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Humoral Immune Response against Lipopolysaccharide and Cytoplasmic Proteins of *Brucella abortus* in Cattle Vaccinated with *B. abortus* S19 or Experimentally Infected with *Yersinia enterocolitica* Serotype 0:9

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The humoral immune responses against three different antigens of *Brucella abortus* were monitored by enzyme-linked immunosorbent assay in cattle vaccinated with *B. abortus* S19 or experimentally infected with *Yersinia enterocolitica* serotype 0:9. Immunoglobulin G (IgG) and IgM responses against (i) *B. abortus* lipopolysaccharide (LPS), (ii) total cytoplasmic proteins depleted of LPS (LPS-free CYT), and (iii) *B. abortus* 18-kDa cytoplasmic protein were measured. Vaccinated animals and *Yersinia*-infected animals developed high anti-LPS IgM and IgG titers, which overlapped with those obtained with sera from *B. abortus* 544-infected animals used as positive controls. In contrast, only a slight or negative IgG and IgM response against LPS-free CYT and the 18-kDa protein was detected in vaccinated or *Yersinia*-infected cattle, although its levels were always significantly lower than those of *B. abortus* 544-infected animals. These data indicate that cytoplasmic proteins of *B. abortus* could be useful for the differential diagnosis of bovine brucellosis.

One of the most difficult tasks in the serological diagnosis of bovine brucellosis has been the discrimination of infected from vaccinated animals (2, 21). Conventional serologic methods, such as agglutination or complement fixation tests, principally measure antibodies to smooth lipopolysaccharide (LPS). Since animals vaccinated with *Brucella abortus* S19 and animals naturally infected with field strains develop similar anti-LPS responses, it is difficult to establish their status by means of conventional tests (22).

Agglutination in the presence of 2-mercaptoethanol (1) or a radial immunodiffusion test using the poly B antigen of *Brucella melitensis* has been proposed as a means to solve this problem (7, 14).

A competitive enzyme-linked immunosorbent assay (ELISA) using an anti-LPS monoclonal antibody improved the sensitivity of the diagnosis but failed to differentiate vaccinated from infected animals (27). Although a *B. abortus*-soluble fraction was also used as a solid-phase antigen in ELISA tests (11, 24), it contained LPS, which may lead to the undesired reactivities mentioned above. However, a competitive ELISA with *Brucella* O polysaccharide as the antigen and monoclonal antibodies showed promising results (22, 23).

In addition, it is very difficult to differentiate cattle naturally infected with smooth strains of *Brucella* from those infected with *Yersinia enterocolitica* serotype 0:9 by means of techniques based on the detection of anti-LPS antibodies (5, 6, 13, 19). Various strategies to avoid this cross-reactivity have been proposed (6, 20). These tests, however, have not been satisfactory in terms of specificity and/or sensitivity (19).

The usefulness of antigens other than LPS for the differential diagnosis of bovine brucellosis has been poorly investigated. We have recently shown that the measurement of immunoglobulin G (IgG) reactivity against an extract of cytoplasmic proteins of *Brucella* or against an 18-kDa cytoplasmic protein made possible the specific diagnosis of active human brucellosis (8, 10). We also showed preliminary results suggesting the potential usefulness of determining anti-18-kDa-protein antibodies for differentiating infected from vaccinated cattle. More recently, Hemmen et al. (12), working with the same protein (cloned in *Escherichia coli*), obtained similar results for cattle.

In the present study, the humoral immune responses to total cytoplasmic proteins of *Brucella* and to the 18-kDa protein have been measured during the course of infection in *Y. enterocolitica* 0:9-infected and *B. abortus* S19-vaccinated cattle and compared with those obtained against LPS.

