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Diagnosis of Contagious Caprine Pleuropneumonia by Detection and Identification of *Mycoplasma capricolum* subsp. *capripneumoniae* by PCR and Restriction Enzyme Analysis

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Contagious caprine pleuropneumonia (CCPP), one of the most serious and dramatic diseases of goats, is caused by *Mycoplasma capricolum* subsp. *capripneumoniae* (*M. capripneumoniae*). This organism is very difficult to isolate and to correctly identify. In a previous report we described a method for the rapid detection and identification of *M. capripneumoniae*. This method is based on a PCR system by which a segment of the 16S rRNA gene from all mycoplasmas of the *M. mycoides* cluster can be amplified. The PCR product is then analyzed by restriction enzyme cleavage for the identification of *M. capripneumoniae* DNA. This system has now been further evaluated with respect to specificity and diagnostic efficacy for the identification and direct detection of the organism in clinical material. Identification by restriction enzyme analysis of amplified DNA from mycoplasmas of the *M. mycoides* cluster was verified for 55 strains, among which were 15 strains of *M. capripneumoniae*. The PCR was applied to clinical samples from the nose, ear, pharynx, pleural fluid, and lung tissue containing *M. capripneumoniae* or other mycoplasmas. As expected, mycoplasmas belonging to the *M. mycoides* cluster could be detected by the PCR. Restriction enzyme analysis of the PCR products could then be applied for the identification of *M. capripneumoniae*. Clinical samples and cultures containing *M. capripneumoniae* were dried on filter paper, to try an easier sample transport method, and were tested by PCR. *M. capripneumoniae* DNA could be detected in the dried specimens, but the sensitivity of the PCR test was reduced.

The goat is an important production animal for milk and meat in many countries. One of its most serious diseases is contagious caprine pleuropneumonia (CCPP). This is a devastating disease causing a high mortality rate and is a major cause of loss in goat rearing in many countries in Africa and Asia. *Mycoplasma capricolum* subsp. *capripneumoniae* (*M. capripneumoniae*), formerly known as *Mycoplasma* sp. strain F38, is generally believed to be the etiological agent of the classical form of the disease (21, 27), and it can be isolated from the lungs and pleural fluid of affected animals.

M. capripneumoniae belongs to the so-called *M. mycoides* cluster, which also comprises *M. mycoides* subsp. *mycoides*, small colony (SC) and large colony (LC) types, *M. mycoides* subsp. *capri*, *M. capricolum* subsp. *capricolum* (*M. capricolum*), and *Mycoplasma* sp. bovine group 7. All of these are pathogenic and can cause disease among ruminants. The most important is *M. mycoides* subsp. *mycoides* SC type, which causes contagious bovine pleuropneumonia. The members of the *M. mycoides* cluster are closely related and are therefore difficult to identify by the conventional serological methods used for mycoplasma typing.

The diagnosis of *M. capripneumoniae* infection in animals with CCPP is largely hampered by difficulties in isolating the organism from clinical material. *M. capripneumoniae* is fastidious, grows slowly in broth media, and produces only minute colonies on solid media. Furthermore, it is also frequently overgrown by other common mycoplasmas such as *M. ovipneumoniae*. Consequently, the geographical spread of *M. capripneumoniae* infection has not been clearly delineated. CCPP was reported from 23 countries, mainly in Africa and Asia (2), but isolation of *M. capripneumoniae* has been reported from only eight countries, namely, Kenya (26), Sudan (15), Tunisia (29), Chad (22), Oman (19), Turkey (41), Ethiopia (38), and Uganda (8). The disease exclusively affects goats, and *M. capripneumoniae* has only been found in goats, except for a report of isolations from healthy sheep in goat herds affected by CCPP (24) and another from sick sheep mixed with goats suffering from CCPP (8). The difficulties of using microbiological culture for diagnosis also explain why our knowledge of the animal carrier state and epidemiology in general is so sparse for this disease.

