

Screening for Chagas disease should be recommended to all Latin American migrants, especially those from Bolivia. This screening would enable early treatment for persons in the chronic asymptomatic phase or those with mild cardiac involvement, persons for whom treatment has been recommended (9).

Current legislation in Spain makes screening all at-risk blood donors mandatory (10). However, screening of pregnant women from Chagas disease–endemic countries is not compulsory, although 46.8% of immigrants in Spain are female and birth rates in this group are higher than the national average for Spain (5). Detection of antibodies to *T. cruzi* during pregnancy would also be a useful public health strategy because it would enable early specific treatment of affected newborns. Screening of blood or organ donors would also be necessary in countries where there is no transmission by vectors.

T. cruzi infection may become a public health problem in countries in Europe that receive immigrants from disease-endemic areas. Thus, chagasic cardiomyopathy may soon have a serious effect on public health in Spain.

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Lethal Bluetongue Virus Serotype 1 Infection in Llamas

To the Editor: Since 1999, of the 24 known bluetongue virus (BTV) serotypes, five (1, 2, 4, 9, and 16) have spread extensively throughout portions of the Mediterranean basin. Since 2006, the range of serotype 8 (BTV-8) has extended northward into areas of Europe never before affected, causing the greatest epizootic of the disease on record. In 2008, a severe epizootic of serotype 1 (BTV-1) occurred in southwestern France; >3,340 outbreaks occurred in <4 months. We report 2 of these outbreaks, which indicate that BTV-1 can infect llamas and induce a lethal disease.

The first outbreak occurred in September 2008 on a sheep breeding farm in Crampagna, France. After bluetongue disease was suspected on the basis of clinical signs, all sheep and all 9 healthy llamas on the farm were tested for BTV by quantitative real-time reverse transcription–PCR (qRT-PCR) (Taqvet BTV “All genotypes,” LSI, Lissieu, France). Of the 9 llamas, 7 had positive results; cycle threshold (Ct) values ranged from 28.1 to 36.2, indicating that these animals were sensitive to BTV infection. Serotype 1 was confirmed by a specific qRT-PCR. After 6 weeks, all infected llamas showed serologic response to BTV (ELISA ID Screen; Bluetongue, ID-Vet, Montpellier, France), and 1 llama remained positive by qRT-PCR, which suggests that viremia lasted at least 42 days in this animal. None of the 9 llamas showed clinical signs during the outbreak.

The second outbreak, also during September 2008, was identified on a llama breeding farm in Auzat, France. Two days before the onset of the disease, 1 female had aborted a 10-month-old fetus; she had no additional clinical signs. Of the 20 llamas on the farm, clinical signs were observed for

1 female and 1 male. The signs were anorexia and lethargy followed by acute respiratory distress with polypnea, dyspnea, hiccup-like breathing, and edema. The legs of the male became rigid with moderate paresis. Both animals died <24 hours after the onset of respiratory signs.

Postmortem examination of each of the 2 dead llamas 6–16 hours after death showed similar results: acute and severe hydrothorax, severe congestion and edema of the lungs, and fibrinous pericarditis. Histopathologic examination showed diffuse and severe congestion of the lungs, heart, and kidneys. Virus detection by qRT-PCR was positive for all postmortem tissue samples (spleen, lungs, kidneys, heart); Ct values ranged from 23.5 to 33.7. A 7-cm-long fetus that had been in utero in the female was also positive by qRT-PCR (Ct 38.6). From the aborted fetus, viral RNA was detected in the heart (Ct 39.4) and spleen (Ct 38.3); the blood of the female that had aborted was also positive. Infectious BTV was isolated (in a BioSafety Level 3 laboratory) from the lungs and the spleen of the 2 dead llamas and from both fetuses after 4–6 passages on baby hamster kidney–21 cells (ATCC-CCL10). The specificity of the cytopathic effect was confirmed by BTV-1 qRT-PCR and immunoperoxidase detection using a rabbit polyclonal serum directed against the VP7 viral protein.

The natural host range of BTV is limited to domestic and wild ruminants (1,2), although seroconversion with no disease in carnivores (3) and BTV-8 lethal disease in Eurasian lynx (4) have been reported. To date, serologic prevalence of BTV has been demonstrated in alpacas (5) but not in llamas or in guanacos (6,7). South American camelids have been considered to be resistant to the disease, although lethal bluetongue infection in 1 alpaca was suggested in a recent report (8).

Our isolation of the virus and detection of large amounts of viral genomes in blood and postmortem samples suggest lethal BTV-1 infection in llamas. In contrast to the clinical signs of paresis, acute respiratory distress syndrome was not frequently reported in domestic ruminants during the BTV-1 epizootic. Nevertheless, pulmonary edema is considered to be a feature of severe bluetongue disease because the lungs of ruminants are most susceptible to permeability disorders of the vasculature after BTV infection (9,10). Finally, bacterial isolation attempts from the lungs of the 2 llamas that died and PCR tests for bovine viral diarrhea virus and ovine herpesvirus type 2 were all negative. Thus, results of gross examination, histopathologic examination, and virus detection and isolation from postmortem samples indicate that BTV-1 infection was responsible for the death of the 2 llamas.

During the first outbreak, high prevalence of subclinical BTV-1 infection in llamas was detected. BTV-1 was also detected by qRT-PCR in 50% of the healthy llamas tested in the second outbreak and in 60% of those tested in a recent third outbreak (Escalquens, France, November 2008). Healthy llamas in northern France were also found to be positive for BTV-8 (B. Giudicelli, pers. comm.). BTV-1 isolation from 2 fetuses indicates that the strain currently circulating in southwestern France is competent to cross the placental barrier. These findings clearly indicate that llamas are currently infected with BTV-1 and that, although infrequently, the disease can be fatal.

