JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 1995, p. 675–679 Vol. 33, No. 3
0095-1137/95/$04.00+0

Detection of *Anaplasma ovis* Infection in Goats by Major Surface Protein 5 Competitive Inhibition Enzyme-Linked Immunosorbent Assay

LEAH W. NDUNG'U,[1] CARMEN AGUIRRE,[2] FRED R. RURANGIRWA,[1,2] TERRY F. MCELWAIN,[2,3] TRAVIS C. MCGUIRE,[2] DONALD P. KNOWLES,[2,4] AND GUY H. PALMER[2]*

Veterinary Research Center, Kenya Agricultural Research Institute, Kabete, Kenya,[1] and Department of Veterinary Microbiology and Pathology[2] and Washington Animal Disease Diagnostic Laboratory,[3] Washington State University, and Animal Disease Research Unit, Agricultural Research Service, U.S. Department of Agriculture,[4] Pullman, Washington

Received 21 October 1994/Accepted 12 December 1994

A competitive inhibition enzyme-linked immunosorbent assay (ELISA) based on a major surface protein 5 (MSP5) B-cell epitope conserved among *Anaplasma* species was used to detect goats infected with *Anaplasma ovis*. We examined strains of *A. ovis* isolated from goats in Kenya and demonstrated that MSP5 and the target B-cell epitope, bound by monoclonal antibody ANAF16C1, were conserved. Sera from 149 goats in four regions of Kenya and from 302 goats in six U.S. states were tested for the presence of epitope-specific antibodies with the MSP5 competitive inhibition ELISA. Evidence that the assay can be used to detect *A. ovis*-infected goats includes the following: (i) 53 goats raised in confinement with arthropod control were all seronegative; (ii) six goats experimentally infected with *A. ovis* seroconverted at the same time that they developed detectable rickettsemia; (iii) seroconverted goats remained seropositive, consistent with the persistence of *A. ovis* in goats and the presence of anti-MSP5 antibody in cattle persistently infected with *Anaplasma marginale*; and (iv) 119 of 127 known *A. ovis*-infected goats in Kenya were seropositive. *A. ovis* infection, as determined serologically and by demonstration of infected erythrocytes, in goats from the four regions in Kenya was highly prevalent. In contrast, despite the presence of *A. ovis* and competent arthropod vectors in the United States, the prevalence of infection appeared to be very low. The high prevalence in Kenya and the occurrence of anemia in persistently infected goats may be impediments to current efforts to increase milk yields on small farms.

Anaplasma ovis is an arthropod-borne rickettsial pathogen that induces acute anemia in sheep and goats following invasion and replication within erythrocytes (2, 10, 28). Experimental inoculation of goats with *A. ovis* has been shown to induce an acute disease characterized by depression, anorexia, fever, and progressive anemia (28, 33). Although *A. ovis* infections in goats have been reported throughout the world (6, 14, 17, 18, 25–28, 33) and natural arthropod vectors have been described (9, 11, 24, 30), the extent of infection and the loss of livestock productivity remain poorly understood. Clinical reports of acute infections in tropical and subtropical countries (6, 14, 18, 25, 33), where goats are an important source of meat, milk, and manure for subsistence farmers, suggest that the disease may be widespread and of economic importance. Efforts to increase goat milk yield in Kenya have been addressed primarily by improvement of genetics. However, a high prevalence of infectious diseases that cause anemia, which markedly decreases milk yield, would represent a constraint to improved productivity. While *A. ovis* infection in sheep within the United States has been described (7, 13, 29), the prevalence of infection within goat herds is unknown.

The diagnosis of *A. ovis* in goats has been based primarily upon the identification of acute infections, using microscopic examination of Giemsa-stained blood smears. However, rickettsemia levels of less than 0.1% infected erythrocytes in goats are not reliably detected by this method (20). A DNA probe reactive with *A. ovis* has been used to identify infected goats in Kenya, although the limited sensitivity of the probe may prohibit detection of persistently infected carrier goats with very low-level rickettsemia (25). In contrast, the development and persistence of antibodies following *Anaplasma* infection provide a means to detect infected animals at all stages of infection (15).

