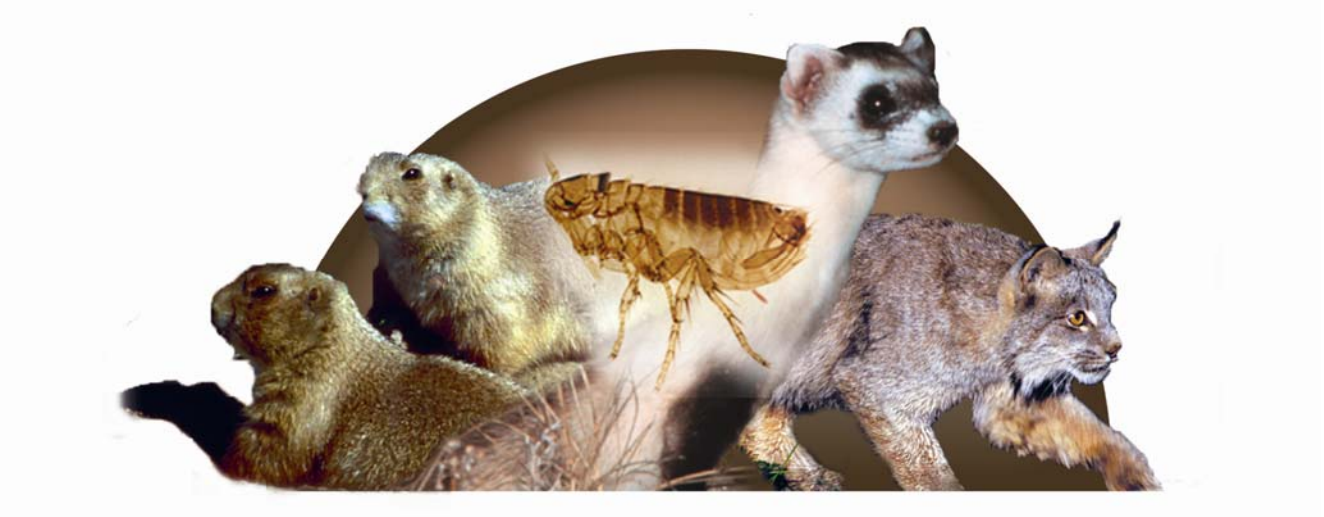
Symposium on the Ecology of Plague and its Effects on Wildlife

Abstracts



Fort Collins, Colorado
November 4 – 6, 2008

Abstracts for the
Symposium on the Ecology of Plague and its Effects on Wildlife
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Oral and Poster Presentations

Abstracts are listed alphabetically by presenter (underlined) at this Symposium with associated affiliation. Please see Participant List provided in Symposium Packet for more detailed contact information.

RESPONSE BY SWIFT FOXES TO PLAGUE EPIZOOTICS IN BLACK-TAILED PRAIRIE DOGS

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An analysis of the relationships between swift fox (*Vulpes velox*) home range distribution and prairie dog colonies (*Cynomys ludovicianus*) on the Central Plains Experimental Range (CPER) in northeastern Colorado is presented. Data from the years 2003 – 2007 is used in conjunction with home range kernel density estimates illustrating swift fox home range distribution and overlap onto prairie dog colonies. The intent of the study is to determine swift fox dependence on prairie dogs for their burrows and food resources. Plague epizootics caused by *Yersinia pestis* occurred in 2005 and again in 2007, and dramatically altered the distribution of prairie dog colonies on the CPER. This event offers a unique opportunity to compare swift fox distribution from the pre- and post- plague time periods. Future work will entail scat analysis to determine the presence of prairie dogs found in swift fox diet, ectoparasite collection and analyses using PCR to determine if foxes are carrying plague positive fleas.

IDENTIFICATION AND CHARACTERIZATION OF *IN VIVO*-INDUCED CONSERVED SEQUENCES (IVICS) FROM *YERSINIA PESTIS* DURING INFECTION IN DIFFERENT SUSCEPTIBLE HOST SPECIES

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Most genes and their products associated with *Yersinia pestis* virulence have been identified using laboratory-grown bacteria. Host factors that up-regulate some virulence genes may or may not be present in these laboratory-grown cultures. One approach that overcomes this problem uses immune sera processed to specifically remove antibodies to constitutively expressed bacterial proteins, while retaining those antibodies to bacterial proteins expressed exclusively during infection. This technique, known as *In-vivo* Induced Antigen Technology (IVIAT) has been successfully used to identify *in vivo*-induced genes in many different bacterial pathogenic species. In parallel studies with collaborators in the Department of Defense, we applied this

gene "discovery" methodology to *Y. pestis* to identify > 20 genes up-regulated *in vivo* in a laboratory rabbit model for bubonic plague. Subsequent sequence analysis revealed that the majority of *in vivo*-induced (IVI) proteins identified to date fall into one of two National Center for Biotechnology Information (NCBI) computer databases: "COGs", or Clusters of Orthologous Groups [of proteins])" or the conserved domain database (cdd). Furthermore, putative *in vivo* up-regulated gene products of more frequently encountered COG/cdd functional categories have emerged from our analyses. Our laboratory has also begun identification of IVI genes using adsorbed sera from *Y. pestis*-infected ferrets, prairie dogs, and coyotes (a species refractory to disease but not infection). In this regard, two genes (encoding COGs) of particular interest have been identified from coyotes that do not appear to be immunogenic in the rabbit, supporting the utility of IVIAT in the identification of host species-specific antigens. We hypothesize that the profiles/patterns of *in vivo*-induced conserved sequences, or IVICS, may represent unique immune "signatures" among different host species susceptible to infection. Thus, the gathering of additional data and analysis of the intact genes and the expressed IVICS products should provide insight into the unique biologic processes of *Y. pestis* during infection, and reveal the genetic patterns of the pathogen's survival strategy in different hosts.

Vernati, G., J.E. Lowry, W.H. Edwards, T.E. Rocke, and G.P. Andrews. 2007. *Yersinia pestis*, the etiology of plague, displays differentially up-regulated genes during infection in different mammalian hosts. The 88th Annual Meeting of the Conference of Research Workers in Animal Diseases (CRWAD), Chicago, IL.

Vernati, G., R. Ulrich, J. Adamovicz, and G.P. Andrews. 2006. Identification of novel *in vivo*-induced virulence genes from *Yersinia pestis* using sera from experimentally infected rabbits. IICAB Symposium on Virulence Mechanisms of Bacterial Pathogens, Ames, IA.

WHAT IS A PLAGUE FOCUS?

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Ecological and epidemiological studies of plague use the term "focus" to describe persistence of *Yersinia pestis* in wild (sylvatic) populations of rodents and their fleas, with occasional spillover to humans. Geographically, numerous plague foci have been identified based on the predominant rodent host in each region: For instance, gerbils (*Rhombomys opimus*) in the desert focus in Kazakhstan, marmots (*Marmota himalayana*) in the Qinghai-Gansu-Tibetan grasslands, black rats (*Rattus rattus*) in Madagascar, and prairie dogs (*Cynomys ludovicianus*) in the western Great Plains in the United States. Phenotypic and genotypic differences between foci are apparent at many levels, from bacterial physiology and pathogenicity to genetic differences based on simple sequence repeats, IS100 and SNP genotypes, plasmid variation, and genomic changes such as insertions, inversions, and gene rearrangements. Geographic and host-species boundaries between foci are often poorly defined, and genotyping of plague isolates shows that foci identified by physiological traits of the bacterium may be epidemiologically linked. The presumption in Asia is that *Y. pestis* is specifically adapted to hosts in each geographic focus, although this has not been experimentally determined, and whether similar adaptation is occurring in the introduced parts of the range of *Y. pestis* is unknown. Plague foci also differ in whether *Y. pestis* remains enzootic while continually cycling at low levels, or are characterized

by sporadic epizootics. Here we describe a new analysis of VNTR genotypes from Colorado and New Mexico indicating that multiple clones likely co-circulate within populations of wild rodents.

METAPOPULATION PERSPECTIVES ON THE DYNAMICS OF PLAGUE: NEW EXPLORATIONS

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In ecology generally, 'metapopulation dynamics' has taken on an increasingly wide range of meanings. This seems to be reflected in the study of plague dynamics, where a number of groups have (apparently independently) developed a metapopulation approach. I briefly review these studies, with the aim of clarifying the different meanings that have been given by plague ecologists to the term 'metapopulation'. I briefly review, too, empirical work, including our own work on plague in great gerbil populations in Central Asia that justifies a metapopulation perspective (see Davis et al. 2007). I then describe some new theoretical explorations that acknowledge both the metapopulation structure of many plague systems and that many (perhaps most) plague systems comprise more than one important host species. Particular attention will be paid to 'Allee effects' (inverse density dependence at low densities – see Harding & McNamara 2002)), both in the colonization of empty sub-populations and in the transmission dynamics of plague itself, and to the important effects these may have on plague dynamics, including the introduction of multiple stable states and an increased sensitivity to the effects of second (and further additional) host species.

Davis, S., N. Klassovskiy, V. Ageyev, B. Suleimenov, B. Atshabar, A. Klassovskaya, M. Bennett, H. Leirs, and M. Begon. 2007. Plague metapopulation dynamics in a natural reservoir: the burrow system as the unit of study. Epidemiology and Infection 135: 740-748.

Harding K.C., and J.M. McNamara. 2002. A unifying framework for metapopulation dynamics. American Naturalist 160:173-185.

REGIONAL CLIMATE AND THE DYNAMICS OF HUMAN PLAGUE IN WESTERN US

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Bubonic plague caused by the gram-negative bacterium *Yersinia pestis* is a vector borne, zoonotic disease where wild rodents are the reservoir and fleas (the vector). This rodent-flea system is subject to climate forcing locally (*1, 2*) in Western United States (US) (i.e., precipitations and temperatures) but no link to regional climate has been found in this region. Such link is nevertheless expected since much of the climate variability in this region is explained by El Nino Southern Oscillation (ENSO). Our hypothesis is that plague foci and dynamics are consistent enough over the western US to significantly interact with climate at a large scale. Using statistical modeling and wavelet analysis we investigate the effect of large-

scale climate variability on human plague infection in the western United States by analyzing a 56-year record of county-based human plague reports (1950-2006). We report that regional climate variability accounts for much of the plague dynamics and levels in the studied area. We furthermore report that these linked are to be explained locally by temperatures and precipitation patterns. We finally investigate the possible effects of climate change on the dynamics and location of plague.

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Parmenter, R., E.P. Yadav, C.A. Parmenter, P. Ettestad, and K.L. Gage. 1999. Incidence of plague associated with increased winter-spring precipitation in New Mexico. American Journal of Tropical Medicine and Hygiene 61:814-821.

RODENT HOST RESPONSES TO VIRAL-VECTORED VACCINES AGAINST PLAGUE

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We are currently conducting laboratory studies to develop and evaluate the efficacy of mucosal plague vaccines that utilize recombinant raccoon poxvirus (RCN) as a vector for various antigens of *Yersinia pestis* (Osorio, *et al*. 2003, Mencher *et al.,* 2004). Because it infects mucosal tissue, RCN is ideally suited for use as an oral vaccine vector. However, the mechanisms by which RCN vaccines elicit an effective immune response are poorly understood. Using a combination of *in vivo* and *in vitro* tools, we are measuring several aspects of rodent host responses to our RCN vaccine constructs. In previous studies, we demonstrated 25-50% protection against plague challenge (700,000 cfu) in mice using a raccoon pox (RCN) virus-vectored vaccine that expressed the F1 capsular antigen of *Yersinia pestis*. We have now found increased protection (up to 89%) for a similar challenge dose by co-administration of a truncated form of the *Y. pestis* V protein (RCN-Vt). Interestingly, the survival of mice vaccinated with RCN-Vfull in combination with RCN-F1 was not as high (50% at a challenge dose of 700,000 cfu). The V antigen has been shown by others to induce production of interleukin-10 (IL-10), which has been linked to the impairment of the host immune response after plague infection. Using Elispot analysis of stimulated splenocytes from immunized animals, we found that RCN-Vt results in lowered production of IL-10 compared to RCN-Vfull. This finding may explain the improved protection afforded with this construct. To better understand the mechanism by which RCN vaccines induce protection against disease, we constructed an RCN virus that expresses the luciferase gene (RCN-luc), allowing us to monitor the course of RCN infection *in vivo* using biophotonic imaging. Our imaging studies demonstrated that RCN exposure via the intramuscular route in mice or the oral route in prairie dogs results in a localized infection that does not progress systemically. We then evaluated the effect of RCN on the mucosal immune response by measuring the production of the chemokine, CCL20, by mouse epithelial cells *in vitro*. Compared to protein-based plague vaccines, we found that RCN vaccines induced significantly higher levels of CCL20. This chemokine recruits dendritic cells (DCs) to the site of

contact with foreign antigens. As professional mucosal sentinels and antigen presenting cells, DCs play a predominate role in linking the innate and adaptive immune response and inducing a protective immune response via the mucosal route. Thus, our findings could explain the efficacy of RCN as an oral vaccine vector. Developing effective vaccines against plague is a challenging problem, particularly for wild animals. A better understanding of the host immune response after vaccination and against specific antigens of *Y. pestis* will aid in developing the most appropriate and efficacious vaccine. RCN-vectored vaccines against *Y. pestis* offer a promising approach.

Osorio, J.E.,T. D. Powell, R. S. Frank, K. Moss, E.J,.Haanes, S. R Smith, T. E. Rocke and D.T. Stinchcomb. 2003. Recombinant raccoon pox vaccine protects mice against lethal plague. Vaccine 3505: 1-7.

Mencher, J., S R. Smith, J. E. Osorio, D. Stinchcomb and T. E. Rocke. 2004. Protection of black-tailed prairie dogs (*Cynomys ludovicianus*) against plague after voluntary consumption of baits containing recombinant raccoon poxvirus vaccine. Infection and Immunity 72:5502-5505.

