

## Acknowledgments

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## Unexpected *Brucella suis* Biovar 2 Infection in a Dairy Cow, Belgium

**To the Editor:** Belgium was declared free of bovine brucellosis by the European Union in 2003 (<http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2003:156:0074:0078:EN:PDF>). To maintain this status, the Federal Agency for the Safety of the Food Chain implemented a monitoring program, approved by the European Union, that consists of random serologic surveys and mandatory reporting of spontaneous abortion. This reporting enabled the detection of 2 outbreaks of bovine brucellosis in cattle caused by *Brucella abortus* biovar 3, in 2010 and 2012, but the origin of these outbreaks has not been identified.

As part of an epidemiologic survey conducted to prevent the spread of the infection, ELISA testing (Brucellosis Antibody Test Kit; Idexx, Hoofddorp, the Netherlands) was performed on bulk milk samples from 9,013 dairy farms in the country; 75 farms had positive test results and were classified as reactor farms. All cows in

milk production on these farms were serologically tested, first by using slow agglutination test with the addition of EDTA to the antigen, and then, if results were positive, by a commercial ELISA. If results of the ELISA were positive, a confirmatory internal ELISA was performed at the national reference laboratory. A total of 41 seropositive cows from 27 farms were identified. All confirmed seropositive cows were slaughtered for bacteriologic investigation; all had negative test results for *B. abortus*.

On March 23, 2012, bulk milk sample testing for a farm in the province of Namur showed positive results. Testing performed in January 2011 on milk collected from the same farm had yielded negative results. The 150 cattle (including 55 dairy cows) on this farm were further serologically tested. One nonpregnant dairy cow had positive test results by slow agglutination test and ELISAs and was slaughtered on April 23, 2012. The cow was >4 years old, born in the farm, last calved in March 2011, and showed no clinical sign of brucellosis.

Bacteriologic examination was conducted on spleen, uterus, lymph nodes, and udder tissue samples; *Brucella* spp. were cultured from the spleen and uterus. Bacterial colonies grew on *Brucella* agar supplemented with 5% horse serum in the presence of basic thionine and safranin O; CO<sub>2</sub> was not required for growth, and H<sub>2</sub>S was not produced. The isolates showed catalase, oxidase, and urease activity, a biochemical profile typical of *B. suis* biovar 2; identity was confirmed by real-time PCR on DNA extracted directly from the uterus (1).

A stamping out with compensation policy was implemented for this farm by the Federal Agency for the Safety of the Food Chain, according to European Union regulations, and subsequent epidemiologic investigations were performed. The farm owner is not a hunter. The culture-positive cow originated from a group of 10

nonpregnant or dry dairy cows that had been held in the same pasture, distant from the main farm structures, during October 15–November 15, 2011; during the stamping out process, a second dairy cow from this group had a positive test result by ELISA.

Hunting of wild boar (*Sus scrofa*) had been organized during September–December 2011 in the adjacent forest, and wild boar offal was discarded in a corner of the pasture, with no biosecurity precautions. A recent study confirmed the high prevalence of *B. suis* biovar 2 infection in wild boars in this province (2). These findings suggest that these animals were naturally infected with *B. suis* biovar 2; because of the period between infection and testing, the results indicate that antibodies can be detected in cattle by ELISA performed on milk or serum >16 weeks after infection.

Blood samples were taken from the farmer, his wife, and their 2 children, all of whom regularly consumed raw milk. No clinical signs or symptoms suggestive of brucellosis were reported, and slow agglutination test results for all family members were negative (titer <160), which suggests they had no exposure to *B. suis* biovar 2 (3). A total of 111 cattle carcasses, including that of the second seropositive cow, were sampled at the abattoir, and all other samples were negative for *Brucella* spp.

Our findings indicate that preventive measures against the spread of pathogens such as *Brucella* spp. must be implemented by hunters (i.e., awareness campaigns, biosecurity education, and responsible hunting practices). In addition, biochemical typing of *Brucella* spp. is necessary to trace the source of infections (4,5), and epidemiologic inquiry of positive test result(s) should be conducted to identify or exclude bovine brucellosis and to investigate possible *B. suis* biovar 2 infections. Our bacteriologic results (absence of isolation of *B. suis* biovar 2 from all samples collected

at the abattoir) suggest that stamping out is not necessary because *B. suis* biovar 2 is not likely to be transmitted between cattle because they are spill-over hosts, not preferential hosts for *B. suis* biovar 2, and are thus not likely to sustain the infection. Finally, from a veterinary public health perspective, *B. suis* biovar 2 has a low residual pathogenicity in humans (5,6).

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## Hepatitis E and Lymphocytic Leukemia in Man, Italy

**To the Editor:** Hepatitis E is an enterically transmitted infection with worldwide distribution and high prevalence in developing countries. This disease can occur as large water-borne epidemics associated with hepatitis E virus (HEV) genotypes 1 and 2. Hepatitis E is less common in industrialized countries, including Italy (1), where sporadic autochthonous cases associated with genotypes 3 and 4 have been reported. Virus strains of these genotypes are widespread in different mammalian species, including wild boar (2).

We report a case of hepatitis E in a 60-year-old man born and living in Vicenza, Italy, who was admitted to the Emergency Department of Vicenza Hospital on May 9, 2012 with symptoms of acute icteric hepatitis. He had been given a diagnosis of chronic lymphocytic leukemia and hemolytic anemia in 2003 and underwent 8 treatment cycles of cyclophosphamide and steroids, which were completed 20 days before he came to the Emergency Department.

His liver function test results at admission were the following: alanine aminotransferase 1,804 IU/L, total bilirubin 24.1 mg/dL, and alkaline