Evaluation of *Anaplasma phagocytophilum* infection in experimentally inoculated sheep and determination of *Anaplasma* spp seroprevalence in 8 free-ranging sheep flocks in California and Oregon

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**Objective**—To evaluate disease progression in sheep experimentally inoculated with *Anaplasma phagocytophilum* and determine the *Anaplasma* spp seroprevalence in sheep in free-ranging flocks in the Sierra Nevada foothills and Oregon Coast Range.

**Animals**—10 mature ewes seronegative for *Anaplasma* spp and 251 sheep from 8 flocks.

**Procedures**—10 ewes received 1 of 3 treatments: *A phagocytophilum* Webster strain (n = 4), *A phagocytophilum* MRK strain (4), or human promyelocytic leukemia cells (control treatment [2]). Sheep were monitored for signs of clinical disease, and blood samples were obtained for serologic and PCR assay evaluation intermittently for 48 days. From a subsample of sheep from each of 8 free-ranging flocks, blood samples were obtained to determine *Anaplasma* spp seroprevalence.

**Results**—Sheep inoculated with *A phagocytophilum* developed subclinical or mild disease, whereas sheep inoculated with the control treatment did not develop any signs of disease. Only 2 ewes seroconverted; both had received the MRK strain. *Anaplasma*-specific DNA was detected in blood samples from 1 sheep in the Webster strain–inoculated group and 3 sheep in the MRK strain–inoculated group. Sheep seropositive for *Anaplasma* spp were detected in 5 of 8 flocks, and flocks in the Sierra Nevada foothills had higher within-flock seroprevalence (22%) than did flocks in the Oregon Coast Range (6.4%).


Sheep are exposed to a number of tick-borne bacterial pathogens, which has important implications for disease and loss of production. Granulocytic anaplasmosis, or tick-borne fever, in domestic sheep in Europe is caused by the rickettsial pathogen *Anaplasma phagocytophilum*. That pathogen targets host neutrophils, and severity of illness can range from subclinical to fatal with hematologic abnormalities and nonspecific signs of fever and weakness.1 Infected lambs generally develop subclinical or mild disease, whereas sheep inoculated with the control treatment did not develop any signs of disease. Only 2 ewes seroconverted; both had received the MRK strain. *Anaplasma*-specific DNA was detected in blood samples from 1 sheep in the Webster strain–inoculated group and 3 sheep in the MRK strain–inoculated group. Sheep seropositive for *Anaplasma* spp were detected in 5 of 8 flocks, and flocks in the Sierra Nevada foothills had higher within-flock seroprevalence (22%) than did flocks in the Oregon Coast Range (6.4%).

*Anaplasma phagocytophilum* is also a common tick-borne pathogen of cattle, horses, dogs, humans, and small wildlife species in the western United States,5 but to our knowledge, it has not been investigated in domestic sheep in North America. In infected humans and dogs, clinical signs of disease and hematologic abnormalities are nonspecific and commonly include fever, lethargy, and thrombocytopenia.6 Infection with *A phagocytophilum* is common in horses in California and is associated with high fever, thrombocytopenia, lameness, and neurologic abnormalities.7 In cattle, anaplasmosis has been associated with failure to gain weight in at least 200 years.1 Infected sheep may become immunosuppressed and persistently infected and have recurrent bacteremia.1,3 Bacteremia generally develops when there is recombination in the major surface protein of *A phagocytophilum*, MSP2(P44), which generates novel antigens that enable the bacteria to avoid and manipulate host immunity.6
weight and can result in substantial loss of production for the cattle industry.9 White-tailed (Odocoileus virginianus), fallow (Dama dama), and roe (Capreolus capreolus) deer have also been infected with A phagocytophilum.10 Other rickettsial pathogens that affect domestic sheep in western North America include Anaplasma ovis and Anaplasma marginale. Anaplasma marginale infects RBCs and can cause fatal anemia, icterus, fever, and weakness with recurring, often subclinical, bacteremia.11 In cattle on range in the western United States, A marginale infection is common, with 100% seroprevalence in cattle in some herds in the Sierra Nevada foothills and Oregon Coast Range.14,15 Deer can also become chronically infected with A marginale.16 Results of 1 study17 indicated that A ovis infection in domestic sheep was often subclinical, and if left untreated, infected sheep could become persistently infected, which predisposed them to potentially fatal secondary bacterial infections and parasitic infestations.