Recently, a competitive inhibition enzyme-linked immunosorbent assay (CI-ELISA) based on antibody binding to recombinant DNA-derived *Anaplasma marginale* major surface protein 5 (MSP5) has been developed and shown to detect *A. marginale*-infected cattle, including persistently infected carriers (22, 32). The anti-MSP5 monoclonal antibody (MAb) ANAF16C1 used in the assay reacts with all strains of *A. marginale* examined (4, 16, 21, 22, 31, 32) and has also been shown to bind an Idaho strain of *A. ovis* (32). Consequently, we determined the conservation of this epitope among *A. ovis* strains and have adapted the assay for detection of *A. ovis* infection in goats. In this paper, we describe the conservation of MSP5 and the MAb ANAF16C1-reactive epitope among *A. ovis* strains, tests of the ability of the assay to detect acute and persistent infections in goats, and use of the assay to compare the seroprevalence of infection in goats from regions in Kenya and the United States.

MATERIALS AND METHODS

Organisms. Four *A. ovis* strains were used in this study. The Dubois Idaho strain was kindly provided by David Stiller (Animal Disease Research Unit, Agricultural Research Service, U.S. Department of Agriculture, Pullman, Wash.). This strain was isolated in 1988 by allowing *Dermacentor andersoni* male ticks, collected from naturally infected sheep, to feed on splenectomized recipient sheep. Three *A. ovis* strains were isolated from infected goats in 1987 and

* Corresponding author. Mailing address: Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, WA 99164-7040. Phone: (509) 335-6033. Fax: (509) 335-8328. Electronic mail address: gpalmer@vetmed.wsu.edu.

were designated, on the basis of the regions of Kenya in which they were isolated, Ngong, Rumururti, and Timau (25). The Kapiti strain of *A. marginale*, isolated from an infected cow, was used as a positive control for presence of MSP5 and was kindly provided by Joseph Katende (International Laboratory for Research on Animal Diseases, Nairobi, Kenya). All strains of *Anaplasma* were maintained as liquid nitrogen-cryopreserved stabilates of infected erythrocytes in dimethyl sulfoxide–phosphate-buffered saline (PBS) (12). Recombinant MSP5 was obtained from *Escherichia coli* expressing the Florida strain *A. marginale msp-5* (32). Briefly, the *msp-5* gene was cloned into pBluescript to create the recombinant plasmid pAM104A, which was used to transform *E. coli* XL-1 Blue cells (32). The pAM104A-transformed *E. coli* expresses a full-length MSP5 polypeptide that binds the anti-MSP5 MAb ANAF16C1 and polyclonal bovine antibody induced by immunization with native MSP5 (32). *E. coli* XL-1 Blue transformed with nonrecombinant pBluescript was used as a control.

Conservation of MSP5 among *A. ovis* strains. The presence of the MSP5 epitope bound by MAb ANAF16C1 and the conservation of MSP5 molecular size was examined by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis of *A. ovis* lysates followed by immunoblotting. *A. ovis* organisms were isolated from thawed, infected caprine erythrocytes by sonication and differential centrifugation as previously described (23). Isolated organisms were solubilized by boiling in a 25 mM Tris-HCl (pH 6.8) buffer that contained 2% SDS, 15% glycerol, 2.5% β-mercaptoethanol, and 0.02% bromophenol blue. As positive controls, lysates from isolated Kapiti strain *A. marginale* and from pAM104A-transformed *E. coli* XL-1 Blue were prepared. As negative controls, lysates of uninfected goat erythrocytes and *E. coli* XL-1 Blue transformed with nonrecombinant pBluescript were prepared. Lysates were electrophoresed in a 7.5 to 17.5% polyacrylamide gel containing SDS and then electrophoretically transferred to 0.45-μm-pore-size nitrocellulose. The nitrocellulose was blocked with a 1-h incubation in PBS containing 5% nonfat dry milk and 0.05% Tween-20. The anti-MSP5 MAb ANAF16C1 or a negative control MAb of the same isotype was added at 3 μg/ml in PBS containing 0.05% Tween 20 and incubated overnight. The nitrocellulose was washed three times with PBS containing 0.05% Tween 20. Bound MAb was detected following addition of horseradish peroxidase-labeled goat antibody against mouse immunoglobulin and processed with enhanced chemiluminescence according to the manufacturer's protocol (Amersham International, Amersham, United Kingdom).