YERSINIA PESTIS SEROPREVALENCE IN PUMAS (*PUMA CONCOLOR*) AND BOBCATS (*LYNX RUFUS*)

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Yersinia pestis is a vector-borne bacterium and the causative agent of plague in mammals. Plague is most likely maintained via flea transmission among rodent hosts that are relatively resistant to symptomatic disease; however, spill-over into accidental hosts can result in severe morbidity and mortality. Cats, like humans, can exhibit severe morbidity and mortality when infected with *Y. pestis*, but the prevalence and potential impact of plague on non-domestic felids remains relatively unknown. We examined plague exposure in multiple populations of North American bobcats (*Lynx rufus*) and pumas (*Puma concolor*) as part of our study on the effects of urbanization on diseases in North American felids. Locations differed significantly in amount of plague exposure and adult animals were more likely to have plague antibodies than younger animals. Animals captured in the spring were also more likely to have been exposed to plague, but the duration of feline antibodies to *Y. pestis* can persist for a year or more, so the role of seasonality is unknown. Species and sex were not significant predictors. Data indicate these animals often survive plague exposure, and their role in *Y. pestis* dynamics as well as the impacts *Y. pestis* has on their populations, merits further research.

FLEA CONTROL IMPROVES SURVIVAL OF THREE SPECIES OF PRAIRIE DOGS (*CYNOMYS*): EVIDENCE FOR PRESENCE OF ENZOOTIC PLAGUE?

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Plague causes periodic epizootics that decimate populations of prairie dogs, but the means by which the causative bacterium (*Yersinia pestis*) persists between epizootics are poorly understood. We indirectly examined the possibility that the bacterium persists in prairie dog colonies, affecting prairie dogs populations during inter-epizootic periods. We attempted to reduce the numbers of fleas (the vectors for *Y. pesits*) on 15 areas encompassing three species of prairie dogs (*Cynomys leucurus*, *C. parvidens* in Utah and *C. ludovicianus* in Montana) during 2000-2004 using deltamethrin powder delivered into burrows as a pulicide. We compared overall reencounter rates for prairie dogs on treated plots (n = 1,344 animal-periods) and on paired plots where burrows were not dusted (n = 1,157 animal-periods). Flea numbers were reduced >90% one month after burrow treatment. The overall survival rate for prairie dogs was significantly higher in dusted plots than in non-dusted plots, even though epizootic plague was not detected during the study. *Y. pestis* was rarely found in fleas taken from prairie dogs, and few prairie dogs had serum antibodies for plague (most of the seropositive animals resided in a single colony of *C. parvidens*). Although other explanations are possible, plague appears to be the most plausible cause for the reduced survival of prairie dogs on plots without flea control, suggesting that *Y. pestis* may be resident on prairie dog colonies rather than periodically invading from reservoirs elsewhere. The effects of enzootic plague raise serious concerns for conservation of prairie dogs and species dependent upon them.

RODENT ASSEMBLAGES CHANGE FOLLOWING, BUT NOT PRIOR TO, PLAGUE EPIZOOTICS IN BLACK-TAILED PRAIRIE DOGS

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Small rodents are purported to be enzootic hosts of *Yersinia pestis* and may serve as sources of infection to prairie dogs or other epizootic hosts by direct or flea-mediated pathogen transmission. Recent research has shown that small rodent and flea assemblages are influenced by the presence of prairie dogs, with higher relative abundance of both small rodents and fleas at prairie dog colony sites compared to grasslands without prairie dogs. However, it is unclear if increased rodent or flea abundance predisposes prairie dogs to infection with *Y. pestis*. We tracked rodent and flea occurrence for three years at a number of prairie dog colony sites in Boulder County, Colorado, before, during, and after a local plague epizootic to see if high rodent or flea abundance was associated with plague-affected colonies when compared to colonies that escaped infection. We found no difference in pre-epizootic rodent abundance or flea prevalence or abundance between plague-positive and plague-negative colonies. Further, we saw no significant before plague/after plague change in these metrics at either plague-positive or plague negative sites. We did, however, find that small rodent species assemblages changed in the year following prairie dog die-offs. In light of previous research from this system that has shown that landscape features and proximity to recently plagued colonies are significant predictors of plague occurrence in prairie dogs, we suggest that landscape context is more important to local plague occurrence than are characteristics of rodent or flea species assemblages.

MONITORING PLAGUE EPIZOOTICS IN BLACK-TAILED PRAIRIE DOG COLONIES WITH A SENSITIVE PCR-BASED PROTOCOL

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Since the 1940s, sylvatic plague, caused by the bacterium *Yersinia pestis*, has extirpated a significant number of black-tailed prairie dog (*Cynomys ludovicianus*) colonies in North America. Although a commonly accepted model for plague maintenance and spread holds that prairie dogs are highly susceptible epizootic hosts that do not maintain *Y. pestis*, there are opposing views about the role prairie dog colonies play as reservoirs and vectors for the plague bacterium. We developed a highly sensitive method for detecting plague-infected fleas using PCR amplification of the *Y. pestis pla* gene from burrow-collected fleas. This protocol was modified from the PCR approach of Hinnebusch and Schwan (1993, Hanson et al. 2007). The specificity of our method to the *Y. pestis pla* gene was verified by sequencing studies that demonstrated that our amplicons have the same sequence as the *Y. pestis pla* gene. We used *Y. pseudotuberculosis* (ATCC#29833), *Y .enterocolitica* (ATCC #23715), and water in our PCR runs as negative controls. This procedure verified that we amplified only *Y. pestis pla* and not contaminating DNA or other bacterial genes. We amplified samples of an avirulent *Y. pestis* strain A1122 var. *orientalis* in each PCR run as a positive control. This procedure allowed us to detect false negative PCR runs. Fleas were collected from a total of 314 burrows in 11 colonies of a large complex of colonies on the Fort Belknap Indian Reservation, MT, (FBIR) in 2002, 2003, and 2005. Fleas were pooled by burrow for analysis. A plague epizootic began in six of the 11 sampled colonies in the FBIR complex in early summer 2005 giving us the opportunity to assess pre- and post-epizootic plague prevalence. Plague prevalence increased from 17.0% plague-positive burrows in 2002 to 29.5% plague-positive burrows in 2003. Plague prevalence was 29.2% plague-positive burrows in 2005. Similarly, we collected 572 fleas from 314 burrows on a single large prairie dog colony on the Pueblo Chemical Depot (PCD) near Pueblo, CO, during an active plague epizootic on a monthly basis over spring and summer 1999. Plague prevalence ranged from 2.6% plague-positive burrows to 38% plague-positive burrows on the PCD colony based on our assay. Our findings suggest that black-tailed prairie dog populations may tolerate low level plague prevalence through inefficient transmission of plague from infected fleas and/or through immunological resistance to plague. Furthermore, these results suggest that there may be a threshold prevalence of plague-positive fleas below which epizootics do not occur. Our data on plague prevalence in burrow-collected fleas indicate that black-tailed prairie dog colonies begin to have widespread plague outbreaks when approximately 25% of burrows show positive PCR-based assays for plague-infected fleas. This method has potential for monitoring plague prevalence and potentially predicting plague epizootics on colonies of interest.

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Hinnebusch, J.B., and T.G. Schwan. 1993. New method for plague surveillance using polymerase chain reaction to detect *Yersinia pestis* in fleas. Journal of Clinical Microbiology 31:1511-1514.

MISSENSE MUTATIONS IN INTERMEDIARY METABOLISM OF *YERSINIA PESTIS* AND EXPRESSION OF ACUTE DISEASE

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Despite the widespread presence of bubonic plague in sylvatic reservoirs throughout the world, the causative agent (*Yersinia pestis*) evolved in its present form within the last 20,000 years from enteropathogenic *Y. pseudotuberculosis*. Comparison of the genomes from the two species revealed that *Y. pestis* possesses only a few unique plasmid-encoded genes that contribute to acute disease whereas this organism has lost about 13% of the chromosomal genes that remain active in *Y. pseudotuberculosis*. As shown by annotation, these losses reflect readily detectable additions, deletions, transpositions, inversions, and acquisition of about 70 IS inserts, none of which are likely to promote increased virulence. In contrast, major enzymes of intermediary metabolism, including D-glucose 6-phosphate dehydrogenase (Zwf) and aspartase (AspA), are present as cross-reacting immune material (CRIM) but are not catalytically functional due to the presence of missense mutations. The latter are generally not detectable by the technology of bioinformatics and, in the case of *Y. pestis*, result in radical changes in the metabolic flow of carbon (e.g., loss of Zwf prevents use of the hexose monophosphate pathway and AspA-deficiency precludes complete oxidation of L-glutamate via the tricarboxylic acid cycle). As an important consequence, plague bacilli exhibit a stringent low-calcium response characterized by conversion of L-glutamate (and metabolically related amino acids) to L-aspartate with secretion of the latter into supernatant fluid at 37°C in culture media containing Na^+ but lacking added Ca^{2+}. This phenomenon also occurs *in vivo* and likely adversely affects the bioenergetics of host amino acid pools. Curiously, AspA is functional in all tested enzootic (pestoides) strains of *Y. pestis*. These isolates are typically restricted to the ancient plague reservoirs of central Asia and Africa and are fully virulent in members of the rodent Superfamily Muroidea but avirulent in guinea pigs and man.

F1-V TRANSGENIC TOMATOES AS AN ORAL VACCINE FOR PLAGUE CONTROL

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Colonization of alveolar spaces by the bacterium *Yersinia pestis* results in pneumonic plague, an extremely contagious disease that develops in 1-3 days and causes a high mortality rate in infected individuals (almost 100%). *Y. pestis*, most well known as the causative agent for the Black Death, remains present in rodent populations in the western US and still has the potential to cause outbreaks of plague. However, perhaps the greater concern is its possible use as an aerosol delivered bio-terrorism agent. Unfortunately, no approved vaccines for protection against plague exist. Our goal was to create a vaccine that addressed several issues: ease of delivery, mucosal efficacy, safety, rapid scalability, and cost. We developed a novel production and delivery system for a plague vaccine consisting of a *Y. pestis* F1-V antigen fusion protein expressed in tomato and orally delivered as a powder. Of all the *Y. pestis*' antigens tested, only F1 and V induce a good protective immune response against a challenge with the bacterium [1].

F1 (Fraction 1) antigen is the major capsular protein. It forms a polymer composed of a protein subunit and plays an important role in inhibiting phagocytosis by macrophages. V antigen is a secreted protein that regulates translocation of the cytotoxic effector proteins from the bacterium into the cytosol of mammalian cells. The effector proteins (termed "Yops") have a range of functions like promoting death of phagocytic host cells and inhibiting normal inflammatory response.We transformed tomato using *Agrobacterium tumefaciens* bearing the plasmid p35SF1-V. Levels of F1-V in the transgenic tomato plants were determined by ELISA and the size and integrity of the F1-V fusion protein was confirmed by Western. We selected nine second generation elite tomato plants expressing high levels of the F1-V fusion protein in fruits [3 - 8 % of total soluble protein (TSP), 600 - 1600 µg per gram of freeze-dried tomato fruits]. In all of these plants, the F1-V fusion protein levels were higher when the fruits were fully developed but completely green. The immunogenicity of these F1-V transgenic tomatoes was confirmed by prime-boost experiments in BALB/c mice. The animals were primed with subcutaneous bacterial F1-V and boosted orally with powdered freeze-dried, transgenic tomatoes. The humoral response was dominated by IgG1 over IgG2a suggesting an antibody mediated Th2 immune response, the preferred response to confer protection against plague. F1- and V-specific mucosal IgA was elicited only in mice boosted with transgenic F1-V tomato. Preliminary results showed that mice were protected against a challenge with s.c. *Y. pestis,* even though challenge was performed almost 18 months after the final boost. We believe that concentration of oral dosage is critical to generate sufficient response and have subsequently developed tomato fruit expressing F1-V at 16% TSP [2]. The expression of the plague antigens in fruits allowed for the production of an oral vaccine candidate without any protein purification and with only minimal processing technology. It represents an economical alternative to fermentation based expression systems, especially for production of high-volume reserves of subunit-vaccines.

[1] Heath D., G. Anderson, M. Mauro, and S. Welkos. 1998. Protection against experimental bubonic and pneumonic plague by recombinant capsular F1-V antigen fusion protein vaccine. Vaccine 16:1131-1137.

[2] Alvarez M.L., H.L. Pinyerd, E. Topal, and G.A. Cardineau. 2008. P19-dependent and P19-independent reversion of F1-V gene silencing in tomato. Plant Molecular Biology (in press and online DOI 10.1007/s11103-008-9352-2).

