Antibody against an *Anaplasma marginale* MSP5 Epitope Common to Tick and Erythrocyte Stages Identifies Persistently Infected Cattle

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A protein epitope of major surface protein 5 (MSP5), defined by monoclonal antibody (MAb) ANAF16C1, is conserved among *Anaplasma* species (E. S. Visser, T. C. McGuire, G. H. Palmer, W. C. Davis, V. Shkap, E. Pipano, and D. P. Knowles, Jr., Infect. Immun. 60:5139–5144, 1992) and is expressed in the salivary glands of infected ticks. A competitive inhibition ELISA (cELISA) for the detection of bovine anti-MSP5 antibodies was developed by using purified recombinant MSP5 fusion protein and MAb ANAF16C1. The specificity of the recombinant-MSP5 cELISA within North America was established by using 261 serum samples from cattle in the regions of Hawaii and Northern Ontario where anaplasmosis is not endemic and from cattle proven by splenectomy or subinoculation of whole blood into susceptible splenectomized recipients to be uninfected. The maximum percent inhibition by these sera was 18%. Sera known to be positive were obtained from 35 cattle either experimentally inoculated with infected erythrocytes or exposed to infected *Dermacentor andersoni* ticks. Thirty-four of the 35 serum samples inhibited MAb ANAF16C1 binding by $\geq 25\%$. During acute infection, the MSP5 cELISA detected antibodies prior to or concomitantly with the appearance of rickettsiae in erythrocytes. Antibodies were detectable in sera from persistently infected cattle inoculated as long as 6 years previously.

Bovine anaplasmosis, a vector-borne rickettsial disease of cattle, is caused by *Anaplasma marginale* (19–21) and *Anaplasma centrale* (21). Ticks biologically transmit *Anaplasma* species, while biting flies and fomites can mechanically transfer infected blood to susceptible cattle (5, 16, 24). Infection occurs in most countries, including the United States (17). Clinical disease, caused by replication of the rickettsiae in erythrocytes, is characterized by anemia, weight loss, and death (19).

Control of *A. marginale* infection will require both an effective vaccine and accurate identification of carrier cattle (11). Two possible methods for routine carrier detection are identification of rickettsial DNA in blood and the detection of *A. marginale*-specific antibodies in serum (11). The ability of an antibody-based test to indicate the true infection status is dependent upon cattle remaining persistently infected. Indefinite persistence of *A. marginale* organisms in infected cattle has been documented (7, 17); however, data supporting lifelong persistence in all cases of untreated infections are lacking.

Current serologic tests for anaplasmosis have a high error rate (11). False-positive results occur when bovine erythrocyte proteins contaminating the *A. marginale* antigen used in some tests are detected by antierythrocyte antibodies in bovine sera (2, 6). Also, false-negative reactions occur in complement fixation tests using guinea pig complement because of failure to detect all bovine immunoglobulin isotypes (12).

Previous work demonstrated that major surface protein 5 (MSP5) is present in all recognized *Anaplasma* spp. and that the MSP5 protein epitope defined by monoclonal antibody (MAb) ANAF16C1 was broadly conserved among *Anaplasma* spp. and isolates (23). The utility of MSP5 and MAb ANAF16C1 in the competitive inhibition enzyme-linked immunosorbent as-

say (cELISA) format (1) was initially demonstrated for the detection of goat anti-*Anaplasma ovis* antibodies in Kenya (14). The data presented here resulted from testing the hypothesis that a cELISA using recombinant MSP5 and MAb ANAF16C1 would detect anti-*Anaplasma* sp. antibodies early in acute infection and in persistently infected cattle. The conclusions drawn from the results presented are as follows: (i) the epitope recognized by MAb ANAF16C1 is present in salivary gland stages of *A. marginale*, (ii) the MSP5 cELISA is highly specific, (iii) the MSP5 cELISA detects antibodies in acute infections prior to or concomitantly with the appearance of rickettsiae in peripheral blood erythrocytes, and (iv) the MSP5 cELISA detects anti-*A. marginale* antibody in cattle persistently infected on a longterm basis.

MATERIALS AND METHODS

Organisms. Two strains of *A. marginale* were used in this study. The Idaho strain was isolated from a naturally infected 13-year-old Hereford cow from a South Idaho herd (24). The Florida strain was isolated in 1955 as previously described (18).

