JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 1984, p. 1159–1164
0095-1137/84/121159-06$02.00/0

Vol. 20, No. 6

Characterization of *Brucella ovis* Lipopolysaccharide and Its Use for Diagnosis of Ram Epididymitis by Enzyme-Linked Immunosorbent Assay

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Received 4 June 1984/Accepted 27 August 1984

Rough lipopolysaccharide, extracted by a mixture of phenol, chloroform, and petroleum ether from freeze-dried *Brucella ovis* cells with a yield of 0.71%, contained relatively small amounts of protein and nucleic acid contaminants as compared with lipopolysaccharides from other *Brucellae*. The crude lipopolysaccharide was suitable as a diagnostic antigen in an enzyme-linked immunosorbent assay for the sensitive and specific detection of ram epididymitis caused by *B. ovis* infection. In comparative serological tests, the enzyme-linked immunosorbent assay with *B. ovis* lipopolysaccharide gave better identification of infections and fewer false-negative results than the enzyme-linked immunosorbent assay with sonicated antigen or the complement fixation test.

Ram epididymitis is an important disease in major sheep-raising countries, including the United States, New Zealand, and Australia. The incidence of the disease varies from 15 to 40% in the western United States (14). Control of the disease has been limited by the lack of an accurate diagnostic test and an effective vaccine. The efficacy of a presently available commercial bacterin (14) varies; in an experimental infection of 8-month-old rams, it gave 56% protection, whereas a new vitamin E adjuvant vaccine of *Brucella ovis* gave 78% protection (M. Afzal, R. P. Tengerdy, R. P. Ellis, C. V. Kimberling, and C. J. Morris, Vet. Immunol. Immunopathol., in press).

Current tests used to diagnose ram epididymitis caused by *B. ovis* include physical palpation of the scrotum for lesions, detection of leukocytes in the semen, isolation of *B. ovis* from the semen, and detection of antibodies in the serum by using the complement fixation (CF) test (10). Recently, Rahaley et al. (25) described an enzyme-linked immunosorbent assay (ELISA) with a sonicated antigen for the detection of antibodies to *B. ovis* in sheep sera. The CF test, the most frequently used serological method for detection of *B. ovis* infection, gives a large number of false-negative and false-positive results (26), and ELISA with a sonicated antigen may yield false-positive results occasionally (25).

B. ovis-infected rams may develop serum antibodies to a wide variety of cellular antigens, including lipopolysaccharide (LPS), outer membrane proteins, and ribosomes. LPS is a potential candidate for diagnostic purposes, since antigens in current use are known to contain LPS (24) and a specific immune response to *B. ovis* LPS can be elicited by whole-cell immunization in rabbits and mice (21). Furthermore, LPS from *Brucella abortus* has been used successfully for the diagnosis of brucellosis in cattle and humans (4, 17, 22, 27).

In the present paper, the isolation and characterization of *B. ovis* LPS and its use in diagnostic ELISA is reported. The CF test and ELISA with LPS and the sonicated antigen used in earlier studies (25) are compared, and their suitability for diagnosis is evaluated by biometric analysis.

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MATERIALS AND METHODS

Bacterial strain and cultivation. *B. ovis* was isolated from the semen of an infected ram with physically palpable epididymitis and identified by morphological, cultural, and biochemical tests (2). The isolated *B. ovis* was grown on tryptose agar (Difco Laboratories, Detroit, Mich.) containing 10% bovine calf serum (K.C. Biologicals, Lenexa, Kans.) for 5 to 7 days in a 10% CO_2 atmosphere. Cells were harvested in distilled water and strained through several layers of sterile cheesecloth. The cells were packed by centrifugation at 6,000 × *g* for 30 min at 4°C and lyophylized.

Isolation and characterization of LPS. LPS from freeze-dried *B. ovis* cells was extracted by phenol-chloroform-petroleum ether (PCE, 2:5:8) as described previously (7, 22). Briefly 2.5 g of freeze-dried cells were extracted three times with 15 ml of PCE as shown in Fig. 1. The extracts were pooled, and the chloroform and petroleum ether were removed under vacuum at 30°C. Distilled water was added dropwise until precipitate started forming, and then the suspension was centrifuged at 5,000 × *g* at 4°C for 40 min. The precipitate was washed with distilled water and freeze-dried. This fraction contained 2-keto-3-deoxyoctonate (KDO) and was designated crude LPS.