To this end, three ELISA systems were used. Serum reactivity against LPS and LPS-associated proteins was determined by capture ELISA with the anti-LPS monoclonal antibody BC68 as described previously (10), with minor modifications. Purified BC68 was adsorbed onto Maxisorp polystyrene plates (Nunc, Roskilde, Denmark). Plates were blocked with a solution (200 μl per well) of phosphate-buffered saline (PBS) containing 0.3% gelatin (Merck, Darmstadt, Germany) and 1.2% chicken egg albumin (Sigma, St. Louis, Mo.). After a wash, the cytoplasmic fraction (5 μg of LPS per well) of *Brucella* (CYT) diluted in PTG (PBS containing 0.05% Tween 20, 30 mM disodium EDTA, 0.1% gelatin, and 0.4% chicken egg albumin) was added. After a wash, bovine sera diluted 1:200 in PTG were dispensed. Specific antibodies were detected with an anti-bovine IgG monoclonal horseradish peroxidase conjugate (Zymed, South San Francisco, Calif.) or an anti-bovine IgM polyclonal horseradish peroxidase conjugate (The Binding Site, Birmingham, United Kingdom). The reaction was developed

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by adding *ortho*-phenylenediamine (2 μg/μl, in 0.1 M citrate-phosphate buffer containing 0.03% H_2O_2).

Serum reactivity against *Brucella* cytoplasmic proteins was assayed by indirect ELISA by using LPS-free CYT as described previously (9, 10). The plates were sensitized with 0.5 μg of LPS-free CYT per well diluted in PBS. Washing, blocking, testing of sera, addition of the conjugates, and development of the reaction were performed as described above.

Serum reactivity against an 18-kDa cytoplasmic protein of *Brucella* was determined by capture ELISA with monoclonal antibody BI24, as described previously (8). Purified BI24 was adsorbed at 1 μg per well in PBS onto Maxisorp polystyrene plates, and then 2 μg of the LPS-free CYT antigen per well diluted in PTG was added. Washing, blocking, testing of sera, addition of the conjugates, and development of the reaction were performed as described above.

In all cases, the specific optical density (OD) of each serum sample was calculated as the difference between the ODs obtained with and without an antigen.

To establish the cutoff value of the assays, 30 serum samples from *Brucella*-free healthy controls from New Zealand and Argentina were assayed at a 1:200 dilution under the same conditions used for sera from infected animals. All these sera were negative in standard tube agglutination, complement fixation, and Rose Bengal plate tests. The cutoff value of each ELISA system was calculated as the mean specific OD plus 3 standard deviations.

A pool of sera from *Brucella*-infected animals was included in all the ELISAs performed. The OD rendered by this pool was used as a reference OD to standardize the results obtained in separate assays.

Antibody response in *B. abortus* S19-vaccinated animals. Six calves (4 to 8 months old) were subcutaneously inoculated with a commercial *B. abortus* S19 vaccine controlled and certified by the Argentine Animal Health Service (SENASA). These animals were born and maintained in a controlled area, free from brucellosis, tuberculosis, foot-and-mouth disease, and other common diseases. Blood samples were obtained monthly after vaccination.

As shown in Fig. 1A, anti-LPS IgG levels were markedly increased in all the animals at day 30 postvaccination and then began to decrease. At day 150, however, three of the six animals still had anti-LPS IgG levels higher than the cutoff. Conversely, the IgG response against the LPS-free CYT extract (Fig. 1B) showed only a slight increase between days 30 and 60 postvaccination and reached normal levels at day 90 in all the animals. Results similar to those obtained with the LPS-free CYT extract were obtained when the IgG response against the 18-kDa protein was measured (not shown).

Anti-LPS IgM antibodies were increased at different levels at day 30 in all the vaccinated animals and then began to decrease, reaching normal levels at day 150 postvaccination (not shown). Conversely, the levels of IgM against the LPS-free CYT extract or the 18-kDa protein showed small variations in all the animals and remained below the cutoff of the assay throughout the follow-up period (data not shown).

Antibody response in *Y. enterocolitica* 0:9-infected animals. The cross-reactivity against different antigens of *Brucella* was investigated in two 5-month-old heifers orally infected, as described previously (16), with 10^9 live *Y. enterocolitica* 0:9 cells.

As shown in Fig. 2A, anti-LPS IgG levels were markedly increased in both animals in the second week after infection and then began to decline, reaching normal levels at week 11. In one animal, IgG antibodies against the LPS-free CYT extract showed minor variations during the follow-up period but always remained below the cutoff. The other heifer showed a slight increase in antiprotein IgG from week 2 to 7. In this case, however, a value above the cutoff was observed only at week 3 postinfection. In contrast, anti-18-kDa-protein IgG antibodies always remained below the cutoff in both animals.

