Characterization of Strains of *Mycoplasma mycoides* subsp. *mycoides* Small Colony Type Isolated from Recent Outbreaks of Contagious Bovine Pleuropneumonia in Botswana and Tanzania: Evidence for a New Biotype

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Four strains of *Mycoplasma mycoides* subsp. *mycoides* small colony type (MmmSC) isolated from recent outbreaks of contagious bovine pleuropneumonia (CBPP) in Africa have been investigated. One Botswanan strain, M375, displayed numerous and significant phenotypic differences from both contemporary field isolates and older field and vaccine strains (African, Australian, and European strains dating back to 1936). Differences include altered morphology, reduced capsular polysaccharide production, high sensitivity to MmmSC rabbit hyperimmune antisera in vitro, and unique polymorphisms following immunoblotting. While insertion sequence analysis using IS1634 clearly indicates a close evolutionary relationship to west African strains, hybridization with IS296 shows the absence of a band present in all other strains of MmmSC examined. The data suggest that a deletion has occurred in strain M375, which may explain its altered phenotype, including poor growth in vitro and a relative inability to cause septicemia in mice. These characteristics are also exhibited by *Mycoplasma capricolum* subsp. *capripneumoniae* (causal agent of contagious caprine pleuropneumonia [CCPP]), against which M375 antiserum exhibited some activity in vitro (unique among the various MmmSC antisera tested). These findings may have evolutionary implications, since CCPP is believed to be lung specific and without a septicemic phase (unlike CBPP). Since M375 was isolated from a clinical case of CBPP, this novel biotype may be fairly widespread but not normally isolated due to difficulty of culture and/or a potentially altered disease syndrome. Bovine convalescent antisera (obtained from contemporary naturally infected cattle in Botswana) were active against strain M375 in an in vitro growth inhibition test but not against any other strains of MmmSC tested. There exists the possibility therefore, that strain M375 may possess a set of protective antigens different from those of other strains of MmmSC (including vaccine strains). These findings have implications for the control of the current CBPP epidemic in Africa.

Contagious bovine pleuropneumonia (CBPP), caused by infection with *Mycoplasma mycoides* subsp. *mycoides* small colony biotype (MmmSC), is one of the major infectious diseases affecting cattle in Africa. CBPP has spread alarmingly during the 1990s, infecting several countries previously free from the disease, and was recently reported by the Office International des Epizooties as causing greater losses in cattle than any other disease, including rinderpest (27). Current losses are estimated to be in the region of $2 billion per annum (23). Contributory factors to this current resurgence are thought to include the breakdown of veterinary services (30), increased and unrestricted cattle movements (due to drought, war, and civil strife (38), and a lack of vaccine efficacy (cited in references 24 and 36). This paper investigates the additional possibility that a new biotype of the causal organism may be involved in current outbreaks.

Studies to date on the molecular epidemiology of MmmSC are consistent with there being two main subtypes: those isolated from recent European outbreaks (post-1980) and those isolated from African and Australian outbreaks, some of which date back to 1936 (e.g., the Australian vaccine strain V5 [6]). This classification is based on a variety of criteria (for review see reference 21). Insertion sequence IS296 banding patterns (3) and restriction fragment length polymorphisms (RFLPs) (29) both suggest that European isolates fall into a single homogenous group, while the African and Australian strains form a separate, more heterogeneous grouping. This heterogeneity is most likely due to the widely separated geographical and temporal origins of the African and Australian strains studied (1931 to 1993), in contrast to those from European outbreaks (post-1980), which have been collected over a relatively short period of time. Similarly, antigenic profiling of European and African and Australian strains has revealed consistent differences between the two types (for example the presence of a 70- to 72-kDa band in African and Australian strains which is absent from European isolates, among other differences (8, 9, 29). Biochemical analyses (1, 12, 13) have also revealed a systematic difference between European and African and Australian strains, as the latter group are able to oxidize glycerol at high rates in vitro.

RFLP analysis of recent African field isolates has revealed some variation in the patterns observed between different strains (36). Although the importance of these findings on virulence and pathogenicity is not known, they do raise the potential that antigenic drift may have occurred among newer field isolates (which could affect current vaccine efficacy). We have conducted a detailed investigation of four strains of MmmSC isolated from the recent outbreaks of CBPP in Botswana and Tanzania. Prior to these outbreaks both countries had been free from the disease for many years (46 and 25 years, respectively [5]). Thus, the isolates should represent...
new, virulent outbreaks of CBPP rather than older endemic types. Findings suggest that while three recent isolates are highly similar to each other (and also display similarities to older strains of MmmSC), a fourth strain, isolated from Botswana in 1995, exhibits considerable phenotypic differences. These differences may have both evolutionary and disease control implications.