For further characterization of the crude LPS, it was dissolved in 0.05 M Tris buffer (pH 8.0) containing 1% sodium deoxycholate and submitted to size-exclusion chromatography on a Sephadex G-100 column (300 by 25 mm) equilibrated with the same buffer. The fractions were monitored by light absorbance at 280 nm for protein, at 260 nm for nucleic acid, at 630 nm for carbohydrates (11), and by the difference in absorbances at 552 and 508 nm for KDO (30). For comparison, the sonicated antigen (described below) was subjected to the same procedure.

Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) was carried out on crude LPS and on pooled fractions 11 to 18 from the Sephadex G-100 run, with 15% minigels at a constant current of 80 V by the method of Laemmli (16). After electrophoresis, the gels were fixed in a 40% ethanol–5% acetic acid solution overnight and stained with silver stain (29).

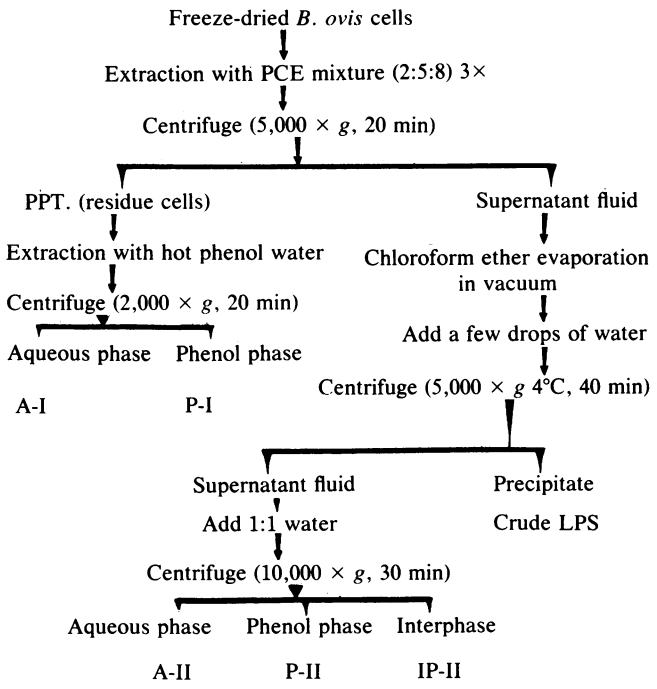

FIG. 1. Extraction of LPS from *B. ovis* cells. PPT., Precipitate.

Immunodiffusion was carried out on 1% agar slides with plexiglass templates to contain the antigen and antisera (5). Slides were dried and stained with Crowle's stain.

Chemical analysis. Protein content was assayed by the Lowry test (18) with bovine serum albumin fraction V (Sigma Chemical Co., St. Louis, Mo.) as the standard. Total carbohydrates were measured by the anthrone assay (11) with dextran (Sigma) as the standard. KDO was measured by the thiobarbituric assay (30) using purified KDO as standard (Sigma).

Antigens for serological tests. (i) CF antigen. *B. ovis* CF antigen was kindly supplied by the National Veterinary Services Laboratory, Ames, Iowa. This antigen is prepared by suspending *B. ovis* cells in normal saline and autoclaving the preparation at 15 lb/in^2 for 20 min and then centrifuging it at 10,000 × *g* for 1 h. The supernatant fluid is used as the antigen. After being diluted 1:64, the supernatant had an absorbance of 0.55 at 280 nm.

(ii) Sonicated antigen. *B. ovis* cells were grown and harvested as described above. Cells were suspended in 0.05 M carbonate buffer (pH 9.6) to give 12% transmittance at 540 nm in a spectrophotometer (Spectronic-20; Bausch & Lomb, Inc., Rochester, N.Y.) and sonicated at 115 W (Sonifier cell disruptor; Heat Systems Co., New York) for 30 min on ice. The sonicated suspension was centrifuged at 10,000 × *g* for 40 min at 4°C, and the supernatant fluid was used as the antigen.

(iii) Crude LPS. LPS was solubilized in 1% SDS at a concentration of 1 mg/ml and diluted to 10 μg/ml in 0.05 M carbonate buffer (pH 9.6) for ELISA.