MSP5 CI-ELISA. The CI-ELISA with recombinant MSP5 was modified from a previously described assay used to detect antibody against MSP5 in *A. marginale*-infected cattle (32). Briefly, pAM104A-transformed *E. coli* XL-1 Blue was grown overnight in 50 ml of Luria-Bertani broth containing 50 μg of ampicillin per ml. Bacterial cells were ultrasonically disrupted in a buffer containing 1% Nonidet P-40 and 1 mg of lysozyme per ml. To minimize enzymatic degradation of recombinant MSP5, bacterial lysates were stored in proteinase inhibitor buffer (50 mM Tris [pH 8.0] containing 5 mM EDTA, 5 mM iodoacetamide, 0.1 mM *N*-α-*p*-tosyl-L-lysine chloromethyl ketone, 1 mM phenylmethylsulfonyl fluoride, 1 mg of lysozyme per ml, and 1% Nonidet P-40) prior to coating of microtiter plates. The appropriate dilution of bacterial lysate, as determined by a checkerboard titration of MSP5 and MAb ANAF16C1, in pH 7.2 PBS containing 20 mM $MgCl_2$ was used to coat individual wells of flat-bottomed 96-well plates (Immulon 2; Dynatech Laboratories, Chantilly, Va.) overnight at 4°C. Coated plates were blocked with 0.5% fraction V bovine serum albumin in PBS for 1 h at room temperature. Blocked plates were washed three times with PBS containing 0.05% Tween 20. Goat sera were diluted 1:10 in PBS, and 50-μl samples were added to duplicate wells and incubated for 20 min at room temperature. Horseradish peroxidase-conjugated MAb ANAF16C1 was added at 0.2 μg per well and incubated for 20 min. Following washing, bound MAb was detected by the addition of 50 μg of *o*-phenylenediamine dihydrochloride in a pH 5.0 0.2 M sodium phosphate–0.1 M citric acid buffer. The detection reaction was stopped by the addition of 3 M HCl, and the optical density at 490 nm was determined with a Dynatech MR 4100 Microplate ELISA Reader. Duplicate serum samples from 10 goats known to be negative were included on each plate and used to determine a mean optical density and standard deviation. A test serum sample was scored as positive for antibody to MSP5 if the optical density was three standard deviations or more below the mean optical density of the known negative samples.

Experimental infection of goats with *A. ovis*. Six Saanen goats, 3 to 7 years old, were used for experimental infection with *A. ovis*. These goats were part of a confinement dairy herd (Washington State University dairy) and were raised in tick-free housing. Prior to experimental infection, all goats were negative by microscopic examination of Giemsa-stained blood smears. Goats were inoculated intravenously with 1.0 ml of Idaho strain *A. ovis* stabilate and then monitored daily for development of rickettsemia and anemia, using microscopic examination of Giemsa-stained blood smears and determination of packed cell volume (PCV), respectively. Sera were obtained daily until the acute infection resolved and then periodically during persistent infection. Sera were stored at −20°C until use.

Testing of field samples from goats in Kenya. Sera were collected from 149 goats in four regions of Kenya (see Table 3) and tested by CI-ELISA. There was no selection of goats on the basis of clinical signs or owner reports of illness. Because of the large number of seropositive animals, blood was obtained from all goats for examination of Giemsa-stained blood smears and from all goats in Machakos and Kiserian for DNA hybridization with a digoxigenin-labeled *A. ovis* DNA probe. The DNA probe was derived from the Idaho strain of *A. ovis* and has been previously described (25). For the experiments described here, pAO12A plasmid DNA was isolated from transformed *E. coli* JM109 by alkaline lysis and digested with *Bam*HI to release the 9.6-kb *A. ovis* insert DNA. The *A. ovis* insert was electroeluted, and 5 ng of the eluted fragment was labeled with digoxigenin by random primer extension. To determine probe sensitivity, *A. ovis*-infected erythrocytes were diluted in uninfected goat blood to obtain a range of 10^1 to 10^7 infected erythrocytes per ml of blood. For test samples, *A. ovis* DNA was isolated from 10 ml of blood as previously described (25) and resuspended in 50 μl of TE buffer (10 mM Tris-HCl and 1 mM EDTA, pH 8.0). Ten microliters of sample DNA was spotted onto a nylon membrane with a manifold and hybridized with the digoxigenin-labeled probe as previously described (25). Genomic DNA isolated from goat leukocytes was used as a negative control.

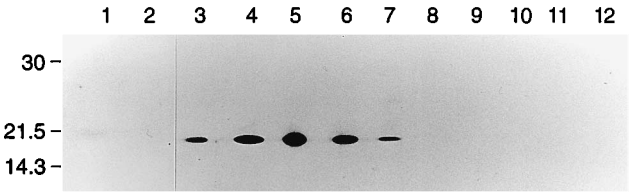

FIG. 1. Conservation of MSP5 in *A. ovis* strains isolated in Kenya. Lysates of the Rumuruti (lanes 5 and 10), Ngong (lanes 6 and 11), and Timau (lanes 7 and 12) strains of *A. ovis*, the Kapiti strain of *A. marginale* (lanes 4 and 9), uninfected goat erythrocytes (lane 2), pAM104A-transformed *E. coli* XL-1 Blue (lanes 3 and 8), and nonrecombinant pBluescript-transformed *E. coli* XL-1 Blue (lane 1) were electrophoresed in polyacrylamide gels containing SDS and transferred to nitrocellulose. Lanes 1 to 7 were reacted with the anti-MSP5 MAb ANAF16C1, and lanes 8 to 12 were reacted with an unrelated MAb of the same isotype. Positions of 30-, 21.5-, and 14.3-kDa molecular size markers are indicated on the left.

