Field Isolation of *Mycoplasma capripneumoniae* in Central Zone of Tanzania  

1E.Y. Noah, 2L.J.M. Kusiluka, 3P. Wambura and 4S.I. Kimera  
1Central Veterinary Laboratory Box, 9254 Dar, Es Salaam, Tanzania  
2Open University of Tanzania Box, 23409 Dar, Es Salaam, Tanzania  
3Department of Veterinary Microbiology and Parasitology,  
4Department of Veterinary Medicine and Public Health, Faculty of Veterinary Medicine,  
Sokoine University of Agriculture Morogoro, Tanzania  

Abstract: An investigation was done on outbreak of acute respiratory disease in goats in Central zone of Tanzania, the investigation involved 3 districts. (Mpwapwa, Dodoma and Kilosa districts). The study involved goats from the field, livestock markets and abattoirs. Samples from clinical and pathological examination of goats with respiratory distress were subjected to microbiological and molecular analysis. A total of 96 lungs samples, 96 lymph node and 59 pleural fluids were collected for microbiological and molecular evaluation. Media used were Hayflick’s medium (H25P) and commercial freeze-dried diagnostic medium for mycoplasma isolation, blood and MacConkey agar for bacteria other than mycoplasma. Mycoplasma isolates were confirmed by Polymerase Chain Reaction (PCR) and Restriction Enzyme Analysis (REA). *Mycoplasma capripneumoniae* was isolated from 47 samples and *Pasteurella multocida* from 13 samples. Eighty one samples subjected to molecular analysis were positive for *M. capripneumoniae*. *M. capripneumoniae* was isolated from samples collected from all the study districts. The results of this study indicate that outbreaks of respiratory disease in the study areas were predominantly due to contagious caprine pleuropneumonia (CCPP). This provides additional information on the distribution of the disease in the country. The results of PCR/REA demonstrate the usefulness of this diagnostic technique in the investigation of field outbreaks of CCPP even when isolation of the causative agent is not possible. The isolation and identification of *M. capripneumoniae* in central zone confirm the presence of contagious caprine pleuropneumonia in the zone.  

Key words: Goats, *Mycoplasma capripneumoniae*, polymarase chain reaction, restriction enzymes  

INTRODUCTION  

The total number of goats and sheep in Tanzania is estimated at 12.7 and 3.5 million respectively. In the Central Zone of Tanzania, it is estimated that there are about 1.7 and 0.75 million goats and sheep, respectively (MAC, 1996).  

Small ruminants in Africa are reared in different production systems ranging from crop/livestock mixed systems in the highlands to pastoral systems in the arid lowlands (Shiferaw et al., 2006).  

In Tanzania, like other African countries, goats are affected by various diseases. These include infectious diseases such as Contagious Caprine Pleuropneumonia (CCPP), pneumonic pasteurellosis (mannheimiosis), dermatophilosis, footrot, caseous lymphadenitis, colibacillosis, orf, goat pox, peste des petits ruminants, helminthosis, external parasites (such as fleas, mange and *Oestrus ovis* larvae) and traumatic injuries (Mboera and Kitalyi, 1991; Thiaucourt et al., 1992; Pettersson et al., 1998; Ibrahim et al., 1999; Heldtander et al., 2001; Yigezu et al., 2004; Shiferaw et al., 2006).  

Goats play a significant role in household economy. Meat and milk are major sources of protein while skins and live animals account for a significant proportion of exports (Shiferaw et al., 2006). In rural households goats are important animals for the peasants for production of milk, meat and as source of income, investment and security.   

Contagious caprine pleuropneumonia is caused by *Mycoplasma capricolum* subspecies capripneumoniae which belongs to the Mycoplasma mycoides (M. mycoidec) cluster. The classical *Mycoplasma mycoides* cluster comprises six species of mycoplasmas which affect ruminants and share multiple phenotypic and genomic properties. These include *Mycoplasma capricolum* subsp. capripneumoniae (M. capripneumoniae), *M. capricolum* subsp. *capricolum* (M. capricolum), *M. mycoides* subsp. capri (M. capri), *M. mycoides* subsp. *mycoides* large colony type (LC) (M. mycoides LC), *M. mycoides* subsp. *mycoides* small colony type (SC) (M. mycoides SC) and Mycoplasma sp. bovine serogroup 7 (Thiaucourt et al., 1996; Pettersson et al., 1998). Unlike classical CCPP, whose pathological...
changes are confined to the thoracic cavity, the disease caused by *M. mycoides* LC, *M. capri*, and *M. capricolum* is characterized by presence of lesions in other parts of the body in addition of those in the thoracic cavity (Shiferaw et al., 2006).