Of California’s approximately 40.5 million hectares, 15.4 million hectares is rangeland, with extensive populations of sheep, cattle, goats, and mule deer (Odocoileus hemionus). The presence of granulocytic and erythrocytic forms of anaplasmosis in deer has created concern that deer could act as a natural reservoir for Anaplasma spp that can infect livestock and result in production losses. California ranks second in the United States for sheep production, with approximately 630,000 sheep and lambs.18 Sheep farming contributes $50 million to the California agricultural industry, yielding ≥1.36 million kg of wool and 325,000 lambs annually.19 Anaplasmosis has the potential to adversely affect the sheep industry in California, and information regarding its prevalence and ecology and the effects of clinical disease in sheep flocks in the United States is lacking. The purpose of the study reported here was to evaluate disease progression in sheep experimentally inoculated with A phagocytophilum and determine the Anaplasma spp seroprevalence in sheep in free-ranging flocks in the Sierra Nevada foothills and Oregon Coast Range.

Materials and Methods

Animals—The study had 2 phases; phase 1 involved the experimental inoculation of domestic sheep with A phagocytophilum to evaluate disease progression, and phase 2 involved a survey of 8 free-ranging domestic sheep flocks in the Sierra Nevada foothills and the northern portion of the Oregon Coast Range to determine seroprevalence to A phagocytophilum and A marginale. Study protocols were reviewed and approved by the University of California-Davis Institutional Animal Care and Use Committee, and written consent was obtained for the use of the privately owned sheep.

For phase 1 of the study, ten 6-year-old Columbia white-faced ewes were obtained from the University of California-Davis sheep herd. The sheep had been born and raised in Davis, Calif, where the western black-legged tick (Ixodes pacificus), a common vector for Anaplasma spp, has not been identified and anaplasmosis has not been reported. For all 10 sheep, results of serologic assays were negative for antibodies against A phagocytophilum, and results of PCR assays for A phagocytophilum–specific DNA were negative. These study sheep were kept in an isolated pen at the University of California-Davis Sheep Farm with free access to inside and outside areas. All sheep had a mild to moderate cough but no other clinical signs of disease prior to the study.

For phase 2 of the study, 28 to 40 sheep were selected from each of 8 free-ranging flocks in the Sierra Nevada foothills and the northern portion of the Oregon Coast Range for evaluation of serum antibodies against A marginale, A phagocytophilum, and A ovis. The first sheep to be processed from each flock were those included in the study. A total of 251 market lambs of mixed breeds and sexes were evaluated. With an assumption of true within-flock Anaplasma seroprevalence of 10%, a sample size of at least 20 would yield ≥95% precision for the estimate with a power of 0.05.

Anaplasma isolates and experimental inoculation (phase 1)—During phase 1 of the study, each of 10 sheep were allocated randomly to receive 1 of 3 treatments: inoculation with A phagocytophilum Webster strain (Genbank accession No. U02521.7) that was cultured in human promyelocytic leukemia (HL-60) cells (0.45 mL, IV [n = 4]), inoculation with A phagocytophilum MRK strain (Genbank accession No. AV330136)9 that was cultured in horse blood (0.45 mL, IV [4]), or inoculation with HL-60 cells (0.45 mL, IV; control treatment [2]). All cell cultures for inocula were grown to achieve a concentration of 1 × 10³ cells/100 mL. For the Webster and MRK strain treatments, 95% of the cells (HL-60 and horse blood cells, respectively) were infected with A phagocytophilum.