PATHOLOGY AND SEROPREVALENCE OF PLAGUE IN WILD FELIDS

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Although plague (*Yersinia pestis*) is generally associated with rodent hosts, carnivores are often monitored as sentinels for plague because evidence of plague exposure can be obtained via antemortem and postmortem sampling. Previous work has shown that there is a continuum of susceptibility among carnivores, with members of the Canidae and Ursidae relatively resistant, and members of the Felidae and some Mustelidae relatively susceptible. Recent detection of fatal plague in free-ranging Canada lynx (*Lynx canadensis*) and mountain lions (*Puma concolor*) has provided the impetus to look more closely at the pathology of plague and its effects on wild felids. In addition, human deaths from exposure to plague-infected domestic cats and wild felids underscore the zoonotic potential of plague from "non-traditional" sources. Goals of our study were to: 1) summarize the pathology of fatal cases of plague in wild felids; 2) compile results from serologic surveys of plague in wild felids; and 3) examine the zoonotic potential of plague from wild felids. Methods: We reviewed published literature and surveyed wildlife veterinarians working in plague endemic areas. Results of previous case reports and studies were compiled and summarized, and supplemented with data from our own research. Results: One bobcat (*Lynx rufus*), 5 lynx, and 5 mountain lions were reported to have died from plague over the last 20 years. Postmortem findings ranged from acute pulmonary hyperemia and congestion to extensive pneumonia affecting most of the lung fields, with variable cranial and cervical lymphadenopathy, sometimes with large abscesses of mandibular and/or retropharyngeal lymph nodes. Microscopic lesions included suppurative to necrotizing bronchointerstitial pneumonia, suppurative to necrotizing lymphadenitis, and lymphoid depletion of splenic tissue. Abundant intralesional coccobacilli were a common finding, and *Y. pestis* was cultured from a number of those lesions. Seroprevalence of plague varied among locations surveyed. In Washington, 8% (31/401) of bobcats, and 9% (2/22) of mountain lions tested positive for plague antibodies since 1975. In Nevada, seroprevalence since 2000 was 7% (1/14) and 14% (13/93) for bobcats and mountain lions, respectively. In Colorado 22% (2/9) and in Montana 5% (2/39) of lynx were seropositive. Domestic cats account for a significant number of human plague cases, and domestic cat-associated plague has caused at least 5 human deaths in the last 30 years. Exposure to 2 plague-infected wild felids (1 bobcat and 1 mountain lion) caused two human deaths since 1981, the most recent being a wildlife biologist in 2007. Conclusions: Plague can be fatal to wild felids, however some survive exposure and seroconvert. Common postmortem findings include pneumonia and cranial/cervical lymphadenomegaly (sometimes with abscesses), although only 1 of 5 lynx exhibited the latter. Plague should be considered as a cause of death in wild felids from plague endemic areas. Future studies should be designed to assess effects of plague on wild felid populations, and to determine the role wild felids may have in plague epidemiology.

PLAGUE IN URBAN PRAIRIE DOG COLONIES

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Yersinia pestis, causative agent of plague, is a bacterium found across the western United States in rodent communities. Urban development throughout the west has influenced the size of these rodent communities and dispersal among them, and also may influence spread of disease. To examine the dispersal and maintenance of plague in Colorado, prairie dogs and associated rodents were trapped and combed for fleas. The trapping sites were mostly in prairie dog colonies in Boulder County, Colorado from June to September 2005. 968 fleas were tested for the presence of *Y. pestis* and 294 were positive. 78 positive fleas had a high enough concentration of *Y. pestis* to support high-resolution genetic analysis. We used the results of these genetic analyses and GPS data to characterize *Y. pestis* population structure across this landscape. We found distinct population structure among the sites. Our results provide insights into movement of plague among urban prairie dog colonies.

PLAGUE REGULATES BLACK-TAILED PRAIRIE DOG POPULATIONS

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Plague is an exotic vector-borne disease caused by the bacterium *Yersinia pestis* that causes mortality rates approaching 100% in black-tailed prairie dogs (*Cynomys ludovicianus*). We mapped black-tailed prairie dog colonies with Global Position System units annually between 1999 and 2006 at 4 complexes of prairie dog colonies in areas with a history of plague, as well as at 2 complexes that are located outside the current distribution of plague and have therefore never been affected by the disease. We expected that the presence of plague would significantly reduce black-tailed prairie dog colony area and alter the size and distribution of colonies on the landscape. Colonies at plague sites were smaller and further apart than at plague-free sites. Populations that have endured plague were composed of fewer large colonies (> 100 ha) than populations that were historically plague-free. Plague significantly altered colony spatial structure, not only by decreasing colony size, but also by creating greater distances between colonies, which may slow recolonization after extinction. At the same time, greater intercolony distances may also reduce intercolony transmission of pathogens. Reduced transmission among smaller and more distant colonies may enhance long-term prairie dog population persistence where plague is present.

DECREASING THE RISKS OF PLAGUE TO WILDLIFE AND HUMANS USING EASILY APPLIED GROUND SQUIRREL BAITS

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The California ground squirrel (*Spermophilus beecheyi*) is known to have been involved in over 50% of the wild rodent-associated human cases of plague in California. In addition, epizootics of plague are known to devastate populations of ground squirrels, as well as numerous other wild rodent species in California, therefore exacerbating the risks for plague transmission to both humans and wildlife. Since the California ground squirrel is considered a pest species, its population control using lethal rodenticides, especially in plague endemic areas, can further increase the risks of plague transmission by releasing potentially plague infected fleas into the environment. Two field studies were conducted in 2005 at Vandenberg Air Force Base, California, to evaluate two different forms of ground squirrel baits. One bait contained an insecticide (imidacloprid) only, with the goal of significantly decreasing flea loads on ground squirrels. The other bait contained an insecticide (imidacloprid) and an anticoagulant rodenticide (diphacinone), with the goal of significantly and simultaneously decreasing both the ground squirrel population and the on-rodent flea populations. Efficacy of the bait at controlling fleas was determined indirectly by assessing free-living fleas in ground squirrel burrows. The insecticide-only bait was effective in significantly reducing the flea populations on ground squirrels between 93.8 and 100% by days 15 and 29 on two different sites during the same trial. The bait containing both insecticide and rodenticide was effective at decreasing squirrel populations between 74.6% and 90.9% on four different sites during the trials. The numbers of fleas collected from burrow swabs and the proportion of burrows positive for fleas decreased markedly after weeks 1 and 2 post-treatment with the combination bait. However, inherent variables in burrow swabbing made statistical analyses difficult. During both studies no non-target species were found to be adversely affected by the baiting. The use of the insecticide-only bait would be beneficial in areas where decreasing plague risk is warranted, but the legal control of squirrels is not supported. However, when controlling ground squirrels in known plague endemic areas, the best management technique would be to control both the fleas and the rodents.

REMOTE SENSING OF PLAGUE EPIZOOTICS IN CENTRAL ASIA

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Great gerbils are desert rodents inhabiting vast areas of central Asia and as a common host of plague (flea-borne *Yersinia pestis* infection), play a central role in many of the plague foci in this region. Their burrow systems are usually large, complex constructions representing the efforts of many generations. The vegetation above and around these burrow systems dissapears and the resulting discs of bare earth, typically 30 metres in diameter, are highly visible on satellite images. Such images bring into clarity the regular spatial arrangement of burrow systems and the extent of the landscapes inhabited by great gerbils. The difference between the scale of these landscapes and the flea movements responsible for transmission of plague between family groups has lead to the finding that the spread of *Yersinia pestis* among great gerbils (*Rhombomys opimus*) is a vast, natural percolation process (Davis et al. 2008). A second line of research that

has arisen is the potential for remote sensing techniques to form the basis of surveillance of plague foci in which great gerbils are the major host. Remote sensing has the potential to assist surveillance indirectly by better defining the desert areas inhabited by great gerbils and directly by providing estimates of the proportion of burrow systems occupied by great gerbils, which in turn is a predictor of epizootic activity. Here we present the progress that has been made in accurately identifying and counting burrow systems on satellite images, the possible applications of burrow system density maps and the difficulties in differentiating empty burrow systems from occupied ones. The surveillance systems established in countries such as Kazakhstan in the late 1940s have been extremely successful in reducing human cases of plague. However, they are labour intensive and these countries face financial pressures such that the possibility of using remote sensing approaches would be a timely and welcome development.

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MOUNTAIN PLOVER RESPONSE TO PLAGUE IN MONTANA

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The Mountain Plover (*Charadrius montanus*) is an uncommon breeding bird of the Great Plains that primarily inhabits active black-tailed prairie dog (*Cynomys ludovicianus*) colonies at the northern limit of its range in Montana. There, the plover depends on the prairie dog to provide the proper mix of short vegetation and bare ground it requires for nesting. The dynamics of sylvatic plague in this ecosystem cause annual variation in local prairie dog abundance and colony size, and thus directly influence the amount of nesting habitat available to plovers (Dinsmore et al. 2005). In turn, nesting plovers quickly disappear from colonies that are lost to plague but are capable of occupying new or expanding colonies post-plague. I used data from a 13-year study of Mountain Plovers in southern Phillips County, Montana to assess annual variation in plover use of prairie dog colonies. I used patch occupancy models (MacKenzie et al. 2002) to evaluate the evidence for acute (1-year post-plague) and chronic (2-4 years post-plague) effects of sylvatic plague, colony size, and two spatial variables (a colony shape index and a measure of the proximity to other colonies) on subsequent occupancy of colonies by plovers. I developed a suite of competing models to explain local extinction (ε) and colonization (γ) rates of plovers on active prairie dog colonies. These meta-population rates describe the probability that plovers 1) colonize a colony that was unoccupied the previous year, or 2) disappear from a colony that was occupied the previous year. During the 13-year study I collected plover use data from >130 prairie dog colonies that ranged in size from 0.1 to 607 ha (mean = 28.5 ha, SD = 80.3). Modeling results suggested that plovers disappeared from colonies as a function of their size but occupied new colonies in response to plague history and the proximity to other active colonies. The predicted probability of extinction, given that plovers were present the previous breeding season, for a 10 ha colony ($\varepsilon = 0.26$) was more than six times that of a 50 ha colony ($\varepsilon = 0.04$). Similarly, plover colonization of previously unoccupied prairie dog colonies peaked at 3 years post-plague ($\gamma = 0.23$) and was enhanced by a measure of the number of colonies within a 7 km radius. These results illustrate how the temporal dynamics of an epizootic can have indirect effects on a bird that relies on the host species to create suitable nesting habitat. This

work also provides a clearer picture of Mountain Plover movements in a plague impacted landscape and could be used to suggest prairie dog management scenarios to benefit the plover.

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IS SOIL AS A RESERVOIR FOR *YERSINIA PESTIS*?

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The current epidemiology model for plague relies upon its dynamics preservation into plague-resistant and plague-susceptible wild rodents, with rodent fleas being the vectors of transmission. Eventual contacts of persons with infected rodents and rodent fleas may result in flea-borne plague, with buboe (enlarged, painfull lymph node) being the clinical characteristic of this form of disease. Such epidemiological model however may not fully explain the fact that plague persists in long-term geographical foci. This fact has been recently highlighted by re-emergence of plague in Oran's area, almost 60 years after previous outbreak in the 40's [Bitam I. et al., 2006; Bertherat E. et al., 2007]. Investigation of plague epidemics in Oran concluded it was caused by re-emergence rather than introduction of *Yersinia pestis* in this well-known plague focus in Algeria. Partial experimental and observational data gathered in the 50's suggested that *Yersinia pestis* could be found in soil [Mollaret H, 1963; XX]. We investigated the hypothesis that the plague agent *Yersinia pestis*, could persist in soil. After experimental inoculation of *Y. pestis* biovar Orientalis in sterilized soil, soil was regularly sampled for microscopic observation, culture and DNA detection for 9 months. Some samples were inoculated into mice to assess virulence. We present data which demonstrate the long-term persistence of viable and virulent *Y. pestis* organisms in soil [Ayyadurai S et al., 2008]. Recent data indicated that indeed *Yersinia pestis* could persist for a few weeks in soil in natural conditions [Eisen RJ. et al., 2008]. Also, *Yersinia pestis*-specific *caf*1 gene has been detected in a few soil specimens collected in Phoenix, Texas [Kuske CR. et al., 2006]. Following these observational and experimental data, we propose that *Yersinia* pestis should be add to the growing list of soil-borne bacterial pathogens, along with other pathogens such as *Yersinia pseudotuberculosis* [Buzoleva LV. et al., 2003], *Brucella microti* [Scholz HC. et al., 2008], and *Mycobacterium bovis* [Duffield BJ. et al., 1985]. These data suggest that soil is one underreported link in the epidemiological chain of plague [Drancourt M. et al., 2006]. We urge field observations to confirm these data in order to get a better model of plague epidemiology.

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HUMAN BODY LOUSE, THE VECTOR OF *YERSINIA PESTIS* DURING HISTORICAL PLAGUE EPIDEMICS

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Plague has been responsible for huge epidemics in the past two millennia, with reported mortality as high as one third of the Medieval European population [Perry RD. & Fetherston JD., 1997]. Historical records related thousand of victims within short periods of time. *Yersinia pestis*, the bacterium responsible for plague, is transmitted from rodents to human by the bite of the rodent flea. This vector-borne form of plague results in one specific clinical feature, the buboe (enlarged, painful lymph node). This flea-route of transmission however may not explain historical epidemics. Using dental pulp as a specimen of which to base molecular detection of *Y. pestis* in plague victims, we investigated the two historical pandemics. We indeed detected specific *Y. pestis* DNA sequences in these individuals [Drancourt M. et al., 1998; Raoult D. et al., 2000]. Interestingly, multispacer sequence typing indicated that only the biotype Orientalis of *Y. pestis* could be detected [Drancourt M. et al., 2004; Drancourt M. et al., 2007]. We therefore investigated the potential for the human body louse to be a vector of *Y. pestis*. An experimental animal model demonstrated that *Y. pestis* biotype Orientalis could be efficiently transmitted by *Pediculus humanus corporis* [Houhamdi L. et al., 2006]. This human ectoparasite may have played a role in the massive historical epidemics. Also, it could serve as a vector of plague in modern populations infected with *P. humanus*. Altogether, these results suggest that, sporadic cases and limited epidemics, massive plague epidemics may result from human to human transmission of *Y. pestis* biotype Orientalis by *P. humanus corporis*.