CCPP was first confirmed in Kenya by isolation of *M. capripneumoniae* in 1976 and then remain endemic in the country (MacOwan, 1976).

The disease has been suspected to be in Tanzania since the early 1980’s (Nyange and Mbise, 1983; Msami, 1991). But was confirmed by isolation in 1998 (Msami et al., 1998), then *M. capripneumoniae* was isolated and shown to cause CCPP in Tanzania in Morogoro, Mpwapwa and Iringa (Kusiluka et al., 2007). The diagnosis of *M. capripneumoniae* infection in goats is largely hampered by difficulties in isolating the organism from clinical material because *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* (Thiaucourt et al., 1996). Consequently, the geographical distribution of *M. capripneumoniae* infection has not been clearly delineated. The disease exclusively affects goats and *M. capripneumoniae* has only been found in goats, except for a few reports of isolation from healthy sheep in goat herds affected by CCPP (Litamoi et al., 1990) and from sick sheep mixed with goats suffering from CCPP (Bölske et al., 1995). The difficulty of using microbiological culture for diagnosis also explains why knowledge on the animal carrier state and epidemiology of this disease in general is sparse. 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 (Bonnet et al., 1993; DaMassa et al., 1992; Lefèvre et al., 1987). The three members of the *M. mycoides* cluster are also phylogenetically the most closely related ones (Ros Bascûnana et al., 1994). Few biochemical features are useful for the identification of *M. capripneumoniae*. The most important one is arginine catabolism which is lacking in *M. capripneumoniae* but present in *M. capricolum*. However, in some strains of *M. capricolum*, arginine catabolism is reported to be lacking or very difficult to detect (Leach et al., 1993; DaMassa et al., 1992; Jones, 1992).

The objective of this study was to establish and characterize the cause of pneumonia in goats in central zone of Tanzania.

**MATERIALS AND METHODS**

The present study was carried out in central zone of Tanzania involving Mpwapwa, Dodoma rural district and Gairo division Kilosa district in Morogoro region which border Mpwapwa district were also involved. This study was carried out between February 2008 and September 2008.

In Mpwapwa district a total of 18 villages in 4 divisions were visited and samples collected, the divisions were Mpwapwa which include the following village Ising’u, Mang’angu, Kisokwe, Gulwe, Izomvu, Lupeta and Ng’ambo. Rudi division includes the following village Rudi, Chogola, Galigali, Chipogoro, Winza, Kinus and Mzase. In Kibakwe include the following village Kibakwe, Ikuyu and Mtera includes the following villages Wienzele and Mtera. Gairo division were also involved in the study, Gairo is located north of the Kilosa district. In Dodoma Rural samples were taken from Mnadani animal market and Dodoma Abattoir were animals are bought from various villages.

The study area is within an arid agro-ecological zone and is prone to recurrent drought due to erratic rainfall. Agro-pastoral is the predominant system of livestock production and local breeds of goats and sheep predominate.

In all study areas the system of farming was agro pastoralist and the animals were grazing communally where the herd comprises cattle, sheep and goats and donkey.

The zone was selected for this study because there is lack of information on the epidemiology of pneumonia in goats in the area although it has been claimed to be a source of CCPP outbreaks that have been encountered in eastern zone of the country (Kusiluka et al., 2000). The animal health delivery system was poor in the study area with few animal health personnel and lack of transport and veterinary drugs and facilities.

Consequently there are limited reports on outbreak of diseases at district and region level. Sometime the reports about diseases reach the district Veterinary authorities very late or outbreaks occurred without being reported.

Selection of goat herds for study was based on information available in District Livestock and Agriculture Development (DALDO) regarding pneumonia-like disease outbreak in the village and information from animal markets and abattoir. The herd, animal markers and abattoir were visited following consultation with DALDO, management of abattoir, Extension worker and village leaders.