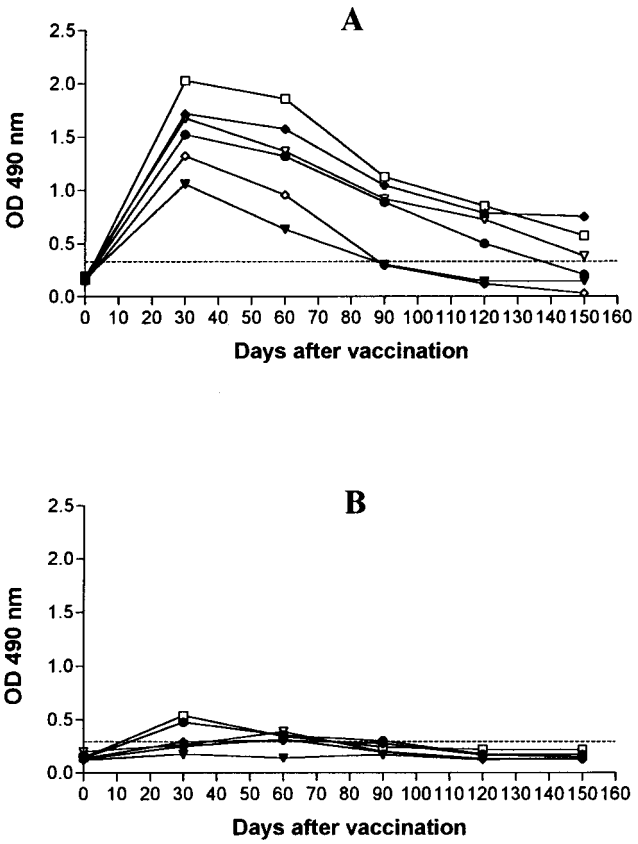

FIG. 1. Evolution of IgG antibodies against *Brucella* antigens in cattle after vaccination with *B. abortus* S19. (A) Anti-LPS antibodies as determined by capture ELISA with monoclonal antibody BC68; (B) antibodies to the LPS-free CYT fraction as determined by indirect ELISA. ----, cutoff of the assay.

Anti-LPS IgM antibodies (Fig. 2B) showed a marked increase at week 2 postinfection, but they reached negativity earlier than anti-LPS IgG antibodies (week 4). On the other hand, IgM antibodies against LPS-free CYT or the 18-kDa protein did not show any significant variation during the study and always remained below the cutoff.

Comparison of the antibody levels observed in different groups of animals. Samples of 20 heifers experimentally infected with *B. abortus* 544 (25) were included as positive controls in ELISA tests. All these sera showed high titers (>1/80) in the standard tube agglutination test and also high titers (>4/64) in the complement fixation test. The anti-LPS, anti-18-kDa-protein, and anti-LPS-free CYT IgG responses of these sera were compared with those of *Yersinia*-infected and *B. abortus* S19-vaccinated animals (Fig. 3).

As shown in Fig. 3A, both *B. abortus* 544- and *Y. enterocolitica* 0:9-infected animals showed high levels of IgG anti-LPS antibodies. In fact, the reactivity of *Yersinia*-infected animals cannot be distinguished from that of some of the heifers infected with *B. abortus* 544. Anti-LPS IgG levels measured in samples obtained at 30 and 60 days after vaccination with *B. abortus* S19 were also indistinguishable from those of *Brucella*- or *Yersinia*-infected animals.

