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Evaluation of a Competitive Enzyme Immunoassay for Detection of *Coxiella burnetii* Antibody in Animal Sera

ATEF K. SOLIMAN,* BOULOS A. M. BOTROS, AND DOUGLAS M. WATTS

Division of Virology, U.S. Naval Medical Research Unit No. 3, Cairo, Egypt

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A competitive enzyme immunoassay (CEIA) was established and compared with other serological techniques for detecting *Coxiella burnetii* antibody in camels, goats, and sheep. This technique was evaluated because a conjugated anti-camel immunoglobulin was not available to serve as a direct signal for the demonstration of antigen-antibody reaction. A *C. burnetii* antibody-positive human serum and a peroxidase-conjugated anti-human immunoglobulin G were used as an indicator system competing against antibody in animal serum or as an indicator of the absence of antibody. Sera were considered antibody positive when the A_{414} of the test sera plus the competing positive antibody was ≤50% of the A_{414} of the negative-control serum plus the competing antibody. Antibody to *C. burnetii* was repeatedly demonstrated in 66% of camel serum samples (n = 200) by the CEIA. Among 48 camel serum samples, 71% were positive for antibody by CEIA versus 65% by EIA using peroxidase-labeled protein A. The CEIA detected *C. burnetii* antibody in 63% of sheep serum samples (n = 40) and in 50% of goat serum samples (n = 96), while the indirect fluorescent-antibody technique detected antibody in 38% of sheep and 34% of goat serum samples and the EIA detected antibody in 50% of sheep and 35% of goat serum samples. These data indicate that the CEIA is a reliable and sensitive technique for demonstrating *C. burnetii* antibody in camels, sheep, and goats.

Wild animals play an important role in the ecology and epidemiology of many infectious agents. Serosurveys are commonly employed to determine the animal species involved, as well as their relative importance as reservoirs of a specific pathogen (3). However, surveys for antibody among wild animals by conventional enzyme immunoassays (EIAs) are often limited because of the unavailability of species-specific diagnostic reagents. The competitive EIA (CEIA) is a two-step procedure in which antibody in the test serum is captured by known antigen and then a probe antibody is added which binds with available binding sites on the antigen and therefore can be detected by an antispecies enzyme-labeled antibody. In this study, the assay was adapted to test for antibody in sera of camels, for which an enzyme-labeled conjugate was not available. Although conjugated protein A has been used extensively as an alternative approach for the detection of immunoglobulin G (IgG) in animal sera (4), the assay is not widely accepted because results can be affected by several variables, such as the pH, ionic strength, conductivity, and detergent level in the buffer, which often present problems (2, 6).

The purpose of this study was to evaluate the CEIA for the detection of *Coxiella burnetii* antibody in camel sera. The sensitivity and specificity of the CEIA were compared with those of the conventional EIA employing antispecies peroxidase or protein A conjugates and the indirect fluorescent-antibody (IFA) technique.

Sheep, goat, and camel sera were obtained during the period from 1987 to 1989 in Egypt for other studies and stored at −20°C until tested for *C. burnetii* antibody. Phase I *C. burnetii* antigen (Nine Mile strain) was obtained from J. C. Williams of the U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Md.

The conventional EIA and the EIA using protein A conjugate (EIA-PA) were performed as described previously (1, 12). The IFA technique was also performed as previously described (5), with *C. burnetii* antigen spot slides (INDX Integrated Diagnostics, Inc., Baltimore, Md.).

The CEIA was performed as described by Van Oirschot et al. (11), with minor modifications. Alternate rows of polystyrene 96-well microtiter plates were coated with an inactivated *C. burnetii* antigen diluted 1:40 in a carbonate-bicarbonate buffer. The remaining wells received only the buffer. The contents of the wells were allowed to dry at room temperature. The buffer plus 0.25% gelatin was then added to each well for blocking, and the mixture was incubated at 37°C for 30 min. The wells were washed three times with phosphate-buffered saline (PBS) containing 1% Tween 20. The test sera were diluted 1:5 in PBS containing 0.05% Tween 20 and 0.5% gelatin (serum diluent), each diluted serum was added separately to individual wells with and without antigen, and the mixture was incubated at 37°C for 60 min. Subsequently, a known *C. burnetii* antibody-positive human serum diluted 1:500 in serum diluent was added to all wells of each plate, and the mixture was incubated at 37°C for another 60 min. The plates were washed for 10 min, a peroxidase-conjugated goat anti-human IgG was added, and the mixture was incubated at 37°C for 60 min. After the plates were washed five times, the substrate [2,2′-azinodi-(3-ethylbenzthiazoline sulfonate); Kirkegaard & Perry Laboratories, Inc., Gaithersburg, Md.] was added, and the mixture was incubated at 37°C for 20 min. Color change was recorded by an automatic spectrophotometer microplate reader (Titertek Multiskan MC/340) at a 414-nm wavelength. Control *C. burnetii* antibody-positive and antibody-negative animal sera were included in each run. Also, positive- and negative-control human sera were included in each test. Serum was considered positive for *C. burnetii* antibody when the A_{414} of the test serum plus the competing antibody-positive human serum was ≥50% less than the A_{414} of the control antibody-negative serum plus the antibody-positive human serum (9).

Camel serum specimens (n = 200) collected during 1989 in

* Corresponding author.