**MATERIALS AND METHODS**

**Mycoplasma strains and growth conditions.** *M. mycoides* subsp. *mycoides* SC strains used in this study are shown in Table 1. Current field strains (N6, M375, Tan1, and Tan8) were clonally isolated three times, and their identity as MmmSC strains used in this study are shown in Table 1. Current vaccine stocks, obtained from the Botswana Vaccine Industry (BVI), Gaborone, Botswana.

**Growth curves.** A 1-ml aliquot of frozen culture (approximately 10^8 CFU) was removed from −80°C storage, thawed for 1 h at room temperature, and transferred into 10 ml of fresh Gourlay’s broth (GB) prewarmed to 37°C. The culture was then incubated for a minimum of 16 h at 37°C to ensure that bacteria were in the logarithmic phase of growth. Every 2 h thereafter, 0.1-ml samples were removed for estimation of viable counts by dilution into fresh GB in the range 10^2 to 10^7 and by plating 0.25 ml onto prewarmed Gourlay’s plates. Plates containing between approximately 10 and 1,000 colonies were counted with the aid of a hand-operated counter to estimate the titer at the time of sampling. Growth curves were performed on three separate occasions for each strain, with the exception of Gladysdale (five times) and 6479 (a single occasion).

**Statistical analysis.** Multiple-comparison analysis of variance was performed using Tukey’s family error approach on the growth curve data. Significance was assessed at the 5 and 1% levels.

**Antisera and GI tests.** Bovine sera NK6 (Complement Fixation [CF] titer, 1:640) and N28 (CF titer, 1:320) were gifts from Willie Amanfu, (National Veterinary Laboratory [NVL], Gaborone, Botswana) and came from unvaccinated, naturally infected cattle (Botswana). Rabbit hyperimmune sera against MmmSC strains were produced by two subcutaneous injections of inactivated mycoplasma in adjuvant (Montanide ISA50), followed by one intravenous injection of an aqueous suspension. Growth medium containing porcine serum was used to culture the mycoplasma prior to immunization; for all subsequent manipulations mycoplasmas were grown in medium containing horse serum to minimize cross-reactivities to serum components. Two rabbits were immunized for each mycoplasma strain, and sera were tested individually and pooled (results from all serum samples were highly similar for each strain tested). Rabbit hyperimmune *M. capricolum* subsp. *capripneumoniae* antiserum was raised against type strain F38 using the same procedure as that described for MmmSC strains (this serum has been described in detail [19]). Bovine serogroup 7 antiserum (rabbit hyperimmune) was obtained from Aarhus, Denmark. For preabsorption of antiserum with capsular polysaccharide (CPS), 10 μg of purified CPS (22) was added to 0.1 ml of serum and the mixture was left at room temperature for 1 h and clarified by centrifugation. This procedure was repeated four times until the CPS antibody titer dropped to background levels (measured using a CPS enzyme-linked immunosorbent assay). Growth inhibition (GI) tests were performed by spotting 20 μl of undiluted serum onto 5-mm-diameter filter paper discs (Mast Diagnostics, Merseyside, United Kingdom), placing the discs onto suitable dilutions of mycoplasma cultures, and incubating at 37°C for 4 days prior to measurement of the zone of inhibition under a light microscope.