Serum samples. Serum samples were collected from 20 experimental rams in a vitamin E adjuvant vaccine study (Afzal et al., in press) and from 27 field cases. Ram sera were divided into three groups: group I, positive sera, where *B. ovis* was isolated from the semen; group II, negative sera, where semen appeared normal and *B. ovis* was not isolated; and group III, suspected sera, where *B. ovis* was not isolated but the epididymis was abnormal or the semen had an increased number of leukocytes or separated heads.

Serological tests. (i) Micro-CF test. The CF test was performed in U-bottom polyvinyl microtiter plates (Dynatech Laboratories, Inc., Alexandria, Va.) as described previously (3). Briefly, sheep sera were diluted 1:10 in modified Veronal buffer and inactivated at 60°C for 1 h. The inactivated sheep serum was serially diluted twofold so that each well contained 0.025 ml of diluted serum, and then 0.025 ml of *B. ovis* CF test antigen and 0.025 ml of 1:35 diluted guinea pig complement containing five 50% hemolytic complement units were added to each well. The plates were incubated overnight in a refrigerator (4°C); then 0.025 ml of sensitized sheep erythrocytes (3% [vol/vol]–2 U of hemolysin in an equal volume) were added, and the plates were shaken on a reciprocal shaker at 150 rpm for 45 min at 37°C. The titer is the serum dilution giving 50% or less hemolysis by visual observation. An animal with a CF titer of 10 or higher was considered positive.

(ii) ELISA. ELISA was carried out in quadruplicate tests in polystyrene microtiter plates (Dynatech). A 100-μl volume of antigen (sonicated antigen or 10 μg of LPS per ml) was dispensed into each well and incubated overnight (16 to 20 h) in a constant humidity chamber at room temperature for LPS or at 4°C for sonicated antigen. Plates were washed once with physiological buffered saline (PBS; 0.14 M sodium chloride containing 0.01 M phosphate buffer [pH 7.2] and 0.5 ml of Tween 20 per liter) and then incubated with 100 μl of 10% fetal calf serum for 45 min at 37°C to block nonspecific adsorption of serum proteins. Plates were washed five times with PBS. A 50-μl volume of 1:100-diluted ram serum in PBS was added, and the plates were incubated on a shaker (ca. 100 rpm) at 37°C for 1 h. Plates were washed five times with PBS, and 50 μl of 1:750-diluted rabbit anti-sheep immunoglobulin G–peroxidase conjugate (Cappel Laboratories, Inc., Malvern, Pa.) was added. Plates were incubated at 37°C for 1 h and then washed five times with PBS. A 50-μl volume of substrate (34 mg of *o*-phenylene-diamine hydrochloride in 100 ml of phosphate-citrate buffer [0.05 M citrate, 0.1 M phosphate {pH 5.0}] containing 0.01% H_2O_2) was added, and the plates were incubated for 30 min. The reaction was

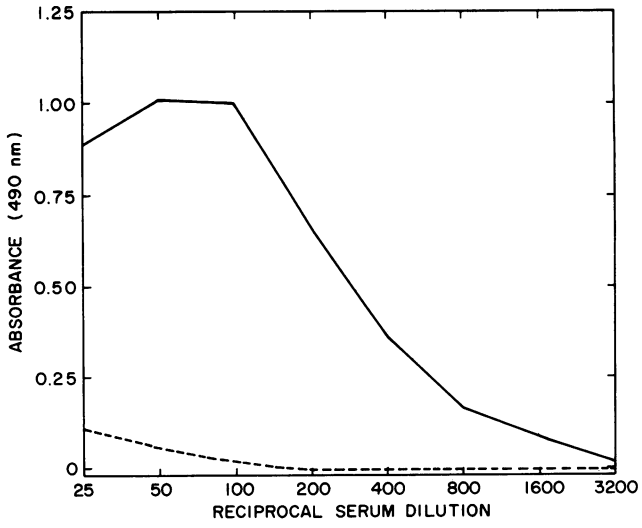

FIG. 2. Effect of serum dilution on ELISA. Symbols: ——, pooled positive sera; – – – –, pooled negative sera.

TABLE 1. Chemical analysis of PCE-extracted fractions from the *B. ovis* fraction

Fraction	Yield (%)[a]	Protein (%)[b]	Carbohydrate (%)[b]	KDO (%)[b]
Crude LPS	0.71	Tr	25.1	3.25
Aqueous II	Tr	—[c]	—	—
Phenol II	4.55	50.6	5.2	0
Interphase II	0.27	30.4	8.3	Tr
Aqueous I	Tr	—	—	—
Phenol I	—	—	—	0

[a] The percentages were measured by using gram/gram (dry weight) of cells.
[b] The percentages were measured by using gram/gram (dry weight) of fractions.
[c] —, Not done.

stopped by the addition of 50 μl of 2 N sulfuric acid, and absorbances were read at 490 and 630 nm on a dual-beam ELISA reader (Dynatech).

