JOURNAL OF CLINICAL MICROBIOLOGY, Nov. 2000, p. 4152–4159 Vol. 38, No. 11
0095-1137/00/$04.00+0

Rapid Detection of Contagious Caprine Pleuropneumonia Using a *Mycoplasma capricolum* subsp. *capripneumoniae* Capsular Polysaccharide-Specific Antigen Detection Latex Agglutination Test

J. B. MARCH,[1]* C. GAMMACK,[1]† AND R. NICHOLAS[2]

Department of Bacteriology, Moredun Research Institute, Penicuik EH26 0PZ, Scotland,[1] and Veterinary Laboratory Agency, New Haw, Addlestone KT15 3NB,[2] United Kingdom

Received 10 May 2000/Returned for modification 29 July 2000/Accepted 27 August 2000

Latex microspheres (diameter, 8 μm) were coated with anti-*Mycoplasma capricolum* subsp. *capripneumoniae* polyclonal immunoglobulin G (IgG) antiserum (anti-F38 biotype). The coated microspheres, when used in a latex agglutination test (LAT), detected *M. capricolum* subsp. *capripneumoniae* antigen in the serum of goats with contagious caprine pleuropneumoniae (CCPP). Beads also agglutinated strongly in the presence of purified *M. capricolum* subsp. *capripneumoniae* capsular polysaccharide (CPS). Preabsorption of CPS-specific antibodies prior to coating of the beads removed agglutinating activity in the presence of *M. capricolum* subsp. *capripneumoniae*, strongly suggesting that CPS is the likely soluble antigen recognized by the test. In addition, the specificity of the LAT exactly mirrored that of an *M. capricolum* subsp. *capripneumoniae* CPS-specific monoclonal antibody (WM25): of the 8 other mycoplasma species tested, agglutination was observed only with bovine serogroup 7. The LAT detected all 11 strains of *M. capricolum* subsp. *capripneumoniae* examined in this study, with a sensitivity level of 2 ng of CPS, or the equivalent of 1.7×10^4 CFU, in a reaction volume of 0.03 ml of serum. With field sera from goats with CCPP, the results of the LAT exhibited a 67% correlation with the results of the currently used complement fixation test (CFT), with the main discrepancy in diagnosis resulting from the increased sensitivity of the LAT compared to that of CFT. This antigen-detection LAT should prove particularly useful in identifying animals in the earliest stages of CCPP and combines sensitivity and low cost with ease of application in the field, without the need for any specialist training or equipment.

Mycoplasma capricolum subspecies *capripneumoniae* is the causative agent of contagious caprine pleuropneumonia (CCPP), a significant disease of goats in Africa, the Middle East, and western Asia, with mortality rates being up to 80% in susceptible herds. While clinical disease has so far been reported in 38 countries, only 11 countries have isolated the causative organism, principally because *M. capricolum* subsp. *capripneumoniae* is difficult to culture (26). *M. capricolum* subsp. *capripneumoniae* is a mycoplasma of the "*M. mycoides* cluster," a taxonomic grouping of six closely related mycoplasmas which are all pathogenic in ruminants. Serological cross-reactivities between members of the *M. mycoides* cluster (3), together with similarities in the clinical diseases that they cause, all play a part in hindering accurate diagnosis of CCPP (19).

A number of serological tests currently exist, but most are difficult to use in situ, lack specificity, or require resources unavailable in many countries affected by the disease. These include the complement fixation test (CFT; the prescribed test for international trade [17, 19]), passive hemagglutination (17), competitive enzyme-linked immunosorbent assay (ELISA) (27), and the latex agglutination test (LAT; with beads coated with *M. capricolum* subsp. *capripneumoniae* capsular polysaccharide [CPS]) (20). Antigen detection tests include immunoblotting (7) and sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis with mycoplasmas concentrated from pleural fluid (28). In addition, a PCR test for the detection of *M. capricolum* subsp. *capripneumoniae* has been developed (8). However, all these tests exhibit certain limitations in either specificity, sensitivity, ease of application, cost, or the requirement for specialist equipment or expertise.

The antibody-detection LAT with *M. capricolum* subsp. *capripneumoniae*-derived CPS-coated beads is now routinely applied in Kenya (19). While the test is inexpensive and simple to perform, animals in the early or acute stages of the disease (prior to the appearance of circulating antibodies or when high levels of circulating antigen may eclipse the immune response [17, 29]) may be misdiagnosed as negative for CCPP. In this respect, the CFT exhibits clear limitations: in published reports, CCPP in between 80 and 100% of acute-phase animals experimentally infected with *M. capricolum* subsp. *capripneumoniae* was not detected by the CFT (10, 17, 19), demonstrating the potential limitations of antibody detection as a sole diagnostic technique. Furthermore, significant complement fixation titers in infected goats were not observed until 21 days postinfection (17). This could be significant in the field, since the incubation period for CCPP can be as little as 6 days, with death in acute cases following only 2 days later (16). Thus, at an early stage in a field outbreak false-negative results based upon serological diagnosis are a real possibility. In contrast, positive reactions may also occur with circulating antibodies long after an *M. capricolum* subsp. *capripneumoniae* infection has been cleared, thus giving an inaccurate diagnosis of the likely infective state of an animal.

The development of a LAT which detects circulating

* Corresponding author. Mailing address: Department of Bacteriology, Moredun Research Institute, Pentlands Science Park, Bush Loan, Penicuik EH26 0PZ, Scotland, United Kingdom. Phone: (44) 131 445 5111. Fax: (44) 131 445 6111. E-mail: marcj@mri.sari.ac.uk.

† Present address: Centre for Veterinary Science, Department of Clinical Veterinary Medicine, University of Cambridge, Madingley Road, Cambridge CB3 0ES, United Kingdom.

TABLE 1. Mycoplasma strains used in this study

Mycoplasma species	Strain
M. capricolum subsp. *capripneumoniae* (11 strains)	Type strain F38[a] (NCTC 10192), 19/2[a], 4/2LC,[a] 7/1a,[a] KD,[b] G1943,[b] M74/93,[b] Baragoi,[b] M159/96,[b] M79/93,[b] M149/96[b]
Mycoplasma bovine serogroup 7 (3 strains)	Type strain PG50[c] (NCTC 10133), RQ1-92,[c] L29-7[c]
M. mycoides subsp. *mycoides* SC (8 strains)	Tan8,[d] BF138,[e] KH_3J,[e] Gladysdale,[c] N6,[f] M375,[f] B820,[e] Afadé[c]
M. ovipneumoniae (3 strains)	20/2,[a] 30/3,[a] 642/9[a]
M. mycoides subsp. *mycoides* LC	Type strain Y-goat[a]
M. capri	Type strain PG3[a] (NCTC 10137)
M. capricolum subsp. *capricolum*	Type strain California Kid[a] (NCTC 10154)
M. bovis (2 strains)	Type strain Donetta,[a] OB-4[a]
M. agalactiae	Type strain PG2[a]

[a] Obtained from Gareth Jones, Moredun Research Institute, Edinburgh, United Kingdom.
[b] Obtained from Göran Bölske, National Veterinary Institute, Uppsala, Sweden.
[c] Obtained from Robin Nicholas, Veterinary Laboratory Agency, Addlestone, United Kingdom.
[d] Obtained from Benedict Lema, Animal Diseases Research Institute, Dar-es-Salaam, Tanzania.
[e] Obtained from Rosário Gonçalves, Laboratorio Nacional de Veterinaria, Lisboa, Portugal.
[f] Obtained from Willie Amanfu, National Veterinary Laboratory, Gaborone, Botswana.