Clinical observations and sample collection (phase 1)—All sheep were inoculated on day 0 during phase 1 of the study. Clinical observations were made every other day after inoculation, and the last observation was made on day 48. Observations included determination of weight and assessment of attitude, hydration status, capillary refill time, rectal temperature, respiratory rate and character, and heart rate (determined during a physical examination performed by a veterinarian [BRH or JEF]). For each sheep, blood samples were collected via jugular venipuncture into a 10-mL tube containing EDTA (for PCR assay) and a 10-mL tube containing no anticoagulant (for serologic evaluation) prior to inoculation on day 0 and on days 3, 9, 15, 22, 28, 37, and 48 after inoculation. Sheep were euthanized on day 48 by use of 20 mL of a barbiturate euthanasia solution injected into a jugular vein. All internal organs were grossly examined by a veterinarian (BRH or JEF) for abnormalities. Within 1 hour after sheep were euthanized, tissue specimens, each weighing approximately 10 g, were harvested from the spleen, heart, lungs, a mesenteric lymph node, liver, and bone marrow. Tissue specimens were frozen and stored at −20°C until analyzed for Anaplasma DNA.

Blood sample collection from sheep in free-ranging flocks (phase 2)—From each sheep selected for serologic screening for exposure to Anaplasma spp, a blood sample (10 mL) was obtained via jugular venipuncture into a tube containing EDTA. The flock location was provided for each sheep; however, signalment and disease status were unavailable.
Serologic evaluation—All blood samples were centrifuged at 1,800 × g for 10 minutes and then plasma or serum was harvested. Antibodies against "A. phagocytophilum" and "A. ovis" (which cross-react with "A. phagocytophilum") were detected via an indirect IFA assay that used "A. phagocytophilum"–infected HL-60 cells as substrate and fluorescein isothiocyanate–labeled goat anti-sheep heavy- and light-chain IgG. Samples were tested at dilutions of 1:25, 1:100, and 1:200, and positive and negative control sera were included in each batch. Samples were considered to have positive results if intense green fluorescence was visually detected within cellular morulae via fluorescent microscopy. Because there is also some serologic cross-reactivity between "A. phagocytophilum" and "A. marginale" on the IFA test that was used, samples with positive results on the IFA test were also assayed for antibodies against "A. marginale" by use of a competitive ELISA that was specific for the detection of antibodies against the MSP5 protein of "A. marginale." The competitive ELISA was performed in accordance with the manufacturer's instructions, and samples with ≥30% inhibition were considered to have positive results. Samples that had positive results for "A. phagocytophilum" or "A. marginale" were tested with a PCR assay in an attempt to detect DNA from "A. marginale," "A. phagocytophilum," and "A. ovis."

DNA extraction and PCR assays—The DNA was extracted from all blood samples and tissue specimens that were obtained from the experimentally inoculated sheep during phase 1 and from blood samples that were obtained from free-ranging sheep during phase 2 that were seropositive for "A. phagocytophilum" or "A. marginale."

The DNA was extracted by use of a kit in accordance with the manufacturer's instructions. For all blood samples and tissue specimens, a real-time PCR assay for "A. phagocytophilum" DNA was performed with primers and probes as described in a combined thermocycler-fluorometer. For blood samples obtained from sheep seropositive for "A. marginale" or "A. phagocytophilum" during phase 2, a real-time PCR assay for "A. marginale" DNA was performed with primers and probes described in a report of one study, and a traditional PCR assay for "A. ovis" was performed by use of primers and probes described in a report of another study.

Statistical analysis—Data were analyzed with statistical software. For all analyses, values of P < 0.05 were considered significant. For phase 1, changes in weight and maximum rectal temperature over the duration of the study period were compared among treatments by use of an ANOVA. The number of sheep with at least 1 positive result on a PCR assay at 1 or more time points was compared between the Webster strain– and MRK strain–inoculated groups by use of a Fisher exact test. For phase 2, the "Anaplasma" spp seroprevalence in sheep from the Sierra Nevada foothills was compared with that in sheep from the Oregon Coast Range by use of a χ² test with Yates continuity correction.