At the consent of the of the goats owners, herds with active suspected pneumonia like outbreaks were sacrificed.

Samples were taken from farmsteads, markets and abattoir facilities in Mpwapwa, Dodoma Rural and Gairo in Kilosa district. Then samples were taken to Sokoine University of Agriculture (SUA) for analysis. Samples collected were lungs with pneumonic lesions, mediasternal and bronchial lymph node and pleural fluid. Samples were collected from the field cases, livestock markets and abattoir the with pneumonia like history and signs.
Animal markets and Abatoir were involved in the study because animals from different location are brought. Due to this reason it is easier to get information of the disease and areas where the disease a present.

Before any samples were taken, history and clinical examination of the herds was carried out with special emphasis on the respiratory diseases. The history taken was number of animals in the herd, how many diseased, death, treatment given any new introduced animals in the herds any neighbors animal that are sick and were recorded into check list. Animals with pneumonia-like lesions were examined in detail in which the following were recorded, rise in body temperature, respiratory distress, coughing, dullness, depression tachypnoea, dyspnoea and nasal discharge. Then post mortem examination was carried out in died goats, purchased and sacrificed goats, in which lungs, pleural fluid, mediasternal and bronchial lymph nodes samples were sampled for study.

In the animal markets and abattoir it was not easy to know the origin of the goats because one goat can be solid in more than one market before final marker. Ante mortem is performed and record is taken from all animals with pneumonia like lesion so that they can be easily followed for sampling during post mortem examination. The lung tissue collected were divided into two portions, one for histopathology which was kept in 10% formalin and the other to be used for bacteriological study with pleural fluid and lymph node was kept in a sterile bottle containing transport medium and put in cool box for sampling during post mortem examination.

Laboratory analysis: The surface of lung and lymph node samples were sterilized by flaming and the crust trimmed off. The tissues were ground using a stomacher and grinding medium was added to facilitate grinding. The grinding medium does not contain antibiotics in order to permit examination of bacteria other than mycoplasma. A total of 96 samples were collected of which were 94 from Dodoma and 2 Morogoro (Table 1).

Media: The media used for microbiological study were Hayflick’s Medium broth (H25P) broth and agar, CCPP diagnostic media, blood and MacConkey agar.

Hayflick’s medium broth (H25P): The Hayflick medium containing 25% horse and porcine serum and pyruvate here in abbreviated as H25P (Bölske et al., 1996) were used for isolation of M. capripneumoniae and other mycoplasmas. The broth H25P medium is composed of 17.5 g of Bacto PPLO Broth without crystal violet (Difco Detroit); 650 ml of glass distilled water; 100 mL of yeast extract (Sigma, St. Louis); 125 ml of horse and 125 ml of porcine serum inactivated at 56ºC 250 mL; 4 mL of 50% w/v glucose (Merek, Darmstadt); 8 ml of 25%/w/v sodium pyruvate (BDH, Poole); 4 mL of 5% w/v thallium acetate (Sigma, St Louis); 250 mg ampicillin (Calbiochem, La Jolla) and 4 ml of 0.5% w/v phenol red (BDH, Poole). The pH was adjusted to 7.8 with 0.1 sodium hydrochloride or hydrochloric acid.

The media was then kept into sterile 1.8 mL tubes then were incubated at 37ºC for 24 h to check for bacterial contamination. The uncontaminated media were stored at -20°C until used.

H25P solid medium: This was prepared by mixing 46 ml of Hanks’ balanced salt solution without dextran and 3.6 g purified agar (Oxoid, Hampshire). The mixture was autoclaved at 120ºC for 2 min cooled slowly in the autoclave to 100ºC and then mixed with 400 mL of H25P pre- heated at 56ºC for 15 min in a water bath. The mixture was then poured into petri dishes and allowed to solidify for 30 min at room temperature. The plates were incubated at 37°C for 24 h to check for bacterial contamination. The uncontaminated media were stored at 4°C until used.