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EARLY-PHASE TRANSMISSION OF *YERSINIA PESTIS* BY UNBLOCKED FLEAS AS A NOVEL MECHANISM EXPLAINING RAPIDLY SPREADING PLAGUE EPIZOOTICS

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Plague is a highly virulent disease believed to have killed millions during three historic human pandemics. World-wide, it remains a threat to humans and is a potential agent of bioterrorism. Dissemination of *Yersinia pestis*, the etiological agent of plague, by blocked fleas has been the dominant paradigm for flea-borne transmission. However, this mechanism, which requires a lengthy extrinsic incubation period prior to a short infectious window followed by death of the flea, cannot sufficiently explain the rapid rate of spread that typifies plague epidemics and epizootics. Inconsistencies between the expected rate of spread by blocked rat fleas and that observed during the Black Death has even caused speculation that plague was not the cause of this medieval pandemic. We used the primary vector to humans in North America, *Oropsylla montana*, which rarely becomes blocked, as a model for studying alternative flea-borne transmission mechanisms. Our data revealed that, in contrast to the classical blocked flea model, *O. montana* is immediately infectious, transmits efficiently for at least 4d after infection (early phase) and may remain infectious for a long time because the fleas do not suffer block-induced mortality. These factors are consistent with criteria required to drive plague epidemics and epizootics. In follow-up studies with five additional flea species, we demonstrated the generality of this phenomenon and explore its implications in transmission cycles in the United States and in Uganda. The scenario of efficient early-phase transmission by unblocked fleas described in our study calls for a paradigm shift in concepts of how plague is transmitted during rapidly spreading epizootics and epidemics.

FACTORS THAT INFLUENCE THE NUMBERS OF FLEAS COLLECTED FROM WHITE-TAILED PRAIRIE DOG (*CYNOMYS LEUCURUS*) BURROWS

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The endangered black-footed ferret (*Mustela nigripes*) and its prairie dog (*Cynomys*) prey are highly susceptible to plague, a contagious infectious disease caused by the bacterium *Yersinia pestis* and transmitted by direct contact and by bites of infected fleas. Ecological factors affecting plague epizootics are not fully understood. Evidence from the literature correlates climatic parameters with epizootics in some black-tailed prairie dog (C. *ludovicianus*) complexes. While other models exist, favorable climate conditions might trigger epizootics by influencing prairie dog population sizes. We tested the hypothesis that prairie dog colonies expand into adjacent areas that are more likely to introduce plague from an unidentified reservoir. This hypothesis predicted increased frequency of small rodent hosts, flea species, or host infestation in expansion areas relative to habitat typically occupied by prairie dogs. We tested this hypothesis by comparing small mammals and their fleas collected in a white-tailed prairie dog (*C. leucurus*; WTPD) colony to those in potential expansion habitat at a black-footed ferret reintroduction site in Coyote Basin, Utah. From 2000-2006, we sampled two permanent live-trapping grid locations within the colony boundary and two outside the colony two to four times each season. We collected fleas from small mammal hosts and from WTPD burrows. We used mark-recapture techniques to estimate small mammal population sizes. The diversity of small mammal hosts (95% *Peromyscus maniculatus*) and their fleas (89% *Aetheca wagneri*) at our study area was low. We observed that the distribution of Ord's kangaroo rats (*Dipodomys ordi*) and certain flea species were not independent of location. However, our hypothesis was not supported because distributions of small mammals, fleas, and host infestation rates did not correlate with the presence or absence of prairie dogs. We also investigated the relationship between host population size and the extent of flea infestation over time at our study site. We tested a series of covariates that were expected to influence variation in the number of fleas from burrows. Logistic regression analysis demonstrated a significant relationship between the numbers of fleas collected from burrows and the following three covariates: spring (April – July) precipitation of the same year, deer mouse infestation, and deer mouse population estimates. High flea infestation of deer mice combined with high WTPD population density and a dry previous month are associated with high numbers of fleas in WTPD burrows. Whether increased numbers of burrow fleas correlates with epizootics in WTPD populations remains to be determined; during our study, no evidence of epizootic activity was observed in the colony. Increased flea infestation of burrows is expected to increase the likelihood of introducing plague into the WTPD population. Precipitation correlates with plague epizootics in some black-tailed prairie dog colonies, and it may influence outbreaks in WTPD colonies as well. This interpretation is consistent with trophic cascade and other climate related models for triggering plague epizootics in prairie dogs.

FEATURES OF RODENT RESERVOIR AND FLEA VECTORS CONTRIBUTING TO ENZOOTIC SYLVATIC PLAGUE IN HIGHLY BIODIVERSE CALIFORNIA COMMUNITIES

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Plague is an enzootic disease in the far-western US, in communities of rodents and fleas notable for considerable biodiversity. Among the numerous rodents known to be susceptible to *Yersinia pestis* infection, there is none that has been reported as a classical reservoir in that it experiences long-term, persistent infection. Rather, the enzootic persistence of plague appears to occur as a function of dynamic interactions of moderately competent flea vectors with transiently infected hosts. We predict that there may be several features of rodent hosts that contribute to their reservoir capacity for plague: failure to develop sufficient herd immunity to block local plague persistence, likely caused by rapid population fluctuation and turnover, and support of a high density, diverse and cosmopolitan flea fauna. Among fleas, characteristics that support enzootic plague include lack of host specificity, low to moderate vector competence, the capability of achieving large population sizes, and persistence year-round (or, alternatively, functioning with a "partner" species where the combination persists year-round). We discuss members of the rodent-flea community in several plague-enzootic sites in California that behave according to our predictions for how enzootic plague persists.

A MATRIX MODEL FOR COMMUNITY RODENT-FLEA-PLAGUE DYNAMICS: IMPLEMENTATION IN R AND ESTIMATION OF THE PARAMETERS

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In California and much of western North America, there does not appear to be a single maintenance host or vector for plague. To explain and predict plague dynamics in several sites in northern and central California, we developed a multidimensional community SIRS model with **v** vector species and **h** host species. The model began as a generalization of Bailey's host-vector SIRS model for malaria. The dynamics of our model are written out as a system of differential equations with a dimensionality of 3(v+h), since we keep track of **N**, **S**, **I** and the redundant **R** classes. To add seasonality, mortality and fecundity, we implemented the model as a discrete time simulation, coded in the statistical language S (or its equivalent R). A few tricks made the implementation a good approximation to the original model. It is not trivial to estimate all the parameters we need: a vector-to-host transmission matrix β, a host-to-vector transmission matrix β_V, a vector of host recovery rates γ, and so forth. We made use of data from the literature, but had to make some additional, plausible inferences to do so, especially for the transmission matrices. Our goal is to compare model results for a variety of California localities with actual plague data. This will allow us simultaneously to test the model and to adapt our parameterization strategies.

SOME RESULTS OF PLAGUE INVESTIGATION IN MONGOLIA

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Mongolia is a country of Central Asia, which is bordered by Russia and Kazakhstan to the north and northwest, and China to the south and east. At 1,564,116 square kilometers, Mongolia is the nineteenth largest, and the least densely populated independent country in the world with a population of around 2.6 million people. Approximately thirty percent of people are nomadic or semi-nomadic. The geography of Mongolia is varied with the Gobi desert to the south and with cold and mountainous regions to the north and west. The rest of Mongolia consists of relatively flat steppes. Most of the country is hot in the summer and extremely cold in the winter, with January averages dropping as low as -30°C (-22°F). It has an extreme continental climate with long, cold winters and short summers, during which most of its annual precipitation falls. Precipitation is highest in the north (average of 20 to 35 centimeters per year) and lowest in the south, which receives 10 to 20 centimeters annually. Plague is enzootic in wild rodent populations over large rural areas of Mongolia. Plague human cases were registered in Mongolia since 1897. Human cases, registered in Mongolia from 1971-2005 and data from plague investigations from 1954-2005 were reviewed and analyzed. Mongolians hunt marmots and use marmot's fur and meat, the risk of plague infection is very high in Mongolia. Human plague has been identified in Mongolia almost every year. Since 1931, approximately 600 human plague cases were registered in Mongolia, of which 70% were fatal. World wide, the fatality rate for the period of 1992-2001 was 7.1%, while in Mongolia 40% of reported cases for the same period died. Bubonic plague accounted for 89.9 % of all human plague cases registered during the 1971-2005 period, while the pneumonic and septic forms accounted for the remaining 4.2 % and 5.9 % of cases respectively, and 42.5 % of the bubonic cased developed subsequent pneumonic, septic or intestinal forms. In 28.3% of territory of Mongolia exist natural plague foci and 47.1% of the natural foci are highly active. Plague field investigations are conducted in about 4000 ha every year and more than 3000 plague strains were isolated from natural foci. High active plague foci exist mainly in western part of Mongolia. In Mongolia, 3 highly pathogenic subspecies of plague agent circulate; *Y. pestis pestis, Y. pestis altaica and Y. pestis ulegeica*. The main hosts for plague in Mongolia are marmots (*Marmota sibirica)*, sousliks (*Citellus undulatus)*, pikas (*Ochotono pallasi*) and Brandt's voles (*Lasiopodomys brandti*) and more than 50% of all plague cultures were isolated from marmots. The main vectors are fleas, especially marmot flea (*Oropsylla silantiewi*). Plague has been idenitified in 28 species of fleas, 4 species of ticks and 2 species of lice. 27% of all plague cultures were isolated from ectoparasites and 91.5% of them were from fleas. 64.5% of all flea cultures were isolated from marmots fleas. According to the geographical coordination studies of 5000 points of human cases and plague cultures in Mongolia since 1890, we determined that the plague agent circulates between $50^0 00 – 43^0 00$ and $88^0 00 – 120^0 00$ of longitude and latitude coordination and plague natural foci locate at altitude between 640-3500 meters in Mongolia. There were noted some correlations between climatic factors such as temperature and precipitation and plague foci activation in Mongolia.

METAPOPULATION DYNAMICS OF PRAIRIE DOGS AND PLAGUE: IMPLICATIONS FOR PERSISTENCE

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Plague, caused by the bacterium *Yersinia pestis*, spread into the range of black-tailed prairie dogs (*Cynomys ludovicianus*) in North America during the 1930s. As a result, prairie dog spatial distributions changed from large, contiguous populations to small, isolated patches in metapopulations. This spatial shift raises fundamental questions of whether prairie dogs will remain viable indefinitely, and whether and how metapopulation dynamics can allow this highly virulent pathogen to persist within prairie dogs populations. To investigate we developed a stochastic patch occupancy model to compare three hypothesized mechanisms of plague spread at the landscape level including (1) size of local populations (target effect), (2) target effects mediated by ENSO events, and (3) connectivity to recently plagued towns. We estimated parameters using data from 1981-2000 on the Pawnee National Grassland in Colorado, selected among competing models using AIC, and validated our model with data from 2001-2005. Our model demonstrates that metapopulation structure allows prairie dog persistence, and provides evidence that host spatial structure can facilitate maintenance of this pathogen in an epizootic host. Our results suggest that prairie dogs are unlikely to disperse *Y. pestis* among towns, but alternative hosts for plague vectors may play an important role in landscape-level plague movement.

THE EVOLUTION OF STABLE FLEA-BORNE TRANSMISSION CYCLES OF *YERSINIA PESTIS*

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Yersinia pestis, uniquely among the enteric group of Gram-negative bacteria, is an arthropod-borne pathogen. This transmission route appears to be an evolutionarily recent adaptation, because the closely related *Yersinia pseudotuberculosis*, from which *Y. pestis* diverged only within the last 10,000 to 20,000 years, is an enteric pathogen transmitted by the fecal-oral route. In one well-characterized mechanism of biological transmission by its flea vector, *Y. pestis* colonizes the proventriculus, a structure in the foregut that functions as a valve during blood feeding. Adherent bacterial growth can interfere with the normal function of the proventricular valve, preventing it from closing completely, which allows bacteria in the flea gut to be expelled into the bite site. In some fleas, bacterial growth eventually fills the valve, blocking the flow of blood into the midgut. In these cases, transmission occurs by reflux of bacteria from the proventriculus into the bite site. Proventricular colonization depends on the formation of a *Y. pestis* biofilm, in which a dense bacterial aggregate contained within an extracellular polysaccharide matrix adheres to the spines that line the interior surface of the valve. Genetic changes that led to the ability to produce this type of transmissible infection have been identified, using the rat flea *Xenopsylla cheopis* as an infection model. For example, *Y. pestis* acquired two new plasmids since it diverged from *Y. pseudotuberculosis*, and each contains genes that function to greatly enhance flea-borne transmission efficiency and the capacity for epidemic spread. In addition, two chromosomal genes that negatively regulate the synthesis of the extracellular matrix required for biofilm formation are pseudogenes in *Y. pestis*, and restoration

of the intact, ancestral *Y. pseudotuberculosis* alleles results in greatly diminished proventricular colonization. Thus, both gene acquisition and gene loss were important evolutionary steps leading to flea-borne transmission cycles. The vector competence of fleas is relatively poor, however, and one consequence of this may have been selective pressure for the emergence of highly virulent *Y. pestis* strains able to cause high-density bacteremia.

SPATIAL ANALYSIS OF PLAGUE IN CALIFORNIA: CURRENT DISTRIBUTIONS AND POTENTIAL RESPONSE TO CLIMATE CHANGE

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The plague bacillus (*Yersinia pestis*) is a public and wildlife health concern in California and the western United States. Spatial analysis of plague clusters, as well as models of the potential distribution of plague foci could aid public health agencies in deciding where to allocate limited surveillance resources. This study tests Maxent, a species distribution modeling technique, for mapping potential distribution of plague foci in California. Models were constructed using geocoded seroprevalence data from surveillance of California ground squirrels (*Spermophilus beecheyi*) as case points, and using Worldclim bioclimatic data as predictor variables. Model results were compared and validated using area under the ROC curve (AUC) statistics. Additionally, model results were compared to locations of positive coyote samples, in order to determine the relationship between model predictions and areas of plague risk as determined by the California carnivore surveillance program. Models based on recent climate conditions accurately identified case locations, and these validated models were used to identify potential plague risk areas based on a future (carbon-doubling) climate scenario. We discuss implications of these results in terms of insight into plague ecology, and for public health decision making.