The optimal serum dilution used in ELISA was determined by comparing pooled sera from six positive and six negative rams. These pooled sera were serially diluted starting at a 1:25 dilution and tested in ELISA. The dilution 1:100, where the maximum absorbance difference was observed between positive and negative sera, was regarded as the optimal working dilution (Fig. 2) at which all test sera were compared. With each test, one pooled negative (CF titer, 0), one pooled positive (CF titer, 80), and three positive sera (CF titers, 10, 20, and 50 [National Veterinary Services Laboratory, Ames, Iowa]) were run for reference. A serum sample with an ELISA absorbance reading equal to or greater than the absorbance of a positive reference serum (CF, ≥10) was regarded as positive.

Semen evaluation. Semen was collected by electroejaculation on the same day that blood was collected for serology. Motility and the presence of leukocytes were examined immediately. Semen morphology was studied after cells were stained with Hancock's stain (Society of Theriogenology, Hastings, Nebr.). Each semen sample was cultured on 10% serum tryptose agar and vancomycin chocolate agar. Plates were incubated at 37°C for 3 to 5 days in a 10% CO_2 atmosphere. Isolated colonies were further examined for positive identification of *B. ovis*.

RESULTS

Characterization of the crude LPS extracted from *B. ovis*. The PCE extraction of *B. ovis* cells yielded crude LPS precipitated from the extract, containing mostly rough LPS. The aqueous phase of the hot phenol-water extraction (A-I) of the residual cells, which is supposed to contain smooth LPS, yielded only a trace amount of material (Fig. 1). The chemical composition of the different fractions obtained in the extraction process is shown in Table 1. The comparison of the chemical composition of LPS preparations from

TABLE 2. Chemical composition of LPS from *Brucellae* spp.[a]

Species	LPS form	Yield (%)	Protein (%)	KDO (%)	Fatty acid (%)	Total carbohydrate (%)	Extraction method	Reference
B. abortus								
1119-3	Smooth		20.00	0.60		17.20	Dimethylsulfoxide	8
1119-3	Smooth		7.00	0.54		20.00	Modified hot phenol	8
1119-3	Smooth		24.0	0.86		31.10	Phenol-water	20, 23
1119-3	Smooth		6.30	0.9	26.4	32.5	Phenol-water	21
1119-3	Smooth		35.2	0.31		11.42	Modified phenol-water	28
11-19	Smooth	0.77	24.0	0.32	19.00	10.7	Modified phenol-water (phenol phase)	22
544	Smooth	1.53	15.63	6.5		34.8	Hot phenol-water (aqueous phase)	9
	Smooth	4.39	21.25	8.4		19.6	Hot phenol-water (phenol phase)	9
544	Smooth		11.40	9.5		44.10	Phenol-water (phenol phase)	19
14	Smooth		33.8	0.52		10.4	Modified phenol-water	1
45/0	Smooth		42.00	0.61	1.0	21.63	Modified phenol-water (aqueous phase)	15
45/0	Smooth		30.00	0.38	2.0	11.40	Modified phenol-water (phenol phase)	15
45/20	Rough		18.00	0.46	0.5	17.94	Phenol-water (aqueous phase)	15
45/20	Rough		10.00	9.5		8.60	Phenol-water	15
45/20	Rough		2.00	3.1	30.0	16.8	Phenol-water	21
45/20	Rough	0.40	1.3	0.25	10.00	13.6	Phenol-water (aqueous phase)	22
B. melitensis								
16M	Smooth					40.10	Modified phenol-water	20, 23
565	Smooth		5.34		10.00	82.5	NaOH hydrolysis of Boivin antigen	6
B115	Rough		2.0	3.4	52.0	15.8	Phenol-water	21
B. canis	Rough		0.0	2.3	29.0	11.8	Phenol-water	21
B. ovis REO	Rough		0.0	5.5	50.0	19.0	Phenol-water	21
strain	Rough	0.71	Tr	3.25		25.1	PCE	This work

[a] It is assumed that the chemical composition was calculated on the dry weight basis of the LPS fraction in most of the studies.