Testing of field samples from goats in the United States. Sera were obtained from 302 goats within the United States. Sera were obtained from 53 adult Saanen goats raised in a confinement dairy (Washington State University dairy) that maintained a consistent control program for arthropod parasites. In addition, blood was obtained from each of the 53 goats for microscopic examination of Giemsa-stained blood smears. The other 249 sera were randomly selected from sera submitted to the Washington Animal Disease Diagnostic Laboratory to be tested for antibody to caprine arthritis encephalitis virus. The 249 sera were obtained from goats in the following locations: six counties in Washington state (176 goats), three counties in Idaho (18 goats), Texas (45 goats), Louisiana (8 goats), and New Mexico (2 goats). Sera were stored at −20°C until use. To determine if seronegative goats represented true negative goats, 10 ml of blood from each of five seronegative goats in Washington was collected into heparin. The blood was pooled and inoculated intravenously into a confinement-raised, seronegative, splenectomized goat. To determine if seropositive goats represented true positive goats, 10 ml of blood was collected from a seropositive goat in Washington and similarly inoculated intravenously into a confinement-raised, seronegative, splenectomized goat. The recipient splenectomized goats were monitored for *A. ovis* infection by microscopic examination of Giemsa-stained blood smears and determination of PCV.

RESULTS

Conservation of MSP5 among *A. ovis* strains. MAb ANAF 16C1 specifically bound an approximately 19-kDa polypeptide in the Rumururti, Ngong, and Timau strains of *A. ovis* (Fig. 1, lanes 5, 6, and 7). Expression of this polypeptide in the Idaho strain has been previously demonstrated (32). MSP5 was also detected in the Kapiti strain of *A. marginale* (Fig. 1, lane 4) and in pAM104A-transformed *E. coli* XL-1 Blue (Fig. 1, lane 3), which were used as positive controls. Uninfected goat erythrocytes (Fig. 1, lane 2) and *E. coli* XL-1 Blue transformed with nonrecombinant pBluescript (Fig. 1, lane 1) were unreactive with MAb ANAF16C1. An isotype control MAb was unreactive with the *A. ovis* strains (Fig. 1, lanes 10 to 12), the Kapiti strain of *A. marginale* (Fig. 1, lane 9), and pAM104A-transformed *E. coli* XL-1 Blue (Fig. 1, lane 8).

Experimental infection of goats with *A. ovis*. Six seronegative adult Saanen goats were inoculated with the Idaho strain of *A. ovis*. All six goats developed acute anaplasmosis characterized by intraerythrocytic rickettsemia and severe anemia (Table 1). As assessed by the MSP5 CI-ELISA, all goats seroconverted

TABLE 1. Experimental infection of goats with *A. ovis* (Idaho strain)

Animal no.	Days p.i.[a] to:		Maximum rickettsemia (%)[b]	Maximum decrease in PCV (%)[c]
	Rickettsemia[d]	Seroconversion[e]		
90G08	20	19	2.3	60
90G14	21	17	1.2	46
90G21	21	20	1.2	51
91G34	24	19	1.4	62
87G09	17	18	2.4	67
87G12	21	19	0.5	65
Group[f]	21 ± 2	19 ± 1	1.5 ± 0.7	58 ± 8

[a] p.i., postinoculation.
[b] Percentage of infected erythrocytes.
[c] Decrease from preinoculation values.
[d] Microscopically detectable rickettsemia.
[e] By the MSP5 CI-ELISA.
[f] Group means ± standard deviations.

within 1 to 5 days prior to or following microscopic detection of intraerythrocytic rickettsemia (Table 1). Intraerythrocytic *A. ovis* could be detected microscopically for a mean of 17 ± 2 days prior to resolution of acute rickettsemia. No infected erythrocytes were observed in any of the goats later than 52 days postinoculation. All goats remained seropositive when examined at approximately monthly intervals after resolution of the acute rickettsemia (Table 2). Although no microscopically detectable rickettsemia was observed, many of the infected goats remained mildly to moderately anemic as determined by PCV compared with preinfection PCV (Table 2).