All members of the *M. mycoides* cluster have two rRNA operons (11), and there are sequence differences in the 16S rRNA genes of the two operons (16, 30, 31). We have earlier developed a PCR system by which a segment of the two 16S rRNA genes from all members of the *M. mycoides* cluster can be amplified. The PCR products from the two operons of this gene were analyzed by restriction enzyme cleavage with *Pst*I. A unique cleavage pattern was found for *M. capripneumoniae*, thus distinguishing it from the other members in the *M. mycoides* cluster. The combined use of PCR and restriction enzyme analysis (REA) with *Pst*I could therefore be used for the identification of *M. capripneumoniae* (31). This system has now been applied to the identification of several *M. capripneumoniae* strains isolated from goats. Furthermore, it has also been applied directly on various clinical samples and has proved to be useful for the diagnosis of CCPP.

MATERIALS AND METHODS

Mycoplasma strains and growth conditions. The mycoplasma strains used in the present work and their origins are listed in Table 1. The growth medium used for *M. capripneumoniae* (here called H25P medium) was a modification of the

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TABLE 1. Mycoplasma strains analyzed by PCR for detection of members of the *M. mycoides* cluster and REA with *Pst*I for identification of *M. capripneumoniae*

Strain	Geographical origin	Supplier[a]	Reference	Animal host, recovery site	PCR result/no. of bands by REA
M. capripneumoniae					
F38[T]	Kenya	WHO/FAO		Goat	+/3
9231	Ethiopia	EMVT	38	Goat, lung	+/3
M74/93; M76/93	Uganda	SVA	8	Sheep, lung	+/3
M79/93	Uganda	SVA	8	Goat, lung	+/3
G338/80; G176/79; 1943	Kenya	HE	11	Goat, lung	+/3
KD	Tunisia	HE	11	Goat	+/3
4/2 LC	Oman	HE	11, 19	Goat	+/3
Baringo	Kenya	HW		Goat, lung	+/3
9081	Oman	EMVT	36	Goat	+/3
7/1a	Oman (Turkey)	JT	6, 19	Goat	+/3
G1943/80; G280/80	Kenya	JT	6	Goat	+/3
M. capricolum					
California Kid[T]	United States	WHO/FAO		Goat	+/2
4214; 4550; 4551; 5407; 1456-1	Israel	SL		Goat	+/2
GM262G; GM13	United States	AD	13	Goat	+/2
7714	France	EMVT	28	Goat	+/2
E 570	Britain	GJ	18	Sheep, vagina	+/2
B 304	Portugal	JR		Bovine, lung	+/2
M153/81b; M144; O528/81	Sweden	SVA	9	Goat, lung	+/2
2712/77; 74/3220	Australia	JT	6	Goat	+/2
M4528/76	Zimbabwe	JT	6, 35	Sheep, joint	+/2
Mycoplasma sp. bovine group 7					
PG50[T]	Australia	WHO/FAO	20	Bovine, joint	+/2
CalfI	Nigeria	HE	11	Bovine, blood	+/2
D 318 b	Germany	HE	11	Bovine, semen	+/2
C 2306	Portugal	HE	11	Bovine	+/2
D 424	Germany	HE	11	Bovine, preputium	+/2
CP 291	Portugal	MM	3	Goat, lung	+/2
QR1	Australia	DP			+/2
4055	France	DP		Bovine, lung	+/2
B144P (bovine group L)	United States	WHO/FAO	1	Bovine	+/2
M. mycoides subsp. *mycoides*					
Y-goat[T] (LC type)	Australia	WHO/FAO		Goat	+/2
UM 30847 (LC type)	United States	JT	40	Goat	+/2
1456-11 (LC type)	Israel	SL		Goat	+/2
M522/89, M508/89 (LC type)	Tanzania	SVA		Goat, lung	+/2
M149h/83; M28/83a (LC type)	Sweden	SVA		Goat, milk	+/2
B3 (LC type)	Sweden	SVA	40	Swine, nose	+/2
PG1[T] (SC type)	?	WHO/FAO		Bovine	+/2
B 421 (SC type)	Portugal	JR		Bovine	+/2
M223/90 (SC type)	Tanzania	SVA	10	Bovine, lung	+/2
M. mycoides subsp. *capri*					
PG3[T]	Turkey	WHO/FAO		Goat	+/2
M133FgII	Tanzania	SVA		Goat	+/2
Other					
M. auris GM 623	United States	AD	12	Goat	−/ND
M. cottewii GM 612	United States	AD	12	Goat	−/ND
M. yeatsii GM 624	United States	AD	12	Goat	−/ND
Mycoplasma sp. HRC 689	United States	WHO/FAO	32	Dog	−/ND

