Detection of Contagious Caprine Pleuropneumonia and Concurrent Diseases in Outbreaks Presenting with Respiratory Signs in Small Ruminants in Tanzania

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Authors’ contributions

This work was carried out in collaboration among all authors. Author AC designed the study, performed the field and lab work, analysed data, wrote the first draft of the manuscript and incorporated all the comments from the supervisor’s authors GS and LK. Author GS approved the study designs, performed and supervised the laboratory work, advised on data analysis and scrutinized the first manuscript. Author LK approved the study design, advised on the laboratory work, supervised on manuscript preparation and scrutinized the manuscript. All authors read and approved the final draft of the manuscript.

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ABSTRACT

**Aims:** To establish the prevalence and concurrent diseases in outbreaks presenting with respiratory signs, major associated clinical signs and postmortem lesions and proportions of those diseases in clinically and autopsied small ruminants for a proper diagnosis and control strategies.

**Study Design:** Purposive outbreaks investigation.

**Place and Duration of Study:** Department of Global Health, School of Life Science and Bio-
**1. INTRODUCTION**

Outbreaks of diseases presenting with respiratory signs are reported in communities keeping small ruminants. Recently, reported outbreaks in Tanzania are characterized by persistency with continuous mortality resulting in significant losses due to loss of animals, low productivity and management costs [1]. In concurrent infections, the aetiological agents of involved diseases may act independently or interact with each other through various mechanisms, synergize or inhibit one another resulting into altered transmission dynamics, clinical presentation and severity and confounding effects of one aetiological agent to another at the individual or flock level [2,3,4]. For example, in controlled trials with multiple infecting parasites like *Theileria parva* and *Haemonchus contortus* [2]. In another study, individual level concurrent diseases, bovine tuberculosis and brucellosis and how they scaled up to produce population infection patterns [3]. Similarly, [4] proposed a study where two interacting nematodes effects were followed. In all these multiple infections, varying results were observed and these interactions altered the general outcome of the disease resulting in complicated diagnoses. This could be the case in the current persisting outbreaks which present with respiratory signs.

In recent studies, concurrent infections in outbreaks of diseases presenting with respiratory signs in small ruminants have been given a consideration [1,5,6]. However, in these studies, there were no considerations of the alterations in clinical signs, pathological lesions and epidemiological dynamics in some concurrent infections of the major transboundary diseases like CCPP and PPR. Furthermore, the role played by the respiratory bacterial pneumonia caused by the normal commensals of the respiratory tract *Pasteurella multocida* (*P. multocida*) and *Mannheimia haemolytica* (*M. haemolytica*) in disease dynamics, clinical presentation and severity has not been evaluated.

Contagious caprine pleuropneumonia is a classical disease of the respiratory system caused by *Mycoplasma capricolum* subsp. *capripneumonia* (*M. capripneumoniae*), a member of *Mycoplasma mycoides* (*M. mycoides*) “cluster” [7,8]. Contagious caprine pleuropneumonia lesions are localized within the respiratory system causing acute to chronic pneumonic conditions, the main clinical signs include, nasal discharges, coughing, respiratory distress, emaciation, posture with extended elbow whereas, pathological lesions are, serofibrinous pleurisy, serosanguinous hydrothorax, unilateral...
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lung inflammation and lung hepatization [1,9,10].

Paste des petits ruminants is caused by small ruminants morbillivirus (previously peste des petits ruminants virus) a member of genus Morbillivirus [1,11]. Peste des petits ruminants affects wild and domestic artiodactyls, including goats and sheep while pigs, and wild boars are considered as SRMV reservoirs [11]. Peste des petits ruminants primarily affects the digestive system [12], the major clinical signs are pyrexia, oculo-nasal discharge, necrotizing and erosive stomatitis, and diarrhea. Pathologically, PPR is presented with gastroenteritis and zebra stripes in the caecocolic junction whereas bronchopneumonia set in following secondary bacterial infection [13].

Pneumonic pasteurellosis and pneumonic mannheimiosis caused by the P. multocida and M. hemolytica respectively, the opportunistic bacteria of the respiratory system that cause bronchopneumonia under stressful conditions including infection with viruses and Mycoplasmas [1,14,15].

