Susceptibility of cattle to infection with *Ehrlichia equi* and the agent of human granulocytic ehrlichiosis

Nicola Pusterla, DVM, MedVet; Randall J. Anderson, DVM, MPVM; John K. House, BVMS, PhD, DACVIM; Jeannine Berger Pusterla, DVM, MedVet; Elfriede DeRock; John E. Madigan, DVM, MS, DACVIM

**Objective**—To determine susceptibility of cattle to infection with *Ehrlichia equi* and the agent of human granulocytic ehrlichiosis (HGE).

**Design**—Experimental disease and prevalence survey.

**Animals**—6 cattle, 2 horses, and 2,725 serum samples from healthy cattle.

**Procedure**—2 cattle and 1 horse were inoculated with *E equi*; 2 cattle and 1 horse were inoculated with the HGE agent, and 2 cattle served as sham-inoculated controls; inoculated animals were evaluated via clinical, hematologic, serologic, and real-time polymerase chain reaction tests. Prevalence of antibodies against *E equi* in 2,725 healthy cattle was determined by use of an indirect immunofluorescent technique.

**Results**—No abnormal clinical or hematologic findings or inclusion bodies within granulocytes were observed in the cattle after inoculation, and results of polymerase chain reaction tests were negative. Serocconversion in inoculated cattle developed 10 to 12 days after inoculation (reciprocal titer, 160). Both horses developed clinical signs of ehrlichiosis. Five of 2,725 (0.18%) cattle were seropositive for *E equi*, with titers ranging from 20 to 80. All seropositive cattle originated from the same tick-rich region in the Sierra Nevada foothills.

**Conclusions and Clinical Relevance**—Results suggest that cattle are not susceptible to infection with *E equi* or the agent of HGE and that prevalence of exposure to *E equi* in healthy cattle is low. Therefore, *E equi* and the agent of HGE are likely of negligible importance for cattle in North America. (J Am Vet Med Assoc 2001;218:1160–1162)

*xodes pacificus* is the most commonly encountered *Ixodes* tick species in California and is the vector of 2 closely related *Ehrlichia* spp: *E equi* (the cause of equine granulocytic ehrlichiosis) and the agent of human granulocytic ehrlichiosis (HGE). These agents are members of the *E phagocytophila* genogroup, which also includes *E phagocytophila*, the cause of tick-borne fever in goats, sheep, and cattle in Europe. Each of these 3 *Ehrlichia* spp infects a different host species, has a different geographic distribution, and may cause different clinical signs. However, research indicates that on the basis of 16S rRNA gene analysis, they are nearly identical species.

Although large numbers of cattle in California are commonly bitten by adult *I pacificus* ticks that are potentially infected with *E equi* or the agent of HGE, to the authors’ knowledge, disease in cattle attributable to these agents has not been reported in the United States. Possible explanations could be that these agents are not pathogenic for cattle or that the disease is self-limiting. However, it is possible that the disease is prevalent and responsible for fever and nonspecific clinical signs in cattle without having been diagnosed. Therefore, the purposes of the study reported here were to determine susceptibility of cattle to infection with *E equi* and the agent of HGE and determine prevalence of antibodies against *E equi* in healthy cattle from California.

**Materials and Methods**

Experimental study—Six healthy 15-month-old steers and two 4-year-old horses were used. The animals were raised and kept in a vector-free environment and determined to be seronegative for antibodies against *E equi* by use of an indirect immunofluorescent assay (IFA) prior to inoculation. Procedures for inoculation and care of the animals were approved by the Animal Care and Use Committee at the University of California, Davis.

Steers 1 and 2 were inoculated IV with 1.5 × 10⁷ equine leukocytes (78%) infected with *E equi* (MRK strain). Steers 3 and 4 were inoculated IV with 1 × 10⁷ human leukemia (HL)-60 cells (99%) infected with HGE agent (Webster strain). Steers 5 and 6 served as negative controls and were inoculated with 1.5 × 10⁷ uninfected equine leukocytes and with 1 × 10⁷ uninfected HL-60 cells, respectively. The 2 horses served as positive controls and were inoculated IV with *E equi*-infected leukocytes (horse 1) and HGE agent-infected HL-60 cells (horse 2). Animals were monitored daily for clinical signs of illness. Blood samples were collected for hematologic, serologic, and molecular examinations beginning on the first day of experimental inoculation and every day thereafter for 30 days. The Hct, erythrocyte, platelet, and leukocyte counts were determined. Blood smears were prepared and stained with modified May-Grünwald Giemsa stain; 1,000 leukocytes were examined for characteristic inclusion bodies (morulae) at 1,000× magnification to determine the number of infected cells. An IFA was used to test for antibodies against *E equi* in serum samples, as described. A real-time polymerase chain reaction (PCR), specific for the *E phagocytophila* genogroup, was used to detect ehrlichial DNA in circulating leukocytes; DNA was extracted from blood leukocytes by use of a DNA extraction kit, according to manufacturer’s instructions.
Serologic study—Blood samples were collected at random from 2,725 healthy adult cattle during the spring of 1999 by field veterinarians performing routine health care. The cattle originated from 111 ranches (mostly dairies). The cattle had access to local pastures and had contact with ticks. Distribution of the cattle throughout California was as follows: coastal ranges (n = 308 cattle), Sierra Nevada foothills (460), central valley (1,621), and southern California (336). Mean ± SD number of cattle tested per ranch was 24.5 ± 4.3.