Conversely, a clear difference in anti-LPS-free CYT IgG

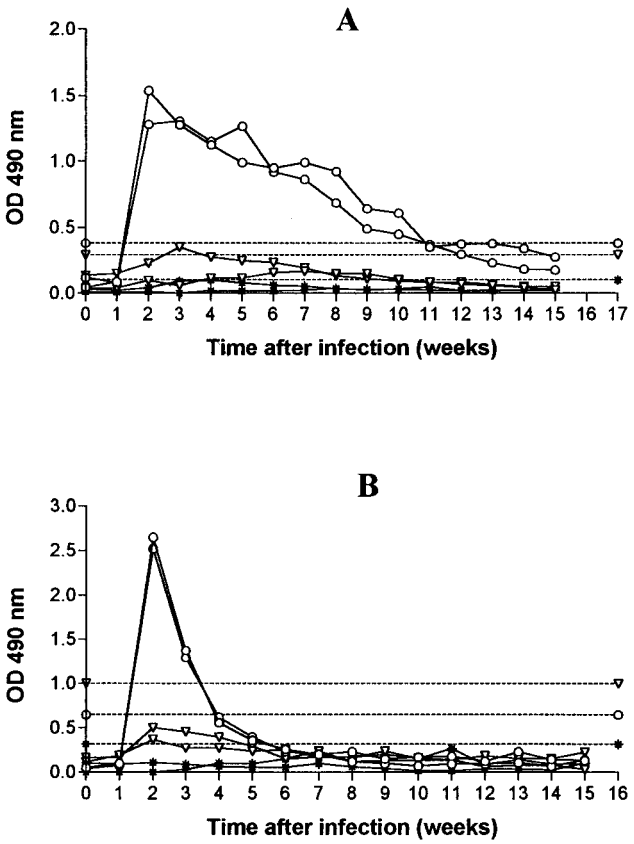

FIG. 2. Evolution of IgG (A) and IgM (B) antibodies against *Brucella* antigens in two heifers orally infected with *Y. enterocolitica* 0:9. Antibodies were assayed by ELISA (see legend to Fig. 1). —*—, anti-18-kDa protein; —▽—, anti-LPS-free CYT; —○—, anti-LPS; ---*---, anti-18-kDa-protein cutoff; ---▽---, anti-LPS-free CYT cutoff; ---○---, anti-LPS cutoff.

levels between *Brucella*-infected animals and all the other groups is observed (Fig. 3B). While the majority of the *Brucella*-infected animals developed high levels of IgG against LPS-free CYT, *Yersinia*-infected animals showed negative values, even at the time of the peak of the anti-LPS response (Fig. 2). As mentioned above, at day 30 after vaccination, vaccinated heifers showed a slight response which is clearly lower than that of *Brucella*-infected animals. Similar results were obtained when anti-18-kDa-protein IgG levels were compared (not shown).

In the present study we have found that anti-LPS IgG antibodies, as measured by ELISA, showed maximal titers 4 weeks after vaccination with S19, in accordance with results reported by Nielsen et al. (22). A wide range of reactivities was observed in vaccinated and experimentally infected cattle, which is in agreement with previous reports (22, 24). Anti-LPS IgG levels observed in the vaccinated group overlapped with those of cattle experimentally infected with either *B. abortus* 544 or *Y. enterocolitica* 0:9.

Although it has been reported that anti-LPS IgM persists at high levels in sera from vaccinated cattle for long periods after vaccination (1, 17), we found that this response had dropped to normal values at day 150 postvaccination. At that time, in contrast, anti-LPS IgG persisted at positive levels in half of the vaccinated healthy animals.

These results confirm previous reports (6, 13, 22) showing that when *B. abortus* infection is diagnosed by measuring anti-LPS antibodies, at least two interfering groups exist: animals recently vaccinated with *B. abortus* S19 and those infected with *Y. enterocolitica* 0:9.

Several authors have proposed the use of antigenic components different from LPS as a means to improve the diagnosis of bovine brucellosis (4, 8, 12). As far as we know, however, only two studies (3, 26) have been performed to measure the humoral immune response of vaccinated cattle against cytoplasmic proteins of *Brucella*. In those studies, no antibody response to recombinant *B. abortus* S19 CuZn superoxide dismutase was detected in either S19-vaccinated or S2308-infected animals, indicating that this protein is not a suitable antigen for differentiating vaccinated from infected cattle. Limet et al. (18) suggested that antigens not present in S19 should be used to differentiate vaccinated from infected animals. Another possibility, however, is that because of transient exposure to *Brucella* antigens in S19-vaccinated cattle, poorly immunogenic components fail to significantly impact the immune system. We have previously shown that the 18-kDa cytoplasmic protein is present in all *Brucella* species, including S19. As shown here, although vaccinated animals developed a slight IgG response against cytoplasmic proteins of *Brucella*, their levels were much lower than those found in infected animals. The marked difference in antiprotein IgG levels between vaccinated and infected cattle is probably explained by differences in the duration of the exposure to *Bru-*

