Rapid Detection of Contagious Bovine Pleuropneumonia by a *Mycoplasma mycoides* subsp. *mycoides* SC Capsular Polysaccharide-Specific Antigen Detection Latex Agglutination Test

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A latex agglutination test (LAT) has been developed for the diagnosis of contagious bovine pleuropneumonia (CBPP). The latex microspheres were coated with MmmSC polyclonal immunoglobulin G antisera and detected MmmSC antigen in the serum of cattle infected with CBPP and in growth medium containing MmmSC. The specific antigen recognized by this test appeared to be the capsular polysaccharide (CPS). The LAT recognized all 23 strains of MmmSC examined in this study, with a sensitivity level of 2 ng of CPS, or the equivalent of $5 \times 10^4$ CFU, in a reaction volume of 0.03 ml. Therefore, rapid identification of MmmSC cultures should be possible. Agglutination was also observed with the related goat pathogens and “*Mycoplasma mycoides*” cluster members *Mycoplasma mycoides* subsp. *mycoides* large colony biotype (four of six strains positive) and *Mycoplasma mycoides* subsp. *capri* (three of six strains positive), in agreement with the suggestion that these latter two mycoplasmas may in fact represent a single species (although collectively exhibiting two capsular serotypes). Comparisons in diagnosis with the complement fixation test (CFT) were made by using African field sera from CBPP-infected cattle. After 2 (or 3) min of incubation, the test detected 55% (or 61%) of CFT-positive sera and 29% (or 40%) of CFT-negative sera, with an overall correlation in diagnosis of 62% (or 61%). The rates for false-positive diagnoses made by using “known” CBPP-negative sera from the United Kingdom were 3 or 13% after 2 or 3 min of incubation, respectively. The data agree with previous findings that some CBPP CFT-negative misdiagnoses may occur due to “antibody eclipsing” by excess circulating antigen. The LAT combines low cost and high specificity with ease of application in the field, without the need for any special training or equipment.

*Mycoplasma mycoides* subsp. *mycoides* small colony biotype (MmmSC) is the causal agent of contagious bovine pleuropneumonia (CBPP), currently the most economically serious disease of cattle in Africa, with losses per annum estimated to be in the region of $2$ billion (19). The disease is now endemic in large areas of sub-Saharan Africa and can rapidly spread over large areas as a result of unrestricted cattle movements. Diagnosis and control can be hampered due to long incubation periods when cattle may express no overt disease symptoms. A number of diagnostic tests currently exist, but most are difficult to use in situ, lack sensitivity, or require resources unavailable in many countries affected by the disease.

The current Office International des Épizooties-prescribed test for the diagnosis of CBPP is the complement fixation test (CFT) (5, 25). Although the test is highly specific, it is relatively expensive to perform, it is slow, and it requires trained personnel and laboratory facilities. In addition, it is less effective at diagnosing animals in the early stages of the disease or of animals with chronic lesions (25). A number of more modern tests have recently been described, including biochemical (28), indirect and competitive enzyme-linked immunosorbent assay (ELISA) (12, 24), immunoblotting (24, 27), and PCR (3, 20). Although these tests can offer advantages in terms of sensitivity, specificity, or reproducibility, they still require considerable investment in equipment and trained personnel and, most importantly, cannot be used for rapid “penside” diagnosis of CBPP, since they must be performed in the laboratory.

As an aid to effective field control, a rapid and inexpensive penside test for the accurate diagnosis of CBPP was recently identified as a top priority by the OIE/FAO/OAU Consultative Group on CBPP (2). Rapid slide agglutination tests for CBPP diagnosis by using MmmSC CFT antigen mixed with a drop of whole blood or serum have previously been described (26, 34). Although these tests were reported to be very sensitive, their use is not recommended due to an unacceptable number of false-positive reactions (1, 34). Since control measures for CBPP can include both quarantine and slaughter, it is vital for a test to exhibit a low number of false-positive reactions if the confidence of the local farmer is to be maintained.

Latex agglutination tests (LATs) for the diagnosis of contagious caprine pleuropneumonia caused by the related *Mycoplasma capricolum* subsp. *capripneumoniae* have been described previously (15, 31). These tests are designed to detect either serum antibodies against the mycoplasmal capsular polysaccharide (CPS) or, alternatively, to detect this antigen directly. The first test was made by coating latex particles with purified CPS, whereas the second test used specific anti-*M.
capricolum subsp. capripneumoniae immunoglobulin G (IgG) to bind to circulating CPS. In use, a positive reaction is signaled by a rapid agglutination signal (1 to 5 min).