Results

Experimental inoculation of sheep with "A. phagocytophilum" (phase 1)—The 2 sheep in the control (HL-60) group did not seroconvert to "A. phagocytophilum" and had no clinical signs of disease except for a chronic cough throughout the duration of the study that was thought to be associated with Corynebacterium pseudotuberculosis or exposure to hay or dust. These sheep did not have positive PCR assay results at any point during the study. For the sheep that received the control treatment, weight change was minimal (1 gained 1.4 kg and 1 lost 1.8 kg) and mean ± SD maximum rectal temperature was 39.6 ± 0.07°C. Sheep inoculated with "A. phagocytophilum" had no apparent signs of depression, weakness, ataxia, or anemia, although all had a chronic cough. Weight change over the observation period varied among sheep, and the mean weight change was not significantly different between the 2 groups inoculated with "A. phagocytophilum."

Rectal temperature ranged from 38.3°F to 40.8°F for the sheep inoculated with the Webster strain and from 38.7°F to 40.0°F for sheep inoculated with the MRK strain. Mean ± SD maximum rectal temperature was 40.4 ± 0.48°C for the sheep inoculated with the Webster strain and 39.8 ± 0.18°C for the sheep inoculated with the MRK strain, and mean maximum rectal temperature was significantly (P = 0.036) different between the 2 "A. phagocytophilum"–inoculation groups and the control group. Rectal temperatures of sheep inoculated with the Webster strain reached maximum values on day 0 (n = 1), 7 (1), and 22 (2) after inoculation. Rectal temperatures of sheep inoculated with the MRK strain reached maximum values on day 7 (n = 1), 16 (2), and 22 (1) after inoculation.

Seroconversion was detected in only 2 of the "A. phagocytophilum"–inoculated sheep; each had an antibody titer of 1:100. Both of those sheep were inoculated with the MRK strain, and seroconversion was detected on day 9. One of those sheep also had an "A. phagocytophilum"–positive PCR assay result on day 9, and 2 other sheep inoculated with the MRK strain also had "A. phagocytophilum"–positive PCR assay results (one on day 22 and the other on day 48). Seroconversion was not detected in any sheep inoculated with the Webster strain, and only 1 had an "A. phagocytophilum"–positive PCR assay result (on day 22). Positive results for "A. phagocytophilum" via PCR assay were obtained only from blood samples; no tissue specimens yielded positive results for "A. phagocytophilum" via PCR assay. The number of PCR assays that yielded positive results did not differ significantly (P = 0.48) between the 2 groups inoculated with "A. phagocytophilum."

During necropsy, 1 sheep that was inoculated with the Webster strain had evidence of focal pneumonia in the lungs, but all other organ systems were grossly normal. One sheep that was inoculated with the MRK strain had focal hepatized lesions in the lungs. Both sheep that received the control treatment had thickened encapsulated granulomas in the lungs and liver that were consistent with "C. pseudotuberculosis" infection.

"A. phagocytophilum" seroprevalence in sheep in free-ranging flocks (phase 2)—Thirty-two of 251 (12.7%) sheep were seropositive for "Anaplasma" spp (Table 1). Sheep that had positive test results for antibodies against "A. phagocytophilum" via IFA originated from 5 of the 8 flocks located in Placerville, Calif; Amador Valley, Calif; Hopland, Calif; Sonoma, Calif; and Brownsville, Ore. Two sheep from the flock in Amador Valley,
Calif, were seropositive for *A. marginale*. Overall, the mean within-flock seroprevalence to *A. phagocytophilum* was 22% for flocks located in the Sierra Nevada foothills and 6.4% for flocks located in the northern portion of the Oregon Coast Range, and the within-flock seroprevalence was significantly ($P = 0.006$) different between the 2 regions. All PCR assays for *A. marginale*, *A. ovis*, and *A. phagocytophilum* yielded negative results.

### Discussion

In the present study, experimental inoculation of mature ewes with 1 of 2 strains of *A. phagocytophilum* of North American origin resulted in transient and mild infections. Also, a seroprevalence survey of 8 free-ranging sheep flocks in the northwestern United States revealed that sheep in 5 of the 8 flocks had been exposed to *Anaplasma* spp, and flocks located in the Sierra Nevada foothills had a higher within-flock seroprevalence to *Anaplasma* spp than did flocks located in northern portions of the Oregon Coast Range.