LANDSCAPE GENETIC CONCORDANCE BETWEEN THE BLACK-TAILED PRAIRIE DOG AND ITS ASSOCIATED FLEA, *OROPSYLLA HIRSUTA*

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It has been long postulated that species-specific parasites should exhibit strong population genetic associations with their hosts (McCoy et al. 2005). We tested the hypothesis that the population genetic structures of fleas somewhat specialized on black-tailed prairie dogs (BTPD) will exhibit concordant population genetic structures with that of their host. If there is strong concordance, then it can be concluded that BTPDs play more of a role in the distribution of fleas that transmit sylvatic plague among BTPD colonies than previously suggested. We captured 89 BTPDs and collected 67 fleas on 5 BTPD colonies in 2005. We estimated population genetic structure of BTPDs with 22 microsatellite loci from previously developed primers. We used 11 microsatellite markers developed for the prairie dog flea, *Oropsylla hirsuta*, and 2 developed for the hen flea, *Ceratophyllus gallinae* (Binz et al. 2003), to elucidate the population genetic structures of fleas sampled from the same 5 BTPD colonies. *O. hirsuta* was the most common flea we found on the study colonies. We analyzed BTPD and *O. hirsuta* genotypic data using

Popgene ver. 1.32, STRUCTURE ver. 2.2, and Arlequin ver. 3.11. BTPDs showed moderate genetic differentiation and gene flow (Fst = 0.2013; Nm = 0.9919) among the five colonies. This finding was further supported by results from AMOVA and STRUCTURE which indicated moderate variation among populations. The results supported the conclusion that BTPD colonies may be acting as somewhat genetically separate populations most likely due to their social structure. Flea population genetic structure showed significant deficiencies in heterozygotes from Hardy-Weinberg expectations at several loci probably due to high levels of inbreeding. Fleas showed low genetic divergence (Fst = 0.132) and fairly high gene flow (Nm = 1.4955) between BTPD colonies. AMOVA also indicated low variation among flea populations. STRUCTURE results indicate that flea populations are structured at a larger spatial scale than BTPDs. These results suggest that fleas disperse readily among nearby BTPD colonies, but that they may encounter dispersal barriers at larger spatial scales.

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POPULATION GENETIC STRUCTURE OF THE BLACK-TAILED PRAIRIE DOG FLEA, *OROPSYLLA HIRSUTA*, IN NORTH-CENTRAL MONTANA WITH A PANEL OF 13 MICROSATELLITE LOCI

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The population genetic structure of the flea, *Oropsylla hirsuta,* a potentially important vector for sylvatic plague among black-tailed prairie dogs (BTPD), was estimated using 13 microsatellite markers. Fleas were collected from a total of 5 BTPD colonies (67 total fleas) on the Fort Belknap Indian Reservation and Bureau of Land Management land in north-central Montana during May - July of 2005. An objective of this study was to determine if the spatial distribution and population genetic structure of *O. hirsuta* can predict how the plague bacterium, *Yersinia pestis*, is distributed among BTPD colonies. The results illustrated a mean number of alleles per locus of 11.9 with moderate gene flow between populations (Nm = 1.4955; Fst = 0.1432). This finding was further supported by the results from an AMOVA which showed less variation in allele frequencies among the populations (21%) than within them (79%). These results establish support for the hypothesis that fleas are not strongly genetically isolated by BTPD populations and that they probably can disperse to nearby colonies. Because the population genetic structure of this key flea species has never been studied, the information presented will be valuable to biologists in understanding the dispersal patterns exhibited by this plague vector and by vectors of highly infectious pathogens in general. Furthermore, these data will be used to estimate the concordance between the population genetic structures of *O. hirsuta* and BTPDs to establish the role BTPDs in the dispersal of fleas that can carry *Y. pestis*.

LONGITUDINAL STUDY OF PLAGUE IN NEW MEXICO: SPATIAL AND TEMPORAL PATTERNS IN EPIZOOTIC DYNAMICS

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Very little is known or understood about the dynamics of plague in its natural reservoirs and about the maintenance cycle of plague in North America. Among the few studies that have investigated the course of plague infection in individual rodent hosts, none have provided clear evidence of rodent resistance to plague. We have initiated a longitudinal study of plague dynamics at two trapping sites in the Eldorado community outside of Santa Fe, New Mexico, an area characterized by frequent plague epizootics. The primary goal of our study was to identify which species in the community were infected with plague; to determine the spatial and temporal patterns of the dynamics of plague epizootics; and to describe the dynamics of *Yersinia pestis* infection within individual hosts. At the first site, 64 permanent trapping stations were sampled every month, and at the second site, 25 trapping stations were sampled at 2-4 month intervals. Rodents were live-trapped by a combination of three different kinds of traps and were marked individually by ear-tag or/and sub-cutaneous transponders. Blood and flea samples were taken from each captured animal. Fleas were also collected from selected burrows. A total of 2,800 fleas collected from 460 small mammals of eight species were tested for *Y. pestis* DNA by PCR. Nine fleas collected from six southern plains woodrats (*Neotoma micropus*) and from one rock squirrel (*Spermophilus variegates*) were positive for the *pla* gene of *Y. pestis*. None of 125 fleas collected from 17 woodrat nests were positive. A serological survey for plague was conducted by passive hemagglutination and inhibition tests on blood samples collected from 893 small mammals of 13 species. Hemagglutinating antibodies (titer $\geq$1:32) to the *Y. pestis*-specific F1 antigen were detected in 11 rodents of six species. One rock squirrel remained seropositive for at least a 13-month period, and one grasshopper mouse (*Onychomys leucogaster*) remained seropositive for at least 2 months. Our observations suggest that small-scale epizootics in woodrats can support maintenance of plague in the active U.S. southwestern focus.

PLAGUE IN TANZANIA : FROM A HOST AND VECTOR PERSPECTIVE

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Today, in Tanzania *Yersinia pestis* apparently persists in sylvatic enzootic cycles, sometimes lasting for decades. Plague circulation remains undetected until an epizootic or an epidemic is observed. In 1982, Akiev wrote “The evidence available concerning plague in Africa indicates that the infection is enzootic throughout the continent and that under certain conditions cases of human plague might occur in any African country”. This single sentence summarizes what we don’t know about plague. In fact, we still know very little about the “certain conditions” that favour long-term maintenance of plague. in African foci. In Mbulu district for example, in northern Tanzania, a sudden plague outbreak in February 2007 reminded the Tanzanians that plague was still present, despite the fact that no plague cases had been reported since 1977. In the Lushoto district, the first recorded plague outbreak occurred in 1980. The outbreak began in a single village and rapidly spread to more than 50 villages. By 2004, 7,603 cases had been

reported from this region. There was great variation in the number of cases of plague among the villages. Although evidence of infection with *Y. pestis* has been observed in several wild rodent and flea species during epidemics, the actual reservoir in which the infection survives has not yet been identified. The ecology of plague and the source from which humans acquire infection are poorly understood. We compared the domestic and sylvatic ecology in villages having frequent plague outbreaks with those in villages where plague is relatively rare. In particular, we studied the prevalence of the infection in small mammals including their species composition and distribution, and the seasonal and spatial pattern of host-flea association. During this 4 years study we found no seropositive animals and no difference in sylvatic rodent and flea diversity between the two sets of villages. The reason for the differences in plague incidence between villages may not be the difference in species composition of either rodents or fleas, but rather difference in relative abundances of those rodent and flea species that possess ecological characteristics that facilitate transmission of the plague pathogen. Within affected villages, socio-cultural factors might be responsible for the observed differential plague incidence in females and different age groups recorded in the Lushoto hospital dataset. Interviews suggest that the risk of exposure to domestic fleas during daily activities is higher in children and women. Moreover, we found that *Pulex irritans*, the human flea, was the predominant flea species in houses and that *P. irritans* index was strongly positively correlated with plague frequency and with the logarithmically transformed plague incidence. These observations suggest that in Lushoto District, at the domestic level, human fleas may play a role in plague epidemiology in the domestic environment. However, this doesn't explain how plague reaches the village, nor does it answer where and how do plague persists between outbreaks, especially on the long term.

EMERGENCE AND GROWTH OF PLAGUE FOCI IN AFRICA

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Plague is globally distributed but in most cases occurs locally in relatively small areas. These areas are linked to enzootic foci that seem to be stable and from where plague rarely moves away. This is remarkable for a disease which through history has become iconic for a rapidly spreading infection travelling over large distances. It is usually assumed that ecological conditions determine local persistence and that human movements are responsible for long-range spreading. Well established plague foci exist in eastern Africa. Their borders are fairly well documented but the underlying ecology for their persistence remains unsure. Ecological Niche Modeling (ENM) suggests that plague foci is found in highly diverse ecological conditions, and hence predicts enormous areas where plague could occur, but nevertheless also equally large areas where plague is unlikely to persist. At a regional scale, predictions show more patchy patterns that are confirmed by recently emerged epidemic areas and observations of plague infected rodents in the absence of human plague. The ecological mechanisms that are responsible for these patterns remain elusive, however, and the niche dimensions selected in the ENM are not easy to interpret. At the local scale, assemblages of rodents and fleas may differ in a complex way between sites with high and low plague incidence but patterns seem to differ between separate foci. As a result, it is extremely hard to convincingly explain the patchy distribution of plague, or to predict how foci may change or where they could emerge. Anthropogenic spreading of the infection may play a role but is certainly not sufficient for the

establishment of foci, as illustrated by the numerous outbreaks along Africa's coast that did not result in the persistent infection of local rodent populations. Climatic factors have been shown in several studies to have an effect on the temporal variation in plague transmission in an area, and therefore it has been suggested that climate change would also affect the spatial characteristics of the foci. Data to support this are mostly lacking, but we make attempts to see whether climate variation can be linked to the growing foci in northeastern R.D.Congo, or the emergent foci of Mbulu and Karatu in Tanzania. Based on the available information, we make a hesitant prediction of areas and situations where plague foci could emerge or grow, with the explicit warning that for now, such predictions must still be used as the testing of hypotheses rather than practical instruments for public health.

SINGLE NUCLEOTIDE POLYMORPHISM DISCOVERY TO DETERMINE *YERSINIA PESTIS* POPULATION STRUCTURE IN THE WESTERN UNITED STATES

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Yersinia pestis, the etiological agent of plague, originated on the central-Asian plateau between 1,500 and 20,000 years ago. It has since spread around the world and occupies every continent except Australia and Antarctica. In the central-Asian countries where *Y. pestis* has existed the longest, plague foci have been spatially delineated based on unique ecological attributes such as vegetation type, elevation, and rodent species. Studies have indicated that these foci each harbor unique *Y. pestis* phenotypes and genotypes (Martinevsky, 1973). *Yersinia pestis* entered the United States just over 100 years ago and has since migrated west to about the hundredth meridian. Rodent epizootic activity is regularly observed in the western states where plague foci were previously defined using the same criteria as those in central-Asia, but in the U.S. there is no evidence that unique *Y. pestis* genotypes correspond to areas that differ ecologically. Studying the genetic diversity of *Y. pestis* in the western U.S. is expected to provide information on bacterial population structure and migration patterns, and to further delineate U.S. plague foci. We used DNA microarray technology (Hinds et al., 2004) to discover single nucleotide polymorphisms (SNPs) in the genomes of *Y. pestis* isolates collected from nine western states and two different predefined plague foci in CO. Isolates from AZ, CA, CO, NE, NM, NV, MT, SD, and WY were compared using parsimony analysis to determine whether these regions harbor unique *Y. pestis* genotypes and to assess directional spread across the U.S. In addition, CO isolates were compared to test whether during epizootic events, *Y. pestis* spreads clonally from the mountainous front range of northern CO to the eastern plains, or if plague epizootics are independent events. Finally, we examined the presence of non-synonymous SNPs (nsSNPs) compared with synonymous SNPs (sSNPs) for indications of purifying selection or niche adaptation within distinct CO foci. Microarray analyses yielded 207 SNPs unique to North American *Y. pestis* isolates. Phylogenetic inference indicated that *Y. pestis* genotypes on the eastern and western plains of CO differ from each other and also from mountainous genotypes. Distinct eastern and western plains clades were supported by nsSNPs indicating that *Y. pestis* niche adaptation may have occurred in CO on a relatively small scale and that epizootic events and plague foci are spatially isolated. In addition, phylogenetically informative SNPs for SD, and NM were identified. Discovery of SNPs in this set of *Y. pestis* isolates provided molecular markers informative for *Y. pestis* population studies. These markers will provide a valuable tool for future studies of *Y. pestis* evolution and population genetics in the U.S.