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[b] ND, not done; REA was carried out only on PCR products from those mycoplasmas that proved to be positive in the PCR experiments.

pyruvate-enriched medium described by Thiaucourt et al. (39) and contained the following ingredients: 17.5 g of Bacto PPLO Broth without crystal violet, 650 ml of glass-distilled water, 100 ml of yeast extract, 250 ml of horse serum inactivated at 56°C, 4 ml of 50% glucose, 8 ml of 25% sodium pyruvate P2256 (Sigma, St. Louis, Mo.), 4 ml of 5% thallium acetate, 250 mg of ampicillin (Astra, Södertälje, Sweden), and 4 ml of 0.5% phenol red. The pH was adjusted to 7.8 with NaOH or HCl. Other mycoplasma strains were grown in the same medium or in the F medium described earlier (7).

Clinical samples. Some of the clinical specimens used in the study originated from animals in areas without CCPP (see Table 2). These specimens had earlier been submitted to the laboratory for mycoplasma culture and had subsequently been stored at −70°C for various periods for up to 12 years. Mycoplasma culture

was performed before freezing, as described earlier (9), and after thawing it was also performed with the H25P medium.

Specimens from goats and sheep from East Africa (see Table 3) showing signs of CCPP were kept at −20°C for 2 weeks or at −70°C for 6 months before they were sent by air transport to the National Veterinary Institute, where they were cultured. Herds 1 and 2 (Table 3) were kept in Uganda, and these outbreaks have been described previously (8). Herd 3, kept in Kenya, held at the time of sampling only 10 surviving animals from an original herd of 620 goats. The remainder had died from what was believed to be CCPP in connection with long-distance transport. Necropsy of one goat (goat G4 in Table 3) revealed a lesion in the left diaphragmatic lung lobe, consistent with fibrinous pleuropneumonia. It consisted of a firm fibrous pleural adhesion surrounded by a zone of grey hepatization, interpreted as a chronic active inflammation.

Mycoplasma isolation from clinical samples. The technique for mycoplasma culture from clinical samples has been described previously (9). Isolation of *M. capripneumoniae* was done both by serial dilution in H25P broth and by streaking onto an agar plate with the same medium. Incubation was at 37°C in 5% CO_2. Broth tubes were, in addition, subcultured onto agar plates after 4 and 8 days. Samples from East Africa were also cultured onto F medium enriched with 0.2% sodium pyruvate.

Dried samples. Samples of about 100 μl from cultures or clinical specimens with *M. capripneumoniae* were applied to filter paper (Whatman 3MM), air dried, and analyzed by PCR. Two cultures of *M. capripneumoniae* and one specimen of pleural fluid with *M. capripneumoniae* were serially 10-fold diluted and were analyzed by PCR directly from the dilutions or from the same dilutions air dried on filter paper as described above.

Samples dried on filter papers were also sent from East Africa by airmail to obtain confirmation of CCPP tentatively diagnosed by clinical and postmortem observations. Nasal samples, dried on cotton-tipped swabs, were also obtained from one culturally confirmed CCPP outbreak (herd 3 in Table 3).