**Western blot analysis.** Whole mycoplasmas were pelleted from growth medium, washed twice in phosphate-buffered saline (PBS) (supplemented with 5% [wt/vol] glucose to prevent cell lysis), and resuspended in 5 volumes of sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer (0.1 M Tris-HCl [pH 6.8], 40% [vol/vol] glycerol, 4% [wt/vol] SDS, 0.25% bromophenol blue, 2% β-mercaptoethanol). Samples were boiled for 5 min and separated by SDS-PAGE using a 12% homogeneous polyacrylamide gel followed by electrophoretic transfer to nitrocellulose membranes (Hybond C-Pure; Amersham, Little Chalfont, United Kingdom) using standard techniques (4). Rainbow markers (Amersham) were run alongside the samples. Efficiency of transfer was estimated by staining the membranes with 0.1% Ponceau red (Sigma) in 1% acetic acid solution, followed by destaining in 1% acetic acid. The membrane was then rinsed twice in PBST (PBS plus 0.05% Tween 20) and blocked in 5% (wt/vol) dry skim milk in PBST for 1 h before primary antibody was added at a dilution of 1:400. The membrane was subsequently incubated for 1 h at room temperature and rinsed three times in PBST, and then secondary antibody (rabbit anti-bovine horseradish peroxidase [HRP] conjugate [DAKO, Cambridge, United Kingdom] or donkey anti-rabbit HRP conjugate [SAPO, Lanarkshire, United Kingdom]) was diluted 1:400 into 5% (wt/vol) dry skim milk in PBST, and the membrane was incubated for a further hour at room temperature. Three 5-min washes in PBST were performed before incubation of the membrane in 0.1 mg of diaminobenzidine (Sigma)/ml in PBST containing 0.1 ml of 30% H2O2 per 100 ml of substrate solution.

**Insertion sequence analysis.** Standard procedures were used for DNA manipulations and agarose gel electrophoresis (33). DNA extraction and insertion sequence analyses were performed as previously described (3, 7). Briefly, DNA
was extracted using guanidinium thiocyanate, ammonium acetate, and phenol-
chloroform-isooamy alcohol. Aliquots of this DNA (200 ng) were then digested
using either HindIII or EcoRI, and the resulting fragments were separated on a
0.7% agarose gel. A digoxigenin-labeled 1-kb ladder (Gibco-BRL) was used as a
molecular weight standard. Southern blotting was performed to transfer the
digested DNA onto nylon membranes (Amersham; Hybond-N). Membranes
were then incubated with either IS1286 (3) or IS1634 (37) digoxigenin-11–dUTP-
labeled insertion sequence probes (supplied by Joachim Frey) using standard
conditions, prior to visualization following incubation of the membrane in ni-
troblue tetrazolium-BCIP (5-bromo-4-chloro-3-indolylphosphate) solution.

RESULTS

Morphological data. MmmSC cultures were diluted to give approximately equal densities of cells and plated onto Gourlay’s agar. The field isolates fell into two distinct types (Fig. 1). N6, Tan1, and Tan8 all gave large homogenous colonies, while M375 produced a mixture of both small and large colonies (approximately 5% of the total were of the large colony type). These larger colonies of M375 were still noticeably smaller than the average colonies of the other field strains (Fig. 1) and were observed at both high and low colony densities. Subculturing of individual small or large colonies of M375 on Gourlay’s agar resulted in progeny displaying the same heterogene-

TABLE 2. Doubling times of various strains of MmmSC in GB at 37°C

<table>
<thead>
<tr>
<th>Strain</th>
<th>Source</th>
<th>Mean doubling time (min) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>M375</td>
<td>Africa, recent isolate</td>
<td>346 ± 44</td>
</tr>
<tr>
<td>KH1J</td>
<td>Africa, vaccine strain</td>
<td>228 ± 44</td>
</tr>
<tr>
<td>B820/124</td>
<td>Europe</td>
<td>201 ± 19</td>
</tr>
<tr>
<td>N6</td>
<td>Africa, recent isolate</td>
<td>168 ± 12</td>
</tr>
<tr>
<td>6479</td>
<td>Europe</td>
<td>189</td>
</tr>
<tr>
<td>Gladysdale</td>
<td>Australia, challenge strain</td>
<td>101 ± 14</td>
</tr>
</tbody>
</table>

* M375, KH1J, B820, and N6, three growth curves; Gladysdale, five growth curves; 6479, one growth curve. Gladysdale exhibited a significantly faster doubling time than KH1J, B820, N6, and M375 (P < 0.01). M375 was significantly slower than B820, N6, and Gladysdale (P < 0.01) and KH1J (P < 0.05). Strain 6479 was not included for statistical analysis.

ity of colony size and appearance. This morphology appears to be unique to M375 and has not been observed in this laboratory for any other strains of MmmSC tested (we have examined upwards of 20 strains originating from Europe, Africa, and Australia).