Despite the similarities in clinical signs and postmortem lesions for the diseases presenting with respiratory signs, the approach in investigations were limited to targeted diseases without consideration of possible concurrent infections. For instance [12,22] diagnosed only PPR, a disease known mostly to affect the digestive system despite, the presence of respiratory signs which could be due to secondary bacterial infections. Only recently, concurrent infections are being considered especially when obvious clinical signs suggestive of other diseases like Lumpy skin disease or goat and sheep pox [1,6].

Generally, in outbreaks of the diseases presenting with respiratory signs, the clinical signs and postmortem lesions at one time, are similar for different diseases but these diseases have not been investigated in the same outbreaks and the role the concurrent infections play in clinical presentation, disease severity and persistence have not been examined. This study examines the concurrent infections and the alterations in clinical and pathological changes associated, this will help in diagnosis and putting down of the proper interventions and control strategies.

2. MATERIALS AND METHODS

2.1 Study Areas

The areas involved in the study were all those from which outbreaks or sporadic outbreaks of diseases presenting with respiratory signs were reported. Outbreaks were investigated in the Southern highland zone in Chunya and Mbarali in Mbeya region, Iringa municipality and Kilolo in Iringa region. From the central zone, outbreak reports were received from Dodoma municipality whereas eastern zone reports were received from Mvomero in Morogoro region and Bagamoyo in Pwani region. Lake zone outbreak reports were received from Serengeti and northern zone (Korogwe in Tanga region and, Monduli in Arusha (Fig. 1).

2.2 Study Animals

Small ruminants from the areas reported to have outbreaks of diseases presenting with respiratory signs. Inclusion criteria were presence of clinical signs or pathological lesions suggestive of involvement of the respiratory system.

2.3 Study Design

Outbreak investigation and individual animal examination were carried out between 2016 - 2018 following the procedures as described in the previous works [16,17]. A data sheet was prepared in Microsoft Word™ 2007, tested and used to record each case, individual animal data, observed clinical signs and pathological lesions during clinical and postmortem examination of cases during every call for outbreak investigation.

2.4 Sample Collection

Samples collected during clinical examination were whole blood collected using EDTA vacutainers tubes, nasal, oral and eye swabs and synovial fluid and from autopsied animals, pleural fluid were collected in RNA shield (Zymo Research Corporation, Irvin California). Also, from autopsied animals, intestinal, lung and lymph node sections were collected in Stuart Medium (HiMedia Laboratories Pvt Limited, India). Whole blood was briefly stored at 4°C and samples stored in RNA shield and Stuart Medium was stored at -20°C, before use. Extracted RNA and DNA were briefly kept at -80°C before analysis.
2.5 Laboratory Testing of the Samples

2.5.1 DNA and RNA extraction

Genomic DNA (gDNA) was extracted using Quick-DNA™ Miniprep plus Kit Catalog No D4069 (Zymo Research Corporation, Orange, California). RNA was extracted using Quick-RNA™ Viral Kit Catalog No. R1035 (Zymo Research Corporation, Orange, California). Extractions were done according to manufacturers’ instructions. Extracted RNA was used in the amplification of the SRMV nucleoprotein (N) gene and extracted gDNA were used to amplify the M. mycoides “cluster” 16S rRNA gene and specific gene for M. capri, M. capricolum, P. multocida and M. haemolytica.

