

sented in mean  $\pm$  standard deviation or percentage when appropriate. Statistical analysis was performed by SPSS Windows version 12.0 (SPSS Inc., Chicago, IL, USA) using the  $\chi^2$  test; p value was set at 0.05.

Two hundred thirty-four of 252 second- and third-year medical students completed the questionnaire. The mean age of the respondents was  $19 \pm 0.87$  years (range 18–23). Twenty-nine percent ( $n = 68$ ) of the students were male and 71% ( $n = 166$ ) were female.

The mean knowledge score was 4.76 of 18 (total of correct and incorrect responses) (range –6 to 11). Second- and third-year students comparably responded to 16/18 questions ( $\chi^2$  test). A list of questions and the percentage of students' responses are provided in the Table.

Most of the respondents (67.2%) indicated that mass media (radio, television, and newspapers) was their major source of information about avian influenza, followed by scientific books and journals (8.3%), the Internet, (13%), and family and friends (10.4%). Only 1.1% of the medical students mentioned "school educational materials" as the source of their information.

Our study shows a relatively low level of knowledge of avian influenza among a group of Iranian medical students. Surprisingly, mass media was the main source of information in our study. Training health care professionals as well as medical students is of great importance in controlling infectious diseases. The findings of this study should be considered seriously by local health centers and disease control agencies because preparing health care professionals with sufficient knowledge is essential to confronting a potential pandemic. We believe that the low level of knowledge about avian influenza among these medical students is primarily a reflection of insufficient academic courses in the medical school curriculum.

We strongly recommend improving the quality of education on this topic through access to textbooks, articles, seminars, and specific courses.

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## Lorraine Strain of *Legionella pneumophila* Serogroup 1, France

**To the Editor:** Legionellosis is a pneumonia caused by inhalation of *Legionella* spp. in aerosol water particles. *Legionella pneumophila* is responsible for  $\approx 90\%$  of cases; serogroup 1 alone accounts for  $\approx 85\%$  of cases (1). Epidemiologic analyses based on pulsed-field gel electrophoresis (PFGE) and sequence-based typing of clinical isolates of *L. pneumophila* serogroup 1 have detected sporadic, epidemic, and endemic strains (2). Most cases

are sporadic and are associated with strains that have not been identified. A strain is considered endemic to an area when several isolates that have identical PFGE patterns and that cause several epidemiologically unrelated cases of legionellosis are detected in that area. Since 1998, the most prevalent strain endemic to France has been the Paris strain (3), which was responsible for 12.2% of culture-confirmed cases of legionellosis from 1998 through 2002 (3). The Paris strain has also been detected in clinical samples from several other European countries (Switzerland, Italy, Spain, and Sweden) and in environmental samples (3,4).

We identified a new endemic clone of *L. pneumophila* serogroup 1, the Lorraine strain, and report its spread throughout France. The French national reference center for *Legionella* collects all clinical isolates of *Legionella* spp. as part of an epidemiologic surveillance system. All *L. pneumophila* serogroup 1 isolates are typed by PFGE methods as described (4). When necessary, sequence-based typing (5,6) and monoclonal antibody-based (MAb) subgrouping are also used (7).

From 1995 through 2006, the reference center typed 1,768 clinical *Legionella* isolates by means of PFGE. Most PFGE patterns were unique and thus corresponded to sporadic cases. Another 145 (8.2%) patterns were identical and corresponded to the endemic Paris strain. An identical PFGE pattern was also found for 80 (4.5%) isolates from epidemiologically unrelated patients; these isolates were further characterized by sequence-based typing and MAb subgrouping. Sequence type was deduced for the following genes: *flaA*, *pilE*, *asd*, *mip*, *mompS*, *proA*, and *neuA* (6). The sequence type was obtained for 78 of the 80 isolates and was 5, 10, 22, 15, 6, 2, 6. The sequence type of the remaining 2 isolates differed from that of the other 78 by 2 alleles (*pilE* and *proA*) and was 5, 1, 22, 15, 6, 10, 6

(**boldface** indicates differences). All but another 2 isolates (which belonged to the Benidorm subgroup) belonged to the Allentown MAb subgroup. Hence, the new endemic strain, Lorraine, was represented by 76 isolates that had an identical PFGE pattern, sequence type, and MAb subgroup.

Isolation of the Lorraine strain was reported anecdotally before 2002. Since 2002, the prevalence of this strain in France has increased considerably, accounting for 10.5% clinical isolates in 2005 and 9.0% in 2006 (Figure). In contrast, prevalence of the Paris strain was  $\approx$ 10% from 1998 through 2002 and peaked in 2000 (16.9%) in association with a hospital outbreak in Paris. From 2003 through 2006, prevalence of the Paris strain fell to  $\approx$ 6.5%.

The Lorraine strain has caused 2 outbreaks. In the first, 3 isolates were recovered from respiratory samples of 34 patients for whom legionellosis was diagnosed by urinary antigen testing in Lyon in 2005. The second outbreak occurred in a western suburb of Paris in 2006, when 1 isolate was cultured from respiratory samples of 12 patients whose diagnoses were also made by urinary antigen testing.

From 1995 through 2006, >4,000 environmental *Legionella* isolates in France were typed by PFGE, and >700 types were identified. The Paris strain type was identified >500 times, but the Lorraine type was identified in only 3 water samples, including 1 from the cooling tower responsible for the outbreak in the Paris suburb. The Lorraine strain is thus rarely found in water samples, which hinders environmental investigations of its sources in outbreaks of legionellosis.