Artificially mixed samples. Clinical samples, with artificially mixed infections of *M. capripneumoniae* and *M. mycoides* subsp. *mycoides* LC, were prepared with a pleural fluid sample from an experimentally infected goat with CCPP (kindly provided by F. Thiaucourt, Montpellier, France). The sample contained 3×10^8 organisms of *M. capripneumoniae*, as determined by culture of serial dilutions. It was prepared in different dilutions in combination with added dilutions of a culture of *M. mycoides* subsp. *mycoides* M149h/83.

Identification of mycoplasmas. Mycoplasma strains and isolates were identified by growth inhibition test (5) and immunofluorescence of unfixed colonies (33). The antisera were produced in rabbits (25) against the following mycoplasma strains: PG1 (*M. mycoides* subsp. *mycoides* SC), Y-goat (*M. mycoides* subsp. *mycoides* LC), California Kid (*M. capricolum*), GM262G (*M. capricolum*), F38 (*M. capripneumoniae*), and PG50 (*Mycoplasma* sp. bovine group 7). Biochemical tests (glucose fermentation, arginine hydrolysis, and serum digestion) were performed as described by Freundt et al. (14). The growth characteristics of *M. capripneumoniae* (slow growth, small colonies, and growth stimulation by pyruvate-enriched medium) were also useful adjuncts for the identification.

Preparation of mycoplasma strains for PCR. A 1-ml broth culture of the mycoplasma strain was centrifuged, washed in phosphate-buffered saline (PBS), resuspended in 1 ml of water, and lysed by being heated at 100°C for 10 min.

Preparation of clinical samples for PCR. Tissue samples of lung material were triturated with sterile sand in F broth, and 1 ml of the supernatant was used, after gravity sedimentation of sand and large tissue pieces. After washing in PBS and centrifugation at 10,000 × *g* for 10 min, the pellet was resuspended in lysis buffer (10 mM Tris-HCl [pH 7.4], 10 mM NaCl, 10 mM EDTA), and sodium dodecyl sulfate was added to a final concentration of 1% and proteinase K was added to a final concentration of 200 μg/ml. The reaction mixture was incubated for 1 h at 50°C and was then extracted with an equal volume of phenol-chloroform-isoamyl alcohol (25:24:1). The aqueous phase was extracted with chloroform-isoamyl alcohol (24:1), from which the aqueous layer was retained. DNA was precipitated with cold ethanol after the addition of 2 M sodium acetate (pH 6.5). After washing of the pellet with ethanol, it was resuspended in 20 to 30 μl of water.

Samples that had been dried on filter paper were cut out and transferred to 0.5 ml of PBS, left at room temperature for about 30 min, and then heated in a boiling water bath for 5 min before performing the PCR. The samples on the cotton tips of the swabs were transferred to 0.5 ml of PBS and were treated in the same way.

PCR and REA. PCR was carried out on DNA from 5-μl samples of undiluted and 10-fold-diluted lysed cells prepared from cultures or from clinical material. A fragment of the 16S rRNA genes from the members of the *M. mycoides* cluster was amplified by PCR with the forward primer MmF and the reverse primer MmR. The experimental details are described elsewhere (31). PCR products of the appropriate size (548 bp), as determined by agarose gel electrophoresis, indicated the presence of DNA from a mycoplasma of the *M. mycoides* cluster in the sample.

The PCR products were then analyzed without further purification by REA with *Pst*I to differentiate *M. capripneumoniae* from the other species. The presence of three bands, the uncleaved 548-bp fragment and the two cleavage products of 420 and 128 bp, showed that *M. capripneumoniae* was present in the sample. The appearance of only two bands of 420 and 128 bp showed that another member of the *M. mycoides* cluster was present (31). The uncleaved DNA fragment of 548 bp originates from the *rrnB* operon of *M. capripneumoniae*, which lacks the restriction site for *Pst*I in this mycoplasma.