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Spotted Fever Group *Rickettsia* sp. Closely Related to *R. japonica*, Thailand

To the Editor: In response to a recent report that suggested human infection with *Rickettsia japonica* in northeastern Thailand (1), we phylogenetically reexamined spotted fever group rickettsiae (SFGR) from Thailand. The organism had been isolated from a male *Haemaphysalis hystricis* tick found on Mt. Doi Suthep, Chiang Mai, northern Thailand, in December 2001. The strain was designated TCM1 and was not distinguishable from *R. japonica* by indirect immunoperoxidase stain using monoclonal antibody (2).

After propagating strain TCM1 in L-929 cell culture, we extracted DNA by using a Wizard Genomic DNA Purification Kit (Promega, Madison, WI, USA). We subjected the DNA to sequencing that targeted a 491-bp fragment of rickettsial outer membrane protein A (*ompA*), a 394-bp fragment of the rickettsial genus-specific 17-kDa antigen gene, and a 1,250-bp fragment of citrate synthase gene (*gltA*). Direct sequencing of amplicons was performed as previously described (3). Phylogenetic analyses based on

ompA indicated that strain TCM1 was closely related to and clustered within the same clade as *R. japonica* strain YH (98.4% identity) (Figure, panel A). Also, a 17-kDa antigen gene obtained from strain TCM1 showed 99.5% identity to the corresponding gene of *R. japonica* (Figure, panel B). Our phylogenetic analysis with *ompA* and 17-kDa antigen gene showed that strain TCM1 was closely related to *R. japonica* but distinguished from *Rickettsia* sp. PMK94 (which was closely related to *R. heilongjiangensis* from northeastern China) (3); another SFGR

agent, *R. honei* from *Ixodes granulatus* ticks in Thailand (4), was apparently different from strain TCM1 (Figure). Phylogenetic analyses based on *gltA* (99.4%–99.6% identity) showed that strain TCM1 is also closely related to *R. japonica* and *Rickettsia* sp. strain PMK94 (data not shown). Thus, we describe the *R. japonica* group in Thailand. DNA sequences of strain TCM1 were determined and deposited in GenBank/EMBL/DBJ under the following accession nos.: *ompA*, AB359459; 17-kDa antigen, AB359457; *gltA*, AB359458.

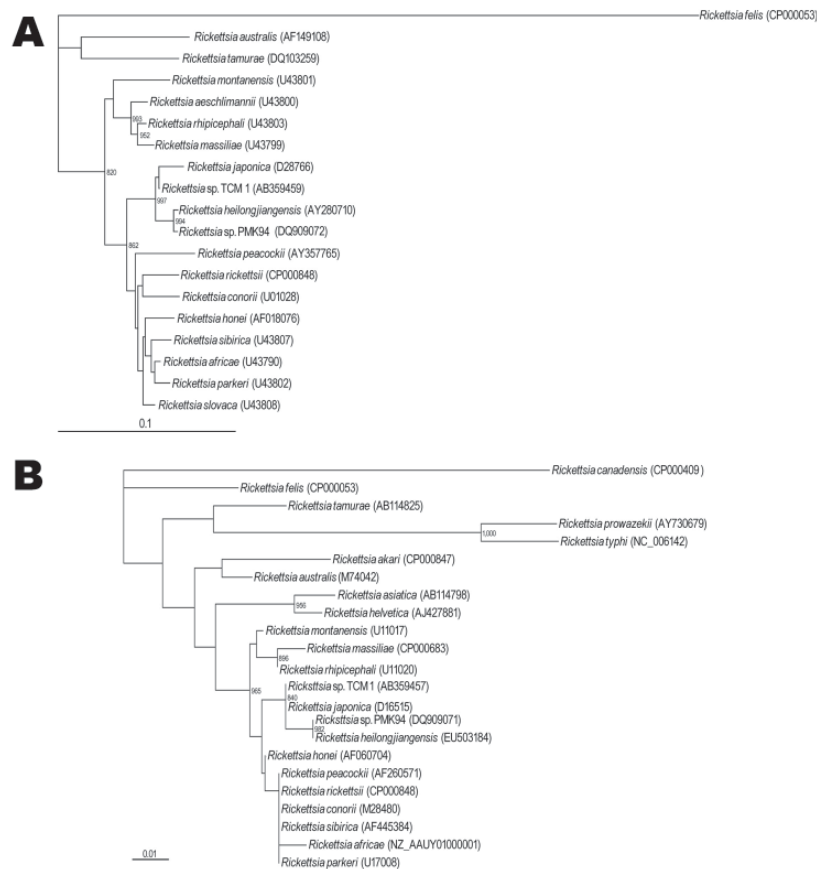


Figure. Phylogenetic analysis based on *ompA* gene (A) and rickettsial genus-specific 17-kDa antigen gene (B). Sequences were aligned by using the ClustalW software package (<http://clustalw.ddbj.nig.ac.jp/top-j.html>), and neighbor-joining phylogenetic tree construction and bootstrap analysis were conducted according to the Kimura 2-parameter method (www.ddbj.nig.ac.jp). Pairwise alignments were performed with an open-gap penalty of 10, a gap extension penalty of 0.5, and a gap distance of 8. Multiple alignments were also performed with the same values, and the phylogenetic branches were supported by bootstrap analysis with 1,000 replications (>800 were indicated). *Rickettsia felis* (CP000053) and *R. canadensis* (CP000409) were used as outgroups for *ompA* and 17-kDa antigen gene, respectively. The phylogenetic tree was constructed by using TreeView software version 1.5 (<http://taxonomy.zoology.gla.ac.uk/rod/treeview.html>). Scale bars indicate nucleotide substitutions (%) per site.