TABLE 1. Agreement between CEIA and IFA, EIA, and EIA-PA for the detection of *C. burnetii* antibody in sheep, goat, and camel sera

Assay	% Agreement with CEIA (no. of samples in agreement/ no. of samples tested) for:		
	Sheep	Goats	Camels
IFA	70 (28/40)	80 (77/96)	ND
EIA	78 (31/40)	75 (72/96)	ND
EIA-PA	ND	ND	60 (29/48)

Egypt were tested five times by the CEIA for *C. burnetii* antibody, and 66% (n = 131) of them were repeatedly positive. For comparison, camel serum specimens (n = 48) were tested for *C. burnetii* antibody by EIA-PA and by CEIA. Results showed that the CEIA detected 71% (n = 34) of the camel serum samples positive for *C. burnetii* antibody while 65% (n = 31) were detected by the EIA-PA, with 60% agreement (Table 1). Of 40% with discordant results, 11 camel serum specimens were positive by CEIA and negative by EIA-PA. Ten each of the positive and negative camel serum specimens by CEIA were coded and retested on 10 different occasions by more than one investigator. Results were in agreement in 95% or more of the test runs. Also, the A_{414} values on the screening dilution of positive sera achieved by the CEIA were highly predictable of the titer of the antibody (r = 0.992, $P < 0.001$) (Fig. 1).

The CEIA was also compared with the conventional EIA and the IFA for the detection of *C. burnetii* antibody in sheep (n = 40) and goat (n = 96) serum samples. Agreement of the results of the CEIA with those of IFA and EIA is summarized in Table 1. Among sheep serum samples, CEIA detected 63% antibody positive, while EIA and IFA detected 50% and 38% antibody positive, respectively. Of the 96 goat serum samples, 50, 35, and 34% were positive by CEIA, EIA, and IFA, respectively (Table 2). Of the 15 IFA antibody-positive sheep serum specimens, 14 were positive by CEIA, whereas 31 of the 33 goat serum specimens that were positive by IFA were positive by CEIA.

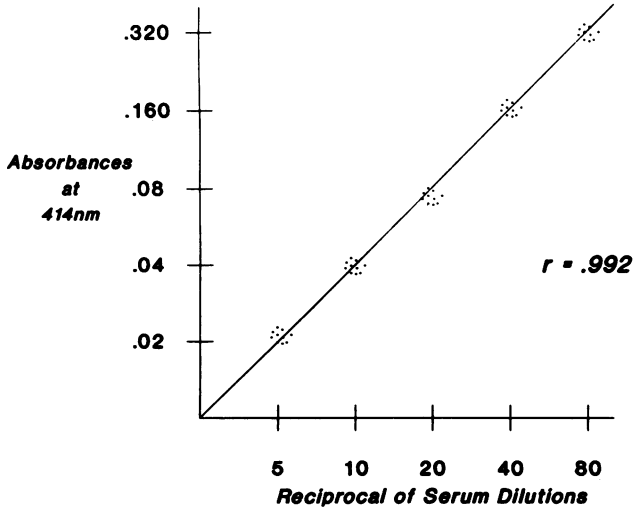

FIG. 1. Comparison of CEIA A_{414} values with dilutions of camel sera positive for *C. burnetii* antibody.

TABLE 2. Comparison of IFA, EIA, and CEIA for the detection of *C. burnetii* antibody in sheep and goat sera

Species	No. of samples tested	% Agreement (no. of samples positive) by:		
		IFA	EIA	CEIA
Sheep	40	38 (15)	50 (20)	63 (25)
Goat	96	34 (33)	35 (34)	50 (48)

The CEIA has been reported as a reliable, sensitive, and specific test for the detection of certain viral proteins (8) or specific antibodies to those proteins (10, 11). The test was modified in this study for the detection of *C. burnetii* antibody in camel sera, for which commercial conjugates were not available. An alternative assay (EIA-PA), in which a protein A conjugate was substituted for the antispecies conjugate, was also evaluated. Similar EIA-PAs have been used to detect antibody in the sera of many species of animals (4). Protein A binds only to the Fc portion of the IgG molecule and, therefore, was less sensitive than the conventional antispecies conjugates (12). Also, protein A has a high affinity to only human and rabbit IgG and, to a lesser extent, to IgG of other animal species (2).

The CEIA procedure was optimized by using sheep and goat sera negative and positive for *C. burnetii* antibody in order to compare results with those of a well-established EIA and IFA. Results of these comparisons indicated that the CEIA and the more standard IFA share similar specificities. However, the CEIA was more sensitive than either IFA or EIA.

EIAs were compared by using either antispecies peroxidase conjugate or protein A-peroxidase to detect *C. burnetii* antibody in sheep and goat sera. Data indicated that protein A was ≥50% less sensitive than the antispecies peroxidase conjugate. Comparing CEIA with EIA-PA for the detection of *C. burnetii* antibody in camel sera demonstrated the lack of reproducibility and lower sensitivity of the EIA-PA.

The CEIA was used to test 200 camel serum samples collected in Egypt during 1989 and successfully detected *C. burnetii* antibody in 131 (66%) of the samples. The prevalence of *C. burnetii* antibody among camels in the present study is considerably higher than the 13% found in a previous study conducted in 1973, in which a micro-agglutination test was used as the screening assay (7). The wide discrepancies between these two results probably is a further reflection of the greater sensitivity of the CEIA versus the micro-agglutination test rather than an indication of a higher prevalence of infection among camels in the present study.

In conclusion, the CEIA was reliable for detecting antibody in species for which there are no commercially available conjugates and may be applicable for the detection of antibody in other wild animals or even birds for which commercial diagnostic reagents are not available.

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