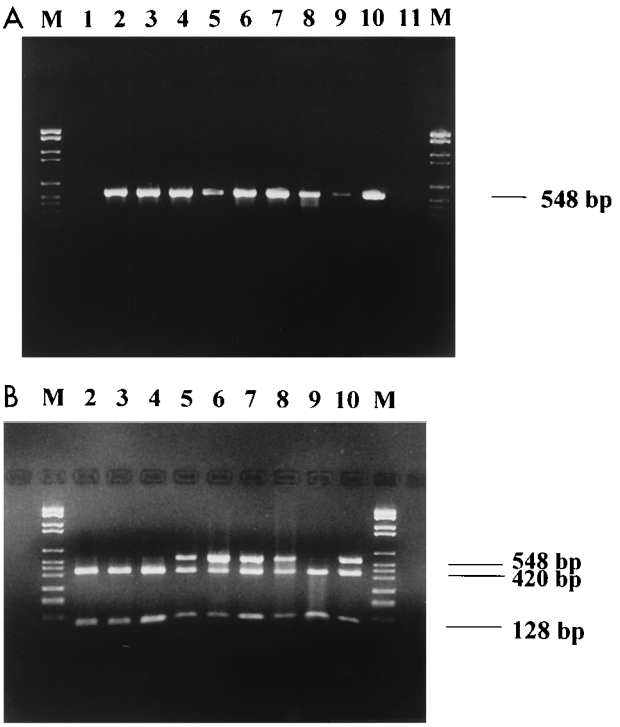

FIG. 1. Agarose gel electrophoresis of PCR products (A) and the corresponding restriction cleavage fragments (B) from some representative strains (strains 1 to 7) and clinical samples (samples 8 to 11). Lane 1, *M. auris* GM 623; lanes 2, *M. mycoides* subsp. *capri* M133FgIII; lanes 3, *Mycoplasma* sp. bovine group 7 CP291; lanes 4, *M. capricolum* 0528/81; lanes 5, *M. capripneumoniae* M74/93; lanes 6, *M. capripneumoniae* 4/2LC; lanes 7, *M. capripneumoniae* F38; lanes 8, lung sample S1 in Table 3 (*M. capripneumoniae* + *M. ovipneumoniae*); lanes 9, nasal swab sample in Table 2 (*M. mycoides* subsp. *mycoides* LC + *M. ovipneumoniae*); lanes 10, lung sample on filter paper from a herd with suspected CCPP; lane 11, lung G2 in Table 3 (*M. ovipneumoniae*). Lanes M, molecular size markers (*Bgl*I-cleaved pBR 328 DNA and *Hin*fI-cleaved pBR328 DNA from Boehringer Mannheim, Mannheim, Germany). Note the difference in proportions between the two largest fragments from strain 4/2LC (lane 6) compared with those from the other *M. capripneumoniae* strains.

RESULTS

Analysis of laboratory strains. The results of the PCR-REA of the different mycoplasma strains are provided in Table 1 and Fig. 1. These results are in agreement with the identifications made by the suppliers of the strains (see Table 1 for references) and with the conventional identification tests performed by us. All *M. capripneumoniae* strains were correctly identified by PCR-REA. Three bands were obtained by REA of the PCR products. However, *M. capripneumoniae* 4/2LC differed from the others, because two of the three bands, 420 and 128 bp, were comparatively weak (Fig. 1). All other strains belonging to the *M. mycoides* cluster proved to be positive in the PCR system, but only two bands were obtained after REA of the PCR products. Other mycoplasma strains, not belonging to the *M. mycoides* cluster, proved to be negative in the PCR and could therefore not be analyzed by REA.

Direct detection in clinical material. The results of the PCR-REA analysis of different kinds of clinical material from both healthy and affected goats are compared in Table 2 with the culture results. Five swab samples from which *M. mycoides* subsp. *mycoides* LC had been isolated were also positive in the