A similar disparity between the clinical and environmental distribution of *Legionella* strains has been reported (8). In a collection of 284 unrelated clinical isolates and 117 unrelated environmental isolates, Harrison et al. found that 3 types, identified by restriction fragment length polymorphism, accounted for 40% of clinical isolates but only 18% of environmental isolates (8).

The high prevalence of the Lorraine strain in clinical samples and its extremely rare detection in water samples have several possible explanations: 1) this strain could be related to specific host factors; 2) it could be highly virulent even in low amounts, below the culture detection limit; and

3) it could be more susceptible than other strains to different stressors (e.g., biocide treatment, selective preplating techniques, environmental medium specific components).

In conclusion, prevalence of a new *L. pneumophila* serogroup 1 strain, Lorraine, endemic to France, is increasing in clinical samples although rarely detected in water samples. The type strain, Lorraine (CIP108 729), is available from the strain collection of the Pasteur Institute (Paris, France).

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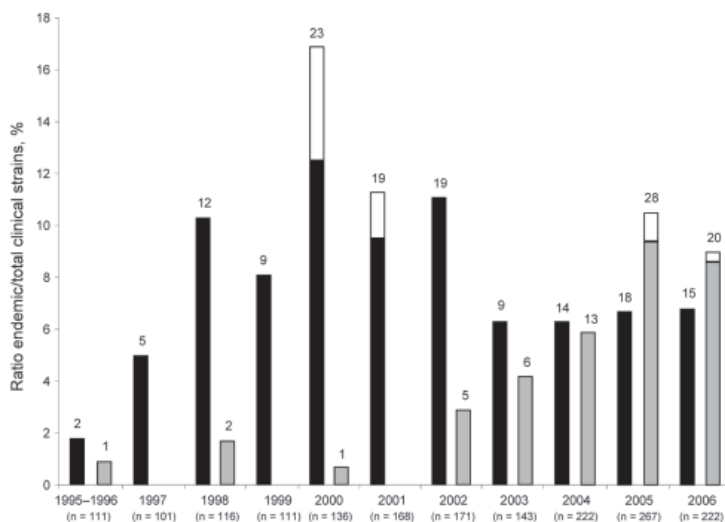


Figure. Prevalence of the *Legionella pneumophila* Paris (black bars) and Lorraine (grey bars) endemic strains, France, 1995–2006. White bar sections represent the proportion of strains isolated during outbreaks. For example, in 2000 the Paris strain accounted for 16.9% of clinical isolates: 12.5% unrelated and 4.4% related to the same outbreak. Numbers above each bar indicate the number of isolates.

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Yaks living in captivity in a Belgian animal park showed clinical signs of BT. A clinical examination performed on 1 yak showed loss of weight associated with a progressive weakness linked to anorexia, ulcerative and necrotic lesions on the muzzle with some crusts and mucopurulent nasal discharge, and udder erythema with papules and crusts. The tongue was severely swollen and cyanotic and protruded from the mouth (Figure). The animal was reluctant to move and was recumbent (possibly as a consequence of podal lesions linked to BT); it died 7 days after examination. Necropsies were performed on carcasses of this and another yak. The main lesions found were severe diffuse congestion of the lungs with edema and emphysema, acute hemorrhagic enteritis restricted to the ileum and jejunum, and petechial hemorrhages on the abomasums. No lesions characteristic of coronitis were noted.

Samples of spleen and bone marrow were taken and prepared according to the method of Parsonson and McColl (4). A real-time reverse transcription quantitative-PCR (RT-qPCR) targeting BTV segment 5 (RT-

qPCR\_S5) was used to detect BTV RNA in tissues samples. Each test was performed in parallel with a RT-qPCR that amplifies  $\beta$ -actin mRNA as an internal control (RT-qPCR\_ACT). Both assays were conducted according to Toussaint and others (5), with slight modifications. Briefly, total RNA was purified from 25 mg of tissue by Trizol extraction (Invitrogen, Carlsbad, CA, USA) and denatured by heating for 3 min at 95°C with 10% dimethylsulfoxide (Sigma-Aldrich, St. Louis, MO, USA). Reverse transcription reactions were conducted by using the Taqman reverse transcription reagents according to the manufacturer instructions (Applied Biosystems, Foster City, CA, USA). RT-qPCR reactions consisted of 1× concentrated Taqman fast universal PCR master mix (Applied Biosystems), 375 nM ( $\beta$  actin) or 500 nM (BTV) of each primer, 250 nM Taqman probe, and 5  $\mu$ L cDNA. Cycling conditions were as follows: 1 cycle at 95°C for 20 s, followed by 45 cycles of 1 s at 95°C, and 20 s at 60°C. The specificity of the RT-qPCR used had been previously tested against prototype strains of genetically related viruses (9 strains of epizootic hemorrhagic disease virus

## Bluetongue in Captive Yaks

**To the Editor:** In August 2006, several northern European countries including Belgium reported cases of bluetongue (BT) (1). This noncontagious, arthropod-borne animal disease is caused by *Bluetongue virus* (BTV), genus *Orbivirus*, family *Reoviridae*. The genome of BTV consists of 10 segments of double-stranded RNA; 24 serotypes have been reported (2). Serotype 8 (BTV-8) was implicated in the emergence in Belgium (3). All ruminant species are thought to be susceptible to BT (2). We report laboratory-confirmed clinical cases of BT in yaks (*Bos grunniens grunniens*).



Figure. A captive yak infected with bluetongue virus. Tongue is swollen, cyanotic, and protruding from the mouth.