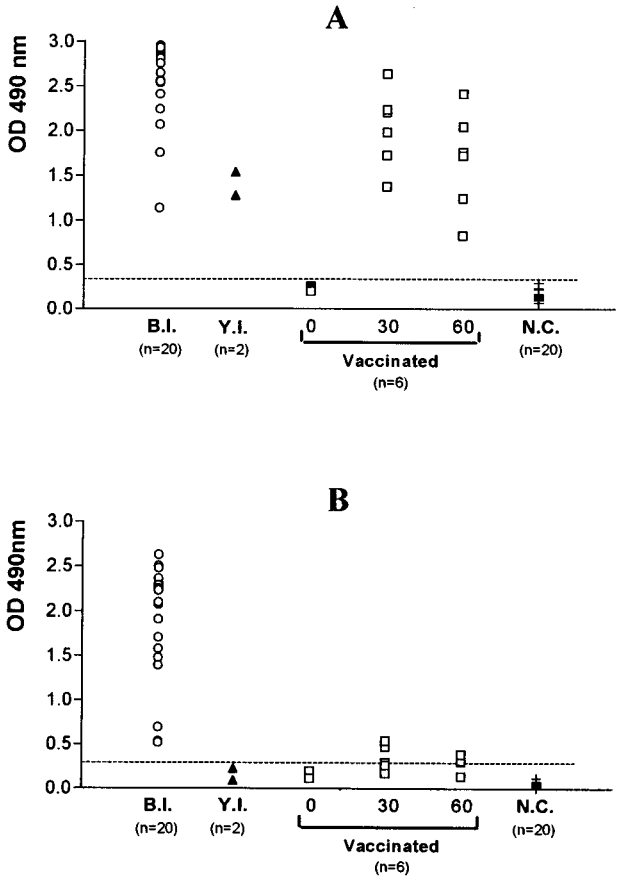

FIG. 3. Comparative analysis of levels of IgG against *Brucella* antigens in serum from cattle experimentally infected with *B. abortus* 544 (B.I.) or with *Y. enterocolitica* 0:9 (Y.I.), cattle vaccinated with *B. abortus* S19, and normal controls (N.C.). Time after vaccination is indicated in days. Antibodies against LPS (A) and the LPS-free CYT fraction (B) were assayed by ELISA (see legend to Fig. 1).

cella antigens. In the case of infection with virulent strains, the exposure to *Brucella* antigens persists for longer periods, producing a stronger antibody response. This fact indicates the potential usefulness of protein antigens for diagnosing active brucellosis.

The present results confirm our preliminary findings (8), since vaccinated animals developed a transient anti-18-kDa-protein response that was much lower than that of infected animals. These results partially agree with those of Hemmen et al. (12), who could not find, by competitive ELISA, any anti-18-kDa-protein response in S19-vaccinated cattle as late as 88 days postvaccination. In the present study, however, a slight IgG response against the 18-kDa protein could be detected by capture ELISA. This quantitative difference in the detection of anti-18-kDa-protein antibodies could be explained by the higher sensitivity usually obtained with the capture ELISA.

This could also explain the higher rate (95%) of serum samples positive for anti-18-kDa-protein antibodies found here in *Brucella*-infected cattle compared with that found by Kittelberger et al. (15) by using immunoblotting techniques (61.5%). Similar differences in sensitivity between these techniques were observed by Hemmen et al. (12) in working with the same protein.

Our results agree with those of Hurvell (13), who showed that the antibodies cross-reacting between *Y. enterocolitica* 0:9 and smooth *Brucella* strains were present both in the IgM and the IgG fractions of sera.

The slight IgG response against the LPS-free CYT extract observed in one of the *Yersinia*-infected heifers could be attributed to the 50- to 80-kDa cross-reacting antigen previously described by Kittelberger et al. (15).

In contrast, no response against the 18-kDa protein could be detected in *Yersinia*-infected animals, which is in agreement with results reported by Hemmen et al. (12). These results also agree with our previous finding showing that the 18-kDa protein is not detectable by immunoblotting in *Y. enterocolitica* 0:9 total extracts (8).