M. capricolum subsp. *capripneumoniae* antigen should allow the diagnosis of a current infection, since antigen has been detected within 3 to 9 days of the onset of clinical signs following infection with *M. capricolum* subsp. *capripneumoniae* (23). Used in conjunction with the current antibody-detection LAT, this should allow effective field screening at all stages of infection. LATs are very rapid, are relatively inexpensive, and can be used in situ by untrained personnel. The aim of the work described in this paper was to investigate the potential of an LAT with anti-*M. capricolum* subsp. *capripneumoniae* immunoglobulin-coated latex microspheres to diagnose clinical and subclinical *M. capricolum* subsp. *capripneumoniae* infection in goats.

MATERIALS AND METHODS

Mycoplasma strains and growth conditions. The mycoplasma strains used in this study are shown in Table 1. All strains were grown in Mycoplasma Experience (ME) broth and agar medium (Mycoplasma Experience, Reigate, United Kingdom). To measure the titer of broth cultures used in agglutination reactions, serial dilutions were made in fresh medium to measure color-changing units or, alternatively, were plated onto solid medium followed by colony counting (18). 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.

Antisera. *M. capricolum* subsp. *capripneumoniae* polyclonal antiserum (F38) was raised in rabbits following vaccination with whole sonicated F38 type strain, while a membrane fraction antiserum (R37) was raised against a Triton Tx114-phase (30) fraction of F38. Both antisera have previously been described in detail (13). The immunoglobulin G (IgG) fraction was purified on a protein A-Sepharose column by a standard methodology (5) and was eluted at a final concentration of 14.5 mg/ml in phosphate-buffered saline (PBS). A negative control rabbit IgG fraction (R46) was similarly purified at a final concentration of 7.5 mg/ml.

Sequential serum samples were obtained from experimentally infected goats (12). *M. capricolum* subsp. *capripneumoniae* strain 19/2 (9) was used for experimental infections. The passage level was about 25 at the time of infection (Gareth Jones, personal communication). The strain was grown in ME medium (Mycoplasma Experience) to the late logarithmic phase, concentrated 10-fold in fresh medium, and then frozen at −70°C. Three animals were infected (animal 1864, a female; animal 1855, a female; and animal 1884, a male). The goats were anesthetized with 4 ml of intravenously administered Saffan (Schering-Plough Animal Products) and were then endobronchially inoculated with 15 ml of freshly thawed culture. The titer at inoculation was measured to be 10^9 CFU per ml. Blood samples were taken weekly until week 8 (with the exception of 1 week postinfection, for which a blood sample was not available). Animal 1855 displayed the most intense clinical signs, with an elevated temperature and persistent cough (days 5 to 22). The other animals exhibited transient clinical signs (2 to 4 days), although all animals were clearly positive by Western blotting and ELISA. Field sera were obtained from naturally infected goat herds with CCPP from Eritrea (T. Tekleghiorghis, Program Abstr. 3rd Workshop Mycoplasmas of Ruminants of the COST Action 826, p. 45, 1998). Negative control goat antisera were obtained from herds located in several outdoor locations in the United Kingdom.

Purification of CPS. CPS was purified from spent medium by a modification (14) of a previously published method (21). Mycoplasmas were removed by centrifugation, the pH of the cleared supernatant was adjusted to 5.0 with glacial acetic acid, and then the cleared supernatant was heated to 100°C for 30 min, adjusted back to pH 7.5 with NaOH, filtered through Whatmann 3MM paper, and then precipitated with 2 volumes of ethanol. The precipitate was resuspended in 0.1 volume of distilled H_2O, and RNase A and DNase I and II (Sigma) were added to a final concentration of 1 μg/ml (each). This mixture was left for 2 h at 37°C, and then SDS was added to 0.5%, followed by the addition of proteinase K (Sigma) to a final concentration of 1 mg/ml. The mixture was left for 24 h at 45°C and was then dialyzed against running tap water for 3 days with a 15-kDa molecular mass cutoff membrane. The dialysate was then extracted once with phenol-chloroform (50:50), extracted once with chloroform, ethanol precipitated, rinsed in 80% ethanol, and dialyzed overnight against several changes of distilled H_2O. CPS was further purified by gel filtration twice through a Sephacryl S-300 column, where it was eluted early, immediately after the void volume. No protein could be detected in the purified CPS by a variety of assays (Bradford assay, Lowry assay, and following silver staining of SDS-polyacrylamide gels). The amount of carbohydrate present was measured by the phenol-sulfuric acid method (4).

Measurement of CPS antibody titer. ELISA analysis was used to estimate the *M. capricolum* subsp. *capripneumoniae* CPS antibody titer in the IgG fraction used to coat the latex particles (to allow standardization between different antibody batches). Microtiter plates (Greiner) were coated with a saturating amount of antigen (0.1 ml of antigen solution at a concentration of 10 μg/ml in PBS left overnight at 4°C). Antigen was either purified CPS or whole sonicated *M. capricolum* subsp. *capripneumoniae* that was pelleted out of the 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 blocked in a 5% solution of dry skim milk in PBS plus 0.05% Tween 20 (PBST; Sigma) for 2 h at room temperature. Primary antibody (either anti-*M. capricolum* subsp. *capripneumoniae* IgG or a negative control IgG) was standardized at an initial concentration of 1 mg/ml in PBS and was then diluted 1:100 in blocking solution. Twofold serial dilutions in blocking buffer were then made across the plate (1:100 down to 1:102,400). 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. Four 5-min washes in PBST were performed, and then secondary antibody (pig anti-rabbit horseradish peroxidase conjugate; DAKO A/S, Glostrup, Denmark) was added at a 1:1,000 dilution in blocking solution. After incubation at room temperature for 1 h, the plates were washed four times in PBST and developed with *o*-phenylenediamine dichloride (Sigma). The optical density was measured at 492 nm with an IEMS plate reader (Labsystems).

Coating of microspheres. A 10% solution of 0.8-μm-diameter polystyrene latex microspheres (LB8; 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-*M. capricolum* subsp. *capripneumoniae* IgG, and the volume was made up to 1 ml with PBS. (For experiments in which the ratio of antibody to beads was altered, the volume of beads and the final reaction volume were kept constant, while the amount of added antibody was varied.) Negative control beads were produced by an identical protocol but with IgG purified from a nonimmunized rabbit. The mixture was incubated for 120 min at room temperature on an end-to-end shaker and was then centrifuged at 15,000 rpm (20,000 × *g*) for 3 min in a microcentrifuge. The supernatant was removed and was stored at 4°C for ELISA analysis, and the beads were resuspended in 1 ml of PBST. The mixture was centrifuged as described above, 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 to be useful in difficult cases. For preabsorption of polyclonal anti-*M. capricolum* subsp. *capripneumoniae* IgG with CPS, 10 μg 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 left to incubate at room temperature for 30 min, followed by centrifugation at 15,000 rpm (20,000 × *g*) in a microcentrifuge. The supernatant was added to 15 μl of beads (15 mg) 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.