During phase 1 of the present study, mature sheep were inoculated with 1 of 2 strains of *A. phagocytophilum* and only minor differences were detected between the treatment groups. Older ewes were used rather than lambs because of reports that older humans and horses are more susceptible to clinical disease caused by *A. phagocytophilum*. During the present study, there were no clinical signs of disease except minor coughing and mildly increased rectal temperatures, which could also have been caused by *C. pseudotuberculosis* infection. There was no apparent pattern for day after inoculation on which a sheep had a positive *A. phagocytophilum* PCR assay result or increased rectal temperature, although peak rectal temperature for all sheep was recorded 22 days after inoculation.

In contrast to results of studies conducted on sheep in Europe, in the present study, most of the *A. phagocytophilum*–inoculated sheep failed to seroconvert, *A. phagocytophilum*–positive PCR assay results were sporadic, and recurrent *A. phagocytophilum* bacteremia was not recorded. All sheep in the present study received approximately the same dose of *A. phagocytophilum*, but only a small proportion seroconverted or developed bacteremia. Investigators of another study in which a large sample of inoculum of *A. phagocytophilum* was subdivided into small aliquots for the inoculation of individual sheep (a procedure similar to that used in the study reported here) found that a few aliquots lacked a sufficient infective dose because of minor stochastic differences. An insufficient infective dose of *A. phagocytophilum* seems unlikely in the present study because the volume of inoculum used for each sheep was larger than that used in the other study; however, failure of the experimental inoculation to induce infection cannot be ruled out. It is also possible that brief periods of bacteremia and hyperthermia were missed because the sheep were not monitored and samples were not obtained every day. It is unlikely that sheep in the present study had been previously exposed and were immune to *A. phagocytophilum* because they all had negative results when tested for antibodies against *A. phagocytophilum* prior to inoculation and had lived only in an environment reportedly free of *Ixodes* spp ticks, the vector for *A. phagocytophilum*. Other potential reasons for the lack of bacteremia and seroconversion in the sheep in phase 1 of the study reported here could be that old (6 years) sheep were included or that we used *A. phagocytophilum* strains that originated from North America and that had poor tropism for sheep. It is also possible that the inocula were attenuated by repeated passage on cells in vitro, but that seemed unlikely because the Webster strain used originated from a low-passage cell line and the MRK strain used was obtained from a horse that had severe clinical disease. Thus, on the basis of these facts, it is most likely that the *A. phagocytophilum* infection was transient and frequently insufficient to induce detectable antibodies in the inoculated sheep of the present study.

For sheep in phase 1 of the study reported here, the only pathological change observed that might have been associated with anaplasmosis was a consolidated pneumatic lung lobe in 1 ewe that received the Webster strain. However, *A. phagocytophilum* PCR assay results were negative for that sheep’s lung specimen, and pneumonia is relatively common in older sheep. Pneumonia is not a characteristic response to anaplasmosis in other animals, including humans, although atypical pneumonia was reported in a case series involving humans in France and pneumonia caused by secondary infections in humans infected with *A. phagocytophilum*.

Table 1—Within-flock seroprevalence of antibodies against *Anaplasma* spp for 8 free-ranging sheep flocks located in the Sierra Nevada foothills and the northern portion of the Oregon Coast Range.