TABLE 2. PCR and REA performed on different kinds of specimens from goats

Type of sample	No. of samples	Result of mycoplasma culture	PCR result[a]	No. of bands by REA
Nasal and pharyngeal swabs	2	*M. mycoides* subsp. *mycoides* LC and *M. ovipneumoniae*	+	2
	1	*M. arginini*	−	ND[b]
	6	*M. ovipneumoniae*	−	ND
	5	*M. ovipneumoniae* and *M. arginini*	−	ND
Ear swabs	3	*M. mycoides* subsp. *mycoides* LC	+	2
	1	*M. ovipneumoniae*	−	ND
	3	*M. ovipneumoniae* and *M. arginini*	−	ND
	2	−	−	ND
Lung tissue	3	*M. mycoides* subsp. *mycoides* LC	+	2
	3	*M. mycoides* subsp. *mycoides* LC	(+)	ND
	1	*M. capricolum*	+	2
	2	*M. ovipneumoniae*	−	ND

[a] +, positive in PCR; (+), weak band, too little PCR product to be used for REA; −, negative in culture or PCR.
[b] ND, not done.

PCR. The swab samples from which *M. ovipneumoniae* and/or *M. arginini* were isolated were all negative in the PCR. All five lung tissue samples containing *M. mycoides* subsp. *mycoides* LC proved to be positive by PCR, but in three of those, the band was too weak to be analyzed by REA. All of the samples with *M. mycoides* subsp. *mycoides* LC, for which it was possible to perform REA on the PCR products, showed two bands and could thereby be differentiated from *M. capripneumoniae*. The lung tissue sample containing *M. capricolum* was also positive in the PCR and gave two bands by REA, as expected. An analysis of necropsy samples from animals involved in outbreaks of CCPP in East Africa is provided in Table 3. Four of five cases of *M. capripneumoniae* infection identified by culture could also be confirmed by PCR-REA.

Analysis of dried samples. Three cultures of *M. capripneumoniae* dried on filter paper proved to be positive in the PCR-REA. Cultures of *M. capripneumoniae* dried on filter paper had to contain 10 to 1,000 times more mycoplasma than cultures analyzed directly to give positive results in the PCR. Two dried samples of lung suspension (from goat G4 in Table 3), containing *M. capripneumoniae*, also proved to be positive in the PCR-REA.

Dried clinical samples of lung or pleural fluid from animals in herds with signs of CCPP were received on seven occasions. Seven of 12 proved to be positive for *M. capripneumoniae* in the PCR-REA. Three were weakly positive in the PCR but could not be further analyzed by REA, and two were negative in the PCR. All of nine dried nasal swab samples from one of the herds with CCPP proved to be negative in the PCR.

Imitation of clinical samples with mixed *M. capripneumoniae-M. mycoides* infection. In the samples with different dilutions of *M. capripneumoniae* mixed with *M. mycoides* culture, the PCR-REA clearly revealed three bands, typical for *M. capripneumoniae*, even in mixtures in which the numbers of *M.*

TABLE 3. Detection and identification of *M. capripneumoniae* in goat and sheep lungs from CCPP outbreaks in East Africa by PCR and REA

Herd	Sample no.	Animal	Tissue	Mycoplasma isolation		PCR result[a]	No. of bands by REA[b]
				Species	Quantity[c]		
1	S1	Sheep	Lung	*M. capripneumoniae* *M. ovipneumoniae*	10^4 10^7	+	3
	G1	Goat	Pleural fluid	*M. ovipneumoniae*	10^2	−	ND[d]
2	S2	Sheep	Lung	*M. capripneumoniae* *M. ovipneumoniae*	10^2 10^4	+	3
	S3	Sheep	Lung	*M. capripneumoniae* *M. ovipneumoniae*	10^2 $>10^4$	−	ND
	G2	Goat	Lung	*M. ovipneumoniae*	$>10^4$	−	ND
	G3	Goat	Lung	*M. capripneumoniae*	10^4	+	3
3	G4	Goat	Lung	*M. capripneumoniae*	10	+	3

[a] +, positive; −, negative.
[b] REA with *Pst*I.
[c] Number of mycoplasmas per milliliter of sample, as estimated by culture of serial 10-fold dilutions.
[d] ND, not done.

mycoides exceeded the numbers of *M. capripneumoniae* 20-fold (for instance, at 3×10^5 CFU of *M. mycoides* and 1.5×10^4 CFU of *M. capripneumoniae*). With an excess of 200- to 250-fold *M. mycoides* (for instance, 8×10^8 CFU of *M. mycoides* and 3×10^6 CFU of *M. capripneumoniae*), the 548-bp band could only be seen very faintly, making the interpretation ambiguous. With an even greater excess of *M. mycoides*, the 548-bp band was not visible in the PCR-REA.