Testing of field samples from goats in Kenya. Examination of goats from four regions of Kenya revealed a high prevalence of *A. ovis*-seropositive goats as determined by the MSP5 CI-ELISA (Table 3). Blood was obtained from all goats for examination of Giemsa-stained blood smears and from each goat in Machakos and Kiserian for DNA hybridization with a digoxigenin-labeled *A. ovis* DNA probe (Table 3). The DNA probe was capable of detecting 1 ng of *A. ovis* DNA or $\geq 10^4$ infected erythrocytes per ml (data not shown). *A. ovis*-infected erythrocytes could be detected either microscopically or with the DNA probe in 127 of 149 goats from Kenya (Table 3). Of these 127 goats known to be positive, 119 were seropositive by the MSP5 CI-ELISA. The eight seronegative goats represented false-negative CI-ELISA results.

A. ovis could be detected, either microscopically or with the DNA probe, in 120 of 125 seropositive goats. This suggested that most infected goats were persistently rickettsemic with $\geq 10^4$ infected erythrocytes per ml. Five seropositive goats were negative by both microscopic examination and DNA hybridization, suggesting either persistent infection with $<10^4$ infected erythrocytes per ml, clearance of the infection, or a false-positive serologic result.

Testing of field samples from goats in the United States. The 53 adult Saanen goats raised in confinement at the Washington State University dairy were all seronegative and were negative by microscopic examination of Giemsa-stained blood smears (Table 3). Examination of 249 sera submitted to the Washington Animal Disease Diagnostic Laboratory from five states revealed a very low prevalence (2 of 249) of seropositive goats (Table 3). Inoculation of blood from five seronegative goats into a splenectomized recipient goat did not transmit *A. ovis* infection during the 45-day examination period. Two seropositive goats were located in Washington and Texas. A second serum sample from the goat in Washington (obtained 3 months after the initial sample) was negative in the CI-ELISA. Inoculation of blood from this goat, collected at the same time as the second serum sample, into a splenectomized recipient goat did not transmit *A. ovis* during the 45-day observation period. The seropositive goat from Texas was not available for further studies.

TABLE 2. Serologic reactivity of goats persistently infected with *A. ovis* (Idaho strain)

Animal no.	Days p.i.[a]	Rickettsemia[b]	Decrease in PCV (%)[c]	MSP5 CI-ELISA[d]
90G08	73	0	35	Positive
	111	0	NT[e]	Positive
	136	0	28	Positive
	165	0	30	Positive
	192	0	35	Positive
90G14	73	0	29	Positive
	111	0	NT	Positive
	136	0	29	Positive
	165	0	31	Positive
	192	0	33	Positive
90G21	73	0	35	Positive
	111	0	NT	Positive
	136	0	34	Positive
	165	0	35	Positive
	192	0	35	Positive
91G34	73	0	40	Positive
	111	0	NT	Positive
	136	0	24	Positive
	165	0	21	Positive
	192	0	27	Positive
87G09	87	0	35	Positive
	108	0	39	Positive
	157	0	30	Positive
	185	0	37	Positive
87G12	87	0	22	Positive
	108	0	39	Positive
	157	0	35	Positive
	185	0	41	Positive

[a] p.i., postinoculation.
[b] Microscopically detectable rickettsemia.
[c] Decrease from preinoculation values.
[d] Scored as positive when the optical density was three standard deviations or more below the mean optical density of the known negative samples.
[e] NT, not tested.

DISCUSSION

MSP5, including the epitope bound by MAb ANAF16C1, is encoded by a single gene copy and is expressed as an approximately 19-kDa polypeptide in the outer membrane of all *A. marginale* strains examined (4, 16, 21, 31, 32). Similarly, the Idaho strain of *A. ovis*, the only strain previously examined, expresses a 19-kDa polypeptide that binds MAb ANAF16C1 (32). In this study, both the ANAF16C1-reactive epitope and the polypeptide size were shown to be conserved among three strains of *A. ovis* isolated in Kenya. The conservation of MSP5 molecular size among strains is in marked contrast to the size polymorphism observed for other *Anaplasma* MSPs, notably MSP1a (1) and MSP2 (19). The uniform conservation of the ANAF16C1-reactive epitope among both species and strains