<table>
<thead>
<tr>
<th>Date of sample collection</th>
<th>Flock location (latitude and longitude)</th>
<th>Geographic region</th>
<th>No. of seropositive sheep (total No. evaluated)</th>
<th>Prevalence (%)</th>
<th>95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>May 13, 2008</td>
<td>Brownsdale, Ore (44° 33' 36&quot; N, 122° 59' 5&quot; W)</td>
<td>Oregon Coast Range</td>
<td>1 (30)</td>
<td>3.3</td>
<td>0.2–19.1</td>
</tr>
<tr>
<td>May 21, 2008</td>
<td>Sonoma, Calif (38° 17' 30&quot; N, 122° 27' 4&quot; W)</td>
<td>Oregon Coast Range</td>
<td>5 (30)</td>
<td>16.6</td>
<td>6.3–35.4</td>
</tr>
<tr>
<td>Sep 29, 2008</td>
<td>Langlois, Ore (42° 55' 24&quot; N, 124° 27' 3&quot; W)</td>
<td>Oregon Coast Range</td>
<td>0 (30)</td>
<td>0.0</td>
<td>0–14.1</td>
</tr>
<tr>
<td>Oct 21, 2008</td>
<td>Hopland, Calif (38° 58' 23&quot; N, 123° 6' 59&quot; W)</td>
<td>Oregon Coast Range</td>
<td>4 (33)</td>
<td>12.1</td>
<td>3.9–29.1</td>
</tr>
<tr>
<td>Apr 3, 2009</td>
<td>Vacaville, Calif (38° 21' 24&quot; N, 121° 59' 12&quot; W)</td>
<td>Oregon Coast Range</td>
<td>0 (28)</td>
<td>0.0</td>
<td>0–15.0</td>
</tr>
<tr>
<td>Jun 26, 2008</td>
<td>Placerville, Calif (38° 43' 47&quot; N, 120° 47' 51&quot; W)</td>
<td>Sierra Nevada foothills</td>
<td>9 (40)</td>
<td>22.5</td>
<td>11.4–38.9</td>
</tr>
<tr>
<td>Jul 31, 2008</td>
<td>Amador Valley, Calif (37° 40' 59&quot; N, 121° 52' 56&quot; W)</td>
<td>Sierra Nevada foothills</td>
<td>13 (30)</td>
<td>43.3</td>
<td>25.9–62.3</td>
</tr>
<tr>
<td>Sep 9, 2008</td>
<td>Auburn, Calif (39° 52' 40&quot; N, 121° 4' 25&quot; W)</td>
<td>Sierra Nevada foothills</td>
<td>0 (28)</td>
<td>0.0</td>
<td>0–14.1</td>
</tr>
</tbody>
</table>

Blood samples were obtained from 28 to 40 mixed-breed market lambs from each flock; the lambs selected for testing were the first lambs to be processed from each flock. Serologic evaluation was performed via an indirect IFA (*Anaplasma phagocytophilum* and *Anaplasma ovis*) and competitive ELISA (*Anaplasma marginale*).

On the basis of these facts, it is most likely that the *A. phagocytophilum* infection was transient and frequently insufficient to induce detectable antibodies in the inoculated sheep of the present study.
has been described.4 Immunosuppression is an important sequel of anaplasmosis.5 Sheep clinically affected with anaplasmosis commonly develop pyemia,24 and humans and cats infected with anaplasmosis often develop unusual secondary infections, which in humans include cryptococcosis and herpetic esophagitis.25,26 Secondary infections were not detected in the sheep inoculated with *A phagocytophilum* during phase 1 of the present study.

Changes in body weight for the sheep in phase 1 of the present study were limited to minor fluctuations and did not differ significantly among the control and *A phagocytophilum*-inoculated groups. Results of other studies9,26 indicated that immature cattle and lambs infected with *A phagocytophilum* had substantial reductions in weight gain; however, the sheep used for phase 1 of the present study were mature animals instead of growing animals and only had subclinical disease or minor morbidity.