DISCUSSION

Our PCR-REA system has earlier been shown to differentiate *M. capripneumoniae* from all other mycoplasmas described from goats (31). The characteristic restriction enzyme cleavage pattern of *M. capripneumoniae*, differentiating it from the other members of the *M. mycoides* cluster, is based on a single nucleotide position. It is therefore important to validate the method on a broad variety of strains, which has been done in the present work (Table 1). The results were in agreement with previous identifications made by others (see references in Table 1) and with our own identification of the strains by conventional methods. Altogether, 15 strains of *M. capripneumoniae* have been analyzed. These strains originated from at least six different countries in North Africa, East Africa, and the Middle East, the three main areas from which *M. capripneumoniae* has been isolated so far. The analyzed material also included 39 other strains from the *M. mycoides* cluster, from different animal host species, and from widely different parts of the world. All of these strains showed two bands in the REA, which means that they were completely cleaved at the restriction sites in the PCR products of the 16S rRNA genes from both rRNA operons. On the other hand, all *M. capripneumoniae* strains showed three bands because of the nucleotide substitution at the restriction site in one of the operons, preventing the cleavage of the PCR product from that operon. These results confirm the assumption that this nucleotide substitution in *M. capripneumoniae* is unique within the *M. mycoides* cluster. *M. capripneumoniae* 4/2LC, which showed an atypical REA pattern, also possesses this unique nucleotide substitution. It was the two cleaved fragments from the other operon that appeared as comparatively weak bands in the REA. This can be explained by the presence of another nucleotide substitution located in the target region of the reverse primer (MmR) in the *rrnA* operon, an A instead of a G at position 1259 (F38 numbering), which probably causes a less efficient amplification of the segment (30).

The species *M. auris* GM 623, *M. cottewii* GM 612, and *M. yeatsii* GM 624 in Table 1, which do not belong to the *M. mycoides* cluster, were included in the present study because they represent recently described mycoplasma species in goats not previously tested with the PCR system described here. Strain HRC 689 is an unclassified mycoplasma from dogs which was included because it is reported to cross-react serologically with the two subspecies of *M. mycoides* (32). However, it was not amplified in our PCR system and should probably not be regarded as a member of the *M. mycoides* cluster.

The identification of *M. capripneumoniae* is often very difficult by conventional methods. Serological cross-reactions have been reported for all members of the *M. mycoides* cluster, but they are particularly pronounced between *M. capripneumoniae*, *M. capricolum*, and *Mycoplasma* sp. bovine group 7 (6, 13, 23). These three members of the *M. mycoides* cluster are also phylogenetically the most closely related (30, 31). Few biochemical features are useful for the identification of *M. capripneumoniae*. The most important one is the presence of arginine catabolism in *M. capricolum*, which is lacking in *M. capripneumoniae*. However, in some strains of *M. capricolum*, arginine catabolism is reported to be lacking or very difficult to detect (13, 17).

Monoclonal antibodies have been produced by which *M. capripneumoniae* can be more reliably differentiated from the other mycoplasmas. The specificities of some monoclonal antibodies are much higher than those of rabbit antisera. However, cross-reactions to some strains of *Mycoplasma* sp. bovine group 7 have been reported (4, 34, 37).