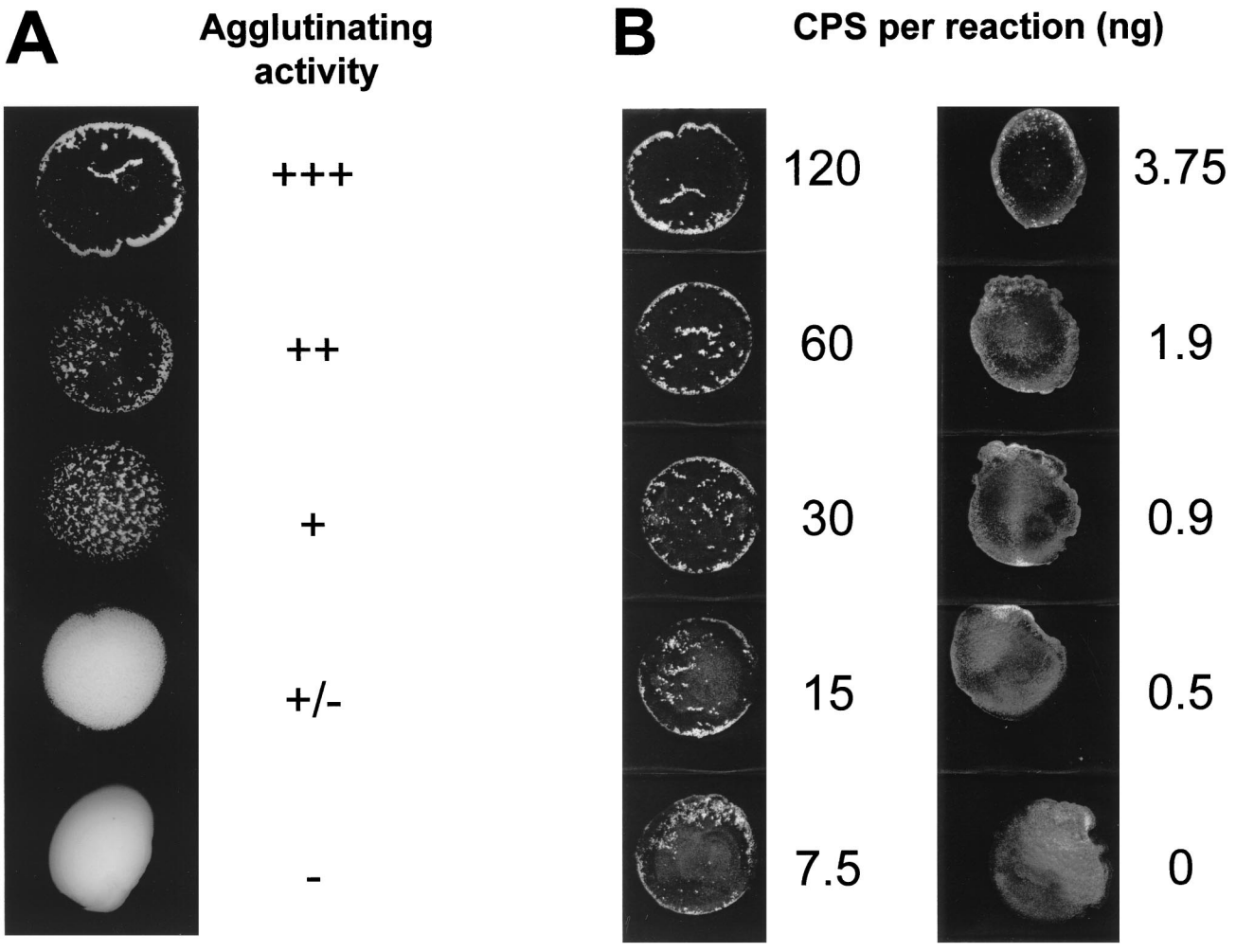

FIG. 1. Visual definition of agglutination reactions. (A) Agglutination reactions photographed while still wet with CPS in solution. Agglutination activity is defined from +++ (uppermost reaction) to − (lower reaction). (B) Agglutination reactions photographed after drying by using pure CPS solution in PBS serially diluted from 120 to 0 ng of CPS ml^{-1}. The clarity of the agglutination reaction is reduced upon desiccation, while at 1.9 and 0.9 ng ml^{-1} reactions that were still wet were positive, although this is not apparent in the figure.

Measurement of unbound antibody. ELISA analysis was used to measure the amount of unbound antibody following microsphere coating and thus estimate the binding capacity of the latex microspheres for IgG. A standard curve was produced by diluting anti-*M. capricolum* subsp. *capripneumoniae* IgG to concentrations of 100, 50, 10, 5, and 1 μg/ml in PBST, and 0.1 ml of each aliquot was added (in triplicate) to 96-well microtiter plates. Similarly, aliquots of the bead supernatant were diluted in PBS in the range of 1:10 to 1:100 and were added in triplicate to the wells. Appropriate controls (including primary antibody only and secondary antibody only) were included on the plates. The plates were left overnight at 4°C, washed twice in PBST, washed once in distilled H_2O, and then blocked in a 5% solution of dry skim milk in PBST for 3 h at 37°C. The plates were then washed twice in PBST and once in distilled H_2O, and secondary antibody (goat anti-rabbit horseradish peroxidase conjugate; DAKO A/S) was added at a 1:2,000 dilution in PBST supplemented with 5% dry skim milk. After incubation at room temperature for 1 h, the plates were washed in PBST and developed with *o*-phenylenediamine dichloride (Sigma). The optical density was measured at 492 nm with an IEMS plate reader (Labsystems). The standard curve was found to be linear in the range of 0.1 to 10 μg of IgG per well, and dilutions of bead supernatant which gave readings within this range were used to calculate the amount of IgG remaining in the supernatant compared to that originally added to the latex microspheres.

LATs. LATs were performed with (i) purified CPS diluted in negative goat serum, medium, or PBS; (ii) field and experimentally infected goat 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 the same procedure: 30 μl of test solution was aliquoted onto a glass microscope slide, and 5 μl of the bead suspension (containing 75 μg of beads coated with 12.5 μg of anti-*M. capricolum* subsp. *capripneumoniae* IgG) was added with a pipette. The beads were thoroughly mixed, and the slide was incubated for 1 to 10 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 the negative IgG were mixed with the positive test solution as a negative control, while beads coated with anti-*M. capricolum* subsp. *capripneumoniae* IgG were also mixed with antigen-free test solution (negative goat serum or ME growth medium) to test for autoagglutination. Agglutination (when seen against a dark background) was most obvious when the reaction mixture was still wet, although it could still be clearly ascertained when it was dry in the case of strong positive reactions. In borderline cases, increasing the reaction time by adding distilled H_2O (10 to 15 μl) after 10 to 15 min or by conducting the reaction in a humid atmosphere to prevent desiccation of the mixture was found to be beneficial. It is also possible to perform agglutination reactions in standard 96-well microtiter plates (Greiner). In this case, 6 μl of beads was added to 60 μl of test solution in a well, the plate was sealed with tape or an adhesive plastic cover (BIS Ltd., Lancs, United Kingdom), and the plate was subjected to gentle agitation for 15 to 90 min prior to visualization under a low-power microscope or magnifying glass.

CFT. The CFT was performed according to the Office International des Epizooties manual of standards (19) with antigen and reagents supplied by CIRAD-EMVT.