Growth rates of MmmSC strains. Strain M375 grew noticeably better on ME agar medium than on Gourlay’s agar medium. Colonies were larger and homogenous in nature, although still smaller than colonies of all other strains of MmmSC tested. This effect was not seen with any of the other strains, in which there was no noticeable difference in colony size between the two media. ME agar is a specialist culture medium formulated for the isolation and growth of nutritionally demanding mycoplasmal species, and this presumably indicates a more fastidious nutritional requirement for M375 than for other strains. The basis for this difference is not currently known but is likely to be the low passage level for M375 since this strain was found to be at least four levels of passage higher than strains Tan1 and Tan8 when tested and since poor growth on Gourlay’s agar was still observed when using higher-passage stocks of M375 (data not shown).

To obtain a more quantitative estimate of the growth rate of strain M375, growth curves with GB were performed and the doubling times of various strains were measured (Table 2). Strain M375 grew significantly more slowly than the other strains (P < 0.01 with the exception of KH1J, for which P < 0.05), with a mean doubling time of 346 min. For most other strains the doubling times were in the range of 168 to 229 min. The only exception was Gladysdale, with a mean doubling time of 101 min, significantly faster than the other strains (P < 0.01). It is unclear if this rapid growth rate is due to adaptation to growth in vitro or whether this might indicate increased virulence for this strain (Gladysdale was used as a standard challenge strain in Australia during the 1960s [14]). The addition of 0.2% sodium pyruvate to the growth medium (shown to be necessary for growth of many strains of M. capricolum subsp. capripneumoniae [11], did not improve the growth rate of M375 (data not shown).

GI tests. GI activities of pooled pairs of antisera are shown in Table 3. Results are shown for rabbit hyperimmune sera raised against vaccine strain T1SR and field strains N6 and M375. Also shown are results observed using bovine sera NK6 and N28 from field cases of CBPP (Botswana). Antisera were tested against the four field strains and two vaccine strains (T1SR and T144). An examination of the GI activity of rabbit hyperimmune sera showed that test strains broadly appeared to fall into three categories with respect to their sensitivities to GI antisera: relatively insensitive (vaccine strains T1SR and
TABLE 3. Sensitivity of field and vaccine strains of MmmSC grown on Gourlay's agar medium to GI by hyperimmune and convalescent antisera

<table>
<thead>
<tr>
<th>Host animal for serum production</th>
<th>Strain or serum antiserum raised against</th>
<th>Zone of clearance (mm) in GI test against a:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabbit</td>
<td>T1SR</td>
<td>M375: 4.5, 3.5, 3.0, 3.0, 2.5, 2.3</td>
</tr>
<tr>
<td>Rabbit</td>
<td>N6</td>
<td>M375: 3.0, 2.2, 2.0, 2.0, 0.5, 0.5</td>
</tr>
<tr>
<td>Rabbit</td>
<td>M375</td>
<td>M375: 1.0, 0.0, 0.0, 0.0, 0.0</td>
</tr>
<tr>
<td>Bovine</td>
<td>NK6</td>
<td>M375: 1.5, 0.0, 0.0, 0.0, 0.0</td>
</tr>
<tr>
<td></td>
<td>N28</td>
<td>M375: 1.0, 0.0, 0.0, 0.0, 0.0</td>
</tr>
<tr>
<td>Cumulative total</td>
<td>GI zone</td>
<td>M375: 12.0, 5.7, 5.0, 5.0, 3.0, 2.8</td>
</tr>
</tbody>
</table>

a Data were obtained from pooled antisera (two samples for each antiserum) except for bovine antiserum, which were tested individually. The sensitivity of each strain to antiserum can be estimated by the extent of the zone of inhibition seen. Rabbit antiserum were hyperimmune and were raised against the strains shown. Bovine antisera were obtained from field cases of CBPP in Botswana. Strains M375, Tan1, Tan8, and N6 are field isolates; strains T1SR and T144 are vaccine isolates. M375 is an atypical field isolate.