CCPP diagnostic medium: The CCPP diagnostic medium (Mycoplasma Experience UK) were donated by Prof. Kusiluka was prepared as per instructions from the supplier. The components of the medium included an agar, a diluents and a freeze-dried supplement which were contained in bottles but the individual volumes not indicated. The total volume of the medium when the components are mixed amounted to 25 mL. To reconstitute the medium, the agar was melted in boiling water at 100°C and allowed to cool slightly to 50°C and then placed in a 50°C water bath. The diluent was added to the freeze-dried supplement, the mixture was agitated gently until the supplement was completely dissolved and placed in 50°C water bath for 15 min. The reconstituted supplement was then added to the agar and mixed thoroughly, then 4 mL of the mixture was dispensed into Petri dishes then the medium was dried in an incubator at 37°C for 10 min and stored in the refrigerator before use.

Blood and macconkey agar: Thoracic fluid, grounded lung and lymph node samples were inoculate in the plates of blood and MacConkey agar and incubated at 37°C for 24 h. Bacterial growths were subcultured to obtain pure colonies, which appeared as Gram-negative short rods after Gram staining. On blood agar, the colony characteristics of pure cultures were examined and recorded. There were no growths in the MacConkey agar. The following biochemical tests were subsequently performed: oxidase, catalase, indole, hydrogen sulphide,
urease, glucose, sucrose, salicine, and trehalose. Based on growth characteristics and the results of the biochemical tests, isolates were classified and grouped according to genus and species.

**Isolation of mycoplasmas:** Portion of lung and lymph node were cut and decontaminated by immersed in absolute alcohol then flaming and thereafter peeling off the surface. About 10 g of the tissue was cut into small pieces and placed in a stomacher bag and 3.6 mL of grinding media was added. The sample was grounded in the stomacher for 5 min then the suspension was recovered. For each sample of the homogenized tissue and pleural fluid, a ten-fold serial dilution to 10⁻¹ was prepared in H25P.

The inoculated broth was incubated at 37°C. The culture were examined daily for evidence of growth, which was manifested by a colour change from red to yellow due to acid production from fermentation of glucose and the appearance of flocular materials at the bottom of the culture tube or a swirl from the bottom when it is agitated (OIE, 2004). After the evidence of growth in the broth, the 10⁻² was subculture in another set of broth and inoculated on the H25P agar for observation of colonial morphology. The broth culture was observed for growth and the 10⁻³ and 10⁻⁴ dilutions showing growth were pooled and frozen for further studies.

The inoculated solid media were incubated at 37°C in a humid anaerobic jar with 5% carbon dioxide supplied by candle. Humidity was maintained by placing cotton wool soaked in water. The media was observed under stereomicroscope for evidence of growth from day 3. The media was observed for 21 days for evidence of mycoplasma colonies. The characteristic mycoplasma colonies were subculture onto H25P broth and incubated at 37°C overnight to allow multiplications of the organisms then serial dilution were made as described above. The 10⁻² dilutions was sub cultured on the solid medium and used in the disc growth inhibition test for identification of the mycoplasma isolates.

**Identification of mycoplasmas isolates:** Identification of mycoplasma isolates were done by using disc Growth Inhibition Test (GIT) (Jones and Wood, 1988).

**Preparation of discs:** Discs were prepared by place 1 drop of sterile rabbit hyperimmune antiserum on all 6 mm dry and sterilized antibiotic assay discs. The discs then dried at 37°C for 24 h and stored until use.

**Procedures for the GIT:** Plates contain H25P broth were flooded with 0.5 mL of 10⁻² broth culture and the plate were tilted carefully to ensure that the fluid spreads all over the surface and the excessive broth was sucked off. The plate was allowed to dry at room temperature for 15 min and then the discs were placed on the media, when the disc has been moistened the plate were incubated at 37°C.

Growth inhibition was observed daily for under stereomicroscope for presence of growth and an inhibition zone. The size of the inhibition zone was measured by using ruler from the edge of the disc to where normal colonies started to grow and recorded. The presence of 4-5 mm inhibition zone around the discs impregnated with hyperimmune sera against *M. capripneumoniae* was considered to be a positive for *M. capripneumoniae* growth.