RESULTS

Definition of agglutinating activity. Following the mixing of anti-*M. capricolum* subsp. *capripneumoniae* coated latex microspheres with *M. capricolum* subsp. *capripneumoniae* antigen-positive solution, an agglutination reaction occurred, the rapidity and extent of which depended upon the amount of

antigen present. For the purposes of this study a four-point scale (exhibited pictorially in Fig. 1A) was adopted and was defined as follows: +++, heavy flocculent precipitates forming within 1 min (clear background); ++, heavy flocculent precipitates taking 1 to 5 min to form (clear background); +, light flocculent precipitate against mostly clear background (5 to 15 min), +/−, light flocculent precipitate against cloudy homogeneous background after extended incubation (15 to 30 min); −, no precipitate, cloudy homogeneous background.

Binding capacity of latex microspheres for anti-*M. capricolum* subsp. *capripneumoniae* polyclonal IgG. Variable amounts of IgG (0.01 to 1.0 mg) were incubated with 1.5 mg of latex microspheres in a reaction volume of 0.15 ml. Following centrifugation, the cleared supernatant was tested for the presence of IgG by ELISA analysis. No significant amounts of IgG remained in the supernatant in the range of 0.01 to 0.1 mg of added IgG per 1.5 mg of beads, suggesting that the binding capacity of the beads had not yet been reached. A small amount of unbound antibody (0.04 ± 0.01 mg) was observed following incubation of the latex beads with 0.5 mg of IgG, and a significant amount of free antibody (0.3 ± 0.004 mg) was observed when 1.0 mg of IgG was used in the binding reaction. The results suggest that the binding capacity of the beads is in the range of 0.46 to 0.7 mg of IgG per 1.5 mg of beads (0.33 to 0.5 mg of IgG per mg of beads).

Agglutinating activity of latex microspheres coated with different amounts of anti-*M. capricolum* subsp. *capripneumoniae* IgG. Beads coated with different amounts of anti-*M. capricolum* subsp. *capripneumoniae* IgG (0.001 to 1.0 mg of IgG per 1.5 mg of beads) were tested in agglutination reactions with *M. capricolum* subsp. *capripneumoniae*-positive growth medium (titer, 5×10^8 CFU per ml). Beads coated with higher levels of IgG (0.1 to 1.0 mg of IgG per 1.5 mg of beads) gave consistently optimum agglutination reactions. Beads coated with a lower, nonsaturating level of IgG (0.01 to 0.1 mg) gave more variable results: only two of four preparations gave positive reactions by the LAT, even with high levels of *M. capricolum* subsp. *capripneumoniae* antigen in the test solution. For this reason a higher (but nonsaturating) level of anti-*M. capricolum* subsp. *capripneumoniae* IgG was used to coat the latex microspheres to produce the test for general usage (0.25 mg of IgG per 1.5 mg of beads in a final volume of 0.1 ml). Control IgG-coated beads (prepared with IgG purified from a nonimmunized rabbit) gave negative agglutination reactions with both positive and negative media, while *M. capricolum* subsp. *capripneumoniae*-specific IgG-coated beads gave negative agglutination reactions with negative media.

Effect of excess anti-*M. capricolum* subsp. *capripneumoniae* IgG on agglutination activity. Since the LAT detects circulating *M. capricolum* subsp. *capripneumoniae* antigen, the presence of *M. capricolum* subsp. *capripneumoniae* antibodies in a serologically positive animal might reduce the sensitivity of the test by sequestering free antigen. The effect of excess anti-*M. capricolum* subsp. *capripneumoniae* IgG on the antigen-detection LAT was tested by the addition of 5 or 1 μl of anti-*M. capricolum* subsp. *capripneumoniae* IgG (concentration, 14.5 mg per ml) to *M. capricolum* subsp. *capripneumoniae*-positive growth medium (titer, 5×10^8 CFU per ml) either (i) 10 min before mixing or (ii) immediately following mixing of the medium with 5 μl of coated microspheres in an LAT. Preabsorption of *M. capricolum* subsp. *capripneumoniae* antigen-positive medium with 5 μl of anti-*M. capricolum* subsp. *capripneumoniae* IgG completely removed agglutination activity, while simultaneous addition attenuated, but did not completely remove, agglutination activity. Preabsorption with 1 μl of anti-*M. capricolum* subsp. *capripneumoniae* IgG reduced agglutination activity, while simultaneous addition had no observable affect. Thus, very high levels of anti-*M. capricolum* subsp. *capripneumoniae* IgG in an infected goat might, in theory, affect the sensitivity of LAT, although in practice this did not appear to be a problem (see below).

TABLE 2. Sensitivity of *M. capricolum* subsp. *capripneumoniae* IgG-coated microspheres at detecting *M. capricolum* subsp. *capripneumoniae* in culture

Dilution factor of *M. capricolum* subsp. *capripneumoniae* culture	Time to agglutination (min)	No. of mycoplasma CFU per agglutination reaction	No. of mycoplasma CFU/ml equivalent
1:1–1:128	1–2	1.5×10^7–1.2×10^5	5×10^8–4×10^6
1:256–1:4,096	5–15	6×10^4–3.6×10^3	2×10^6–1.2×10^5
1:4,096 and higher	No agglutination	$<3.6 \times 10^3$	$<1.2 \times 10^5$
Control medium	No agglutination	Negative	Negative

Sensitivity of latex microspheres at detecting *M. capricolum* subsp. *capripneumoniae* in medium. *M. capricolum* subsp. *capripneumoniae* strain F38 was cultured in ME medium to a titer of 5×10^8 CFU per ml (growth was stopped at the mid-logarithmic phase to prevent overgrowth and cell death, which could give an underestimate of the true amount of *M. capricolum* subsp. *capripneumoniae* antigen present). The culture was serially diluted in fresh medium and was tested in an LAT (Table 2). Clear agglutination activity was observed down to a dilution of 1:4,096, which is equivalent to 3.6×10^3 mycoplasmas per agglutination reaction (0.03 ml) or a titer of 1.2×10^5 mycoplasmas per ml. This is the same degree of sensitivity previously reported for an antigen-detection LAT directed at *M. pneumoniae* (15). Similar sensitivity levels were observed with all strains of *M. capricolum* subsp. *capripneumoniae* tested. When the medium was cleared of mycoplasmas prior to testing by LAT (by centrifugation at $15{,}000 \times g$ for 30 min), no difference in sensitivity was observed, strongly suggesting that a soluble antigen rather than (or in addition to) the mycoplasmas themselves was responsible for the agglutinating activity. If the mycoplasma pellet was resuspended in fresh ME medium following centrifugation, a positive agglutination reaction was observed at an approximately 100-fold reduced sensitivity, presumably due to shedding of the CPS coat during resuspension (following a further round of centrifugation, this medium was still positive). No agglutination was observed with negative IgG-coated beads or anti-*M. capricolum* subsp. *capripneumoniae* IgG-coated beads placed in *M. capricolum* subsp. *capripneumoniae*-negative medium.