Transmission of *A. marginale* via *Dermacentor andersoni*. Transmission of the South Idaho strain of *A. marginale* via the tick vector *D. andersoni* (stock origin from Owyhee County, Idaho) was performed as described elsewhere (24). Donor cow C591 was inoculated intravenously with 10^8 infected erythrocytes from a second-passage South Idaho blood stablate (24). Fifty male *D. andersoni* ticks (normal colony) were applied under a cloth patch to C591 when the percentage of parasitized erythrocytes (PPE) was 2.8%. PPE was determined by examining 10^3 erythrocytes per test. Seven days after attachment, 47 fed, live ticks were recovered and held at 26°C for 8 days. The peak PPE during the feeding period was 11.0% on day 6. Twenty-three exposed *D. andersoni* males applied to recipient C592 and 24 applied to C597 were removed 7 days postapplication. Fifteen ticks acquired from each calf were processed for detection of MSP5 within their salivary glands as described below.

Immunoblotting of tick salivary glands for detection of MSP5. Infected ticks acquired after they had been feeding on calves C592 and C597 and normal colony ticks were dissected, and their salivary glands were placed in 490 μ l of proteinase inhibition buffer (50 mM Tris [pH 8.0], 5 mM EDTA, 5 mM iodoacetamide, 0.1 mM Na-p-tosyl-L-lysine chloromethyl ketone, and 1 mM phenylmethylsulfonyl fluoride). Salivary gland protein concentrations were determined

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by the DC Protein Assay (Bio-Rad Laboratories, Richmond, Calif.). Tick salivary gland lysates were boiled for 3 min in sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) sample buffer (25 mM Tris [pH 6.8], 2% [wt/vol] SDS, 15% [vol/vol] glycerol, 2.5% 2-mercaptoethanol [final concentrations], and a few crystals of bromophenol blue), and 25 μ g per lane was electrophoresed in an SDS-7.5 to 17.5% polyacrylamide gradient slab gel with a 5% stacking gel. Transfer to nitrocellulose was performed by standard techniques (22). Membranes were blocked in Tris-Tween 20 buffer containing 5% milk. MSP5 was detected with MAb ANAF16C1, immunoglobulin G2a (IgG2a) (2 μ g/ml) (23); detection was followed by treatment with peroxidase-conjugated donkey anti-murine IgG (Jackson ImmunoResearch Laboratories, Inc., West Grove, Pa.) and enhanced chemiluminescence (kit by Amersham International plc, Amersham, United Kingdom). The negative control was an irrelevant IgG2a MAb at the same concentration.

Subcloning and expression of rMSP5. A PCR-amplified copy of the *mSP5* gene (bp 118 through 753) from clone pAM104 (23) was subcloned into the *Xba*I site of the pMAL-c2 cloning and expression vector (New England BioLabs, Beverly, Mass.) to create recombinant plasmid pMALmSP5. *Escherichia coli*, XL1-blue competent cells (Stratagene, La Jolla, Calif.) were transformed with pMALmSP5 and cultured on LB agar (for 0.5 liter, 5.0 g of Bacto-tryptone, 2.5 g of Bacto-yeast extract, 5.0 g of NaCl [pH 7.5], 7.5 g of agar) containing ampicillin at 100 μ g/ml. A colony containing the insert in the correct orientation was identified by digestion of plasmid minipreps with *Eco*RI.

Expression and affinity purification of the recombinant MSP5-maltose-binding protein (rMSP5-MBP) fusion protein were performed according to the manufacturer's recommendations (New England BioLabs). Briefly, 10 ml of an overnight culture was inoculated into 1.0 liter of medium (10 g of tryptone per liter, 5 g of yeast extract per liter, 5 g of NaCl per liter, 2 g of glucose per liter, and 100 μ g of ampicillin per ml) and grown at 37°C to 2.0×10^8 cells per ml. Following addition of 85.0 mg of isopropyl- β -D-thiogalactopyranoside, bacteria were incubated at 37°C for 2 h. Bacteria were harvested by centrifugation at $4.0 \times 10^3 \times g$ for 20 min and then resuspended in 50 ml of column buffer (20 ml of 1.0 M Tris-Cl [pH 7.4] per liter, 11.7 g of NaCl per liter, 2.0 ml of 0.5 M EDTA per liter, and 154 mg of dithiothreitol per liter). The bacterial suspension was frozen overnight at -20°C, thawed in cold water, and sonicated on ice for 2 min in 20-s pulses (100 W). After sonication, the suspension was centrifuged at $9.0 \times 10^3 \times g$ for 30 min and the supernatant was diluted 1:5 with column buffer and applied to the amylose resin. The resin was washed 10 times with column buffer, and the fusion protein was eluted with column buffer containing 10 mM maltose. Fractions were collected, and the amount of rMSP5 fusion protein was measured by the DC Protein Assay (Bio-Rad Laboratories). Purity of isolated rMSP5 was assessed by SDS-PAGE and silver staining.