Even though no clinical signs of anaplasmosis were observed and only 2 of 8 inoculated sheep seroconverted during phase 1 of the present study, serologic evaluation of sheep during phase 2 of the study revealed that exposure to *Anaplasma* spp was not uncommon in free-ranging sheep flocks in the Sierra Nevada foothills and Oregon Coast Range. Five of 8 surveyed flocks had sheep with antibodies against *Anaplasma* spp, and 32 of 251 (12.7%) sheep tested were seropositive for *A. marginale* spp. Two important differences between the sheep in phase 1 and phase 2 of the present study were route of exposure to *A phagocytophilum* and age at initial exposure. The sheep used during phase 1 of the present study were experimentally inoculated with *A phagocytophilum* via IV injection, whereas the sheep tested during phase 2 of the study were naturally exposed to *A phagocytophilum* via a tick bite. Tick saliva is an important potentiator of the early local inflammatory response in the dermis,30 which may facilitate development of a humoral immune response. Age is also an important risk factor in determining the outcome of *A phagocytophilum* infection. Although results of studies31,32 indicate that *A phagocytophilum* infection causes disease of greater severity in older patients, results of other studies26 indicate that immature cattle and lambs are highly susceptible to *A phagocytophilum* infection. Age was unknown for the sheep tested during phase 2 of the study reported here, but it was assumed they were raised in an area where ticks and *A phagocytophilum* were endemic. Therefore, it is probable that the sheep tested during phase 2 were initially exposed to *A phagocytophilum* at ≤6 years of age. It is possible that *A phagocytophilum* infection in young, naturally infected sheep is more likely to induce an antibody response than is experimentally induced infection in mature sheep.

For phase 2 of the present study, the within-flock seroprevalence to *A phagocytophilum* was higher for sheep flocks located in the Sierra Nevada foothills than for sheep flocks located in the northern portion of the Oregon Coast Range. That geographic distribution for *A phagocytophilum* is consistent with the geographic distribution reported in case reports3,32,33 that involved dogs, horses, and humans. Unfortunately, the specific species of *Anaplasma* to which the sheep were exposed could not be determined because of the potential for cross-reactivity among the various *Anaplasma* spp in the serologic tests34 and the lack of positive results on the PCR assays that were used in the present study. However, we suggest that *A phagocytophilum* was the most likely species of *Anaplasma* to which the sheep tested during phase 2 of the present study were exposed because of the geographic distribution and the fact that only 2 sheep were seropositive for *A marginale* (as determined by use of a competitive ELISA). Information on the prevalence and clinical importance of *A ovis* in sheep in the northwestern United States is lacking; therefore, continued surveillance for anaplasmosis in sheep flocks in this region is warranted. In particular, efforts should be focused on the identification of actively infected sheep from which samples can be obtained for DNA sequencing and pathogen identification.

Infection with *A phagocytophilum* in sheep during phase 1 of the present study and in cattle of another study resulted in only subclinical or mild disease. In livestock in Europe, classical tick-borne fever is associated with severe clinical disease, hematologic abnormalities, immunosuppression that results in secondary infection, and loss of production.35 In North America, granulocytic anaplasmosis can cause severe disease in horses and occasionally severe or fatal disease in dogs and humans.3,35 The results of phase 1 of the present study do not support the hypothesis that anaplasmosis causes severe disease in sheep in California. Reasons for the difference in incidence and severity of *Anaplasma* infections between North American and European livestock include vector exposure, abiotic factors that affect vectors, and virulence differences among strains of *Anaplasma* spp. Evaluation of DNA sequences from strains of *A phagocytophilum* obtained from infected lambs in Norway and North America revealed substantial differences in the central variable regions of the MSP2 gene. Therefore, differences in virulence among strains of *Anaplasma* spp may be caused by genetic differences.2

Anaplasmosis can adversely affect sheep production and is an emerging zoonotic disease in the United States. Many opportunities exist for transmission of anaplasmosis from wildlife reservoirs to domestic livestock. Additional studies are necessary to enhance the understanding of anaplasmosis in domestic sheep in North America. These studies should focus on the extent of immunosuppression caused by anaplasmosis in infected lambs, role of tick saliva in the establishment of *Anaplasma* infection, identification of genetic variations among different strains of *A phagocytophilum* that naturally infect sheep in North America, and the impact of these variations on pathogenicity. More extensive surveillance for anaplasmosis in domestic livestock is also necessary, preferably coupled with surveillance for anaplasmosis in sympatric deer. Data from these studies could then be used to better define the losses attributable to anaplasmosis and aid in the development of management strategies to minimize the impact of anaplasmosis on the North American sheep industry.

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a. HL-60 cells, American Type Culture Collection, Rockville, Md.
References


35. Stuen S. Anaplasma phagocytophilum—the most widespread tick-borne infection in animals in Europe. Vet Res Commun 2007;31:79–84.