MmmSC is known to produce large quantities of extracellular CPS (11) and, during the early or acute stages of infection, this antigen can “eclipse” the antibody response and lead to a false-negative diagnosis for CBPP with the CFT (35). In one study, up to 36% of CBPP-positive animals were undetected by the CFT (4). The development of a LAT that detects this antigen could offer significant advantages in terms of diagnosis of early or acute stages of infection. We describe here the development and testing of such an LAT for the diagnosis of CBPP that is based upon the detection of serum CPS by using latex particles coated with MmmSC-specific IgG.

### MATERIALS AND METHODS

**Mycobacteria strains and growth conditions.** The mycobacteria species and strains used in the present study are shown in Table 1. All mycobacteria were grown in Mycobacteria Experience (ME) broth and agar medium (Mycobacteria Experience, Reigate, Surrey, United Kingdom) apart from MmmSC strain N6 for the production of CPS (grown in modified Newing’s tryptose broth [10]). To measure the titre of broth cultures used in agglutination reactions, serial dilutions were made in fresh medium to measure color changing units, or alternatively, plated onto solid medium, followed by colony counting (22). Sodium azide (0.05%) was added to broth cultures at the time of sampling, and cultures were subsequently stored at 4°C prior to agglutination reactions.

**Antiserum.** Rabbit hyperimmune serum (R54) against MmmSC strain N6 (14) was produced by two subcutaneous injections of mycoplasma (glutaraldehyde inactivated, followed by quenching with glycine). The final inoculum concentration was 5.6 mg/ml of protein in phosphate-buffered saline (PBS; pH 7.5) containing 0.01% glutaraldehyde-0.01% thimerosal in an equal volume of oil adjuvant (Montanide ISA50; Seppic, Paris, France), followed by one intravenous injection of an aqueous suspension. Injections were given every 3 weeks. The IgG fraction was purified on a protein A-Sepharose column by standard methodology (7) and eluted in a final volume containing 7.8 mg/ml in PBS. Negative control rabbit IgG fraction (R46) was similarly purified at a final concentration of 7.5 mg/ml.

**CBPP field sera.** Seropositive sera were obtained from naturally infected cattle herds from the Iringa, Mbeya, and Morogoro regions of Tanzania. Seronegative control bovine antisera were obtained from herds located in several outdoor locations in the United Kingdom, a country that has been free from CBPP for more than 100 years. (The sera were supplied by Robin Nicholas, Veterinary Laboratories Agency, Addlestone, United Kingdom.)

**Purification of CPS.** CPS was purified from spent medium by using a previously published technique (36). No protein could be detected in the purified CPS by a variety of assays (Bradford assay, Lowry assay, and after silver staining of sodium dodecyl sulfate-polyacrylamide gel electrophoresis gels). The amount of carbohydrate present was measured by the phenol-sulfuric acid method (6).

