

Serodiagnosis of Ram Epididymitis by Counterimmunoelectrophoresis, Using *Brucella ovis* Surface R Antigen

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The counterimmunoelectrophoresis (CIE) technique was developed as a diagnostic procedure for ram epididymitis caused by *Brucella ovis*. CIE test results with sera from naturally and experimentally infected male sheep compared favorably with those obtained by gel diffusion and complement fixation employing the same *B. ovis* surface R antigen. The main advantage of CIE over gel diffusion consists in a significant reduction of the time required to detect precipitin formation, whereas both methods obviate several of the difficulties encountered with complement fixation tests for *B. ovis*.

Serodiagnosis of ram epididymitis caused by *Brucella ovis* infection has been carried out by complement fixation (CF) and gel diffusion (GD) techniques (1, 3, 4, 9). Use of *B. ovis* surface R antigen (8) has improved the specificity of these tests (5, 7). Although this rough-phase antigen is common to *B. ovis* and *B. canis*, it does not cross-react with smooth-phase *Brucella*, other *Brucellaceae*, or *Enterobacteriaceae*. It has the added advantage of producing a single precipitin band in GD tests and not being anti-complementary.

The present report deals with the development and application of the counterimmunoelectrophoresis (CIE) test, using the surface R antigen, for diagnosis of natural and experimental *B. ovis* infection. Test results are compared with those obtained with the GD and CF tests.

MATERIALS AND METHODS

The specific surface R antigen employed in the CIE, GD, and CF tests in the present study was prepared as described previously by Myers et al. (8). A phosphate-buffered saline (pH 7.2) suspension of freshly harvested *B. ovis* cells, which had been kept at 80°C in a water bath for 2 h, was centrifuged. The supernatant was frozen, thawed, and recentrifuged. The clear antigenic preparation was then dialyzed against phosphate-buffered saline at 4°C and stored in small frozen or lyophilized portions until ready for use. The preparation was standardized by titrating antigen dilutions against positive control sheep serum and rabbit antiserum to *B. ovis*. The antigen concentration which produced the clearest precipitin band in GD after 24 h at room temperature was used.

The CIE test employed was similar to those used for the diagnosis of other infectious diseases (6). After preliminary studies to determine the optimal test con-

ditions for use in this system, the CIE procedure selected for use in this study was carried out as follows. Glass slides measuring 75 by 50 mm were covered with a thin coat of 0.9% melted agar (Difco Laboratories, Detroit, Mich.) in 0.05 M Veronal buffer (pH 8.2) containing 1:10,000 Merthiolate, and allowed to dry. Eight milliliters of the same agar solution was layered onto each slide with a pipette and allowed to gel at room temperature. Slides were placed in petri dishes and refrigerated at 4°C for at least 10 min.

A cardboard template was then placed under each glass slide, and wells 3 mm in diameter were punched in the gel with a corkborer. The design consisted of two parallel rows separated by a distance of 10 mm. Within each row the distance between wells was 6 mm.

Agar was removed from the wells at the anodic side of the slide. Sera were added and then electrophoresed for 30 min at a potential difference of 10 V between both extremes of each slide. Subsequently, agar was removed from the wells in the cathodic side of the slide. These were charged with the antigen, and electrophoresis was continued for an additional period of 90 min. Cooling of the agar was achieved by maintaining a constant current and periodically moistening the connecting filter paper wicks with the buffer during the run.

Positivity of the CIE test consisted, by visual detection, of a characteristic precipitation band at the end of the run.

The GD test for *B. ovis* was conducted as described previously (7). Briefly, 8.0 ml of 1.25% agar (Difco) in 0.85% phosphate-buffered saline containing 1:10,000 Merthiolate was layered onto glass slides (75 by 50 mm) as described above for the CIE test. A punch was used to cut a central horizontal antigen trough of approximately 60 mm in length and 4 mm in width. A row of five serum wells, 6 mm in diameter and 4 mm apart, was set at a distance of 3 mm from each side of the trough. Readings were made at 24, 48, and 72 h of

incubation, and the presence of the characteristic precipitation band diagnostic for *B. ovis* infection was recorded.

The CF test described by Biberstein and McGowan (1) was performed employing the *B. ovis* surface R antigen as reported previously (7, 8). A 1:10 serum dilution showing complete fixation was considered as a positive reaction, whereas partial or complete lysis was taken as indicative of a negative reaction.

Known positive sera from confirmed *B. ovis* cases and rabbit antiserum to this organism (8) were included as controls for each of the three tests. Negative test controls included similar *B. abortus* and *B. melitensis* sera (8).