TABLE 3. Detection of *A. ovis*-infected goats in Kenya and the United States

Location	No. of goats examined	No. positive by:		
		Microscopic examination[a]	DNA hybridization	MSP5 CI-ELISA[b]
Kabete	23	14	NT[c]	17
Kiserian	75	68	72	67
Machakos	22	20	21	21
Naivasha	29	20	NT	20
WSU[d] dairy	53	0	NT	0
Washington	176	NT	NT	1
Texas	45	NT	NT	1
Idaho	18	NT	NT	0
Louisiana	8	NT	NT	0
New Mexico	2	NT	NT	0

[a] Microscopically detectable rickettsemia.
[b] Optical density three standard deviations or more below the mean optical density of the known negative samples.
[c] NT, not tested.
[d] WSU, Washington State University.

of *Anaplasma* indicates that this epitope may be an invariant target for serologic diagnosis.

The utility of the MSP5-based CI-ELISA in detecting cattle infected with *A. marginale* suggested that the assay could be used to detect goats infected with *A. ovis* (22, 32). The evidence that the MSP5 CI-ELISA can be used to detect *A. ovis*-infected goats includes the following: (i) six experimentally infected goats seroconverted at the same time that they developed detectable rickettsemia, (ii) seroconverted goats remained seropositive, consistent with the persistence of *A. ovis* in goats and the presence of anti-MSP5 antibody in cattle persistently infected with *A. marginale* (22, 32), and (iii) 119 of 127 goats known to be infected with *A. ovis* (positive by either microscopic examination or DNA hybridization) in Kenya were seropositive. The percent inhibition of MAb ANAF16C1 binding by sera from experimentally infected goats progressively increased following infection (data not shown). This suggests that, similar to cattle with *A. marginale* infections (3, 15), goats remain persistently infected with *A. ovis*. Whether seropositive goats may eventually clear *A. ovis* infection is unknown; however, clearance of *A. marginale* from infected cattle appears to be uncommon (3, 5, 15).

On the basis of results for the 127 Kenya goats demonstrated to have *A. ovis*-infected erythrocytes by either microscopic detection or DNA hybridization, the MSP5 CI-ELISA had a sensitivity of 94% (95% confidence limits of 90 to 98%). However, the number of persistently infected goats with rickettsemia levels below the DNA probe detection limit of 10^4 *A. ovis*-infected erythrocytes per ml is unknown. While the minimal rickettsemia levels in *A. ovis*-infected goats have not been described, $<10^3$ *A. marginale*-infected erythrocytes per ml commonly occur in persistently infected carrier cattle (3, 5, 8). The consistent seropositive status of experimentally infected goats during low-level *A. ovis* persistent infection suggests that the MSP5 CI-ELISA may be sufficiently sensitive to detect low-level rickettsemia during persistent infection in the field. Determining the CI-ELISA sensitivity with low-level persistent infection will require definitive identification of naturally acquired persistent infections by enhanced nucleic acid probe sensitivity or by direct transmission of infection to susceptible goats.

The negative CI-ELISA results for all 53 confinement-raised dairy goats suggest that the assay specificity is high. The seronegative results for 247 of 249 U.S. goats in five states also indicate that false-positive reactions do not appear to be a significant problem with the MSP5 CI-ELISA. The single Washington goat with a positive CI-ELISA result was retested with a second serum sample and was negative. This retest serological result and the failure to transmit *A. ovis* to a splenectomized recipient goat by direct blood inoculation indicated that the initial CI-ELISA result was most likely a false positive. The possibility that this individual goat cleared the infection and became seronegative during the 3-month interval between the two sampling dates cannot be definitively excluded. With all 302 U.S. goats included, the specificity was estimated to be >99%. Confirming this high specificity will require testing of additional goats which have been established as true negatives by improved nucleic acid hybridization assays or by transmission studies (5). The five U.S. goats established as uninfected with *A. ovis* (true negative goats) by failure to transmit infection following direct blood inoculation into a susceptible recipient goat were all seronegative.

The contrast in seroprevalence between the four regions in Kenya and the five states in the United States was striking. The high percentage of infected goats in Kenya likely reflects husbandry practices in which goats are brought into contact with arthropod vectors during grazing. There was no arthropod control on any of the farms in Kenya. In contrast, the sampled goats in the United States were predominantly dairy goats and are more likely to be raised in confinement and under conditions that include arthropod control. Although competent arthropod vectors occur within the United States (9, 24, 30) and *A. ovis* has been isolated in several states (7, 13, 28, 29), the current level of infection in goats appears to be low.