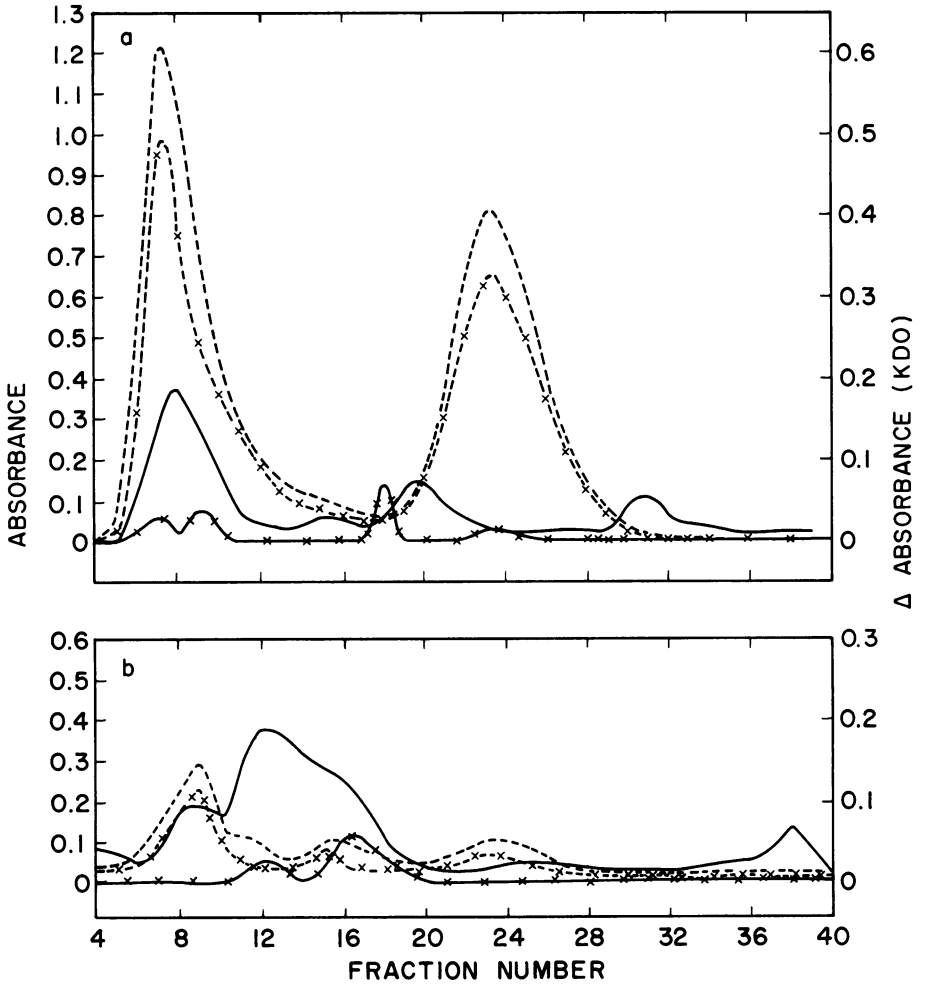

FIG. 3. Sephadex G-100 separation of *B. ovis* antigens. (a) Sonicated antigen; (b) crude LPS. Symbols: ——, carbohydrate (630 nm); —X—, KDO (Δ = 552 to 508 nm); - - - -, nucleic acid (260 nm); - - -X- - -, protein (280 nm).

different *Brucellae* by various extraction methods is presented in Table 2. It is evident that the yield of the PCE-extracted crude *B. ovis* LPS is more than of rough LPS from *B. abortus* but less than of smooth LPS from *B. abortus*. It is clear from Table 2 that the PCE-extracted crude LPS has a superior purity compared with other LPS preparations, it has the high KDO content usually associated with rough LPS and is essentially free of protein.

The result of the separation of the components of the sonicated antigen and crude LPS by Sephadex chromatography can be seen in Fig. 3a and b. Sonicated antigen is included here since it has been used as diagnostic antigen in ELISA previously (25). It can be seen that the sonicated antigen contains LPS (KDO activity) as one of the main antigenic components in various associations with proteins, nucleic acids, and carbohydrates; but in the crude LPS, all of the KDO activity and most of the carbohydrate can be found in fractions 11 to 18. The LPS is not bound to protein and contains relatively small amounts of other materials.