cELISA with rMSP5 fusion protein. The cELISA was performed as previously described, with minor changes (14). Briefly, 1.0 μ g of amylose resin-purified rMSP5-MBP fusion protein in 100 μ l of carbonate-bicarbonate coating buffer (15.0 mM Na₂CO₃, 35 mM NaHCO₃ [pH 9.6]) was applied to individual wells of flat-bottom plates (Immulon 2; Dynatech Laboratories, Chantilly, Va.). Plates were sealed and stored overnight at 4°C. Plates were incubated at room temperature (RT) for 1 h and then incubated for 1 h at RT with 200 μ l of blocking buffer (2×0.5 M K₂HPO₄, 0.5 M KH₂PO₄, 10 g of fraction V bovine serum albumin [BSA] per liter, 15 g of glycine per liter, and 40 g of sucrose per liter) per well. Wells were then washed four times with 200 μ l of phosphate-buffered saline (PBS) per well.

Test sera were adsorbed for 30 min at RT with an equal volume of MBP at 10 μ g/ μ l in 0.5 M PBS, pH 7.2, with 1.0% fraction V BSA. Duplicate aliquots of adsorbed sera were added at 100 μ l per well, and plates were incubated for 30 min at RT. Wells were then washed four times with 200 μ l of PBS per well. Each well received 0.08 μ g of MAb ANAF16C1 conjugated to horseradish peroxidase as previously described (14) in 100 μ l of 0.5 M PBS with 1.0% fraction V BSA, and plates were incubated for 15 min. After wells had been washed four times with 200 μ l of PBS per well, 50 μ l of 0.5- μ g/ μ l *o*-phenylenediamine dihydrochloride in substrate buffer (0.2 M Na₂HPO₄, 0.1 M citric acid [C₆H₅O₇Na₃·2H₂O]) was added to each well, and then plates were incubated for 10 min and the reactions were stopped with 25 μ l of 2 N H₂SO₄.

Specificity of rMSP5 cELISA. The specificity of the rMSP5 cELISA for cattle in North America was defined by evaluating 261 serum specimens obtained from cattle in the state of Hawaii ($n = 46$) and in Northern Ontario ($n = 182$), where anaplasmosis is not endemic, or from experimental cattle ($n = 33$) from the U.S. mainland, including 12 splenectomized calves without detectable rickettsiaemia, 12 Holstein dairy cattle demonstrated to be free of *A. marginale* by subinoculation of splenectomized calves with 50 ml of whole blood, and cattle that developed clinical anaplasmosis following challenge (9 preinfection serum specimens). Postinfection sera from cattle experimentally inoculated with *Babesia bovis* ($n = 5$) and *Babesia bigemina* ($n = 2$) were also tested. The confidence limits for specificity were obtained from tabulated values for binomial proportions (3, 4).

Determination of percent inhibition representing a positive cELISA result. The percent inhibition representing a positive result was determined from the optical density (OD) of the 261 negative bovine serum specimens described above. For these 261 serum specimens, the mean OD was 0.767, with a standard deviation (SD) of ± 0.065 . A value 3 SDs below the mean OD of the negative sera (0.572) represented 25% inhibition. None of the negative sera inhibited binding of the MAb at $\geq 25\%$.

FIG. 1. Immunoblot of tick salivary gland proteins with MAb ANAF16C1 (A) and an isotype control MAb (B). Lanes 1 and 2 (both panels) each contain 25 μ g of protein from infected *D. andersoni* salivary glands. Lanes 3 each contain 25 μ g of protein from uninfected *D. andersoni* salivary glands. Numbers on the left indicate molecular mass in kilodaltons.