As far as we know, this is the first follow-up of the humoral immune response of vaccinated cattle to cytoplasmic proteins of *Brucella*. As shown here, this antiprotein response, as measured by ELISA, could be useful for differentiating vaccinated from infected animals as early as 90 days postinfection. In contrast, the ELISA detecting anti-LPS IgG antibodies would not be useful for testing cattle before 150 days postvaccination. On the other hand, while S19-vaccinated animals developed anti-LPS IgG levels that overlapped with those developed by *Brucella*- or *Y. enterocolitica* 0:9-infected cattle, a significant anti-18-kDa-protein antibody response was detected only in *B. abortus* 544-infected cattle. Thus, the ELISA using the 18-kDa protein could be potentially useful for differentiating *Brucella*-infected cattle from those vaccinated with S19 or infected with *Y. enterocolitica* 0:9.

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REFERENCES

1. **Alton, G. G., L. M. Jones, and D. E. Pietz.** 1975. Laboratory techniques in brucellosis, 2nd ed., p. 149–154. World Health Organization, Geneva.
2. **Chappel, R. J., D. J. McNaught, J. A. Bourke, and G. S. Allan.** 1978. Comparison of the results of some serological tests for bovine brucellosis. J. Hyg. **80:**365–371.
3. **Cheville, N. F., M. G. Stevens, A. E. Jensen, F. M. Tatum, and S. M. Halling.** 1993. Immune responses and protection against infection and abortion in cattle experimentally vaccinated with mutant strains of *Brucella abortus*. Am. J. Vet. Res. **54:**1591–1597.
4. **Cloeckaert, A., P. Kerkhofs, and J. N. Limet.** 1992. Antibody response to *Brucella* outer membrane proteins in bovine brucellosis: immunoblot analysis and competitive enzyme-linked immunosorbent assay using monoclonal antibodies. J. Clin. Microbiol. **30:**3168–3174.
5. **Corbel, M. J.** 1985. Recent advances in the study of *Brucella* antigens and their serological cross-reactions. Vet. Bull. **55:**927–942.
6. **Corbel, M. J., and G. A. Cullen.** 1970. Differentiation of the serological response to *Yersinia enterocolitica* and *Brucella abortus* in cattle. J. Hyg. **68:**519–531.
7. **Diaz, R., P. Garatea, L. M. Jones, and I. Moriyon.** 1979. Radial immunodiffusion test with a *Brucella* polysaccharide antigen for differentiating infected from vaccinated cattle. J. Clin. Microbiol. **10:**37–41.
8. **Goldbaum, F. A., J. Leoni, J. C. Wallach, and C. A. Fossati.** 1993. Characterization of an 18-kilodalton *Brucella* cytoplasmic protein which appears to be a serological marker of active infection of both human and bovine brucellosis. J. Clin. Microbiol. **31:**2141–2145.
9. **Goldbaum, F. A., C. P. Rubbi, and C. A. Fossati.** 1994. Removal of LPS from a *Brucella* cytoplasmic fraction by affinity chromatography with an anti-LPS monoclonal antibody as immunosorbent. J. Med. Microbiol. **40:** 174–178.
10. **Goldbaum, F. A., C. P. Rubbi, J. C. Wallach, S. E. Miguel, P. C. Baldi, and C. A. Fossati.** 1992. Differentiation between active and inactive human brucellosis by measuring antiprotein humoral immune responses. J. Clin. Microbiol. **30:**604–607.
11. **Heck, F. C., B. L. Deyoe, and T. D. Williams.** 1982. Antibodies to *Brucella abortus* in sera from strain 19 vaccinated and non-vaccinated cows as determined by enzyme linked immunosorbent assay and conventional serologic methods. Vet. Immunol. Immunopathol. **3:**629–634.
12. **Hemmen, F., V. Weynants, T. Scarcez, J.-J. Letesson, and E. Saman.** 1995. Cloning and sequence analysis of a newly identified *Brucella abortus* gene and serological evaluation of the 17-kilodalton antigen that it encodes. Clin. Diagn. Lab. Immunol. **2:**263–267.
13. **Hurvell, B.** 1973. Serological cross-reactions between different *Brucella* species and *Yersinia enterocolitica*. Acta. Vet. Scand. **14:**474–488.
14. **Jones, L. M., D. T. Berman, E. Moreno, B. L. Deyoe, M. J. Gilsdorf, J. D. Huber, and P. Nicoletti.** 1980. Evaluation of a radial immunodiffusion test with polysaccharide B antigen for diagnosis of bovine brucellosis. J. Clin. Microbiol. **12:**753–760.
15. **Kittelberger, R., F. Hilbink, M. F. Hansen, M. Penrose, G. W. de Lisle, J. J. Letesson, B. Garin-Bastuji, J. Searson, C. A. Fossati, A. Cloeckaert, and G. Schurig.** 1995. Serological crossreactivity between *Brucella abortus* and *Yersinia enterocolitica* 0:9. I. Immunoblot analysis of the antibody response to *Brucella* protein antigens in bovine brucellosis. Vet. Microbiol. **47:**257–270.
16. **Kittelberger, R., F. Hilbink, M. F. Hansen, G. P. Ross, M. A. Joyce, S. Fenwick, J. Heesemann, H. Wolf-Watz, and K. Nielsen.** 1995. Serological cross reactivity between *Brucella abortus* and *Yersinia enterocolitica* 0:9. II. The use of *Yersinia* outer proteins for the specific detection of *Yersinia enterocolitica* infections in ruminants. Vet. Microbiol. **47:**271–280.
17. **Lamb, V. L., L. M. Jones, G. G. Schurig, and D. T. Berman.** 1979. Enzyme-linked immunosorbent assay of bovine immunoglobulin subclass-specific response to *Brucella abortus* lipopolysaccharides. Infect. Immun. **26:**240–247.
18. **Limet, J. N., P. Kerkhofs, R. Wijffels, and P. Dekeyser.** 1988. Le diagnostic sérologique de la brucellose bovine par ELISA. Ann. Med. Vet. **132:**565–575.
19. **Mittal, K. R., and I. Tizard.** 1981. Serological cross-reactions between *Brucella abortus* and *Yersinia enterocolitica* serotype 09. Vet. Bull. **51:**501–505.
20. **Mittal, K. R., and I. R. Tizard.** 1979. A simple technique to differentiate between animals infected with *Yersinia enterocolitica* IX and those infected with *Brucella abortus*. Res. Vet. Sci. **26:**248–250.
21. **Nicoletti, P.** 1980. Epidemiology of bovine brucellosis. Adv. Vet. Sci. Comp. Med. **24:**69–98.
22. **Nielsen, K., J. W. Cherwonogrodzky, R. J. Duncan, and D. R. Bundle.** 1987. Enzyme-linked immunosorbent assay for differentiation of the antibody response of cattle naturally infected with *Brucella abortus* or vaccinated with strain 19. Am. J. Vet. Res. **50:**5–9.
23. **Nielsen, K. H., L. Kelly, D. Gall, P. Nicoletti, and W. Kelly.** 1995. Improved competitive enzyme immunoassay for the diagnosis of bovine brucellosis. Vet. Immunol. Immunopathol. **46:**285–291.