In the pleural fluid specimens containing *M. capripneumoniae* and spiked with *M. mycoides* subsp. *mycoides* LC, the presence of *M. capripneumoniae* could be demonstrated by PCR-REA in all except those containing an approximately 200-fold excess of *M. mycoides*. The lack of visible PCR products from *M. capripneumoniae* in such samples may be explained by the competition for limited amounts of reagents. Thus, if the difference in amounts between the two templates is too large, the product of the major one will reach a plateau, at which limited reagents become depleted, before the product of the minor template has reached detectable amounts.

Because of the difficulties associated with collecting clinical material from animals with CCPP, the material is bound to be limited. To broaden the study of PCR with clinical materials, we also used old frozen material from goats infected with other mycoplasmas belonging to the *M. mycoides* cluster. The present study demonstrates the usefulness of PCR for the direct detection of mycoplasmas in swab samples. All lung tissue samples from Swedish goats, shown by culture to contain members of the *M. mycoides* cluster, also proved to be positive in the PCR, but in three of these samples the paucity of PCR products precluded REA (Table 2). Further identification was therefore impossible for the PCR products in these cases. The reason for this may be shortcomings in the DNA isolation or the presence of inhibitors for the amplification. Use of a reamplification of the PCR products to increase the amounts might solve this problem, but this was not investigated in the present study.

In a previous report (31), we estimated the sensitivity of the PCR under optimal conditions to be 5 CFU per 5-μl test volume (31). This means that the sensitivity would theoretically be 1,000 CFU/ml if no concentration step is introduced into the pretreatment procedure. This applies, for example, to the pleural fluid specimens. Samples from lung tissue, on the other hand, were extracted, which resulted in samples that were concentrated about 40 times. The detection limit would therefore be 25 CFU/ml. However, from several positive lung samples, it was only possible to see the PCR product in the material diluted 10 times, and the detection limit would then be correspondingly higher.

Among the samples from animals involved in the CCPP outbreaks in East Africa, *M. capripneumoniae* was detected and identified by PCR-REA in four of five samples in which it was detected by cultivation (Table 3). The number of organisms determined by culture (Table 3) is probably underestimated because of the fastidious growth requirements of *M. capripneumoniae* and possibly also because of a high proportion of dead organisms in the tissue. According to the clinical data (8), most cases of infection were probably subacute or chronic.

It is remarkable that three of the five positive samples came from sheep. Sheep are known to be refractive to CCPP, and the carrying of *M. capripneumoniae* among sheep has only been reported on one earlier occasion (24).

It proved to be possible to detect *M. capripneumoniae* DNA from dried cultures or dried clinical samples (lung or pleural fluid). A proportion of the dried samples obtained by mail

from East Africa were also positive in the PCR-REA, which provides some further indications on the usefulness of the method. Nine dried nasal swabs originating from a herd with CCPP (including a nasal swab from the culturally positive animal G4 in Table 3) were, however, all negative in the PCR. The presence of *M. capripneumoniae* in the noses of these animals has, however, not been proven. The reason for the failure to detect *M. capripneumoniae* in any of the dried nose swabs could be attributable to a probable low prevalence of the organism in the nose late in the disease or to a low sensitivity of the test for this type of sample. The presence of inhibitors for the PCR is another possibility, which was not further investigated. For samples of lung or pleural fluid from animals with acute cases of CCPP, *M. capripneumoniae* can still be detected because there are large numbers of organisms. This method for the preservation of samples, combined with PCR, could therefore be very useful for the confirmation of new cases of CCPP in many areas where poor communication make the sending of fresh samples to the laboratory impossible.

The PCR system for the detection of mycoplasmas belonging to the *M. mycoides* cluster proved to be reliable for swab samples from the nose, pharynx, and ears of goats. Whether it is useful for nose swab sampling of goats with CCPP is not known, because there is to our knowledge only one report of the isolation of *M. capripneumoniae* from that site (24). Pleural fluid often harbors large numbers of mycoplasmas in animals with acute CCPP and can be used in the PCR technique described here. Lavage samples from the trachea would probably also be appropriate, but these samples were not tested. It was also possible to use PCR to demonstrate the presence of *M. capripneumoniae* DNA in lung tissue samples from animals with CCPP.

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