Effect of preabsorption with CPS on agglutinating activity. *M. capricolum* subsp. *capripneumoniae* has been reported to produce large quantities of extracellular CPS, which is shed into the growth medium (21). It seemed likely that the soluble antigen recognized by the test was CPS. Anti-*M. capricolum* subsp. *capripneumoniae* IgG was preabsorbed with purified CPS to remove CPS-specific antibodies and was then used to coat the beads, as described above. All agglutination activity was now lost from these beads, not only with purified CPS in solution (66 μg per ml) but also with *M. capricolum* subsp. *capripneumoniae* culture medium (titer, of 5×10^8 CFU per ml) and previously LAT-positive serum samples from CCPP-positive animals. 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

TABLE 3. Sensitivity of IgG-coated latex beads in detecting *M. capricolum* subsp. *capripneumoniae* capsular polysaccharide in solution

M. capricolum subsp. *capripneumoniae* CPS concn (ng/ml) in test solution	Amt (ng) of CPS per reaction mixture	Agglutination activity of 1.5 mg of latex microspheres coated with the following different amounts of anti-*M. capricolum* subsp. *capripneumoniae* IgG[a]:			
		No IgG	0.01 mg	0.1 mg	0.25 mg
≥8,000	≥240	−	+++	+++	+++
4,000	120	−	++	+++	+++
2,000	60	−	+	+++	+++
1,000	30	−	+/−	++	+++
500	15	−	−	+	++
250	7.5	−	−	+	++
125	3.75	−	−	+/−	++
62.5	1.88	−	−	−	+
31.25	0.94	−	−	−	+/−
16	0.47	−	−	−	−
8	0.24	−	−	−	−
0	0	−	−	−	−

[a] Definitions of agglutination activity are provided in the text (see also Fig. 1). Data for beads coated with negative IgG and beads coated with 0.01 mg of *M. capricolum* subsp. *capripneumoniae* IgG come from a single batch of beads, and data for beads coated with 0.1 and 0.25 mg of IgG come from two different batches of beads. In all instances fresh dilutions of CPS were made, and all agglutination reactions were performed in triplicate.

with rabbit anti-*M. capricolum* subsp. *capripneumoniae* IgG did not result in agglutination, as would be expected if the beads were coated with *M. capricolum* subsp. *capripneumoniae* CPS. These findings strongly suggest that CPS is the major or single antigen recognized by the test.

Sensitivity of latex microspheres at detecting pure CPS in solution. Twofold serial dilutions of *M. capricolum* subsp. *capripneumoniae* CPS in *M. capricolum* subsp. *capripneumoniae*-negative goat serum were made, and aliquots were incubated (in triplicate) with microspheres coated with different amounts of anti-*M. capricolum* subsp. *capripneumoniae* IgG. The results are shown in Table 3 and Fig. 1B. Beads coated with 0.25 mg of IgG per 1.5 mg of beads were slightly more sensitive than beads coated with smaller amounts and were able to reproducibly detect amounts as as low as 2 to 4 ng of CPS in a reaction volume of 0.03 ml (equivalent to a concentration of 60 to 120 ng of CPS per ml). This level of sensitivity was noted for CPS dissolved in *M. capricolum* subsp. *capripneumoniae*-negative goat serum, ME growth medium, and PBS. The reaction time in PBS was considerably shortened, however, and a higher level of background agglutination (exhibited as a grainy background) was noted. The maximum concentration of CPS tested by LAT was 0.5 mg/ml, which still gave a strong positive signal. This finding suggests that saturation of antibody-coated microspheres due to excessive CPS levels in infected goats is unlikely to occur.

Measurement of CPS-specific antibody titer in *M. capricolum* subsp. *capripneumoniae* IgG fraction. To enable reproducibility between different batches of antisera, 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 (with purified CPS used as the antigen) and the second to measure the overall anti-*M. capricolum* subsp. *capripneumoniae* titer (with whole sonicated *M. capricolum* subsp. *capripneumoniae* used as the antigen). The titer was expressed per milligram of IgG. Thus, the specific anti-CPS titer could be estimated and taken into account (if necessary) for different batches of serum.

The concentration of purified IgG was standardized at 1 mg/ml and was then serially diluted twofold from 1:100 to 1:102,400 and incubated with antigen-coated plates. The results are plotted graphically in Fig. 2A (with purified CPS used as the antigen) and Fig. 2B (with whole *M. capricolum* subsp. *capripneumoniae* used as the antigen). By using the linear portion of the graph to estimate the antibody titer, 1 mg of F38 antiserum (which was used to produce LAT) can be seen to detect whole sonicated *M. capricolum* subsp. *capripneumoniae* down to a dilution of 1:9,600 and purified CPS to a dilution of 1:12,800 (a ratio of 1:1.334). In contrast, R37 antiserum, raised against the membrane fraction of *M. capricolum* subsp. *capripneumoniae* (13), has a much lower titer against whole *M. capricolum* subsp. *capripneumoniae* but a considerably higher anti-CPS titer (1:2,400 against whole sonicated *M. capricolum* subsp. *capripneumoniae* and 1:76,800 against purified CPS, a ratio of 1:32). Negative control IgG (R46) exhibited a negligible titer against both antigens. Despite the relatively low CPS antibody titer of F38 antiserum compared to that of R37 antiserum, F38 antiserum performed well in the LAT for CPS detection reported here, suggesting that specific antigen purification procedures prior to immunization are not necessary.

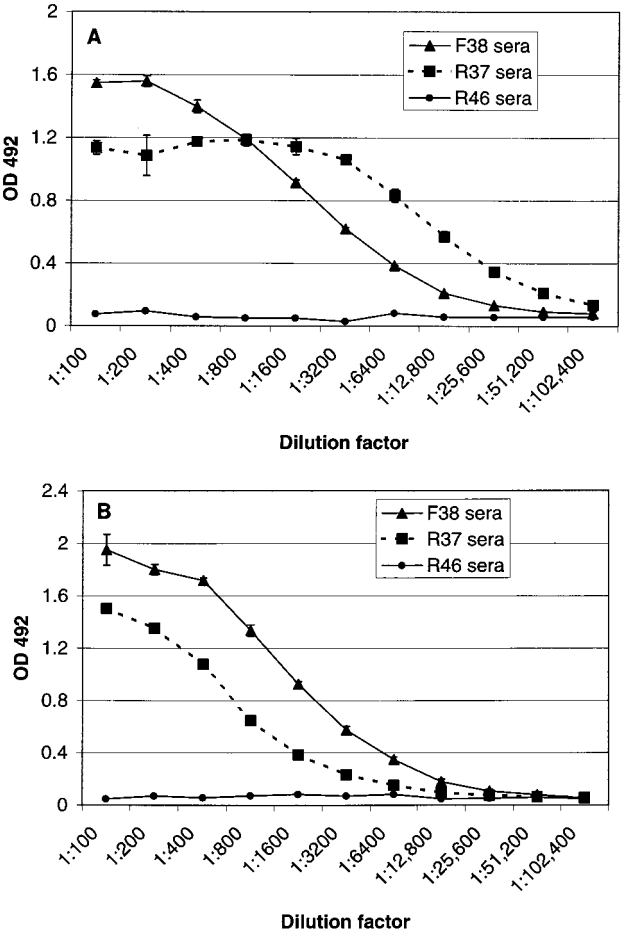

FIG. 2. ELISA data showing antibody titers of various sera against purified CPS antigen (A) and whole *M. capricolum* subsp. *capripneumoniae* antigen (B). Serum was initially diluted 1:100 and was then serially diluted twofold down to 1:102,400. The linear portion of each graph was used to estimate the point of intersection with the *x* axis (titer of F38-positive serum against whole antigen, 1:9,600; R37-positive serum against whole antigen, 1:2,400; titer of F38-positive serum against CPS, 1:12,800; titer of R37-positive serum against CPS, 1:76,800). OD 492, optical density at 492 nm.