TABLE 4. Growth inhibiting activity of MmmSC rabbit hyperimmune antiserum against M. capricolum subsp. capripneumoniae strain 19/2 grown on ME agar medium

<table>
<thead>
<tr>
<th>Organism against which antiserum raised</th>
<th>Zone of clearance (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. capricolum subsp. capripneumoniae</td>
<td>5 (clear zone)</td>
</tr>
<tr>
<td>Bovine serogroup 7</td>
<td>2 (clear zone)</td>
</tr>
<tr>
<td>MmmSC</td>
<td>0</td>
</tr>
<tr>
<td>T1SR (vaccine strain)</td>
<td>0</td>
</tr>
<tr>
<td>C425 (European strain)</td>
<td>0</td>
</tr>
<tr>
<td>M375 (Botswana strain)</td>
<td>3 (reduced colony size)</td>
</tr>
<tr>
<td>N6 (Botswana strain)</td>
<td>0</td>
</tr>
</tbody>
</table>
have entered Botswana from Namibia to the west (24), in agreement with the insertion sequence data presented here. Despite its close relationship to Botswanan strain N6 as shown by IS1634 analysis, M375 could be clearly differentiated from N6 by probing HindIII-digested DNA with labeled IS1296 (Fig. 3, bottom) due to the absence of an insertion sequence in M375. The absence of this band is unlikely to be due to the loss of a restriction site, since this would result in the insertion sequence still being present but at a higher molecular weight (which was not observed). This band was present in every other strain of MmmSC tested (European, African, and Australian).

FIG. 2. (Top) Immunoblot profiles for recent field isolates of MmmSC compared with vaccine strains and older isolates. Whole-cell antigens of the strains shown were run on SDS-PAGE gels and electroblotted prior to incubation with serum NK6 from a naturally infected animal from Botswana. Polymorphisms at 110, 70, and 40 kDa are shown, the last two unique to M375. (Bottom) Immunoblot analysis using rabbit hyperimmune sera raised against the nine strains of MmmSC shown in the top panel. In this case an antigen mixture of all nine strains was run as a single lane on a gel and probed with sera raised against the individual strains (using mixed duplicate serum samples). Arrow, unique polymorphism present in antisera raised against M375 (nonrecognition of a protein at 40 kDa).

FIG. 3. (Top) HindIII-digested genomic DNA of recent field isolates probed with IS1634. Regions of variability are boxed. Similar patterns are observed with the two Tanzanian isolates (Tan1 and Tan8) and the two Botswanan isolates (N6 and M375). (Bottom) HindIII-digested genomic DNA of various MmmSC strains probed with IS1296. The band of approximately 8.5 kb missing in M375 is boxed. Note that the band has not simply shifted in molecular weight, as would be expected if the absence were simply due to the loss or gain of a restriction enzyme site.
DISCUSSION

Strains of MmmSC isolated from recent outbreaks of CBPP in Africa have been compared to vaccine strains and older isolates. Results suggest that Botswanan field isolate M375 differs from all other strains of MmmSC tested by a variety of criteria. Compared with other strains of MmmSC, observed differences include altered colony morphology and poor growth in vitro, unique polymorphisms following immunoblotting, a high degree of sensitivity to growth-inhibiting antisera in vitro, some level of GI activity for M375 antiserum against M. capricolum subsp. capripneumoniae, and the absence of a band following genomic hybridization with insertion sequence IS1296. Other distinctive features of this strain include low CPS production (22), a relative inability to cause septicaemia in mice (20), and unique sugar utilization patterns (13). Some of these characteristics, such as poor growth in vitro (25, 35) and inability to cause septicaemia in mice (17, 31), are also exhibited by M. capricolum subsp. capripneumoniae, with which M375 shares some serological similarity (some cross protection in GI tests).

Strain M375 lacks an IS1296 element present in every other strain of MmmSC tested, which could indicate that M375 is evolutionarily more ancestral than the other strains studied. Alternatively, this missing band could indicate that a deletion event has taken place in M375, removing both the insertion sequence element and portions of the surrounding chromosomal DNA. The latter explanation is more likely given the phenotypic characteristics of M375. The absence of a 40-kDa band following immunoblotting, together with the poor growth observed for this strain, would be consistent with a deletion event removing some chromosomal DNA (and possibly some biosynthetic or metabolic transport capability).

When grown on a standard culture medium for MmmSC, M375 gave very poor growth compared to other strains and displayed a unique morphology. This characteristic was far less obvious when M375 was grown on a specialist mycoplasma isolation medium (ME medium), suggesting that M375 has unusually fastidious nutritional requirements. MmmSC is generally regarded as being easy to culture (28), and the poor growth of M375 bears some resemblance to that of M. capricolum subsp. capripneumoniae, the causal agent of contagious caprine pleuropneumonia (34). Interestingly, antiserum raised against M375 displayed some GI activity against M. capricolum subsp. capripneumoniae. This effect was not observed using antiserum raised against any other strains of MmmSC. Although the inhibitory effect was small compared to that observed with homologous M. capricolum subsp. capripneumoniae antiserum, these data would suggest that M375 may be more closely related to M. capricolum subsp. capripneumoniae than other strains of MmmSC examined.

