primates
Staph Enterotoxin B	No	0.03 mg/person incapacitation	3-12 hours after inhalation	Hours	< 1%	Resistant to freezing	No vaccine
Ricin	No	3-5 mg/kg is LD_{50} in mice	18-24 hours	Days - death within 10-12 days for ingestion	High	Stable	No vaccine
T-2 Mycotoxins	No	Moderate	2-4 hours	Days to months	Moderate	For years at room temperature	No vaccine

[76] http://www.mold-survivor.com/biowarfare.htm

6. REFERENCES

All references, citations, and internet addresses are provided as footnotes within the text.