24. **Ruppanner, R., M. E. Meyer, P. Willeberg, and D. E. Behymer.** 1980. Comparison of the enzyme linked immunosorbent assay with other tests for brucellosis, using sera from experimentally infected heifers. Am. J. Vet. Res. **41:**1329–1332.
25. **Saegerman, C., V. Weynants, T. K. Vo, L. Dewaele, A. Tibor, P. Denoel, J. Godfroid, P. Miche, E. Saman, J. J. Letesson, and J. Limet.** 1994. Evaluation de l'activité protectrice de la fraction de paroi de *Brucella* insoluble dans le SDS et identification d'antigènes de *Brucella* utilisables por le diagnostic, p. 221–233. *In* AUPELF-UREF (ed.), Biotechnologies du diagnostic et de la prévention des maladies animales. John Libbey Eurotext, Paris.
26. **Stevens, M. G., L. B. Tabatabai, S. C. Olsen, and N. F. Cheville.** 1994. Immune responses to superoxide dismutase and synthetic peptides of superoxide dismutase in cattle vaccinated with *Brucella abortus* strain 19 or RB51. Vet. Microbiol. **41:**383–389.
27. **Sutherland, S. S., and L. Den Hollander.** 1986. Comparison of an enzyme-linked immunosorbent assay using monoclonal antibodies and a complement fixation test for cattle vaccinated and infected with *Brucella abortus*. Vet. Microbiol. **12:**55–64.