When the pooled fractions 11 to 18 from the Sephadex G-100 run of the crude LPS were subjected to double immunodiffusion against *B. ovis* antiserum from an infected ram, a single band was observed. The crude LPS and the pooled fraction gave one major band in SDS-PAGE (Fig. 4). The *B. ovis* LPS band had an identical position with the rough LPS band of the reference *Escherichia coli* LPS.

Diagnosis of *B. ovis* infection by serological tests. A positive diagnosis of *B. ovis* infection by CF and ELISA as correlated with established disease criteria is shown in Table 3. The

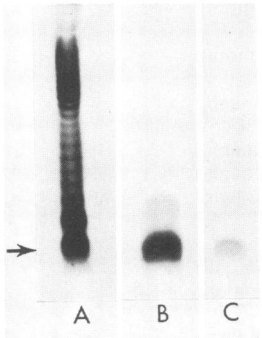

FIG. 4. SDS-PAGE of LPS. Lane A, LPS from *E. coli* 0111:B4; lane B, crude LPS from *B. ovis*; lane C, pooled fractions 11 to 18 from the Sephadex G-100 column. The arrow indicates rough LPS from *E. coli*.

TABLE 3. Detection of *B. ovis* infection by different diagnostic tests

Animals (no.)[a]	No. of animals with				No. of animals with positive titer by[e]		
						ELISA with:	
	Palpable epididymitis	Culture-positive semen[b]	Leukocytes in semen[c]	Separated heads in semen[d]	CF	Sonicated antigen	LPS
Experimental							
Positive (10)	6	10	4	6	8	9	9
Negative (8)	0	0	0	1	0	1	0
Suspected (2)	0	0	1	1	0	2	2
Field cases							
Positive (9)	8	9	6	5	8	9	9
Negative (15)	0	0	0	1	0	0	0
Suspected (3)	1	0	2	1	0	2	2

[a] Animals were designated as follows: positive, *B. ovis* isolated at least once on repeated culturing; negative, *B. ovis* was not isolated from semen and semen appeared normal; suspected, *B. ovis* was not isolated from semen, but other criteria suggested infection.
[b] *B. ovis* was isolated from semen.
[c] Presence of >20 leukocytes per field at a magnification of ×400.
[d] Presence of >30% separated heads.
[e] Positive titers were judged for CF titer of ≥10 or ELISA absorbances equal to or greater than the absorbance of a positive reference serum.

main criterion for infection was the isolation of *B. ovis* from semen at least once in repeated culturing. ELISA was more sensitive than CF, since it detected 18 of 19 positive infections and 4 of 5 suspected infections, whereas CF detected only 16 positive and none of the suspected infections. These differences, however, were statistically not significant ($P > 0.05$). One experimental ram from which *B. ovis* was isolated only once during a 3-month period had neither CF nor ELISA titers. In field cases, the correlation between positive serum titer and infection is better (8 of 9 for CF, and 9 of 9 for ELISA) than that for experimental rams (8 of 10 for CF, and 9 of 10 for ELISA). The ELISA test with sonicated antigen gave 1 false-positive among 23 negative rams tested. This animal had normal semen, *B. ovis* was not isolated upon repeated culturing, and the epididymis appeared histologically normal. This result was expected since the sonicated antigen may contain certain antigens shared by other gram-negative organisms, and thus it is less specific than LPS. The ELISA titer of suspected rams may indicate subclinical exposure to *B. ovis*. This view is supported by the observation that one of the two rams in the experimental group did not develop the disease upon challenge with *B. ovis*, indicating a possible state of immunity.

A biometric analysis of the two serological tests, CF and ELISA, using three different antigens, the CF antigen in the CF test, the sonicated antigen, and LPS in ELISA, is shown in Table 4. It appears that ELISA is a more suitable diagnostic test than CF, especially when a specific diagnostic antigen like LPS is used.