Serum samples to be examined by the three tests were obtained from 14 male sheep inoculated by the intratesticular route with a *B. ovis* strain previously isolated from a naturally infected animal (group I). After experimental infection, serum samples were collected weekly on three occasions and thereafter at monthly intervals for 9 months. The organism was isolated from all animals by semen cultures made on week 2 postinoculation and throughout the course of the study.

Sera from male sheep were also collected on four farms with clinical or cultural (or both) evidence of *B. ovis* infection (group II). These included samples from five animals with clinical epididymitis from which the organism was isolated and samples from eight rams with similar signs whose semen cultures were negative.

Additional serum samples were obtained from 49 rams on a farm with no clinical or cultural evidence of *B. ovis* infection (group III).

RESULTS

Data on the comparative sensitivity of the CIE, GD, and CF tests for the detection of antibody activity in rams with natural and experimental *B. ovis* infection are shown in Table 1.

In general, a high correlation was observed

TABLE 1. Correlation among results of CIE, GD, and CF tests, using the surface R antigen in sera from natural and experimental *B. ovis* infection of sheep

Determination	CIE	
	+	-
Group I		
CF: +	161	0
-	7	0
GD: +	163	0
-	5	0
Group II		
CF: +	12	0
-	1	0
GD: +	13	0
-	0	0

among the results obtained with each test. All but three of the group I animals remained positive to each of the tests during the entire 10-month study period. However, 165 days postinoculation, seven serum samples collected from these three animals were CF negative, while two and seven of these were still positive in GD and CIE tests, respectively. Similarly, one of the group II rams which was positive both clinically and by culture was CIE and GD test positive while CF test negative.

Positive reactions were not observed in 49 rams from group III examined by the three tests.

The characteristic precipitation band of the *B. ovis* surface R antigen was revealed in CIE tests by sera from naturally and experimentally infected rams and by hyperimmune rabbit anti-*B. ovis* sera (Fig. 1). No precipitation bands were observed in CIE tests with *B. melitensis* rabbit antiserum, nor with sera from normal sheep.

DISCUSSION

Control of ram epididymitis caused by *B. ovis* has been achieved through the removal from flocks of CF or GD (or both) test-positive animals (1, 3, 4, 7). In the present study, CIE test results correlated well with those recorded by microslide GD and CF tests. This suggests that the diagnosis of *B. ovis* infection by CIE test may also be of value in the implementation of control measures.

Since the surface R antigen does not cross-react with smooth-phase *Brucella* (5, 8), its use in CIE, GD, or CF tests would render serodiagnosis of *B. ovis* infection applicable in areas in

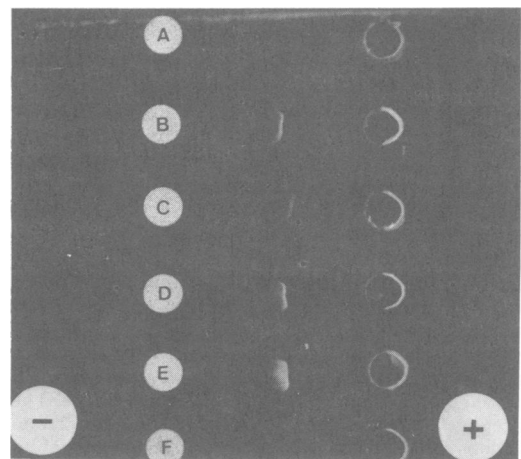


FIG. 1. CIE results with sera from a normal sheep (A), *B. ovis* culture-positive rams (B, C, D), and rabbit antisera to *B. ovis* (E) and to *B. melitensis* (F). Serum wells are located at the anodic (+) position of the slide, and antigen wells are at the cathode (-).

which control measures include immunization with *B. abortus* 19 (2) or *B. melitensis* Rev1 vaccines (11).

During the course of the development of the CIE technique described, a number of test variables were evaluated. These included the use of agar or agarose as supporting medium and the determination of the optimal conditions for electrophoresis. It was found that the distance between the precipitin band and the serum well was greater when agar was used in conjunction with a 30-min delay in the introduction of the antigen.

The CIE test with the surface R antigen is similar to the GD method in that the criterion for test positivity is based on the detection of a single precipitation band, diagnostic for *B. ovis* infection of sheep. It was slightly more sensitive than the CF test in the present study and had the convenience of obviating problems associated with anti-complementary or hemolyzed sera. The CIE technique has the added advantage of considerably reducing the time required to obtain test results, whereas the GD test lends itself for use in field conditions in which laboratory facilities are minimal.

Considering that the *B. ovis* surface R antigen is shared by *B. canis* (8, 10), studies are in progress to determine whether the CIE test is equally applicable to the diagnosis of canine and human infections caused by the latter organism.

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