The experimental infection of goats clearly demonstrated that *A. ovis* is capable of inducing severe anemia during acute anaplasmosis. Interestingly, the goats remained mildly to moderately anemic following resolution of the acute infection. The occurrence of anemia during acute and persistent infection may well represent an infectious disease impediment to improvement of milk and meat production in enzootic regions.

ACKNOWLEDGMENTS

This work was supported by the U.S. Agency for International Development Title XII Small Ruminant Collaborative Research Support Program Grant DAN-1328-G-00-0046-00 and the Kenya Agricultural Research Institute. Carmen Aguirre was supported by NIH grant 1 T35-RR07042.

We acknowledge Joseph Buyu, Beverly Hunter, Lowell Kappmeyer, Emma Karel, Kay Morris, Abuu Oriko, and the staff in the Serology Section of the Washington Animal Disease Diagnostic Laboratory for technical assistance.

REFERENCES

1. **Allred, D. R., T. C. McGuire, G. H. Palmer, S. R. Leib, T. M. Harkins, T. F. McElwain, and A. F. Barbet.** 1990. Molecular basis for surface antigen size polymorphisms and conservation of a neutralization-sensitive epitope in *Anaplasma marginale*. Proc. Natl. Acad. Sci. USA **87:**3220–3224.
2. **Bevan, L. E. W.** 1912. Anaplasmosis of sheep. Vet. J. **68:**392–400.
3. **Eriks, I. S., G. H. Palmer, T. C. McGuire, D. R. Allred, and A. F. Barbet.** 1989. Detection and quantitation of *Anaplasma marginale* in carrier cattle by using a nucleic acid probe. J. Clin. Microbiol. **27:**279–284.
4. **Eriks, I. S., D. Stiller, W. L. Goff, M. Panton, S. M. Parish, T. F. McElwain, and G. H. Palmer.** 1994. Molecular and biological characterization of a new isolated *Anaplasma marginale* strain. J. Vet. Diag. Invest. **6:**435–441.
5. **Eriks, I. S., D. Stiller, and G. H. Palmer.** 1989. Impact of persistent *Anaplasma marginale* rickettsemia on tick infection and transmission. J. Clin. Microbiol. **31:**2091–2096.
6. **Goksu, K.** 1965. A review of anaplasmosis and observations of infections in sheep and goats. Turk. Veteriner. Heklimeri Dernigi Dergisis **35:**399–417.
7. **Jensen, R.** 1955. Suspected anaplasmosis in sheep. N. Am. Vet. **36:**117–118.
8. **Kieser, S. T., I. S. Eriks, and G. H. Palmer.** 1990. Cyclic rickettsemia during

persistent *Anaplasma marginale* infection of cattle. Infect. Immun. **58:**1117–1119.