An IFA that was validated for cattle by use of *E phagocytophila* and *E equi* as antigens was used to test for antibodies against *E equi*. The IFA is specific for *E phagocytophila*, *E equi*, and the HGE agent without cross-reactions against related bovine pathogens such as *Coxiella burnetii*, *Brucella abortus*, *Anaplasma marginale*, *Babesia bovis*, and *B divergens*. Serum samples from cattle naturally and experimentally infected with *E phagocytophila* and serum samples from cattle without reported tick exposures have been used to determine that the IFA is sensitive at a titer ≥ 20. Therefore, all test sera were screened at a dilution of 1:20, and those with positive results were titrated to end point. Positive results were confirmed by use of a commercial membrane-based immunoassay, according to the manufacturer’s recommendations. Positive control sera were obtained from cattle with induced *E phagocytophila* infection.

**Results**

No abnormal clinical signs were found in steers 1 to 4 during the study period, and results of hematologic analyses remained within reference ranges. *Ehrlichia* organisms were not observed in leukocytes of blood smears, and ehrlichial DNA was not detected via real-time PCR. However, seroconversion developed in steers 1 to 4 at 10 to 12 days after inoculation, with reciprocal titers ≥ 20. The titers continued to increase in the 4 steers, with maximum reciprocal values of 80 to 160 observed 30 days after inoculation. Inoculation of uninfected cells into steers 5 and 6 did not induce any abnormal findings.

Both horses developed clinical signs (fever, anorexia, signs of depression, edema of the distal portions of the limbs, icterus, reluctance to move), leukopenia, thrombocytopenia, and parasitemia characteristic of experimentally induced EGE. In both horses, initial appearance of cytoplasmic inclusion bodies correlated closely with the onset of fever (day 7 after inoculation). Inclusion bodies were detected for 6 days in both horses, and percentage of neutrophils that contained inclusion bodies ranged from 1 to 39%. Ehrlichia DNA was detected by use of real-time PCR on blood buffy-coat cells 2 days prior to the microscopic detection of inclusion bodies in both horses. The PCR signals were measured for 8 (horse 1) and 13 (horse 2) days. The serum antibody titer increased to ≥ 20 on days 11 (horse 1) and 14 (horse 2) after inoculation, and a maximum reciprocal value of 320 was observed 30 days after inoculation in both horses.

Among 2,725 healthy cattle tested for antibodies against *E equi*, 5 (0.18%) cattle were seropositive with titers of 20 (n = 2), 40 (2), and 80 (1). The 5 seropositive cattle belonged to the same herd and originated from the Sierra Nevada foothills. Seroprevalence was 9.16% (5/52 tested cattle) within the herd and 1.08% (5/460) when all cattle from the Sierra Nevada foothills were considered. All 5 serum samples reacted against the 44-kd HGE-agent protein used in the immunoassay, confirming exposure to the ehrlichial agents.

**Discussion**

In California, *E equi* and the HGE agent have a broad natural host range that includes humans, horses, dogs, and deer. The distribution of these ehrlichial agents is largely dependent on the distribution of the vector *I pacificus*, which has been reported predominantly from the coastal ranges and the Sierra Nevada foothills. Recent epidemiologic studies conducted in California have found members of the *E phagocytophila* genogroup in 0.3 to 6.7% of adult *I pacificus* from the coastal area and the Sierra Nevada foothills. Adult *I pacificus* ticks commonly bite cattle, making exposure to *E equi* and the HGE agent inevitable.

Although our sample size was small, our findings suggest that cattle are not susceptible to these agents despite having a weak immune response to them. Recently, Stuen and Artursson reported that even 1 *E phagocytophila*-infected cell may be sufficient to transmit the infection to susceptible lambs. In the study reported here, *E equi* and the HGE agent used in a standard dose caused disease in susceptible horses but not in steers, which are nonspecific hosts. Our results are in agreement with those of studies performed in Europe and the United States, which indicate that inoculation of *E phagocytophila* into horses, inoculation of a Swiss HGE-agent strain into cows, and inoculation of *E equi* and a Swedish HGE-agent strain into sheep induced asymptomatic seroconversion. At present, it is not clear whether seroconversion was caused by transient multiplication of the agents or resulted from the immune response to the initial antigen load. A similar situation may be expected in the field, where cattle may eventually become asymptatically infected and seroconvert, depending on the number of exposures and the amount of antigen that is transmitted.

A seroepidemiologic survey of antibodies against *E equi* in horses from northern California revealed that 10.4% of healthy horses from the foothill regions were seropositive. In horses from northern California revealed that 10.4% of healthy horses from the foothill regions were seropositive. In horses from northern California revealed that 10.4% of healthy horses from the foothill regions were seropositive. In horses from northern California revealed that 10.4% of healthy horses from the foothill regions were seropositive. In horses from northern California revealed that 10.4% of healthy horses from the foothill regions were seropositive. In horses from northern California revealed that 10.4% of healthy horses from the foothill regions were seropositive. In horses from northern California revealed that 10.4% of healthy horses from the foothill regions were seropositive. In horses from northern California revealed that 10.4% of healthy horses from the foothill regions were seropositive. In horses from northern California revealed that 10.4% of healthy horses from the foothill regions were seropositive.
Ruminants


References