DISCUSSION

The yield and chemical composition of LPS from *Brucellae* depend on the strain, extraction method, and purification procedure (Table 2). The PCE extraction gave a crude LPS preparation that could be used directly as a diagnostic antigen in ELISA and compared favorably in chemical properties with other LPS preparations obtained by other methods. The crude LPS contained only small amounts of nucleic acids and other contaminants that apparently did not interfere with the specificity of the antigen in ELISA. In SDS-PAGE, the *B. ovis* LPS moved similarly to the rough LPS of *E. coli*. The relatively high KDO content distinguishes the *B. ovis* LPS from other *Brucellae*. This observation also corroborated the data of Moreno et al. (21). A smooth LPS that is present in the phenol phase of the phenol-water extraction of *B. abortus* (22) was present in the corresponding fraction (fraction A-I) from *B. ovis* only in trace amounts, insufficient for further analysis.

Although the LPS from *B. ovis* had an identical position with the rough LPS of *E. coli* in SDS-PAGE, LPS of *Brucellae* are significantly different from LPS of *Enterobacteriaceae*, and cross-reactions caused by enteric infections which might result in false-positive tests have not been reported (8, 12, 13, 15, 20). The *B. ovis* LPS shares some antigenic determinants with rough LPS of other *Brucellae* as was shown by partial identity patterns in immunodiffusion (21). It may be expected, therefore, that ELISA with *B. ovis* LPS would detect other *Brucella* infections too. The sonicated antigen contains other antigens besides LPS, some of which may be shared with other organisms; thus, more cross-reactions are expected. The biometric analysis of the 23 negative cases examined in this study seems to bear out this assumption; the LPS antigen did not give any false-positive results, whereas the sonicated antigen gave one. This assumption has to be further validated by testing a large number of ram sera. The biometric analysis indicates that

TABLE 4. Sensitivity and specificity of the CF test and ELISA for the diagnosis of *B. ovis* infection[a]

Test	Total positive	True positive	False-positive	Total negative	True negative	False-positive	Specificity (%)	Sensitivity (%)
CF	19	16	3	23	23	0	100.0	84.2
ELISA								
Sonicated antigen	19	18	1	23	22	1	95.7	94.7
LPS	19	18	1	23	23	0	100.0	94.7

[a] The percent specificity was defined as [the number of true negatives/(the number of true negatives + the number of false-positives)] × 100. The percent sensitivity was defined as [the number of true positives/(the number of true positives + the number of false-negatives)] × 100.

the ELISA is more sensitive and as specific as the CF test and LPS is more specific for diagnosis than the sonicated antigen.

ACKNOWLEDGMENTS

This work was supported by a grant from Hoffman La Roche, Inc. and by grants from the U.S. Department of Agriculture (Animal Health, Colorado State University Experiment Station Program).

The technical help of Dave Schoen and Matt Sumpter is gratefully acknowledged.