**Measurement of CPS antibody titer.** ELISA analysis was used to estimate the CPS antibody titer in the IgG fraction used to coat the latex particles (to allow for standardization between different antibody batches). Microtiter plates (Greiner) were coated with a saturating amount of antigen, i.e., 0.1 ml of antigen solution at a concentration of 10 μg/ml in PBS (pH 7.5), and left overnight at 4°C. The antigen was either purified IgG, or whole MmmSC, pelleted out of growth medium, washed three times in PBS (supplemented with 5% [wt/vol] glucose), and then resuspended in PBS prior to sonication (three 20-s bursts). The plates were then block in a 5% solution of dry skim milk in PBS (pH 7.4) for 1 h at 4°C, and the plates were incubated overnight at room temperature. For the antibody (either anti-MmmSC IgG [R54] or a negative control IgG [R37]) was standardized at an initial concentration of 7.5 mg/ml in PBS and then diluted 1:200 into blocking solution. Twofold serial dilutions in blocking buffer were then made across the plate (1:200 down to 1:409,600). Incubations were done in a final volume of 0.1 ml. Appropriate negative primary and secondary controls were included. Sodium azide was added to a final concentration of 0.05% (wt/vol), and the plates were incubated overnight at room temperature. For the mixture of beads and antigen was added, the mixture made up to a final volume of 0.1 ml. No further washing was performed in order to prevent loss of the beads and to break up any large particles. To 0.15 ml of beads (15 mg) was added 2.5 mg of polyclonal anti-MmmSC IgG, and the volume was made up to 1 ml with PBS. (For experiments where the ratio of antibody to beads was altered, the volume of beads and final reaction volume were kept constant, whereas the amount of added antibody was varied. In this case, the volume of beads added was 0.015 ml, with the mixture made up to a final volume of 0.1 ml. No further washing was performed in order to prevent loss of excess IgG from the bead solution.) Negative control beads were produced by using an identical protocol but with IgG purified from a nonimmunized rabbit. The mixture was incubated overnight at 4°C on an end-to-end shaker, and then centrifuged at 15,000 rpm (20,000 × g) for 5 min in a microfuge. The supernatant was removed, and the beads were resuspended in 1 ml of PBS. The mixture was centrifuged as before, the supernatant was removed, and the beads were resuspended in a final volume of 1.0 ml of PBS supplemented with 0.05% sodium azide as a preservative. Resuspension of the beads was normally achieved by vortexing, although a brief sonication (5 s) was found be useful in difficult cases. Precipitation of polyclonal anti-MmmSC IgG with CPS was accomplished as follows: 50 μl of purified CPS was added to 100 μg of IgG in a reaction volume of 0.1 ml of PBS. A positive control tube contained only IgG. The mixture was centrifuged as before, the supernatant was removed, and the beads were resuspended in an equal volume of PBS supplemented with 0.05% sodium azide as a preservative. A 10% (wt/vol) solution of 0.8-μm diameter polystyrene latex microspheres (LSB; Sigma) was used in the binding reaction. Prior to use, the bead suspension was vigorously vortexed to ensure even distribution of the beads and to break up any large particles. To 0.15 ml of beads (15 mg) was added 2.5 mg of polyclonal anti-MmmSC IgG, and the volume was made up to 1 ml with PBS. (For experiments where the ratio of antibody to beads was altered, the volume of beads and final reaction volume were kept constant, whereas the amount of added antibody was varied. In this case, the volume of beads added was 0.015 ml, with the mixture made up to a final volume of 0.1 ml. No further washing was performed in order to prevent loss of excess IgG from the bead solution.) 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beads and treated as described above, prior to resuspension in a final volume of 0.1 ml of PBS. The beads were then tested in agglutination reactions.

**Latex agglutination tests.** Agglutination tests were performed on (i) purified CPS diluted in negative bovine serum samples; and (ii) field and experimentally infected bovine serum samples; and (iii) mycoplasma suspensions grown in ME liquid medium, with serial dilutions made in the same medium. Agglutination reactions were performed in triplicate by using the same procedure: 25 to 30 μl of test solution was divided into aliquots onto a glass microscope slide, and 3 to 5 μl of the bead suspension (containing 50 to 75 μg of beads) was added with a pipette. The beads were thoroughly mixed, and the slide was incubated for 1 to 5 min on a rocking shaker with gentle agitation. The key to good agglutination reactions appeared to be side-to-side movement of the reaction solution across the slide. Beads coated with negative IgG were mixed with the positive test solutions to test for autoagglutination. Agglutination (when seen against a dark background) was most obvious when the reaction was still wet, although it could still be clearly observed when dry in the case of strong positive reactions.

**CFT.** The CFT was performed according to the OIE manual of standards (25) with antigen and reagents supplied by Onderstepoort Veterinary Institute of the Agricultural Research Council of South Africa.

### RESULTS

**Definition of agglutinating activity.** After the coated latex microspheres were mixed with positive test solutions (containing CPS antigen), an agglutination reaction occurred, the rapidity and extent of which depending upon the amount of antigen. For the purposes of the present study, a four-point scale was adopted, exhibited pictorially in Fig. 1, and defined as heavy flocculent precipitates forming within 1 min (clear background) (3 [‌++]‌), heavy flocculent precipitates taking 1 to 5 min to form (clear background) (2 [‌++]‌), light flocculent precipitate against mostly clear background (1 to 5 min) (1 [+‌]), or no precipitate, cloudy homogeneous background (0 [‌–‌]).

