Available online at www.sciencedirect.com
SCIENCE DIRECT®
Veterinary Microbiology 106 (2005) 287–292
veterinary microbiology
www.elsevier.com/locate/vetmic

Detection of antibodies against *Anaplasma marginale* in milk using a recombinant MSP5 indirect ELISA

Susana Torioni de Echaide [a,*], María Florencia Bono [b], Cecilia Lugaresi [a], Nerina Aguirre [a], Atilio Mangold [a], Rosalía Moretta [c], Marisa Farber [c], Carolina Mondillo [c]

[a] *Instituto Nacional de Tecnología Agropecuaria, Estación Experimental Agropecuaria Rafaela, Ruta 34 km 227, CP 2300 Rafaela, Santa Fe, Argentina*
[b] *Universidad Nacional del Litoral, Facultad de Veterinaria de Esperanza, Reverendo P. Kreder 2805, CP 3080, Esperanza, Santa Fe, Argentina*
[c] *Instituto Nacional de Tecnología Agropecuaria, Centro Nacional de Investigaciones Agropecuarias Castelar, Los Reseros y Las Cabañas, CP 1712 Castelar, Buenos Aires, Argentina*

Received 22 June 2004; received in revised form 13 December 2004; accepted 21 December 2004

Abstract

An indirect enzyme linked immunosorbent assay (iELISA) for diagnosis of anaplasmosis using undiluted individual milk samples from dairy cows was developed. The recombinant 19 kDa major surface protein 5 (rMSP5) of *Anaplasma marginale* was used as antigen. A monoclonal antibody against bovine IgG1 conjugated with peroxidase and the chromogen 3,5,3′,5′-tetramethylbenzidine were used in the test. Strong and weak, positive and negative milk samples were set up as reference controls. Results were expressed as percentage of positivity (PP) contrasting with the strongest positive control. The test was evaluated in two groups (G1 and G2) of lactating dairy cows from herds located in *A. marginale* non-endemic areas of Argentina. The infection status of both groups, G1 ($n = 128$) sampled after anaplasmosis outbreak, and G2 ($n = 216$) free of anaplasmosis was established by polymerase chain reaction (PCR). Serum samples of cows from G1 and G2 were analyzed by card agglutination test (CAT) and competitive ELISA (cELISA), while the novel iELISA was evaluated in their corresponding milk samples.

At a cutoff of 42 PP, the ELISA has 98% sensitivity and 95% specificity. A significant difference ($P < 0.0001$) was found between the mean PP value of negative samples from G1 (17.4 ± 14.9), and G2 (8.6 ± 7.1). The agreement and kappa (κ) value between iELISA and PCR was 96%, $\kappa = 0.919$; between iELISA and CAT was 97%, $\kappa = 0.880$; and between iELISA and cELISA was 97%, $\kappa = 0.899$. These results strongly support the usefulness of iELISA to detect *A. marginale* antibodies in milk. Additional studies are necessary to define the ability of the milk iELISA to detect not only acutely infected, but also carrier cattle.

Keywords: *Anaplasma marginale*; ELISA; Diagnosis; Milk; Dairy cattle

* Corresponding author. Tel.: +54 3492 440121; fax: +54 3492 440114.
E-mail address: sechaide@rafaela.inta.gov.ar (S.T. de Echaide).

0378-1135/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.vetmic.2004.12.026

1. Introduction

Anaplasmosis caused by *Anaplasma marginale* is an economically important cattle disease transmitted by ticks, hematophagous diptera and fomites (Stiles, 1936; Hawkins et al., 1982; Abdala et al., 1992; Aguirre et al., 1994). It is endemic in tropical and subtropical areas but is frequently reported in temperate regions of the world (Ristic, 1968; Stepanova et al., 1977; Kuttler, 1984; Losos, 1986). In Argentina, *A. marginale* is widespread north of parallel 33°S, exceeding the limit of the *Boophilus microplus* infested area (Anziani, 1979). However, anaplasmosis outbreaks in dairy herds have been detected outside the *A. marginale* endemic zone due to the movement of carrier cattle to non-endemic area (Stiles, 1936; Guglielmone et al., 1997; Abdala et al., 1998). A live vaccine based on *Anaplasma centrale* infected erythrocytes is used mostly in beef herds in endemic areas, where anaplasmosis outbreaks occur. Although *A. centrale* is of low pathogenicity, vaccination in dairy herds is not common in areas with low prevalence (Guglielmone, 1994).

Acute anaplasmosis causes anemia, abortion, decreased milk production and death (Ajayi et al., 1978). Cattle recovered from this stage remain as long lasting reservoirs for *A. marginale*. Fluctuating rickettsemia below 0.1%, often undetectable by direct microscopy (Eriks et al., 1989, 1993), is the distinctive characteristic of carriers. Although PCR and hybridization techniques (Eriks et al., 1989; Barbet, 1995; Gale et al., 1996a) have been applied to identify carriers, these methods are neither suitable for large scale testing nor useful for developing countries because of their cost. Serological tests such as card agglutination test (CAT) (Amerault et al., 1972), enzyme linked immunosorbent assay (ELISA) (Nielsen et al., 1996) and competitive ELISA (cELISA) (Knowles et al., 1996; Torioni de Echaide et al., 1998) have been widely used for anaplasmosis field evaluations (De Wall, 2000; Maloo et al., 2001; Cringoli et al., 2002; Tassi et al., 2002). However, none of these tests have been developed to identify antibodies in milk.

The detection of antibodies in milk would be a valuable tool for diagnosis in dairy herds since it would reduce the possibility of transmission of *A. marginale* and others pathogens through the use of common needles, and would prevent a drop in milk production caused by the stress of bleeding. An indirect ELISA (iELISA) based on the recombinant form of the major surface protein 5 (rMSP5) of *A. marginale* was developed to identify specific antibodies in individual milk samples for epidemiological surveillance of anaplasmosis in dairy herds. The test was evaluated in samples from cows of *A. marginale* infected and uninfected herds, using PCR as a gold standard. Results were compared with those obtained in the corresponding serum samples by CAT and competitive ELISA (Torioni de Echaide et al., 1998).

2. Materials and methods

2.1. Cattle and samples

Two groups (G1, G2) of cows, from different dairy herds, located in an anaplasmosis non-endemic area of Argentina, were analyzed to evaluate the ability of an iELISA to detect antibodies against *A. marginale* in milk samples. G1 comprised 128 dairy cows, from herds where outbreaks of anaplasmosis had been recently diagnosed after the introduction of cows from herds with an unknown history of anaplasmosis status. G2 included 216 cows from a closed dairy herd known to be free of *A. marginale* and *A. centrale*.

Whole blood (blood), blood serum (serum) and milk samples were obtained in parallel from all cows of G1; while serum and milk samples from all cows of G2 were collected in parallel, blood samples for PCR were obtained afterward.

Additionally, blood samples were obtained from a calf experimentally infected with *A. marginale* (≅30% infected erythrocytes) and from two calves experimentally infected with *A. centrale*(≅2% infected erythrocytes).

2.2. Polymerase chain reaction (PCR)

DNA isolated from blood samples collected with EDTA as anticoagulant in tubes and kept at −20 °C was evaluated using PCR. Specific primers to amplify