Temporal development and persistence of rMSP5 cELISA-detectable antibody. Data addressing the ability of the rMSP5 cELISA to detect antibody early after infection and in carrier cattle were obtained by testing 35 bovine serum specimens obtained from experimentally inoculated animals that had rickettsiaemia detectable by light microscopy. Calves B496 to B498, B501, B504, B513, B532, B540, B542, B545, B549, B556, B579, B584, and B585 ($n = 15$) were inoculated intravenously with either 10^4 ($n = 6$), 10^3 ($n = 6$), or 10^2 ($n = 3$) parasitized erythrocytes of *A. marginale* (Florida strain) (15) from an infected donor calf; calves B110, B122, B170, B178, B182, and B185 ($n = 6$) were inoculated intravenously with 10 ml of Florida strain blood stabilate (11); calves B803 to B808 and B810 to B813 ($n = 10$) were inoculated intravenously with 1.0 ml of blood from a splenectomized calf with a PPE of 18.3%; and calf C591 was inoculated intravenously with 0.4 ml of a South Idaho strain blood stabilate (24). Calves C592 and C597 were infected with *A. marginale* (Idaho strain) via transmission from *D. andersoni* ticks as described above. An anti-*A. marginale* reference serum specimen from the Food and Agricultural Organization of the United Nations (9) was also tested.

RESULTS

Expression of the ANAF16C1 MSP5 epitope in salivary glands of infected *D. andersoni* ticks. To test for the presence of MSP5 bearing the ANAF16C1 epitope in infected-tick salivary glands, salivary glands from *D. andersoni* ticks used to transmit *A. marginale* (South Idaho strain) to calves C592 and C597 were solubilized and immunoblotted. Figure 1 shows the presence of an approximately 19-kDa polypeptide in *A. marginale*-infected salivary glands from *D. andersoni* ticks that is recognized by MSP5-specific MAb ANAF16C1 (Fig. 1A, lanes 1 and 2) but not an isotype control MAb (Fig. 1B, lanes 1 and 2). The ANAF16C1 MSP5 epitope was not detected in proteins from uninfected-tick salivary glands (Fig. 1, lanes 3 [both panels]).

Specificity of rMSP5 cELISA. Expression of the *mSP5* gene by the pMAL-c2 expression vector (New England BioLabs) resulted in a fusion protein that migrated with an approximate molecular mass of 67 kDa (43 kDa from MBP and 19 kDa from MSP5) and was specifically bound by MAb ANAF16C1 in an immunoblot assay (data not shown). The OD values for binding of MAb ANAF16C1 to coating- and blocking-buffer controls were 0.04 to 0.05. Negative-control sera from calves B620 and B636 (shown to be negative by splenectomy) were run in duplicate in each plate. A total of 56 plates were used in this study. The mean $\pm$ SD for the 224 control ODs was 0.782 ± 0.084 .

Amylose resin-purified rMSP5 fusion protein was tested with 261 negative bovine serum specimens (defined in Materials and Methods) to determine the specificity of the cELISA. None of these 261 known negative serum specimens, following absorption with MBP, yielded an inhibition rate of $>18\%$

FIG. 2. Percent inhibition frequency distribution of negative and positive sera. Negative sera ($n = 261$) are from cattle from the *Anaplasma*-free state of Hawaii ($n = 46$) and Northern Ontario ($n = 182$) and experimental cattle from the U.S. mainland ($n = 33$) as described in the text. The percent inhibition frequency distribution for positive sera represents 447 independent cELISA determinations with sera from 16 experimentally inoculated cattle. Cattle C591, C592, and C597 were tested weekly for 60 weeks after the time of the first detectable rickettsemia (0.1%); cattle B497, B498, B501, B504, B513, B532, B540, B542, B545, B556, B579, B584, and B585 ($n = 13$) were tested daily for up to 26 days after the time of the first detectable rickettsemia (0.1%). Infection of these cattle is detailed in the text.

when the mean OD of all negative sera was used as a reference. The percent inhibition frequency distribution of these sera is shown in Fig. 2. Seven postinfection serum specimens from cattle inoculated with *B. bovis* or *B. bigemina* hemoparasites, which occur in many countries where *A. marginale* is endemic, yielded $\leq 11\%$ inhibition. On the basis of results for these 268 serum specimens, the specificity of the MSP5 cELISA is 100%, yielding a predictive value of a positive test of 1 (3, 4).