**Isolation of other bacteria:** Blood and MacConkey agar were used to determine bacterial causes of pneumonia other than mycoplasmas. Samples from lungs and lymph nodes were cultured in the above media and examined daily for bacterial growth. Bacterial isolates were identified by their growth characteristics, biochemical tests and gram staining characteristics (Shiferaw et al., 2006).

**Molecular diagnosis of mycoplasma:** *M. capripneumoniae* is one of the most serious and dramatic mycoplasmas which cause fatal disease of goats, is caused by *Mycoplasma capricolum* subsp. *capripneumoniae* (*M. capripneumoniae*). This organism is very difficult to isolate and to correctly identify. But there are 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 (Bölske et al., 1996). In this study PCR was applied to clinical samples from the lung, lymph node, pleural fluid and cultured broth. 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*.

Specimens from goats from Mpwapwa, Dodoma rural and Kilosa showing signs of CCPP were kept at -30 after they were cultured and organs from affected goats were used for PCR and REA.

**DNA extraction:** The lung, lymphnode and broth were used for PCR/REA test and extraction method described below was adopted from Sepa Genex extraction protocol.

DNA extraction was carried out according to above protocol,

Two grams of tissue, 1 mL of broth and thoracic fluid were mixed in 700 μL of sterilized PBS and homogenized...
loading buffer was analyzed by electrophoresis in a 1.5% agarose gel with 0.5 µL of ethidium bromide per mL. In each analysis, one well on the gel was loaded with 1 kb ladder as a molecular marker and was run in electrophoresis machine at 120V for 30 min then viewed in UV light to observe the bands, the photos was taken by using digital camera Olympus.

Restriction enzyme analysis: The PCR products were then digested without further purification by REA with PstI as follows; 1 µL of the PCR product was mixed in a 0.5 mL eppendorf tube with 2 µL of 10X restriction buffer, 0.2 µL of bovine albumin serum, 0.5 µL of PstI and sterile deionized water added to make a 20 µL volume. The digestion mixture was mixed thoroughly by pipetting and centrifugation for a few seconds followed by incubation at 37°C for 4 hours. Then 4µL of 6X loading buffer was added to the PCR product. The digested PCR product were analysed by agarose gel electrophoresis at 120 v for 30 min, then viewed in UV light to observe the bands, the photos was taken by using digital camera Olympus.

RESULTS

Mycoplasma and bacteria isolated from samples: In order to confirm presence of CCPP in suspected cases, organs (lung, lymph node and pleural fluid) were cultured for isolation of causative agent (M. capripneumoniae). All samples collected from the affected herd, animal market and Abattoir, from Dodoma Rural, Mpwapwa districts

Table 3: Mycoplasmas and bacteria isolated

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Count</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. capripneumonia</td>
<td>47</td>
<td>48.96</td>
</tr>
<tr>
<td>Mycoplasma arginin</td>
<td>4</td>
<td>4.17</td>
</tr>
<tr>
<td>Mycoplasma avipneumonia</td>
<td>11</td>
<td>11.46</td>
</tr>
<tr>
<td>Pasteurella spp</td>
<td>13</td>
<td>13.54</td>
</tr>
</tbody>
</table>

Table 4: Isolations according area of sampling

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Field</th>
<th>Market</th>
<th>Abattoir</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. capripneumonia</td>
<td>30</td>
<td>12</td>
<td>5</td>
</tr>
<tr>
<td>M. arginin</td>
<td>3</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>M. avipneumonia</td>
<td>5</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Pasteurella</td>
<td>7</td>
<td>5</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 5: Isolates from samples collected

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Pleural fluid</th>
<th>Lung</th>
<th>Lymph node</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. capripneumonia</td>
<td>41</td>
<td>20</td>
<td>34</td>
</tr>
<tr>
<td>M. arginin</td>
<td>2</td>
<td>6</td>
<td>35</td>
</tr>
<tr>
<td>M. avipneumonia</td>
<td>7</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Pasteurella</td>
<td>6</td>
<td>9</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 6: Results of PCR and REA from clinical materials and cultured broth