9. **Kocan, K. M., and D. Stiller.** 1992. Development of *Anaplasma ovis* (Rickettsiales: Anaplasmataceae) in male *Dermacentor andersoni* (Acari: Ixodidae) transferred from infected to susceptible sheep. J. Med. Entomol. **29:** 98–107.
10. **Lestoguard, F.** 1924. Deuxieme note sur les piroplasmoses du mouton en Algerie. L'Anaplasmoses: *Anaplasma ovis* nov. sp. Bull. Soc. Pathol. Exot. **17:**784–788.
11. **Losos, G. J.** 1986. Anaplasmosis, p. 742–795. *In* G. J. Losos (ed.), Infectious tropical diseases of domestic animals. Longman Press, Essex, United Kingdom.
12. **Love, J. N.** 1972. Cryogenic preservation of *Anaplasma marginale* with dimethylsulfoxide. Am. J. Vet. Res. **33:**2557–2560.
13. **Magonigle, R. A., W. P. Eckblad, S. D. Lincoln, and F. W. Frank.** 1981. *Anaplasma ovis* in Idaho sheep. Am. J. Vet. Res. **42:**199–201.
14. **Mallick, K. P., S. K. Dwivedi, and M. N. Malhotra.** 1979. Anaplasmosis in goats: report on clinical cases. Indian Vet. J. **56:**693–694.
15. **McGuire, T. C., W. C. Davis, A. L. Brassfield, T. F. McElwain, and G. H. Palmer.** 1991. Identification of *Anaplasma marginale* long-term carrier cattle by detection of serum antibody to isolated MSP-3. J. Clin. Microbiol. **29:** 788–793.
16. **McGuire, T. C., G. H. Palmer, W. L. Goff, M. I. Johnson, and W. C. Davis.** 1984. Common and isolate-restricted antigens of *Anaplasma marginale* detected with monoclonal antibodies. Infect. Immun. **45:**697–700.
17. **Mohan, R. A., and R. C. Pathak.** 1966. Prevalence of anaplasmosis in ruminants in northern India. Indian Vet. J. **43:**685–689.
18. **Neitz, W. O.** 1968. *Anaplasma ovis* infection. Bull. Off. Int. Epiz. **70:**359–365.
19. **Oberle, S. M., G. H. Palmer, A. F. Barbet, and T. C. McGuire.** 1988. Molecular size variations in an immunoprotective protein complex among isolates of *Anaplasma marginale*. Infect. Immun. **45:**697–700.
20. **Palmer, G. H.** 1992. Development of diagnostic reagents for anaplasmosis and babesiosis, p. 59–66. *In* T. T. Dolan (ed.), Recent developments in the control of anaplasmosis, babesiosis, and cowdriosis. English Press, International Laboratory for Animal Diseases, Nairobi, Kenya.
21. **Palmer, G. H., A. F. Barbet, A. J. Musoke, J. M. Katende, F. Rurangirwa, V. Shkap, E. Pipano, W. C. Davis, and T. C. McGuire.** 1988. Recognition of conserved surface protein epitopes on *Anaplasma centrale* and *Anaplasma marginale* isolates from Israel, Kenya and the United States. Int. J. Parasitol. **18:**33–38.
22. **Palmer, G. H., T. F. McElwain, T. C. McGuire, L. Kappmeyer, W. C. Davis, D. Stiller, E. Visser, N. Tebele, L. Ndung'u, E. Pipano, V. Shkap, and D. P. Knowles.** 1994. Recent advances in serological diagnosis of anaplasmosis: development of the msp-5 competitive inhibition ELISA, p. 102–104. *In* G. Uilenberg, A. Permin, and J. W. Hansen (ed.), Use of applicable biotechnological methods for diagnosing haemoparasites. Food and Agriculture Organization of the United Nations, Rome.
23. **Palmer, G. H., and T. C. McGuire.** 1984. Immune serum against *Anaplasma marginale* initial bodies neutralizes infectivity for cattle. J. Immunol. **133:** 1010–1015.
24. **Ryff, J. F., J. L. Weibel, and G. M. Thomas.** 1964. Relationship of ovine to bovine anaplasmosis. Cornell Vet. **54:**407–414.
25. **Shompole, S. P., S. D. Waghela, F. R. Rurangirwa, and T. C. McGuire.** 1989. Cloned DNA probes identify *Anaplasma ovis* in goats and reveal a high prevalence of infection. J. Clin. Microbiol. **27:**2730–2735.
26. **Singh, B., and O. P. Gautam.** 1972. Anaplasmosis. IV. Experimental anaplasmosis induced by splenectomisation in sheep and goats. Haryana Vet. **11:**4–10.
27. **Sinha, G. K., and R. C. Pathak.** 1966. Anaplasmosis in goats and sheep. Indian Vet. J. **43:**490–493.
28. **Splitter, E. J., H. D. Anthony, and M. J. Twiehaus.** 1956. *Anaplasma ovis* in the United States: experimental studies with sheep and goats. Am. J. Vet. Res. **17:**487–491.
29. **Splitter, E. J., M. J. Twiehaus, and E. R. Castro.** 1955. Anaplasmosis in sheep in the United States. J. Am. Vet. Med. Assoc. **127:**244–245.
30. **Stiller, D., W. L. Goff, S. P. Shompole, L. W. Johnson, H. Glimp, F. R. Rurangirwa, and J. R. Gorham.** 1989. *Dermacentor andersoni* Stiles: a natural vector of *Anaplasma ovis* Lestoguard of sheep in Idaho, p. 183–193. *In* R. J. Hidalgo (ed.), Proceedings of the Eighth National Veterinary Hemoparasite Disease Conference, St. Louis, Mo.
31. **Tebele, N., T. C. McGuire, and G. H. Palmer.** 1991. Induction of protective immunity by using *Anaplasma marginale* initial body membranes. Infect. Immun. **59:**3199–3204.
32. **Visser, E. S., T. C. McGuire, G. H. Palmer, W. C. Davis, V. Shkap, E. Pipano, and D. P. Knowles.** 1992. The *Anaplasma marginale msp5* gene encodes a 19-kilodalton protein conserved in all recognized *Anaplasma* species. Infect. Immun. **60:**5139–5144.
33. **Zwart, D., and J. Buys.** 1968. Studies on *Anaplasma ovis* infection. II. Pathogenicity of a Nigerian goat strain for Dutch sheep and goats. Bull. Epiz. Dis. Afr. **16:**73–80.