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ENZOOTIC PLAGUE REDUCES BLACK-FOOTED FERRET (*MUSTELA NIGRIPIES*) SURVIVAL IN MONTANA

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Black-footed ferrets (*Mustela nigripes*) are dependent on extensive prairie dog colonies (*Cynomys spp*.) to provide both habitat and prey. Plague kills both prairie dogs and ferrets, and is a major factor limiting establishment of black-footed ferret populations and recovery of this highly endangered species. Plague epizootics are an obvious threat, killing ferrets directly and eliminating virtually all of the prairie dogs in an area. We hypothesized that enzootic plague (i.e. ferret exposure to plague-causing *Yersinia pestis* bacteria in the absence of any noticeable prairie dog die-off) could also be affecting ferret survival in some locations. To evaluate this hypothesis, we conducted a manipulative experiment by reducing the risk of plague on portions of 2 ferret reintroduction sites in north-central Montana through flea control using deltamethrin dust and by vaccinating about half of the ferrets released on dusted and non-dusted colonies with F1-V fusion protein, a plague vaccine shown to protect ferrets in laboratory studies (Rocke, et al. 2008). We evaluated 6-month survival rates for ferrets in 4 treatment groups involving all combinations of vaccine and dust. The study was completed from 2003-2006 and in the absence of any noticeable loss of prairie dogs. We used spring and fall spotlight surveys to locate and individually identify ferrets throughout both study areas. We used logistic regression to assess capture rates, an index to survival, for 208 time intervals on 131 individual ferrets. Ferrets living on deltamethrin-treated sites and/or receiving the vaccine had significantly higher capture rates (0.51) than non-vaccinates living in areas without flea control (0.29) ($P = 0.013$). The general model, however, also provided evidence for two interactions (Vaccine X Dust and Vaccine X Origin) that suggested separate analyses of ferrets of captive and wild origins by separate treatment categories. Analysis of captive-reared animals (156 time intervals on 109 individuals) revealed a significant vaccination effect on colonies without flea control, with the capture-rate higher for vaccinates (0.52) than for non-vaccinates (0.18) ($P = 0.044$). On colonies with flea control, vaccine did not significantly affect capture rate (vaccinates = 0.56, non-vaccinates = 0.35, $P = 0.769$). Analysis of captive-reared animals also revealed a significant flea control effect among non-vaccinates with a higher capture-rate on colonies with flea control (0.42) compared to colonies without flea control (0.23) ($P = 0.027$). Among vaccinates, flea control did not significantly affect capture rate (with flea control = 0.41, without flea control = 0.44, $P = 0.534$). The significant enhancement of survival due to the plague vaccine supports the hypothesis that enzootic plague reduces ferret survival, even when there was no noticeable loss of prairie dogs indicative of an epizootic. The different effect of vaccine on colonies with and

without flea control suggests that fleas are required for transmission and/or maintenance of enzootic plague and that other studies showing similar effects of flea control on survival of prairie dogs are due to enzootic plague. Finally, we demonstrate that the experimental F1-V fusion protein vaccine appears to provide protection to ferrets in the wild, although more evaluations are needed.

FLEA COMMUNITY AND PREVALENCE OF *YERSINIA PESTIS* IN BLACK-TAILED PRAIRIE DOGS (*CYNOMYS LUDOVICIANUS*) FROM NORTHWESTERN MEXICO

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One of the largest areas of native grasslands in northwestern Mexico is in Janos-Nuevo Casas Grandes, Chihuahua. The black-tailed prairie dog (*Cynomys ludovicianus*) is the keystone species and engineer of these native grasslands (Ceballos et al, 1993). Plague, caused by the bacterium *Yersinia pestis*, has decimated entire prairie dog populations in southern USA (Cully and Williams, 2003). However, we don't know if plague is present in Mexico. Similar environmental conditions exist in Janos and southern USA and fragmented prairie dog populations are factors that may enhance local transmission and perpetuation of disease outbreaks in prairie dogs in this area. The main objective of this study is to evaluate occurrence, distribution, and spatial dynamics of *Y. pestis* in black-tailed prairie dogs in the region of Janos-Nuevo Casas Grandes, Chihuahua, Mexico. In 2007, we sampled 13 prairie dog colonies and we captured 272 individuals with Tomahawk traps. Captured animals were handled in canvas bags to collect blood samples and fleas. Blood samples were taken with Nobuto filter strips. Fleas were collected in microtubes with physiological solution and were later identified at the "Universidad Nacional Autónoma de México" (UNAM) in Mexico City. We obtained 182 blood samples and 1,838 fleas. A total of three flea species were identified including *Pulex simulans, Echidnophaga gallinacea*, and *Oropsylla* [*Opisocrostis*] *hirsuta*. Fleas in the genus *Pulex* and *Oropsylla* were the dominant fleas in prairie dogs from Janos, Chihuahua. To further evaluate the flea community structure and plague dynamics we will collect more fleas in future trapping sessions in 2008 and 2009. Plague identification in both blood samples and fleas is in progress at the Medicine School, UNAM in Mexico. We will then analyze the effect of flea community structure and prairie dog population structure on plague dynamics. Final products of this research will help to develop conservation strategies that sustain wildlife populations and ecosystem integrity in one of the top priority areas for conservation of Mexican vertebrate diversity in the region of Janos, Chihuahua.

PLAGUE IN TANZANIA – A LANDSCAPE ECOLOGICAL APPROACH

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Plague remains a public health threat in many parts of the world, but particularly in sub-Saharan Africa. In general, it occurs seasonally and shows a clear geographically disjunct distribution in circumscribed foci. In spite of plague's highly focal nature, the underlying ecology remains often unknown and Ecological Niche Modeling suggests that plague can occur in highly diverse landscapes under wide ranges of environmental conditions (Neerinckx et al. in press). In 1980 a persistent focus of human plague was discovered in Lushoto, northeastern Tanzania. By 2004 >7000 cases had been reported from this region and a strong variation in plague frequency and incidence is seen among neighboring villages in the plague endemic area (Davis et al. 2006). Earlier studies, which focused mainly on the host-vector-parasite system, demonstrated that this striking variation could not be explained by differences in fauna composition or human domestic behavior. Therefore, landscape ecological factors are suspected to determine plague's local persistence and/or to act as disease-provoking factors. In the present study, we report on the link between human plague incidence in Lushoto and data on climate, landforms, land cover, soils and vegetation. We performed a comparative field survey in a number of plague-positive and -negative villages in Lushoto and gathered the collected information in a GIS database, including an elevation model, weather data time series (rainfall and temperature), landform and land cover descriptions, soil physical and chemical properties, and concentrations of chemical elements in the common plant *Rumex usambarensis*. We found a positive correlation between plague incidence and altitude, and the endemic plague area appeared to coincide with an area that had been totally deforested in the early 1960s. Moreover, first observations suggest that villages with a high plague incidence are connected through typical fertile valley bottoms, i.e. Gleysols and Fluvisols, and that hamlets (part of a village) in this valley bottom have had more human plague cases. Soil and plant samples are being analyzed to test if factors that define the microclimate (in this study, bulk density, soil texture, pH, and organic carbon, and concentrations of chemical elements in soil and plant) are linked with plague occurrence in Lushoto. Our results give an indication that a landscape ecological study approach can provide insights into the persistence of plague and how its distribution can be affected by landscape features, and therefore in this case, might open the track towards a better understanding of the underlying ecology of plague's distribution in Lushoto.

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IRON TRANSPORT AND PLAGUE PATHOGENESIS

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Iron acquisition from the host is an important step in the pathogenic process. *Yersinia pestis* has two iron transporters, the yersiniabactin (Ybt) siderophore-dependent system and the Yfe ABC transporter for iron and manganese, with proven roles in the pathogenesis of bubonic plague in mice while the Yfe, Feo (for ferrous iron), and Hmu heme transporters affect intracellular growth in J774A.1 cells (Perry and Fetherston, 2004; Perry *et al.*, 2007). In this study we examined the importance of key iron transport systems in the pathogenesis of bubonic and pneumonic plague. In a BSL3/ABSL3 facility, bacterial cells were grown in Heart Infusion broth at 28^{o}C (for subcutaneous injection; bubonic plague) or 37^{o}C (for intranasal instillation; pneumonic plague) and Swiss Webster mice (Hsd:ND4; Harlan) were infected with 10-fold serial doses. The animals were monitored daily for two weeks and fifty percent lethal doses (LD_{50}s) calculated. In the bubonic plague model, strains with mutations in either the outer membrane receptor (Psn) or a Ybt biosynthetic enzyme (HMWP1/*irp1*) caused some transient disease symptoms but failed to kill mice at a significant rate at doses as high as 10^7. In contrast, an *ybtX* mutant (encoding a putative inner membrane protein of unknown function), had an LD_{50} ~10-fold higher than the wild-type (WT) Ybt^+ strain (LD_{50} = 23 ± 14 cells). While an *feo* mutant was fully virulent in this model, a *yfe* mutant had a modest decrease in virulence (~9-fold lower than WT). A *yfe feo* double mutant was ~90-fold less virulent than WT. These results indicate that the Ybt system is essential while the Feo and Yfe systems serve overlapping functions of moderate importance for bubonic plague in mice. The pneumonic mouse model revealed intriguing differences in the effect of *ybt* mutations. The WT LD_{50} was 329 ± 105 cells while two independent *psn* mutants had an LD_{50} of $1.1 \times 10^4 \pm 2.9 \times 10^3$. However, two independent *irp2* biosynthetic mutants had an LD_{50} ~24-fold and 790-fold higher than the *psn* mutants and WT strain, respectively. With a *Δpgm* mutant (102-kb deletion that includes Ybt, the Hms biofilm genes, and numerous other genes), doses as high as 3.9×10^6 failed to yield an LD_{50} value. In contrast, *feo, yfe,* and *yfe feo* mutants were all fully virulent. Consequently, the Yfe and Feo systems are not important in pneumonic disease. The differences in virulence between mutants which produce but are unable to use the Ybt siderophore (*psn* mutant) and those which cannot produce Ybt suggest that the siderophore is playing a non-iron acquisition role in pneumonic plague. Ybt serves as a signal molecule to activate genes in the Ybt system; perhaps it activates other virulence factors in *Y. pestis*. Alternatively it may alter the lung environment directly or affect cytokine signaling. The further loss of virulence by the *Δpgm* mutant suggests that one or more unidentified factors encoded within this region play a role in the pathogenesis of pneumonic plague.

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PRELIMINARY STUDY OF FLEAS ON RODENTS IN THREE COLORADO COUNTIES

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Some species of fleas are found on multiple species of rodents, thus increasing the likelihood of spreading disease from one host to another. As part of a preliminary study, fleas were collected off live-trapped and kill-trapped rodents from October 2003 through August 2007 in Elbert, El Paso, and Pueblo Counties, Colorado. Twelve species of fleas representing three families were collected off nine species of rodents. The two most common species of fleas, *Aetheca wagneri* and *Orchopeas leucopus* were found on four species of mice (*Peromyscus* spp.), Mexican woodrats (*Neotoma mexicana*) and rock squirrels (*Spermophilus variegatus*). *Oropsylla montana* was found in high numbers on rock squirrels and, because of its high incidence, is an important vector in plague transmission (Lewis 2002). In addition, we found small numbers of *Hoplopsyllus anomalus*, a known plague vector, on rock squirrels. We also found high numbers of *Foxella ignota*, a primary parasite of northern pocket gophers (*Thomomys talpoides*), which is a minor vector of plague (Pigage et al.2005). Although pocket gophers are frequently found in the same areas as other rodents, we did not collect *F. ignota* from any other host. Our current goal is to investigate rodent populations that live in or near black-tailed prairie dog (*Cynomys ludovicianus*) colonies and their fleas as well as some predators of the prairie dogs in order to examine flea exchange.

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EFFICACY OF A SYSTEMIC INSECTICIDE AGAINST FLEAS ON WYOMING GROUND SQUIRRELS (*SPERMOPHILUS ELEGANS*) AND PRAIRIE DOGS (*CYNOMYS LUDOVICIANUS*)

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Thirteen Wyoming Ground Squirrels (*Spermophilus elegans*) and 15 black-tailed prairie dogs (*Cynomys ludovicianus*) were live-trapped in Larimer County, Colorado. The ground squirrels and prairie dogs were randomized into three groups- treatment group I (imidacloprid), treatment group II (imidacloprid and diphacinone) and group III, controls. Both species were administered the solutions via oral intubation, at the rate of 1 mL per animal. Ground squirrel treatment groups were given an amount of active ingredient equivalent to consuming 1 g of formulated bait. The squirrel study was conducted on September 18, 2006. Each squirrel in treatment group I ground squirrels was dosed with 0.25 mg of imidacloprid. Treatment group II ground squirrels were dosed with a combination of imidacloprid (0.025 mg) and diphacinone 0.25 mg). Prairie dogs were dosed with the equivalent of 3 grams of bait: group 1 containing 0.75 mg

imidacloprid, group II having 0.75 mg imidacloprid and 0.075% diphacinone. The prairie dogs were administered the test materials on September 12, 2009. Control animals were dosed with the 1 mL alcohol. Approximately three hours after gavaging, the capsules with fleas, were secured to the rodents after shaving the fur. The fleas were able to take a blood meal through the pervous membrane cloth on the end of the capsule. Each capsule contained from 10-50 fleas (*Oroysylla spp*). Fleas were allowed to feed for three hours, after which the apparatuses were removed. Fleas were then observed at intervals of 24 and 48 hours to determine flea mortality. After 48 hours, flea mortality in capsules placed ground squirrels on treatment groups I and II were 75.7% and 92.0% mortality respectively. Control mortality was 10.4%. In prairie dogs after 48 hours, flea mortality was 97.3% in the imidacloprid group and 96.0% in the imidacloprid-diphacinone group while control mortality was 4.5%.

DETERMINATION OF THE BLOOD TITER LEVELS OF IMIDACLOPRID AND EFFECTIVENESS AGAINST *XENOPSYLLA CHEOPIS* FLEAS ON LABORATORY RATS (*RATTUS NORVEGICUS*)

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Rats have been long considered a reservoir host of flea-born diseases, especially plague. Systemic insecticides commonly used for flea control in veterinary medicine could also be applied to control flea populations on rats. Imidacloprid [1-(6-chloro-3pyridylmethyl)-N-nitroimidazolidin-2-ylideneamine] is a versatile and effective insecticide with diverse and growing number of applications ranging from agricultural uses to the treatment of pets and control of household pests. We report here the evaluation of imidacloprid as an effective insecticide to systemically control *Xenopsylla cheopis* fleas on laboratory rats and thus to mitigate flea-borne diseases. A high performance liquid chromatography (HPLC) method with reverse phase separation for determining imidacloprid level in blood of rats was developed. Imidacloprid was detected by UV at 270 nm with the Limit of Detection at 0.018 µg/mL, Limit of Quantification at 0.051 µg/mL, and mean recovery of 97.6%. The method was validated for imidacloprid concentration range from 0.02 to 0.82 µg/mL. For testing imidacloprid as an insecticide, single doses of 0.2 mg (group 1) and 0.4 mg (group 2) were orally delivered to two treatment groups (n=5) of rats. The control group (n=3) was given an aliquot of pure solvent. After 3 hours of delivery ~20 fleas were applied to each rodent using flea chambers placed on animals. Fleas were allowed to feed for 3 hours and then removed. Blood was collected into 3 mL EDTA tubes. Liquid samples for HPLC were prepared by liquid-liquid extraction of imidacloprid from blood by dichloromethane. The imidacloprid level was found to be 0.47 ± 0.049 µg/mL and 0.89 ± 0.188 µg/mL for group 1 and group 2, respectively. No traces of imidacloprid were found in the control group. Flea mortality in group 1 was 78.0% and 80.5% after 24 hours and 48 hours of imidacloprid exposure, respectively. Flea mortality in group 2 was 72.2% and 77.8% after 24 hours and 48 hours of imidacloprid exposure, respectively. Mortalities of fleas in the control group were 10.5% and 12.6% respectively. The reported HPLC/flea application method allows determination of the effective imidacloprid doses that need to be delivered to host rats to reach effective imidacloprid concentrations in host blood and lethally control *Xenopsylla cheopis* fleas.