TABLE 4. Testing of LAT with sera from goats with CCPP over time

Animal no.	Agglutination activity of sera on the following days postinfection[a]:							
	0[b]	14	21	28	35	42	49	56
1855[c]	−	+++	+	+	+	−	−	+
1864	−	++	−	−	−	−	−	+/−
1884	−	++	−	−	+/−	−	−	−

[a] See Fig. 1 and the text for definitions of agglutination activity.
[b] Blood was taken immediately prior to endobronchial infection.
[c] This animal displayed a persistent cough on days 5 to 22.

Production of a CPS-monospecific polyclonal antiserum for use in LAT was not successful, since immunization of rabbits with purified CPS did not result in a detectable antibody titer (13), a frequent occurrence with carbohydrate antigens when presented in a pure form to the immune system (6, 25).

Specificity of anti-*M. capricolum* subsp. *capripneumoniae* LAT. The LAT was tested against a variety of mycoplasmal species known to be evolutionarily closely related to *M. capricolum* subsp. *capripneumoniae* (including all members of the *M. mycoides* cluster) and mycoplasmas likely to be found as associated pathogens (such as *M. ovipneumoniae*) (Table 1). All mycoplasmal species were grown in ME liquid medium to the mid-logarithmic phase, with titers in the range of 5×10^7 to 8×10^8 per ml. The LAT was specific for *M. capricolum* subsp. *capripneumoniae* and bovine serogroup 7, with all strains testing positive. Similar sensitivity levels were noted for all the strains examined (dilutions in the range of 10^{-2} to 10^{-3} were positive). No agglutination was noted for any other mycoplasmal species tested, even in undiluted growth medium. The specificity of the LAT exactly mirrors that of the CPS-specific monoclonal antibody WM-25 (2, 23), providing further evidence that CPS is the *M. capricolum* subsp. *capripneumoniae* antigen recognized by the LAT.

The LAT was tested against a bank of 52 serum samples from goat herds located at a variety of locations in the United Kingdom (a country which has never experienced CCPP). All samples returned a negative result in agglutination reactions.

Testing of LAT with sera from animals experimentally infected with *M. capricolum* subsp. *capripneumoniae*. The LAT was tested over time with sera from animals experimentally infected with *M. capricolum* subsp. *capripneumoniae* (Table 4). Blood samples were not available at 7 days postinfection, but a positive reaction was observed in all three animals 14 days postinfection (8 to 11 days following the onset of clinical signs). The animal which exhibited the most intense clinical signs also exhibited the longest positive reaction by LAT (goat 1855, which exhibited a persistent cough from days 5 to 22 postinfection). All three animals exhibited a significant humoral immune response (IgG and IgM) against *M. capricolum* subsp. *capripneumoniae* from week 2 onward (by ELISA and immunoblotting) (data not shown). It is unclear why the signal from the antigen-detection LAT increased in intensity at 8 weeks postinfection for animals 1855 and 1864, but it may be related to the fact that the relative levels of anti-CPS IgG and IgM affected test sensitivity.

Correlation with CFT. Experimentally infected animals 1855, 1864, and 1884 were all negative for CCPP by CFT, although they were positive by a variety of other criteria (immunoblotting, ELISA, clinical signs [12], and antibody-detection LAT). This finding suggested that CFT may be less sensitive than the other methods of diagnosis, particularly in the case of low-level infections, such as those observed for the experimentally infected goats. Similar findings have been reported previously following infection of goats with a high-passage strain of *M. capricolum* subsp. *capripneumoniae*, in which only 7 of 20 infected animals exhibited a positive titer by CFT (11). The LAT was used to test 57 field serum samples obtained from CCPP-affected areas in Eritrea (T. Tekleghiorghis, Prog. Abstr. 3rd Workshop Mycoplasmas of Ruminants of the COST Action 826) to determine the level of correlation with the results of CFT (Table 5). An exact correlation in diagnosis was noted for 38 of 57 samples tested (67% overall correlation). Twenty-six serum samples were negative by both CFT and LAT, while 12 serum samples were positive by both tests. Seven serum samples were CFT positive and LAT negative, while 12 serum samples were LAT positive but CFT negative, which would agree with the premise that the antigen-detection LAT may be more sensitive than CFT. However, an exact correlation is unlikely since CFT detects a serological response, in contrast to LAT, which detects *M. capricolum* subsp. *capripneumoniae* antigen and which is therefore likely to be more sensitive at the early stages of infection (CPS antigen has been detected in the serum of experimentally infected animals within 4 to 9 days [23]) or 8 to 11 days [this study], while significant complement fixation titers were not observed until 21 days postinfection [17]).

A comparison of the complement fixation titers and the LAT signals for individual serum samples does not reveal any obvious inverse correlation (i.e., a high CPS antigen signal does not correlate with a low complement fixation titer and vice versa). In general, high and low signals for CPS by LAT can be seen with all intensities of complement fixation titers (ranging from negative to a complement fixation titer of 1:640).

TABLE 5. Comparison of CFT and CPS-LAT with 57 serum samples from animals in a region positive for CCPP[a]

CFT and LAT results (no. of serum samples)	CFT titer	CPS-LAT signal
CFT positive (12) and LAT positive (12)	1:10	+
	1:10	++
	1:20	+++
	1:40	+
	1:40	++
	1:40	+++
	1:80	++
	1:80	++
	1:80	+++
	1:160	+++
	1:320	++
	1:320	++
	1:640	+++
CFT positive (7) and LAT negative (7)	1:10	
	1:20[b]	
	1:40	
	1:160	
	1:320	
CFT negative (12) and LAT positive (12)		5@ +
		5@ ++
		2@ +++

[a] See Fig. 1 and the text for definitions of LAT activity. No titers or signals were detected for 26 CFT-negative animals, and 26 animals were negative for CPS by LAT.
[b] Three serum samples had this titer.

DISCUSSION

An antigen-detection LAT was produced by coating polystyrene latex microspheres with the IgG fraction of *M. capricolum* subsp. *capripneumoniae*-specific rabbit hyperimmune antiserum. Experimental findings are consistent with the fact that the *M. capricolum* subsp. *capripneumoniae* CPS is the soluble antigen recognized by the test. Spent *M. capricolum* subsp. *capripneumoniae* growth medium cleared by centrifugation still gave a positive agglutination reaction (with no decrease in sensitivity), indicating that a soluble antigen rather than the whole organism is responsible. Purified CPS in solution caused strong and rapid agglutination, while preabsorption of anti-*M. capricolum* subsp. *capripneumoniae* IgG with CPS prior to coating of latex microspheres removed all agglutinating activity when the beads were subsequently used in a LAT. In addition, the specificity of the LAT exactly matched that of a CPS-specific monoclonal antibody (WM-25) which only recognizes *M. capricolum* subsp. *capripneumoniae* and bovine serogroup 7 from *M. mycoides* cluster mycoplasmas (2, 22). The LAT was tested against a bank of 52 serum samples from goat herds located at a variety of locations in the United Kingdom (a country which has never experienced CCPP), with all samples returning a negative result in agglutination reactions. Latex microspheres coated with anti-*M. capricolum* subsp. *capripneumoniae* IgG in the range of 0.1 to 1.0 mg of IgG per 1.5 mg of microspheres were found to be effective in the LAT, and the sensitivity of the test was found to be 2 ng of CPS, or the equivalent of 3.6×10^3 CFU *M. capricolum* subsp. *capripneumoniae* per reaction mixture, in a volume of 0.03 ml (equivalent to 125 ng of CPS per ml, or 1.2×10^5 *M. capricolum* subsp. *capripneumoniae* CFU/ml). This is a level of sensitivity similar to that reported previously for an *M. pneumoniae* antigen-detection LAT, for which a detection limit of 2×10^5 CFU was reported (15). However, it should be noted that the amount of CPS produced by *M. capricolum* subsp. *capripneumoniae* may vary in vivo; thus, these sensitivity values must be treated with caution, particularly since soluble CPS rather than whole mycoplasma appears to be the main target for the LAT. A 67% correlation between the results of LAT and the results of CFT for the diagnosis of CCPP was noted with field sera from goat herds naturally infected with *M. capricolum* subsp. *capripneumoniae*, with the main discrepancy in diagnosis being apparently due to the increased sensitivity of the LAT compared to that of CFT; while the LAT detected CCPP in our three experimentally infected goats, CFT failed to do so (although the animals were positive by both ELISA and immunoblotting). This apparently poor sensitivity of CFT at detecting subclinical CCPP has been noted before (11).