Development of anti-MSP5 antibody in acutely infected calves. Using $\geq 25\%$ inhibition as a positive result, the rMSP5 cELISA consistently detected anti-MSP5 antibody in calves acutely infected with *A. marginale* before or concomitantly with the development of a PPE of $\geq 0.1\%$ (Table 1). Calf B496, infected with 10^3 parasitized erythrocytes, developed detectable rickettsemia, but its serum achieved a maximum rate of inhibition of ANAF16C1 binding of only 19% in the cELISA at day 28 postinfection (Table 1). Also, calf B549, challenged with 10^2 parasitized erythrocytes, never developed detectable rickettsemia and its serum reached a maximum rate of inhibition of 13% 18 days postchallenge (Table 1).

Figure 3A shows the kinetics of anti-MSP5 antibody development in calves C592 and C597 over a period of 35 days following exposure to infected *D. andersoni* ticks. C592 and C597 became serologically positive ($\geq 25\%$ inhibition) at 20 and 19 days after tick exposure, respectively (Fig. 3A). Rickettsemia, defined as a PPE of >0.1 by light microscopy, was not detectable until day 26 (C592) and day 24 (C597) after tick exposure.

Detection of anti-MSP5 antibodies in cattle persistently infected with *A. marginale*. Testing for anti-MSP5 antibodies in calves C592 and C597 by cELISA at postinfection weeks 6 through 61 demonstrated persistence of antibodies against the MSP5 epitope defined by Mab ANAF16C1 (Fig. 3B). Although the inhibition rate never decreased below 25%, antibody levels as measured by the rMSP5 cELISA underwent cyclic fluctuation (Fig. 3B).

Table 2 shows the percent inhibition obtained in the cELISA with sera from cattle persistently infected with *A. marginale* for longer periods of time. Although the cattle listed in Table 2 were inoculated once, sera remained positive for anti-MSP5

TABLE 1. Temporal development of anti-MSP5 antibodies in cattle acutely infected with *A. marginale*

Animal ^a	Days to:	
	Rickettsemia ^b	cELISA positivity ^c
B501	26	19 (48%)
B504	26	22 (32%)
B513	26	17 (47%)
B556	23	17 (42%)
B579	27	20 (37%)
B584	27	17 (41%)
B496	30	— ^d
B497	27	27 (38%)
B498	27	23 (43%)
B532	27	23 (35%)
B545	28	24 (35%)
B585	31	23 (32%)
B540	31	25 (52%)
B542	31	27 (53%)
B549	* ^e	—
C591	22	16 (34%)
C592	26	20 (41%)
C597	24	19 (25%)

^a Calves with B designations were infected intravenously with either 10^4 (block from B501 to B584), 10^3 (block from B496 to B585), or 10^2 (B540, B542, and B549) *A. marginale* (Florida strain)-parasitized erythrocytes. Calf C591 was infected intravenously with *A. marginale* (Idaho strain) and served as a donor for transmission of the organism to calves C592 and C597 via *D. andersoni*.

^b Detectable rickettsemia was defined as $\geq 0.1\%$ parasitized erythrocytes.

^c A positive cELISA result was defined as $\geq 25\%$ inhibition; percent inhibition is shown in parentheses.

^d Calf remained negative.

^e *, calf never developed detectable parasitemia.

FIG. 3. Kinetics of anti-MSP5 antibody development in calves C592 and C597 over a 35-day period after exposure to infected *D. andersoni* ticks (A) and persistence of anti-MSP5 antibodies during weeks 6 through 61 (B). PPE data shown are means for C592 and C597 for each day.

antibody in the cELISA for up to 72 months postinoculation. Blood from five cattle (B110, B122, B170, B182, and B185), listed in Table 2, was infectious for splenectomized calves at both 2 and 3 years postinfection (11).

DISCUSSION

Identification of carrier hosts is critical for management of persistent infections like that caused by *A. marginale*. Clinically normal, persistently infected cattle represent a continuous

source of rickettsiae for transmission to other susceptible cattle (8). Collective data presented here indicate that (i) antibodies against the MSP5 epitope defined by MAAb ANAF16C1 are specific for detection of anaplasmosis in North American cattle; (ii) antibodies to this epitope become detectable prior to or concomitantly with detectable parasitemia in calves infected by intravenous inoculation or with infected ticks; and (iii), importantly for disease control, long-term carriers of *A. marginale* maintain anti-MSP5 antibody levels at a range detectable by the rMSP5 cELISA.