RAT MOVEMENT AND PLAGUE INDICATORS IN RURAL HABITATS OF MALAGASY PLAGUE FOCI

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Rattus rattus is the main reservoir host for rural plague in Madagascar. About forty districts are affected by the disease according an inconsistent spatio-temporal distribution. In order to establish the risk of plague diffusion, it is essential to understand the relationships between landscape structure and reservoir hosts dispersion. The study was assessed first at the scale of the habitats where human can be in contact first with rats and their fleas. The typical habitats of the rural plague villages in Madagascar are constituted by the houses, the hedges of sisal and the rice fields. The aim of this work is to evaluate *R. rattus* dispersion and plague indicators at the scale of various habitats during the plague season in rural endemic foci of the highlands in Madagascar. The movements of rats at the scale of the village were followed by using a non-toxic biomarker, the Rhodamine B (RB) incorporated into the baits given in the three habitats. Further baiting, animals were trapped in the studied areas for three months. Indicators for plague risk such as abundance of rats, flea index, *Yersinia pestis* carriage and seroprevalence for anti-F1 antibody were assessed. The study has been carried out since august 2006. *R. rattus* represented more than 86 % of captures. The highest abundance of rats was observed in the hedges of sisal. The highest Flea Index were observed in the houses and the hedges of sisal. Some *Xenopsylla cheopis* and *Synopsyllus fonquerniei* collected in the houses and the hedges of sisal were positive for *Y. pestis* (culture). These fleas were collected on *R. rattus*, *Mus musculus* and *Suncus murinus*. Seropositive animals were found in the three habitats. No significant difference was found in the abundance and the flea index between the localities tests with RB and control without RB. During plague transmission season, movement of rats was observed mainly between houses and hedges of sisal. We found that RB is a practical alternative for studying rodent movement in Madagascar. The hedge of sisal and the house are important habitats for plague transmission. These results may help in designing management strategies to reduce the risk of plague transmission.

INCREASED PROTECTION AGAINST PLAGUE IN PRAIRIE DOGS FOLLOWING ORAL IMMUNIZATION WITH RACCOON POX VIRUS-VECTORED VACCINES

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Prairie dogs (*Cynomys* spp.) are highly susceptible to plague caused by *Yersinia pestis* and the disease often causes local extinctions or regional reductions of populations. Along with other wild rodents, prairie dogs are also considered a significant reservoir of plague for other wildlife, domestic animals, and humans in the western United States. Prevention of plague in wild rodents by immunization could reduce outbreaks of the disease. However, efficient large-scale immunization of free-ranging wildlife populations can only be achieved through voluntary consumption of vaccine. Unfortunately, most of the vaccines formulated to date are poor candidates for oral immunization as they cannot withstand the alimentary tract or do not illicit a strong mucosal immune response. Orthopoxviruses are ideal vectors for vaccines because they have a predilection for mucosal tissue. We previously showed that a recombinant raccoon

poxvirus (RCN) expressing the F1 antigen of *Y. pestis* (RCN-F1) provided partial protection (about 50%) against plague infection when voluntarily consumed by prairie dogs (Mencher, et al. 2003; Rocke et al. 2008). To determine if the addition of a second *Y. pestis* antigen (V) could improve the observed protection after vaccination, we designed and tested a RCN-vectored vaccine construct that expresses a truncated form of the V antigen (RCN-Vt). Both RCN-vectored vaccines (RCN-F1 and RCN-Vt) were incorporated into palatable baits and offered several times over the course of several months to a group of 16 black-tailed prairie dogs (*C. ludovicianus*) for voluntary consumption. For comparison, two additional groups of prairie dogs (n=12 each) were injected subcutaneously one or two times with 40ug of F1-V fusion protein, a vaccine demonstrated to induce immunity to plague in mice and other mammals, even after a single injection (Anderson, et al 1998). A control group of prairie dogs (n=15) received baits containing RCN-Tk- that lacks the inserted antigen. Mean antibody titers to *Y. pestis* F1 and V antigen increased significantly in the immunized groups compared to controls, but titers were significantly higher in those receiving injections of F1-V fusion protein. Even so, upon challenge with 70,000 cfu of virulent *Y. pestis*, the survival rate of the orally immunized group (94%) was significantly higher than the group immunized by injection with 1 or 2 doses of F1-V fusion protein (25% and 58%, respectively) and much higher than the control group (7%). Our findings demonstrate that oral immunization of prairie dogs with RCN-vectored vaccines can provide near full protection against challenge at dosages that simulate simultaneous delivery of the plague bacterium by numerous (3-10) flea bites. The basis for this protection in prairie dogs is unknown at this time, but it did not appear to be correlated to the level of circulating antibodies at the time of challenge.

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THE ROLE OF CARNIVORES IN PLAGUE ECOLOGY AND SURVEILLANCE

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Exposure to plague bacteria (*Yersinia pestis*) by flea-bites or consumption of infected rodents is common in mammalian carnivores in North America. Most carnivore species exhibit seroprevalence rates ranging from 3% to 100% in areas where plague occurs (Salkeld & Stapp, 2006), and because they interact with rodent populations, some carnivores act as good spatio-temporal sentinels of rodent plague activity. For example, swift fox (*Vulpes velox*) seroprevalence strongly correlates spatially with epizootic plague activity in black-tailed prairie dogs (*Cynomys ludovicianus*) colonies in northern Colorado (Salkeld et al., 2007). Coyote (*Canis latrans*) activity was higher on prairie dog colonies than on grasslands based on scat counts between colonies and grasslands. Furthermore, coyotes and swift foxes (*Vulpes velox*) appear to preferentially use prairie dog colonies: rates of removal of rodent carcasses on prairie dog

colonies were significantly greater than on grassland sites where no prairie dogs were found. Therefore, these carnivores may play an important role in transfer of plague between prairie dog colonies. At a larger spatial scale, we compared seroprevalence data of coyotes (*Canis latrans*) and rodents (California ground squirrels, *Spermophilus beecheyi*) in California. Monitoring of plague exposure in ground squirrels is reactive, whereas surveillance of coyote plague exposure is opportunistic. There were no correlations in yearly seroprevalence rates between coyotes and ground squirrels at the state level, or when considering separate Californian bioregions. Nonetheless, both surveillance techniques are valid in highlighting plague activity. Carnivores may only be important in plague ecology as vectors of infective fleas, but animal-to-human (zoonotic) transmission suggests that mammalian carnivores can act as infectious hosts and a review of clinical investigations reveals that plague can be harvested from canid and felid hosts (Salkeld & Stapp 2007). Further study of plague transmission by carnivores in both wild and laboratory conditions is needed to understand the possible role of carnivores as wildlife reservoirs of plague.

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SOCIO-ECONOMIC RISK FACTORS ASSOCIATED WITH HUMAN PLAGUE CASES IN NEW MEXICO

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Plague, caused by the bacterium *Yersinia pestis*, is a zoonotic disease that is rare in the United States, but can be highly fatal in humans, when left untreated. The majority of human cases in recent decades have been acquired in New Mexico. A recently developed habitat suitability model for the disease identified areas which support a diverse assemblage of rodent hosts for plague as those most at risk for human infections in this state (Eisen et al. 2007). Here, we combine known environmental risk factors with socio-economic features of U.S. census block groups in a Geographic Information Systems (GIS) model to further refine the area within New Mexico which poses the highest plague risk for humans. The socio-economic risk factors identified included proportion of housing units with incomplete plumbing, proportion of housing units built 40 or more years prior to a census, and poverty rate. The overall accuracy of our model was about 82%, and reduced the area considered at highest risk from about 17% to between 2 and 7% of New Mexico. This reduction in the area identified as high-risk highlights the potential importance that human behavioral or lifestyle factors play in *Y. pestis* infection risk, particularly when these factors are ones that might influence the likelihood of native rodent species invading peridomestic habitats. Moreover, such integration of environmental and socio-

economic risk factors may aid in the targeting of limited public health resources to areas where prevention and control efforts may be most effective.

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THE ROLE OF ALTERNATIVE RODENT HOSTS IN THE DYNAMICS OF PLAGUE IN BLACK-TAILED PRAIRIE DOG COLONIES

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Plague causes local extinction of colonies of black-tailed prairie dogs (*Cynomys ludovicianus*) in grasslands of eastern Colorado. Although other small rodents may be exposed to the bacterium that causes plague, *Yersinia pestis*, during or after epizootics, the effects of plague on populations of these rodents is not known, and their role in transmitting plague during epizootics, and in maintaining *Y. pestis* in the absence of prairie dogs is unclear. Since 2004, we have studied the ecology of plague in small rodents associated with prairie dog colonies on the Pawnee National Grasslands, Colorado. We live-trapped rodents and collected their fleas in colonies before, during and after epizootics, and swabbed prairie dog burrows for fleas. Serological tests were used to determine exposure of rodents to *Y. pestis*, and PCR-based molecular analyses were used to identify the presence of the bacterium in fleas. Northern grasshopper mice (*Onychomys leucogaster*) were the most numerous species on colonies, representing 36% of individuals captured. Abundance of grasshopper mice was low on sites following epizootics in 2004, and declined markedly (~69%) following the onset of plague on other colonies in 2005. These changes coincided with serological evidence of exposure to *Y. pestis*: 11-23% of grasshopper mice were seropositive during epizootics, indicating that some fraction of the population was resistant to mortality. Declines in grasshopper mice also corresponded to periods when flea loads in prairie dog burrows were extremely high and when grasshopper mice became infested with prairie dog fleas (*Oropsylla hirsuta*), including some that were infected with *Y. pestis*. Additionally, several *Pleochaetis exilis*, a flea found almost exclusively on grasshopper mice and never on prairie dogs, were PCR-positive for *Y. pestis*, indicating that grasshopper mice are capable of infecting their own fleas. In contrast, no changes in thirteen-lined ground squirrel (*Spermophilus tridecemlineatus*) or deer mouse (*Peromyscus maniculatus*) abundance could be attributed to plague, and neither species was seropositive during epizootics, hosted *O. hirsuta* during epizootics, or harbored *Y. pesti*s-infected fleas. In spring 2004, grasshopper mice were most numerous in colonies that suffered plague the following year; logistic regression revealed that grasshopper mouse abundance in 2004, combined with colony area, was a significant predictor of whether a colony suffered plague in 2005. Moreover, the pattern of colony extinctions over a 12-year period mirrored long-term patterns of grasshopper mouse abundance in our study area, suggesting that colonies with high densities of grasshopper mice may be more susceptible to epizootics. We speculate that grasshopper mice help initiate and spread plague during epizootics through their ability to survive *Y. pestis* infection, harbor prairie dog fleas and transport infected fleas among burrows, which functionally connects prairie dog coteries that would otherwise be socially distinct. Although their apparent resistance to plague mortality, combined with the capacity to infect their

own fleas, also supports a possible enzootic role, the fact that we have no evidence of persistent, circulating infections of *Y. pestis* in grasshopper mice suggests that the traditional view of epizootic-enzootic cycles may not apply, and that alternative explanations may be needed to explain the long-term persistence of plague in the Great Plains.

CLIMATE CHANGE AND PLAGUE EPIDEMICS

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The bacterium *Yersinia pestis* causes bubonic plague. A general review of how climate variation may affect plague dynamics is presented. The lecture summarizes a broad spectrum of studies, primarily based upon the analysis of data from Central Asia, data both on the reservoir system as well as data on the human cases. The findings based upon such data from the last few decades are then used to look backward (is there a climate component to the past pandemics?) as well as forward (will there be more or less climate-driven plague in the future?). Published as well as unpublished work will be summarized: in short, climate is concluded to have a driving force on the plague dynamics, but the nature of this does depend upon the geographic location.