Compared to other MmmSC isolates, M375 proved to be unusually sensitive to GI with MmmSC rabbit hyperimmune antiserum raised against a variety of different strains. Preabsorption of these antisera with MmmSC CPS removed most of the GI effect, indicating that CPS must be a target for GI antibodies in vitro. Earlier work by us (22) showed that the GI activity of rabbit hyperimmune serum correlates with its CPS antibody titer, while the sensitivity of a strain to GI antiserum inversely correlates with the amount of CPS produced by that strain (i.e., the more CPS produced by a strain, the less sensitive it is to GI). M375 produces up to sixfold less CPS than other strains of MmmSC when grown in culture (22), indicating a likely cause for the increased sensitivity of this strain to GI using rabbit hyperimmune antiserum. Interestingly, substrate utilization studies (13) showed that strain M375 differed from other isolates of MmmSC (European and African) by its ability to metabolize glucosamine and mannose, two CPS components (22). Oxidation of these sugars, rather than their incorporation into the capsule, may result in the lowered CPS production of strain M375.

The low CPS production of M375 may explain its apparent inability to cause a mycoplasmaemia following experimental infection of mice; in a study by us (20), only 30% of mice infected with M375 exhibited a mycoplasmaemia, compared to 80 to 100% of mice infected with other MmmSC strains. Similarly, the duration of the mycoplasmaemia in M375-infected mice was only 24 h, compared to 3 to 5 days with other strains. Additional support for this hypothesis comes from a study using cattle that were experimentally infected with a low-virulence strain of MmmSC (KHJ) (14), which apparently did not cause a mycoplasmaemia unless additional CPS was added to the inoculum at the time of infection. In another report (26), following experimental infection of cattle with variants of the same strain of MmmSC producing low and high levels of CPS, variants producing low levels of CPS were present only in the lungs, while variants producing high levels of CPS resulted in a more general septicaemia, with mycoplasma being found in many tissues. This is the more usual situation with CBPP, where MmmSC is found in many tissues and bodily secretions (5). The implication is that a strain producing low levels of CPS, such as M375, would be restricted to the lungs following infection. A similar situation is seen during contagious caprine pleuropneumoniae, which is believed to be lung specific (25, 34). Thus both the clinical presentation and the likelihood of isolation of MmmSC from an M375-infected animal may be altered. This latter point could be particularly significant given the apparently poor growth of strain M375 on conventional culture medium.

GI results using bovine field convalescent sera isolated from Botswana (the geographical source of strain M375 itself) showed that this strain alone was sensitive to the inhibitory effects of these antisera. In contrast to results obtained with rabbit hyperimmune sera, preabsorption of bovine convalescent sera with CPS did not remove all GI activity, strongly suggesting that non-CPS (presumably protein) antigens were the target for GI. If so, the implication is that strain M375 exhibits some unique protective epitopes not shared among other strains of MmmSC.

Immunoblot analysis did reveal some unique polymorphisms for M375 compared to other strains of MmmSC (contemporary field isolates, vaccine strains, and older type strains): the absence of a common 40-kDa band and the sole presence of a 70-kDa band. That these field sera recognized the unique 70-kDa band present in M375 is significant, since it suggests that the cattle from which these sera were isolated were infected with a strain similar or identical to M375. No other strains exhibit these polymorphisms. Whether these polymorphisms affect either virulence or immunity is unknown and perhaps merits further investigation, as CPS is the only virulence factor identified to date for MmmSC (18, 22).

What is likely to be of more immediate value, however, is to determine the virulence and pathogenicity of these newer field isolates and the ability of vaccine strains to protect against them. Data to assess how widespread this new variant is would also be welcome. Vaccination using T,S,R was reportedly ineffective in controlling CBPP in Botswana (24) and field reports of a new clinical morphology for CBPP in this region have been made (R. S. Windsor, personal communication), both of which would be consistent with a new biotype. Since M375 is difficult to culture and may be restricted to lung tissue
only, the effect of a new biotype on current diagnosis of CBPP in Africa remains to be determined.

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