TABLE 2. Persistence of anti-MSP5 antibody in cattle chronically infected with *A. marginale*

Animal ^a	Mo persistently infected ^b	cELISA inhibition (%) ^c
B110	24/72	69/66
B122	24/72	51/28
B170	24/72	52/ND ^d
B178	24/72	72/89
B182	24/72	96/96
B185	24/72	32/32
C591	14	67
C592	14	40
C597	14	33
B803	7	72
B804	7	86
B805	7	70
B806	7	87
B807	7	71
B808	7	72
B810	7	96
B811	7	80
B812	7	77
B813	7	92

^a Calves B110, B122, B170, B178, B182, and B185 were inoculated intravenously with 10 ml of Florida strain blood stabilate; calf C591 was inoculated intravenously with *A. marginale* (Idaho strain) and served as the donor for transmission of *A. marginale* to C592 and C597 via *D. andersoni*. Calves B803 to B808 and B810 to B813 were inoculated intravenously with 1.0 ml of blood from a splenectomized calf with a ricketsemia level of 18.3%.

^b Months postinoculation at which sera were tested. A slash separating numbers indicates that sera were tested at two different times.

^c Percent inhibition in rMSP5 cELISA. A cELISA result was considered positive at 25% inhibition. A slash separating numbers indicates that sera were tested at two different times.

^d ND, not determined.

To determine the specificity of the rMSP5 cELISA for North American cattle, 268 known negative bovine serum specimens, including 7 serum specimens from cattle infected with *B. bovis* or *B. bigemina*, were tested. All inhibited MAb ANAF16C1 binding <25%, resulting in a specificity of 100%. Based on tabulated values for binomial proportions, the true specificity is in the range of 98 to 100% with 99% confidence (3, 4).

Sequence comparison of the *msh5* gene and the gene encoding a 21-kDa protein of *Cowdria ruminantium* revealed 55.5% amino acid identity (10). However, preliminary testing of bovine anti-*C. ruminantium* sera indicates that these proteins are unlikely to be antigenically related (13). Furthermore, ANAF16C1 did not bind to recombinant 21-kDa *C. ruminantium* protein (data not shown). Collectively, the data suggest that the rMSP5 cELISA is highly specific and may be useful on a global basis.

Although the sensitivity data presented are preliminary, two conclusions can be drawn. First, in cattle intravenously inoculated with *A. marginale* anti-MSP5 antibodies are detectable early in infection at a time that positively correlates with the dose of the inoculum. Also, the early development of detectable anti-MSP5 antibody following exposure to infected ticks is consistent with the finding of MSP5 in the salivary glands of infected *D. andersoni*.

Secondly, in cattle infected either via *D. andersoni* or intravenously anti-MSP5 antibodies remain detectable for as long as 15 and 72 months, respectively, without reexposure. This finding further supports previous data indicating that in the absence of treatment, *A. marginale* causes a lifelong infection, the consequence of which is the existence of a persistent reservoir for transmission to susceptible animals (8).

One calf (B496) that was inoculated with 10³ parasitized erythrocytes developed detectable ricketsemia and never inhibited MAb ANAF16C1 binding >19%; thus, this represents a false-negative result with a 25% cutoff level. However, there was a consistent increase in the percent inhibition by sera from B496 during the time up to that when parasitemia was detectable (data not shown), suggesting the development of MSP5 antibodies that never reached a level capable of causing ≥25% inhibition. It is unknown whether calf B496 developed a persistent infection or whether perhaps the parasitemia was controlled prior to the development of a higher level of MSP5 antibodies. Additional testing of known positive sera may require the percent inhibition cutoff for a positive result to be lowered so that animals such as B496 are detected as positive. Alternatively, under natural conditions the number of rickettsiae inoculated by biological or mechanical vectors may be significantly greater than 10³, resulting in development of MSP5 antibody levels detectable at the current cutoff.

Data presented in this paper demonstrate that the rMSP5 cELISA is highly specific for serologic diagnosis of anaplasmosis in cattle from North America. Also, the rMSP5 cELISA detects infection with *A. marginale* early and in long-term carriers. Field studies that compare the results of the rMSP5 cELISA with those obtained by direct detection of the rickettsiae in infected cattle are needed to demonstrate the overall accuracy of the rMSP5 cELISA in a region of endemicity.

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