An effective antigen-detection test should be useful in identifying animals at the earliest stages of CCPP, prior to the development of a measurable immune response. This could be significant in the field, since the incubation period for CCPP can be as little as 6 days, with death in acute cases following only 2 days later (16). Previous work has indicated that CFT is unable to detect infection in such animals (thought to be due to the fact that high levels of CPS eclipse the immune response) (10, 17, 19), and even for animals with nonacute cases of CCPP CFT appears to be relatively insensitive prior to about 21 days postinfection (17, 20). In contrast, *M. capricolum* subsp. *capripneumoniae* CPS antigen has been detected in *M. capricolum* subsp. *capripneumoniae*-infected goats 4 to 9 days following the onset of clinical signs (23), suggesting that a suitable antigen-detection test should allow early diagnosis of CCPP. In our hands, using experimentally infected animals, the LAT was able to detect CPS at the first bleed (14 days postinfection), 8 to 11 days following the onset of clinical signs. The data presented in this paper suggest that the period over which the antigen-detection LAT remains effective may extend over several weeks; thus, the test may not be restricted solely to diagnosis at the primary stages of infection. The observed correlation of the results of LAT with those of CFT offers good evidence that the antigen-detection LAT should be able to diagnose both acute and nonacute cases of CCPP over an extended period.

Interestingly, CCPP (unique among diseases caused by mycoplasmas of the *M. mycoides* cluster) does not exhibit a bacteriemic phase and appears to be lung specific (16, 26). Detection of *M. capricolum* subsp. *capripneumoniae* antigen in the sera of infected goats (as reported in this paper and by others [23]) suggests either a hitherto undetected systemic phase to the infection or, perhaps more likely, that soluble antigen (CPS) is entering the circulation from the infected lung. Using both PCR and direct culture we were unable to detect *M. capricolum* subsp. *capripneumoniae* in the sera of infected animals, in support of the latter hypothesis (data not shown). The role (if any) of CPS in disease pathogenesis and the mechanism by which it enters the circulation remain to be determined, however.

A diagnostic test based upon the detection of carbohydrate rather than protein or DNA could offer certain advantages, since polysaccharides are the end product of multienzyme synthesis pathways and random mutations at the DNA level are less likely to affect the overall antigenic structure of the molecule. This is in contrast to a protein- or PCR-based antigen detection system, in which the change of a single nucleotide could result in an altered epitope and destroy the binding of a monoclonal antibody or prevent primer extension during PCR (24). Similarly, the likelihood that antigenic variation between mycoplasmal strains will affect the ability to diagnose CCPP is much reduced (a problem with protein-specific monoclonal antibodies, because a minority recognize epitopes in all strains of *M. capricolum* subsp. *capripneumoniae* and none of the antibodies is completely specific [27]). From the work described in this paper (Table 1) and that of others (2, 22), there is no evidence for antigenic variation of CPS between strains of *M. capricolum* subsp. *capripneumoniae*. The observed cross-reactivity between the CPSs of *M. capricolum* subsp. *capripneumoniae* and bovine serogroup 7 is unlikely to present problems in field diagnosis since both species appear to be host specific (goats and cattle, respectively) (26). There has been a single isolated incident of the isolation of bovine serogroup 7 from a goat (1), but since this animal was also infected with *Pasteurella multocida*, evidence for a significant pathogenic role for bovine serogroup 7 in goats appears to be slight. Apart from this, all current tests for the detection of *M. capricolum* subsp. *capripneumoniae* (with the exception of the PCR test [8]) also show cross-reactivity with bovine serogroup 7, including a monoclonal antibody-based ELISA (27).

For ease of duplication, the CPS antigen-detection LAT was produced by using a simple rabbit polyclonal hyperimmune antiserum. Use of the purified IgG fraction was found to result in an accurate and sensitive test, although a higher titer of CPS-specific antiserum was available (produced against an *M. capricolum* subsp. *capripneumoniae* membrane fraction). Production of a CPS-monospecific antiserum was not successful due to the lack of response in immunized animals (13). While a LAT based upon CPS monoclonal antibodies might give greater specificity, the costs and ease of production are likely to be considerably higher, and it is unlikely that specificity would be increased (all *M. capricolum* subsp. *capripneumoniae* CPS monoclonal antibodies produced to date also

cross-react with bovine serogroup 7 [2]). A good polyclonal antiserum has the additional advantage that it should detect several epitopes, which might increase test sensitivity and reduce the susceptibility of a test to antigenic variations between isolates.

A test which allows rapid and inexpensive primary screening prior to confirmatory laboratory diagnosis should be useful in the field. In particular, such a test used in conjunction with the current antibody-detection LAT (20) (which may be expected to exhibit optimum sensitivity at a later stage of infection) could allow the rapid and inexpensive screening of herds (using latex beads of different colors to differentiate the tests). In contrast to other diagnostic tests, no expensive equipment or reagents (e.g., ELISA plate readers, PCR machines, gel electrophoresis equipment, or enzymes) are required and results can be obtained in situ within minutes. Requirements are minimal: glass slides (which are inexpensive and reusable) and disposable plastic Pasteur pipettes (with a predetermined drop size). The test appears to be more sensitive than CFT (which cannot effectively detect acute cases [10, 19]), is active at an earlier stage of infection, and is also very specific since it detects a conserved soluble antigen (CPS) rather than the whole organism. The stability of the beads after storage for 1 month at 37°C was unaffected (data not shown). The test should allow rapid testing of herds in the field, an important attribute for a disease like CCPP, in which death can occur well before the results of laboratory-based diagnosis are available to aid in field treatment strategies.

ACKNOWLEDGMENTS

This research was funded by the Department for International Development's Animal Health Program of the United Kingdom.

Thanks are due to Frank Jackson for the supply of negative goat sera.