**Identification of the antigen recognized by anti-MmmSC IgG-coated beads.** A similar LAT for the identification of the related goat pathogen *M. capricolum* subsp. *capripneumoniae* has previously been described (15) which was based upon detection of extracellular CPS as the sole or major antigen. Since MmmSC has been shown to produce large quantities of extracellular CPS (11, 35), it seemed likely that the soluble antigen recognized by the anti-MmmSC IgG-coated beads was also CPS. First, MmmSC IgG-coated beads strongly agglutinated pure CPS in solution (see below). Second, when CPS antibodies were preabsorbed from the MmmSC IgG prior to coating of the latex particles, all agglutinating activity was lost with purified CPS in solution (50 μg per ml), with MmmSC culture medium (titer of 5 × 10⁷ per ml), and with MmmSC-positive serum samples. Nonpreabsorbed beads continued to give a positive reaction in these tests. To ensure that CPS had not simply displaced IgG from the latex particles, preabsorbed beads were incubated with goat anti-rabbit IgG. A strong agglutination reaction indicated that the beads were still coated with rabbit IgG. Similarly, incubation of the preabsorbed beads with rabbit anti-MmmSC did not result in agglutination, as would be expected if the beads were coated with MmmSC CPS. These findings strongly suggest that CPS is the major or single antigen recognized by the test.

**Sensitivity of anti-MmmSC IgG-coated latex microspheres at detecting MmmSC in medium.** MmmSC strain N6 was cultured in ME medium to a titer of 5 × 10⁹ CFU/ml. The culture was serially diluted in fresh medium and tested in a LAT (Table 2). Clear agglutination activity was observed down to a dilution of 1: 32,768, which is equivalent to 4.8 × 10³ mycoplasma per agglutination reaction (0.03 ml) or a titer of 1.4 × 10⁹ mycoplasmas per ml. This is the same degree of sensitivity as previously reported for antigen detection LATs directed at both *M. capricolum* subsp. *capripneumoniae* (15) and *M. pneumoniae* (18) and represented an “average” sensitivity level for strains of MmmSC. Sensitivity levels between different cultures of MmmSC varied over a 5- to 10-fold range (data not shown), possibly reflecting differences in the level of CPS production between strains (17). When the medium was cleared of mycoplasma prior to testing in the LAT (by centrifugation at 15,000

### TABLE 2. Sensitivity of MmmSC IgG-coated microspheres at detecting MmmSC in culture

| Dilution factor of MmmSC culture | Agglutination signal after 5 min | No. of mycoplasma CFU/agglutination reaction | No. of mycoplasma CFU/ml eq |
|----------------------------------|---------------------------------|---------------------------------------------|----------------------------|
| 1:1–1:1,024                     | 3                               | 1.5 × 10⁶–1.5 × 10⁷                          | 5 × 10⁴–5 × 10⁶             |
| 1:2,048–1:4,096                 | 2                               | 7.5 × 10³–3.8 × 10⁴                          | 2.5 × 10³–2.1 × 10⁹         |
| 1:8,192–1:32,768                | 1                               | 1.9 × 10³–4.8 × 10³                          | 6 × 10³–4 × 10⁵             |
| 1:32,768 and higher             | NA                              | <4.8 × 10³                                  | <1.4 × 10³                  |
| Control medium                  | NA                              | Negative                                    | Negative                   |

* NA, no agglutination.
× g for 30 min), no difference in sensitivity was observed, strongly suggesting that soluble CPS was responsible for agglutinating activity rather than (or in addition to) the mycoplasmas themselves. If the mycoplasma pellet was resuspended in fresh ME medium after centrifugation, a positive agglutination reaction was observed at ~100-fold-reduced sensitivity, presumably due to shedding of the CPS coat during resuspension (after a further round of centrifugation, this medium was still positive). No agglutination was observed with negative IgG-coated beads or with anti-MmmSC IgG-coated beads placed in negative medium.