<table>
<thead>
<tr>
<th>Sample</th>
<th>Number of sample</th>
<th>PCR positive</th>
<th>Number of bands in REA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Broth</td>
<td>47</td>
<td>47</td>
<td>3 bands</td>
</tr>
<tr>
<td>Pleural fluid</td>
<td>37</td>
<td>31</td>
<td>3 bands</td>
</tr>
<tr>
<td>Lungs</td>
<td>96</td>
<td>59</td>
<td>3 bands</td>
</tr>
<tr>
<td>Lungs</td>
<td>96</td>
<td>81</td>
<td>3 bands</td>
</tr>
</tbody>
</table>
Fig. 1: *Mycoplasma capripneumoniae* colony in CCPP diagnostic medium

Fig. 2: PCR product viewed in the agarose gel sample from Dodoma abattoir, L DNA ladder, C1, C2 and C3 PCR products

Fig. 3: Restriction enzyme analysis viewed in the agarose gel samples from Chogola village in Mpwapwa district

These bacteria were isolated from all samples taken from abattoir, market and field cases Table 4.

A total of 47 broths cultured, 96 lungs, 96 lymph node and 37 pleural fluids were subjected to PCR/REA (Table 6).

The PCR products was visualized in a agarose gel where 548 bp was observed Fig. 2.

The digestion of PCR product produced three fragments of 548 bp, 420bp and 128 bp Fig. 3. 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 uncleaved DNA fragment of 548 bp originates from the rrnB operon of *M. capripneumoniae*, which lacks the restriction site for PstI in this Mycoplasma.

**DISCUSSION**

In microbiology study, *M. capripneumoniae* was isolated from all districts. Bacteriological evaluation revealed few specimens (47) that were positive to *M. capripneumoniae* despite the larger number of animals having typical post-mortem lesions of CCPP. It was observed that large number of isolates was from field cases followed by markets and abattoir this could be attributed by treatment given to the animals in which reduce the rate of isolation.

The low recovery rate of isolation of *M. capripneumoniae* from samples may be attributed to its fastidiousness and easiness of being overgrown by other bacteria and fungi (Thiaucourt *et al.*, 1996).

Indeed, bacterial contaminants and *M. ovipneumoniae* and *M arginini* were encountered in this study and these may have inhibited the growth of *M. capripneumoniae*. However, it is also possible that the frequent use of antibiotics by farmers as observed in the study areas might have contributed to the low level of isolation since antimicrobial treatment significantly lowers mycoplasma recovery rates.

In the microbiological study, *M. capripneumonia* was isolated from clinical cases originated from Dodoma Rural, Mpwapwa and Kilosa districts. The fact that there was at least isolate from each of the study areas confirmed the presence of CCPP in central zone because isolation of *M. capripneumoniae* is required for an official declaration of infection (Thiaucourt *et al.*, 1996). Hence, study findings further confirm the presence of the contagious caprine Pleuropneumonia in Central zone of Tanzania where it was previously being suspected.

The identification of *M. capripneumoniae* by convectional methods is often very difficult. 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 (Bonnet *et al.*, 1993; DaMassa *et al.*, 1992; Lefèvre *et al.*, 1987b). These three
members are also phylogenetically related (Pettersson et al., 1996a; Ros Bascúnana et al., 1994). Therefore, a few biochemical tests are used for identification of *M. capripneumoniae* the most important one is lack of arginine catabolism in *M. capripneumoniae* but present in *M. capricolum*. However, it was reported that the arginine catabolism is lacking or very difficult to detect (DaMassa et al., 1992; Jones, 1992).

Isolation was more in CCPP diagnostic media than H25P and the feature in CCPP media was similar to those growths as described by Bashiruddin and Windsor (1998).

The identification system for *M. capripneumoniae* involves two steps. In the first, which was based on PCR, a 548-bp segment of 16S rRNA genes from the organism was amplified. So far 81 samples have been positive in the PCR and 15 samples were negative. In the second steps, the amplimers were cleaved with the restriction enzymes Pst I (Ros Bascúnana et al., 1994). These results to unique restriction pattern for *M. capripneumoniae*, that differentiating the organism from other members of the cluster. Three bands were generated in those positive samples. The presences of a PstI site in the 16SrRNA genes of the *M. capripneumoniae* distinguish it from other members of the *M. mycoides* clusters. Further more this single site was also used as an internal control for the activity of the restriction enzymes.