REFERENCES

1. **Atalaia, V., M. Machado, and F. F. Frazao.** 1987. Patologia dos pequenos ruminantes infeccoes em ovinos e caprinos, originadas pelo micoplasma do grupo 7, Leach (Pg. 50). Rep. Trab. L.N.I.V. **19:**55–60.
2. **Belton, D., R. H. Leach, D. L. Mitchelmore, and F. R. Rurangirwa.** 1994. Serological specificity of a monoclonal antibody to *Mycoplasma capricolum* strain F38, the agent of contagious caprine pleuropneumonia. Vet. Rec. **134:**643–646.
3. **Cottew, G. S., A. Breard, A. J. DaMassa, H. Erno, R. H. Leach, P. C. LeFevre, A. W. Rodwell, and G. R. Smith.** 1987. Taxonomy of the *Mycoplasma mycoides* cluster. Isr. J. Med. Sci. **23:**632–635.
4. **Dubois, M., K. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith.** 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. **28:**350–356.
5. **Ey, P. L., S. J. Prowse, and C. R. Jenkin.** 1978. Isolation of pure IgG1, IgG2a and IgG2b immunoglobulins from mouse serum using protein A-Sepharose. Immunochemistry **15:**429–436.
6. **Goldblatt, D.** 1998. Recent developments in bacterial conjugate vaccines. J. Med. Microbiol. **47:**563–567.
7. **Guerin, C., F. Thiaucourt, V. Brady, A. Breard, and P. C. LeFevre.** 1993. Rapid diagnosis of contagious caprine pleuropneumonia in pleural fluids by immunobinding assay. Small Ruminant Res. **12:**193–200.
8. **Johansson, K.-E., A. Persson, and M. Persson.** 1998. Diagnosis of contagious caprine and contagious bovine pleuropneumonia by PCR and restriction enzyme analysis, p. 137–158. *In* Proceedings of an International Symposium on Diagnosis and Control of Livestock Diseases using Nuclear and Related Techniques. Towards livestock disease diagnosis and control in the 21st century. International Atomic Energy Agency, Vienna, Austria.
9. **Jones, G. E., and A. R. Wood.** 1988. Microbiological and serological studies on caprine pneumonias in Oman. Res. Vet. Sci. **44:**125–131.
10. **MacOwan, K. J., and J. E. Minette.** 1977. Contact transmission of experimental contagious caprine pleuropneumonia (CCPP). Trop. Anim. Health Prod. **9:**185–188.
11. **MacOwan, K. J., and J. E. Minette.** 1978. The effect of high passage Mycoplasma strain F38 on the course of contagious caprine pleuropneumonia (CCPP). Trop. Anim. Health Prod. **10:**31–35.
12. **March, J. B., N. Foster, J. C. Harrison, C. Gammack, L. Hyndman, S. M. Borich, and G. E. Jones.** 2000. Immune responses following experimental infection of goats with *Mycoplasma capricolum* subsp. *capripneumoniae*, causal agent of contagious caprine pleuropneumonia, p. 205–208. *In* D. Bergonnier, X. Berthelot, and J. Frey (ed.), Mycoplasmas of ruminants: pathogenicity, diagnostics, epidemiology and molecular genetics, vol. 4. European Commision, Luxembourg, Luxembourg.
13. **March, J. B., and G. E. Jones.** 1998. Inhibitory effects of vaccines containing subunit fractions of *Mycoplasma capricolum* subsp. *capripneumoniae*, p. 44–49. *In* G. Leori, F. Santini, E. Scanziani, and J. Frey (ed.), Mycoplasmas of ruminants: pathogenicity, diagnostics, epidemiology and molecular genetics, vol 2. European Union, Luxembourg, Luxembourg.
14. **March, J. B., G. E. Jones, P. Hitchen, H. R. Morris, and A. Dell.** 1999. Analysis of the capsular polysaccharide of Mycoplasma mycoides subsp. mycoides sc, the causal agent of CBPP: purification, composition and its role in infection and immunity, p. 69–72. *In* L. Stipkovits, R. Rosengarten, and J. Frey (ed.), Mycoplasmas of ruminants: pathogenicity, diagnostics, epidemiology and molecular genetics, vol. 3. European Union, Luxembourg, Luxembourg.
15. **Masayoshi, T., Y. Hirai, and Y. Kanemasa.** 1992. A latex agglutination test for the detection of *Mycoplasma pneumoniae* in respiratory exudates: a comparative study with a commercially available DNA-probe test. Microbiol. Immunol. **36:**149–160.
16. **McMartin, D. A., K. J. MacOwan, and L. L. Swift.** 1980. A century of classical contagious caprine pleuropneumonia: from original description to aetiology. Br. Vet. J. **136:**507–515.
17. **Muthomi, E. K., and F. R. Rurangirwa.** 1983. Passive haemagglutination and complement fixation as diagnostic tests for contagious caprine pleuropneumonia caused by the F-38 strain of mycoplasma. Res. Vet. Sci. **35:**1–4.
18. **Nicholas, R., and S. Baker.** 1998. Recovery of mycoplasmas from animals. Methods Mol. Biol. **104:**37–43.
19. **Office International des Epizooties.** 1996. Contagious caprine pleuropneumonia, p. 374–383. *In* Manual of standards for diagnostic tests and vaccines. Office International des Epizooties, Paris, France.
20. **Rurangirwa, F. R., T. C. McGuire, A. Kibor, and S. Chema.** 1987. A latex agglutination test for field diagnosis of contagious caprine pleuropneumonia. Vet. Rec. **121:**191–193.
21. **Rurangirwa, F. R., T. C. McGuire, N. S. Magnuson, A. Kibor, and S. Chema.** 1987. Composition of a polysaccharide from mycoplasma (F-38) recognised by antibodies from goats with contagious pleuropneumonia. Res. Vet. Sci. **42:**175–178.
22. **Rurangirwa, F. R., T. C. McGuire, A. J. Musoke, and A. Kibor.** 1987. Differentiation of F38 mycoplasmas causing contagious caprine pleuropneumonia with a growth-inhibiting monoclonal antibody. Infect. Immun. **55:** 3219–3220.
23. **Rurangirwa, F. R., A. N. Wambugu, S. N. Kihara, and T. C. McGuire.** 1995. A *Mycoplasma* strain F38 growth inhibiting monoclonal antibody (WM-25) identifies an epitope on a surface-exposed polysaccharide antigen. Infect. Immun. **63:**1415–1420.
24. **Sommer, R., and D. Tautz.** 1989. Minimal homology requirements for PCR primers. Nucleic Acids Res. **17:**6749.
25. **Stone, S. S., and S. Razin.** 1973. Immunoelectrophoretic analysis of *Mycoplasma mycoides* var. *mycoides*. Infect. Immun. **7:**922–930.
26. **Thiaucourt, F., and G. Bolske.** 1996. Contagious caprine pleuropneumonia and other pulmonary mycoplasmoses of sheep and goats. Rev. Sci. Tech. **15:**1397–1414.
27. **Thiaucourt, F., G. Bolske, G. Libeau, C. Le Goff, and P. C. LeFevre.** 1994. The use of monoclonal antibodies in the diagnosis of contagious caprine pleuropneumonia (CCPP). Vet. Microbiol. **41:**191–203.
28. **Thiaucourt, F., C. Guerin, V. Mady, and P. C. LeFevre.** 1992. Diagnosis of caprine contagious pleuropneumonia: recent improvements. Rev. Sci. Tech. **11:**859–865.
29. **Turner, A. W.** 1962. Circulating *M. mycoides* antigen as a cause of loss of agglutination and complement fixation reactivity during acute pleuropneumonia. J. Aust. Vet. **38:**401–405.
30. **Wise, K. S., M. F. Kim, and R. Watson-McKown.** 1995. Variant membrane proteins, p. 227–242. *In* S. Razin and J. G. Tully (ed.), Molecular and diagnostic procedures in mycoplasmology, vol. I. Molecular characterization. Academic Press, Inc., San Diego, Calif.