**Agglutinating activity of latex microspheres coated with different amounts of anti-MmmSC IgG and the effect of excess IgG on test sensitivity.** Previous results (15) indicated that beads have a binding capacity of 0.33 to 0.5 mg of IgG per mg of beads. Beads coated with different amounts of anti-MmmSC IgG were tested in agglutination reactions (i) to determine the most sensitive and cost-effective ratio of IgG to latex particles for general use and (ii) to determine whether an excess of CPS antibodies in the agglutination reaction might affect sensitivity by sequestering free CPS antigen. This situation might occur if an animal had a high anti-CPS titer at the time of testing. Beads (1.5 mg) were mixed with 0.001 to 5.0 mg of anti-MmmSC IgG, made up to a final volume of 0.1 ml of PBS and titrated against purified CPS in a solution of PBST (Table 3). At very low IgG concentrations (0.001 to 0.005 mg of IgG per 1.5 mg of beads), the test was ineffective at detecting CPS in solution. Test sensitivity was highest in the range 0.01-0.5 mg of IgG per 1.5 mg of beads (sensitivity of 125 ng/ml or 0.625 mg of CPS/ml or 2.5 ng of CPS per reaction volume of 0.025 ml). The test sensitivity was reduced (4 μg of CPS/ml or 80 ng per reaction) at very high IgG concentrations (1 to 5 mg of IgG per 1.5 mg of beads). This equates to an MmmSC IgG serum concentration of 8.3 mg/ml (5 mg of IgG/15 mg of latex made up to 0.1 ml [i.e., 50 mg/ml] and diluted 1:6 into CPS test solution). This is ~1,000-fold higher than the CPS antigen concentration, suggesting that excess anti-CPS is unlikely to be a problem affecting test sensitivity in practice.

Although this series of experiments suggested that beads coated with 0.05 mg of IgG per 1.5 mg of beads offered the greatest sensitivity in the LAT, previous research (15) has suggested that beads coated with lower levels of IgG sometimes give more variable results. To maintain consistency, a slightly higher level of IgG was used to produce the latex test for general use (0.25 mg of IgG per 1.5 mg of beads).

**Measurement of CPS antibody titer in MmmSC IgG fraction.** To enable reproducibility between different batches of antisera used to produce anti-MmmSC-coated beads, the titer of the CPS-specific IgG used to coat the beads was measured by ELISA. Two ELISAs were used: one to measure the anti-CPS titer (using purified CPS as antigen) and the second to measure the overall anti-MmmSC titer (using whole sonicated MmmSC as antigen). Thus, the specific anti-CPS titer could be estimated and taken into account (if necessary) between different batches of serum.

Purified IgG was standardized at 7.5 mg/ml and then twofold serially diluted from 1:200 to 1:409,600 and incubated with antigen-coated plates. Results are plotted graphically in Fig. 2 (purified CPS as antigen [panel A] and whole sonicated MmmSC as antigen [panel B]). Using the linear portion of the graph to estimate antibody titer, 7.5 mg of R54 antiserum/ml (as used to produce the LAT) can be seen to detect purified CPS down to a dilution of 1:100,000 and whole sonicated MmmSC down to a dilution of 1:300,000 (a ratio of 1:3). Negative control IgG (R37) exhibited a negligible titer against both antigens. Production of a CPS monospecific polyclonal antiserum for use in the LAT was not successful, since immunization of rabbits with purified CPS did not result in a detectable antibody titer (16), a frequent occurrence with carbohydrate antigens when presented in a pure form to the immune system (9, 32).

**Specificity of anti-MmmSC IgG-coated latex microspheres.** The antigen-detection LAT was tested against a variety of mycoplasmal species known to be evolutionarily closely related to MmmSC (including all members of the M. mycoides cluster) and mycoplasmas likely to be found as associated pathogens (such as M. bovis; Table 1). All mycoplasmal species were grown in ME liquid medium to mid-log phase, with titers in the range 5 × 10^7 to 8 × 10^8/ml. The LAT detected all strains of MmmSC with similar sensitivity levels (Table 4; dilutions in the

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**TABLE 3. Sensitivity of the anti-MmmSC IgG-coated microspheres in detecting MmmSC N6 CPS in solution**