SEASONAL AND SPATIAL CHANGES IN FLEA COMMUNITIES OF BLACK-TAILED PRAIRIE DOGS OF NORTHWESTERN MEXICO

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Host population structure and density, and changes in flea community structure and composition, are considered to be important in the dynamics of vector borne diseases in prairie dogs, including plague and tularemia. In Mexico, neither tularemia nor plague is recognized despite the presence of environmental conditions similar to those found in the southern USA where these diseases are frequently reported. We carried out a survey of fleas parasitizing black-tailed prairie dogs at 8 160x160m quadrants in 4 prairie dog colonies in northwestern Chihuahua, Mexico during the dry and rainy seasons of 2006. The colonies of the survey are included at the core of one of the largest black-tailed prairie dog colony complexes in North America, and differ in size, isolation degree of isolation and host density. Fleas were collected directly from the pelage of the trapped prairie dogs, along with some blood samples to search for evidence of plague or tularemia infection. All captured animals were measured and released at the site of capture. We trapped 51 prairie dogs and collected 119 fleas belonging to 5 flea species, including *Echidnophaga gallinacean*, *Pulex simulans*, *Pulex sp*., *Thrassis fotus,* and *Oropsylla hirsuta*. *Pulex* was the dominant genus among the prairie dog colonies surveyed, comprising 57% of the total fleas, followed by *Oropsylla hirsute* with 38%. Seasonal changes in flea community structure were recorded. During the dry season, *Oropsylla hirsuta* was the dominant flea, comprising 42% of all fleas collected, followed by Pulex spp (31 %) and Pulex simulans (24%). During the wet season, *Pulex simulans* dominated the flea community with 70% occurrence, followed by *Pulex* spp. with 20%. The smallest and most isolated colony of prairie dogs exhibited the lowest

diversity of flea species. Changes in flea community structure due to changes in host densities, degree of isolation and changes in flea communities due to seasonal changes may produce changes in vector competence for infectious agents. These changes also may create a different pattern of disease occurrence between prairie dogs colonies from northern Mexico and southern USA. Recognizing the role of flea communities, vector competence and host population structure is the key to understanding and predicting disease outbreaks. Further analyses are needed, including molecular and serologic tests for plague and tularemia in fleas and prairie dogs of northern Mexico. In future studies, we will assess the relationship of flea community dynamics and disease prevalence in both fleas and hosts with different spatial and temporal scales. The results of this research will contribute to the development of predictive models for prairie dogs colonies in northern Mexico.

PLAGUE EPIDEMIOLOGY AND RISK IN THE HETEROGENEOUS RURAL LANDSCAPES OF MADAGASCAR

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Currently Africa faces the greatest threat of human plague outbreaks. Madagascar has one of the highest incidences of human plague in the world, with most cases occurring in rural communities. Rural plague foci in Madagascar are a relatively well-studied epidemiological system and are maintained by populations of black rats (*Rattus rattus*) and two species of flea, *Xenopsylla cheopis* and *Synopsyllus fonquerniei* (Duplantier et al., 2005). Rats are susceptible to infection, suffering high mortality, and hence the distribution of plague is very patchy as infected rat populations are liable to local extinction. Consequently, both dispersal of plague at the regional scale and local transmission from the surrounding habitats to villages appear crucial to disease outbreaks in rats and humans. As *X.cheopis* and *S.fonquerniei* dominate within villages and agricultural areas respectively, both species appear important for plague persistence and transmission to humans. One of the key issues for increased understanding of plague dynamics in Madagascar is how the abundance and movement of rats and vectors influences local disease spread. Two projects are currently investigating how movement patterns of hosts and vectors within and between habitats contribute to disease dynamics and human risk in this system. One project focuses on the rats, whilst the other focuses on the two flea species. Both projects will combine field studies, genetic analyses and epidemiological modelling in order to, first, quantify the typical patterns of rat and vector abundance and movement in plague foci, and, second, determine how these patterns contribute to the transmission of plague. Cross-sectional sampling of rats and fleas is being carried out on 5 occasions during the annual cycle, with 4 villages sampled on each occasion. In each village, traps are set within 3 habitat types (houses, sisal hedges, irrigated lowlands). Flea samples are collected from both trapped rats and burrows. To provide a detailed picture of host and vector movement patterns, microsatellite markers are being used on samples from rats and both flea species. Here we present initial results from the projects and discuss the implications for control strategies in Madagascar.

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ECOLOGY OF RODENTS AND FLEAS ASSOCIATED WITH BLACK-TAILED PRAIRIE DOGS IN AREAS WITH PLAGUE

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The aim of this study was to identify rodent-flea complexes that might be important in the transmission and maintenance of plague in the black-tailed prairie dog (*Cynomy ludovicianus*) ecosystem. We characterized the relationship between fleas and their rodent hosts in the presence of prairie dog colonies (refered to as on-colony grids) and compared them to adjacent assemblages in the surrounding grasslands (refered to as off-colony grids). We evaluated the rodent-flea relationship by quantifying prevalence (proportion of rodents infested with fleas) and estimated the probability of infestation based on observed prevalence, flea load (number of fleas per infested rodent), and mean intensity of fleas on rodents (number of fleas of a given species per infested rodent). Because prairie dog burrows provide refugia for fleas, we hypothesized that prevalence, flea load, and intensity would be higher for rodents that are associated with black-tailed prairie dog colonies. Rodents were trapped at off- and on-colony grids, resulting in the collection of 4,509 fleas from 1,430 rodents in six study areas. The rodent community composition varied between these study areas. Flea species richness was not different between prairie dog colonies and the surrounding grasslands ($p = 0.883$) but was positively correlated with rodent species richness ($p = 0.055$). Prairie dog colonies did not increase the prevalence of fleas ($p > 0.10$). Flea loads on rodents did not vary between off- and on-colony grids at three of the study areas ($p > 0.10$). Based on the prevalence, infestation rates, and flea loads, we identified *Peromyscus maniculatus, Onychomys leucogaster,* and two *Neotoma* species as important rodent hosts for fleas and *Aetheca wagneri, Orchopeus leucopus, Peromyscopsylla hesperomys, Pleochaetis exilis,* and *Thrassis fotus* as the most important fleas associated with these rodents. These rodents and fleas have been implicated in plague (Biggins and Kosoy 2001, Gage and Kosoy 2005) and their presence in areas without a known history of plague suggests that the current distribution of plague is not limited by the distribution of these rodents and fleas. Prairie dog colonies did not seem to facilitate transmission of fleas between rodent hosts, and the few rodent- flea associations exhibited significant differences between off- and on-colony grids. However, the presence of prairie dog fleas on rodents at both off- and on-colony grids suggests the potential for intra and interspecific transmission of fleas between rodent hosts, and between rodents and prairie dogs.

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FLEA LOADS ON BLACK-TAILED PRAIRIE DOGS (*CYNOMYS LUDOVICIANUS*) DURING PLAGUE EPIZOOTICS IN COLORADO

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Plague, caused by the bacterium *Yersinia pestis*, is primarily a disease of wild rodents and their fleas. Black-tailed prairie dogs (*Cynomys ludovicianus*) are highly susceptible and mortality on individual towns often reaches 100%. Flea load, or the number of fleas per host, fluctuates seasonally and transmission of the pathogen during epizootics is likely to become more efficient as flea load increases. Fleas were collected from live-trapped black-tailed prairie dogs on towns before and during plague epizootics and tested by PCR for the presence of *Y. pestis* DNA. The predominate fleas infesting Black-tailed prairie dogs were *Oropsylla hirsuta*, *Oropsylla tuberculata cynomuris*, and *Pulex simulans*, with greatest flea abundance occurring in March and October. Flea load and infestation intensity increased during epizootics and was highest on prairie dogs with *Y. pestis*-infected fleas. The seasonal occurrence of epizootics among black-tailed prairie dogs was found to coincide with seasonal peaks in mean flea load. The ability of individual flea species to transmit *Y. pestis* and the importance of flea-borne transmission during epizootics is discussed.

PLAGUE ACTIVITY IN CALIFORNIA: A SUMMARY OF STATEWIDE PUBLIC HEALTH SURVEILLANCE, 1984-2007

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Yersinia pestis, the causative bacterial agent of plague, entered California by way of infected rats and humans who disembarked at the major port cities during the early 1900s. Over the succeeding decades, plague rapidly adapted to indigenous wildlife and spread throughout the western United States. Since 1930 the California Department of Public Health (CDPH) has maintained an integrated statewide plague surveillance program that encompasses investigations of clinical plague in humans and domestic felids, evaluation of epizootic activity in rodents, and serologic monitoring of wild carnivores. This poster provides a 24-year summary of surveillance data collected in California from 1984 through 2007: Twenty-four human cases of plague occurred from 14 counties, two of which were fatal. *Y. pestis* was isolated from 82 domestic pets in 12 counties. We recorded at least 68 epizootic events among wild rodents in 21 counties. Rodent species most frequently involved in plague maintenance and transmission were California ground squirrels (*Spermophilus beecheyi*), Douglas' squirrels (*Tamiasciurus douglasii*), shadow chipmunks (*Tamias senex*), and yellow-pine chipmunks (*Tamias amoenus*). Carnivores frequently detected with serum antibodies to *Y. pestis* were black bears (*Ursus americanus*), bobcats (*Lynx rufus*), and coyotes (*Canis latrans*). Animals evaluated for plague activity totaled 34,537 and provided 2,355 positive results. Through the use of this cooperative statewide plague surveillance program, CDPH has been able to respond effectively to human cases and epizootic activity when they occur. The program has also enabled CDPH to gain a greater understanding of the epidemiology of the disease.

POPULATION STRUCTURE AND EVOLUTIONARY HISTORY OF *YERSINIA PESTIS* IN NORTH AMERICA

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Yersinia pestis, causative agent of plague, was introduced to North America around 1900. Following its introduction to the native rodent fauna *Y. pestis* spread rapidly across the western United States. *Y. pestis* is now endemic in much of the western United States although it rarely causes disease in humans. Despite its widespread distribution, little is known about the population structure and evolutionary history of *Y. pestis* in North America. We have previously shown that highly mutable genetic markers known as variable-number tandem repeats (VNTRs) can provide unprecedented discrimination among isolates of this pathogen. However, the highly mutable nature of VNTRs makes them especially prone to homoplasy, which can lead to incorrect phylogenetic conclusions, especially at deeper branches. For this reason, use of these markers alone has not provided useful insights into the evolutionary history of *Y. pestis* in North America. Single nucleotide polymorphisms (SNPs) mutate very slowly compared to VNTRs and are rarely subject to homoplasy. As a result, SNPs are very useful for defining major phylogenetic patterns within recently evolved pathogen species, such as *Y. pestis*. We will present preliminary results from our analysis of more than 700 North American isolates using SNP markers. We find evidence of rapid initial spread of *Y. pestis* across North America and two potential introduction events.

EVOLUTION OF RESISTANCE IN NATURAL HOSTS OF PLAGUE

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Prairie dogs exhibit extremely high mortality when infected with plague. Given this strong selection pressure and experimental and correlative evidence for genetic variation in plague resistance (Gage and Kosoy 2005), why have prairie dogs not evolved higher levels of resistance? We use a stochastic simulation modeling approach to investigate this question for prairie dogs specifically and for small mammal hosts more generally. Our results suggest that host species with initially low resistance are so maladapted that they cannot evolve higher levels of resistance because of their susceptibility to extinction from demographic stochasticity. However, host species with higher initial resistance can evolve still higher level of resistance. This result is consistent with evolutionary theory (Gomulkiewicz and Holt 1995) that posits that natural selection can prevent extinction in populations faced with novel selection if the risk of extinction from demographic stochasticity is low. These results also have important implications for wildlife vaccination strategies for prairie dogs. Naively, we might expect a cost of resistance to exist. If prairie dogs could evolve resistance, under a cost scenario, vaccination would reduce selection pressure by plague and contribute to the decay of natural resistance. Instead, because prairie dogs appear unable to evolve higher levels of resistance, partial vaccination could raise the population above the threshold for extinction due to demographic stochasticity and thus actually enhance evolution of natural resistance in prairie dogs.

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FATAL PRIMARY PNEUMONIC PLAGUE CONTRACTED FROM A MOUNTAIN LION CARCASS

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Background: Primary pneumonic plague is a rare manifestation of *Yersinia pestis* infection in humans characterized by a high fatality rate and the potential for person-to-person spread. We describe the first wild carnivore-associated case of primary pneumonic plague, which occurred in a wildlife biologist at Grand Canyon National Park (GCNP), Arizona. The biologist was found deceased in his residence on November 2, 2007, one week after performing a necropsy on a mountain lion (*Felis concolor*) carcass and 3 days after being seen and discharged from the GCNP clinic with a one-day history of fever/chills and cough with blood-tinged sputum. The biologist's work duties included trapping and collaring mountain lions and removing rodents from public buildings. **Methods:** An unexplained death investigation, including post-mortem examination of the biologist's body, was initiated on November 2. Persons in close contact (within 2 meters) to the biologist after he had developed symptoms were identified and offered chemoprophylaxis. Documents and physical evidence were reviewed, including archived specimens of the mountain lion carcass and the biologist's medical chart, camera, and work computer. Human and mountain lion tissues were submitted for testing. Local/regional zoonotic plague activity was assessed. **Results:** Photographs document that the biologist conducted the necropsy with bare hands and likely without the use of masks or other personal protective equipment (PPE). Gross examination of the biologist's body showed multi-focal areas of consolidation and large amounts of fluid in both lungs (right lung, 1.4 kg; left lung, 1.05 kg); no buboes or other abnormalities were noted. A presumptive diagnosis of plague was made on November 6 based on positive polymerase chain reaction (PCR) testing on specimens from the biologist's lung, liver, and spleen. The diagnosis was later confirmed on November 14 by culture recovery of *Y. pestis*. On November 9, PCR samples from the mountain lion's liver and sub-mandibular lymph node tested positive for *Y. pestis*. On November 16, pulsed field gel electrophoresis (PFGE) testing determined that the human and mountain lion strains of *Y. pestis* were indistinguishable and supported epidemiologic evidence that the mountain lion was the source of the biologist's infection. Among 49 contacts who received chemoprophylaxis, none developed symptoms consistent with plague. Though a plague epizootic in prairie dogs was reported in Flagstaff, AZ, in September 2007, unusual mortality was not observed among rodents at GCNP. **Conclusions:** Based on clinical and laboratory evidence, we believe that the biologist died from primary pneumonic plague acquired after inhaling infectious droplets aerosolized during the necropsy of the mountain lion carcass. Though this case illustrates a highly unusual

exposure for pneumonic plague, the biologist's death underscores the need for clinicians to maintain a high index of suspicion for zoonotic diseases, particularly in endemic areas, and to inquire about potential risks faced by biologists and other persons who handle wildlife (e.g. hunters/trappers, taxidermists). Enhanced awareness/recognition of zoonotic diseases and guidelines on the appropriate use of PPE are also needed for persons handling wildlife.