LITERATURE CITED

1. **Birmingham, J. R., L. B. Tabatabai, B. L. Deyoe, E. L. Jeska, and M. E. Nuessen.** 1982. Generation of chemotactic factor for granulocytes and monocytes from serum by fractions of *Brucella abortus*. Immunology **46**:17–22.
2. **Brinley-Morgan, W. L., and N. B. McCullough.** 1974. Genus Brucella, p. 278–282. *In* R. E. Buchanan and N. E. Gibbons (ed.), Bergey's manual of determinative bacteriology, 8th ed. The Williams & Wilkins Co., Baltimore.
3. **Burgess, G. W., and M. J. Morris.** 1982. Evaluation of cold complement fixation test for diagnosis of ovine brucellosis. Aust. Vet. J. **59**:23–25.
4. **Carlson, H. E., B. Hurvell, and A. A. Lindberg.** 1976. Enzyme-linked immunosorbent assay (ELISA) for titration of antibodies against *Brucella abortus* and *Yersinia entercolitica*. Acta Pathol. Microbiol. Scand. Sect. C **84**:168–176.
5. **Crowle, A. J.** 1973. Immunodiffusion, 2nd ed. Academic Press, Inc., New York.
6. **Dranovskaya, E. A., I. V. Shibayeva, H. A. Khabakpasheva, and N. A. Rostovtseva.** 1975. Immunological, biochemical and physico-chemical characteristics of *Brucella* lipopolysaccharide subjected to various doses of gamma radiation. J. Hyg. Epidemiol. Microbiol. Immunol. **19**:116–125.
7. **Galanos, C., O. Lüderitz, and O. Westphal.** 1969. A new method for the extraction of R lipopolysaccharide. Eur. J. Biochem. **9**:245–249.
8. **Hoffmann, E. M., and J. J. Houle.** 1983. Failure of *Brucella abortus* lipopolysaccharide to activate the alternate pathway of complement. Vet. Immunol. Immunopath. **5**:65–76.
9. **Hurvell, B.** 1973. Serological cross reactions between different brucella species and *Yersinia enterocolitica*. Acta Pathol. Microbiol. Scand. Sect. B **81**:105–112.
10. **Jensen, R., and B. L. Swift.** 1982. Diseases of sheep. Lea & Febiger, Philadelphia.
11. **Jermyn, M. A.** 1975. Increasing the sensitivity of the anthrone method for carbohydrates. Anal. Biochem. **68**:332–335.
12. **Jones, L. M., and D. T. Berman.** 1976. Studies of Brucella lipopolysaccharide. Dev. Biol. Stand. **31**:62–67.
13. **Jones, L. M., R. Diaz, and D. T. Berman.** 1976. Endotoxic activity of rough organisms of *Brucella* species. Infect. Immun. **13**:1638–1641.
14. **Kimberling, C. V.** 1980. Ram epididymitis. CSU Extension Bulletin 8-016. Colorado State University, Fort Collins.
15. **Kreutzer, D. L., C. S. Buller, and D. C. Robertson.** 1979. Chemical characterization and biological properties of lipopolysaccharides isolated from smooth and rough strains of *Brucella abortus*. Infect. Immun. **23**:811–818.
16. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
17. **Lamb, V. L., L. M. Jones, G. G. Schurig, and D. T. Berman.** 1979. Enzyme-linked immunosorbent assay for bovine immunoglobulin subclass-specific response to *Brucella abortus* lipopolysaccharide. Infect. Immun. **26**:240–247.
18. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement by the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
19. **Marx, A., J. Ionescu, and A. Pop.** 1983. Immunochemical studies on *Brucella abortus* lipopolysaccharide. Zentralbl. Bakteriol. Hyg. I Abt. Orig. A **253**:544–553.
20. **Moreno, E., D. T. Berman, and L. A. Boettcher.** 1981. Biological activities of *Brucella abortus* lipopolysaccharide. Infect. Immun. **31**:362–370.
21. **Moreno, E., L. M. Jones, and D. T. Berman.** 1984. Immunochemical characterization of rough *Brucella* lipopolysaccharides. Infect. Immun. **43**:779–782.
22. **Moreno, E., M. W. Pitt, L. M. Jones, G. G. Schurig, and D. T. Berman.** 1979. Purification and characterization of smooth and rough lipopolysaccharides from *Brucella abortus*. J. Bacteriol. **138**:361–369.
23. **Moreno, E., S. L. Speth, L. M. Jones, and D. T. Berman.** 1981. Immunochemical characterization of *Brucella* lipopolysaccharides and polysaccharides. Infect. Immun. **31**:214–222.
24. **Myers, D. M., L. M. Jones, and V. M. Varela-Diaz.** 1972. Studies of antigens for complement fixation and gel diffusion tests in the diagnosis of infections caused by *Brucella ovis* and other brucella. Appl. Microbiol. **23**:894–902.
25. **Rahaley, R. S., S. M. Dennis, and M. S. Smeltzer.** 1983. Comparison of the enzyme-linked immunosorbent assay and complement fixation test for detecting *Brucella ovis* antibodies in sheep. Vet. Rec. **12**:467–470.
26. **Ris, D. R.** 1973. Is a *Brucella ovis* control scheme based on the complement fixation test technically feasible? Proc. NZVA Sheep Society's 3rd Seminar, p. 77–79.
27. **Schurig, G. G., L. M. Jones, S. L. Speth, and D. T. Berman.** 1978. Antibody response to antigens distinct from smooth lipopolysaccharide complex in Brucella infection. Infect. Immun. **21**:994–1002.
28. **Spellman, I. M., and N. D. Reed.** 1979. Immune and mitogenic responses by BALB/c, C_3H/H_eJ and nude mice to *Brucella abortus* bacterin and lipopolysaccharide. Infect. Immun. **24**: 371–378.
29. **Tsai, C.-M., and C. E. Frasch.** 1982. A sensitive silver stain for detecting lipopolysaccharides in polyacrylamide gels. Anal. Biochem. **119**:115–119.
30. **Waravdeker, V. S., and L. D. Saslaw.** 1959. A sensitive colorimetric method for the estimation of 2-deoxy sugars with the use of the malonaldehyde-thiobarbituric acid reaction. J. Biol. Chem. **234**:1945–1950.