| N6 CPS solution concn (ng/ml) | Amt of CPS/reaction (ng) | Agglutination reaction with 1.5-mg latex beads coated with different amounts of anti-MmmSC IgG made up in a volume of 0.1 ml of PBS | 0.1 mg of preimmune IgG | 0.001 | 0.005 | 0.01 | 0.05 | 0.1 | 0.25 | 0.5 | 1 | 1.5 | 2 | 5 |
|-----------------------------|-------------------------|-----------------------------------------------------------------------------------------------------------------|--------------------------|-------|-------|------|------|-----|------|------|-----|-----|-----|-----|
| >8,000                      | 160                     | ++                                                                                                              | ++                       | ++    | ++    | ++   | ++   | ++  | ++   | ++  | ++  | ++  | ++  | ++  |
| 4,000                       | 80                      | +                                                                  | ++                       | ++    | ++    | ++   | ++   | ++  | ++   | ++  | ++  | ++  | ++  | ++  |
| 2,000                       | 40                      |                                                                    | +                        | ++    | ++    | ++   | ++   | ++  | ++   | ++  | ++  | ++  | ++  | ++  |
| 1,000                       | 20                      |                                                                    |                          | +     | ++    | ++   | ++   | ++  | ++   | ++  | ++  | ++  | ++  | ++  |
| 500                         | 10                      |                                                                    |                          |       | +     | ++   | ++   | ++  | ++   | ++  | ++  | ++  | ++  | ++  |
| 250                         | 5                       |                                                                    |                          |       |       | +     | ++   | ++  | ++   | ++  | ++  | ++  | ++  | ++  |
| 125                         | 2.5                     |                                                                    |                          |       |       |       | +     | +   | +    | +   | +   | +   | +   | +   |
| 62.5                        | 1.25                    |                                                                    |                          |       |       |       |       | +   | +    | +   | +   | +   | +   | +   |
| 31.25                       | 0.625                   |                                                                    |                          |       |       |       |       | +   | +    | +   | +   | +   | +   | +   |
| 16                          | 0.313                   |                                                                    |                          |       |       |       |       |       | +   | +    | +   | +   | +   | +   | +   |
| 8                           | 0.156                   |                                                                    |                          |       |       |       |       |       |       | +   | +    | +   | +   | +   | +   | +   |
| 0                           | 0                       |                                                                    |                          |       |       |       |       |       |       |       | +   | +    | +   | +   | +   | +   | +   |

* Each reaction combination was executed in triplicate, with the average value shown. The CPS dilutions were made and tested on the same day.
range $10^{-2}$ to $10^{-3}$ were positive. Some strains of *M. mycoides* subsp. *mycoides* large colony biotype [MmmLC] tested positive (four of six), as did some strains of *M. mycoides* subsp. *capri* (three of six strains tested positive). No agglutination was noted for any other mycoplasmal species tested, even in undiluted growth medium.

**Determination of optimum incubation period for the LAT.**

To determine sensitivity and specificity, the LAT was tested against CFT-positive ($n = 44$) or -negative ($n = 35$) sera obtained from African CBPP field outbreaks and against a bank of sera from the United Kingdom ($n = 32$), a country that has been free of CBPP for more than 100 years. The agglutination signal was read every minute for 5 min, and individual samples were recorded as either positive (agglutination $>1$ [+] or negative (agglutination signal 0 [-]). The percentage

![Graph A](image1.png)

**A**

![Graph B](image2.png)

**B**

**FIG. 2.** ELISA data showing antibody titer of R54 and R37 antisera against purified CPS antigen (A) and whole MmmSC antigen (B). Serum was initially diluted 1:200 and then twofold serially diluted down to 1:40,960. The linear portion of each graph was used to estimate the point of intersection with the x axis.

**TABLE 4.** Specificity of MmmSC IgG-coated LAT as indicated by the agglutination signal after mixture with mycoplasma cultures grown in ME broth

| Subspecies and strain | Reaction | Subspecies and strain | Reaction |
|-----------------------|----------|-----------------------|----------|
| *M. mycoides* subsp. *capri* | UM30847..............+ | *M. mycoides* subsp. *mycoides* | G8......................+ |
| 133/87........................... | | G10..........................+ |
| WK354.............................+ | | Y-goat..........................+ |
| PG3b............................... | | 293..........................+ |
| BOT...............................- | | 247/4..........................- |
| L...............................- | | 5307..........................- |

* For *M. mycoides* subsp. *mycoides* SC (MmmSC), there were 23 strains, all of which were positive (see Table 1).

* Type strains for respective mycoplasma species. All other mycoplasma species (Table 1) were negative when tested with the MmmSC IgG-coated LAT.

- Negative agglutination; +, positive agglutination.
of samples within each of the three sera categories that gave a positive agglutination signal at each time point was then plotted (Fig. 3). These data allowed the optimum incubation period (highest signal-to-noise ratio) for the LAT to be determined.

Incubation for 2 min yielded a sensitivity figure of 55% compared to CFT and a specificity figure of 3% false positives (United Kingdom negative sera). After 3 min of incubation, the results were 61% sensitivity and 13% false positives. With African CFT-negative field sera, 29 and 40% of samples were detected as positive by the LAT after 2 and 3 min of incubation, respectively.