The PCR/REA analysis presented here provide specific identification of *M. capripneumoniae* isolates, without the need for downstream processing, and have potential in the diagnostic detection of this species (Böltske et al., 1996). The lack of a digesting site in *M. capripneumonia* results in 3 bands of PCR products after digestion with Pst I becomes the bases for confirmation for the presence of *M. capripneumoniae* (Ros Bascúnana et al., 1994). The lack of digestion of 548 kb of DNA in this region of 16SrRNA gene confirms the species of the organism to be *M. capripneumoniae* in the study area.

Typing of isolates over this gene using PCR/REA analysis considerably reduces the time and sample manipulation required for analysis (Lorenzon et al., 2000) which previously has been based on clinical signs, post mortem results and cultural. Recent publications have highlighted strain differentiation on the basis of PCR/REA analysis. Given the high density of these elements within a number of *Mycoplasma* species this technique could provide a useful method for strain classification.

The presence of *M. capripneumoniae* was confirmed by PCR/REA. 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 uncleaved DNA fragment of 548 bp originates from the rnb operon of *M. capripneumoniae*, which lacks the restriction site for PstI in this mycoplasma (Ros Bascúnana et al., 1994). The combined PCR/REA test was analytically more sensitive in detecting *M. capripneumoniae* than isolation, because 81 samples was positive were by only 47 were positive in isolation.

Respiratory disease caused by mycoplasma in goats is commonly complicated by *Pasteurella* infection (Shiferaw et al., 2006; Brogden et al., 1998) which was also observed in this study. *Pasteurella* were isolated from lower respiratory system in the lung and thoracic fluid samples. The growth of these bacteria could be due to stress associated with the CCPP.

Many strains of *P. multocida* have been implicated as causes of severe outbreaks of respiratory disease in cattle, sheep, goats, pigs, and rabbits, atrophic rhinitis in pigs, and fowl cholera (Jaworski et al., 1993).

Other strains of *Pasteurella multocida* frequently are detected as normal commensals on the mucosa of the upper respiratory tracts of mammals. Many factors such as viral and mycoplasmal infections, poor nutrition, overcrowding, and shipping are associated with reduced physical and immunologic defences which permit these commensal organisms to invade the lower respiratory tract and cause respiratory disease (Jaworski et al., 1993).

*Mycoplasma arginini*, was isolated from 4 samples as a contaminants. It has been reported that *M. arginini* occurs in goats and sheep and can be isolated from various anatomical sites of the hosts. (Jones et al., 1983) and most often the organism is considered nonpathogenic. *M. arginini* has been isolated from cases of ovine keratoconjunctivitis. Leach (1970) and Cottew (1979) reported that *M. arginini* frequently occur in pneumatic sheep lungs, mouth, and esophagus.

*M. ovipneumoniae* plays a role in disease of goats and sheep. This mycoplasma can be isolated frequently from the lung, trachea, and nose and occasionally from the eyes of sheep with pneumonia but can also be found in the respiratory tract of healthy sheep (George and Horsfall, 1973) and principally affecting lambs up to 12 months of age (Jones et al., 1983). Goats can also harbor *M. ovipneumoniae*, and growing evidence incriminates this mycoplasma in goat disease. In an experimental study by Goltz et al. (1986), young goats infected with this agent developed pneumatic signs. The organism was recovered, but not regularly, from the infected goats. *M. ovipneumoniae* displays an uncharacteristic morphology on solid medium as colonies do not have the fried egg appearance typical of mycoplasma rather the colonies on such agar appear granular.

It was observed that pleural fluid yield more isolate than other samples from the same animals the same as observed by Kusiluka et al. (2000).

The present study has confirmed the presence of CCPP in Dodoma, Mpwapwa and Kilosa districts in which, prior to this work, the reports were based on clinical signs and post-mortem features only. Therefore, the results of this study show that the disease is probably
increasingly becoming more widespread and endemic in Tanzania. The endemicity and degree of spread of the disease are attributable to the lack of disease control programmes; poor animal health service delivery in the rural areas; uncontrolled animal movements and use of antimicrobials by farmers that often is associated with under-dosing, use expired drugs and drug abuse. For instance, in this study, it was observed that most livestock keepers had used antibiotics before farm visits.

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