Comparison of the LAT with the CFT. A comparison of the LAT with the CFT was made (Table 5) by using African field sera from a CBPP outbreak. The LAT diagnosis was read at 2 or 3 min (the optimum signal-to-noise ratio determined from Fig. 3). The overall correlation was calculated as the number of sera in which an exact identity in diagnosis was made at the time point shown (i.e., the CFT-positive/LAT-positive value added to the CFT-negative/LAT-negative value divided by the total number of sera tested [n = 79]).

TABLE 5. Comparison of CFT and LAT with 79 sera from animals in a region positive for CBPP

| Incubation period (min) | CFT result | No. of sera found to be LAT | Overall correlation (%) |
|-------------------------|------------|-----------------------------|-------------------------|
|                         | Positive   | Negative                    |                         |
| 2                       | 24         | 20                          | 44                      |
|                         | 10         | 25                          | 35                      |
|                         | 34         | 45                          | 62                      |
| 3                       | 27         | 17                          | 44                      |
|                         | 14         | 21                          | 35                      |
|                         | 41         | 38                          | 61                      |

*a CFT diagnosis compared with LAT diagnosis by testing African field sera from a CBPP outbreak. The LAT diagnosis was read at 2 or 3 min (the optimum signal-to-noise ratio determined from Fig. 3). The overall correlation was calculated as the number of sera in which an exact identity in diagnosis was made at the time point shown (i.e., the CFT-positive/LAT-positive value added to the CFT-negative/LAT-negative value divided by the total number of sera tested [n = 79]).

The sensitivity level of the MmmSC IgG-coated LAT was (the equivalent of) ca. $5 \times 10^3$ mycoplasmas or 2.5 ng of pure CPS per agglutination reaction (volume of 0.025 to 0.03 ml). Since the test detects soluble CPS rather than (or in addition to) the mycoplasmas themselves, and with equal sensitivity, the use of clarified serum, pleural fluid, nasal swabs, or cell medium is possible. The LAT was found to detect all strains of MmmSC, tested, in agreement with data obtained with monoclonal antibodies that CPS is antigenically conserved between all strains of MmmSC (13). In addition to MmmSC, the LAT was also agglutinated by some strains of the closely related M. mycoides cluster members M. mycoides subsp. mycoides large-colony biotype (MmmLC), and M. mycoides subsp. capri. The taxonomic grouping of these latter two mycoplasmal species is the subject of some debate at present, with several recent publications suggesting that on the basis of DNA sequence analysis it may be more appropriate to reclassify them as a single species (8, 21, 33). The data reported here, in which some strains of each species are detected by the LAT, do not disagree with this hypothesis. However, they do suggest that if MmmLC and M. mycoides subsp. capri are indeed the same species of mycoplasma, then at least two serotypes must exist. We have observed similar “serotyping” results with CPS-specific monoclonal antibodies (unpublished results). Whether the presence of common CPS epitopes between MmmSC and the MmmLC-M. mycoides subsp. capri cluster might result in false-positive diagnoses for CBPP remains to be determined from field studies. However, we have been unable to trace any report concerning the detection of CPS antigen from MmmLC-M. mycoides subsp. capri in cattle, and the pathogenicity of these latter species in cattle remains uncertain (23, 29, 30).

It is likely that the LAT will be more effective in diagnosing acutely infected cattle, whereas the CFT will be more effective in diagnosing convalescent animals. The CFT was originally introduced in 1935 (5, 25) and, although the test is highly specific, it is not particularly sensitive, being less effective at diagnosing animals in the early stages of the disease or of animals with chronic lesions (25). High levels of circulating infection (rather than recovered animals, which are likely to be serologically positive but not necessarily infective).

DISCUSSION

A LAT for the diagnosis of CBPP that uses latex microspheres coated with anti-MmmSC IgG and is based upon the detection of MmmSC CPS antigen in the serum of infected animals has been described. Such a test should be useful in diagnosing acutely infected animals in circumstances in which “antibody masking” can occur due to excess circulating CPS (35) or in diagnosing such animals at the earliest stages of infection, prior to a measurable antibody response. From the perspective of CBPP field control, it is important to identify such animals due to their likely role as “active” sources of infection in a region positive for CBPP...
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