2.5.2 Mycoplasma capricolum subsp capripneumoniae detection

Molecular detection of the M. capripneumoniae was carried out using PCR/REA technique [18]. Briefly, the 16S rRNA gene for members of the M. mycoides “cluster” was amplified by PCR. The amplification process was carried out with the set of primers as described in the previous study [19] (Table 1). Master Mix, PCR water and the template were mixed to 25 µl total volume, preheated in C100 touch thermal cycler (BIO-RAD®, Singapore) at 94°C for 5 minutes. This was followed by a 40 cycles of 95°C for 1 minute denaturation, 58°C for 1 minute, annealing 72°C for 2 minute elongation, held at 72°C for 10 minutes and 4°C holding to infinite. The PCR products were visualized in 0.5% DNA loading dye (EZ-Vision®, VWR Life science, California, USA) in 1.5% agarose, after a gel electrophoresis. The amplicons were digested using restriction enzyme PstI, resulting into three fragments of 548 bp of the operon that was not ligated, 420 bp and 128 bp from the ligated operon indicating presence of M. capripneumoniae. The other members of the M. mycoides “cluster” had only two fragments 420 bp and 128 bp from ligated operons of the 16S rRNA gene fragment [18].
Amplified gene fragment expected size was 395 base pairs at 94°C for 30 cycles of denaturation at 94°C, annealing at 55°C by 30 cycles of denaturation at 94°C (BIO-RAD®, Singapore). Initial denaturation was set at 94°C (BIO-RAD®, Singapore). The master mix, PCR water and specific primers (Table 1) targeting the outer membrane protein (Omp87) and specific antigen 1 (SSA) and outer membrane protein 87 (Omp87) genes respectively, were amplified from the extracted gDNA [23] with some modifications. Briefly, the amplification process was done in C100 touch thermal cycler (BIO-RAD®, Singapore), in 25 µl total volume of substrate, Master Mix, PCR water and specific primers (Table 1) targeting the outer membrane protein 87 (Omp87) for Mannheimia hemolytica and serotype specific antigen 1 (SSA-1) for Pasteurella multocida. The amplification cycle started with initial denaturation at 94°C for 2 minutes, followed by 40 cycles of denaturation at 94°C for 45 seconds, annealing at 45°C, and elongation at 72°C for 1 minute. The resulting amplicons were detected in 1.5% agarose gel stained with 0.5% DNA loading dye (EZ-Vision®, WVR Life Science, California, USA).

2.5.5 Detection of small ruminants’ morbillivirus (SRMV)

Small ruminants morbillivirus (formerly peste des petits ruminants virus), a member of the genus Morbillivirus and causative agent of peste des petits ruminants (PPR) was also detected by amplification of the Nucleoprotein (N) gene using specific NP3/NP4 (Table 1) in one-step Reverse Transcriptase Polymerase Chain Reaction (RT-
lesions were observed in goats and sheep. A number of clinical signs and postmortem lesions observed included, anorexia, vesicular lesions on the gums, diarrhea, coughing, nasal discharges, eye discharge (lacrimation), difficult breathing, reluctance to move and emaciation. Generally the clinical signs were equally distributed among goats and sheep except nasal discharge and reluctance to move which were more observed in goats and diarrhea which was more observed in sheep. A combination of nasal discharge, anorexia and coughing were the most prevalent clinical signs in both goats and sheep (Fig. 2). On postmortem examination, the main postmortem lesions observed included, consolidation of lung tissue, froth in the trachea, increased straw coloured serosanguinous fluid (hydrothorax), congested nasal cavity, firm lung lobes, yellowish nodules in congested lung lobes, soft hemorrhagic lymph nodes, lung attachment to the chest wall and unilateral or asymmetric lung inflammation (Fig. 3). High proportions of congested nasal cavity was observed in sheep and a combination of consolidated lungs, vesicular lesions in digestive system, attachment to the chest wall were the most prevalent postmortem lesions in goats (Fig. 4).

2.6 Data analysis
A detailed Data analysis using R version R. 3.5.1 [25] is presented. Odds ratios were calculated using the molecular data to assess the association of the diseases with species and status of animals at examination where p < 0.05 was considered significant.

3. RESULTS AND DISCUSSION

3.1 General Observations of Animals
A number of clinical signs and postmortem lesions were observed in goats and sheep examined. The clinical signs and postmortem lesions observed were suggestive of different diseases. The main clinical signs observed in different cases include, anorexia, vesicular lesions on the gums, diarrhea, coughing, nasal discharges, eye discharge (lacrimation), difficult breathing, reluctance to move and emaciation.

### Fig. 2. Percentage proportions of clinical signs observed in examined cases in small ruminants
Fig. 3. Postmortem lesions picked during outbreak investigations
Legend 3A – 1-areas of attachment, 2&3 - asymmetrical inflamed lung lobes, 3B – 1-serosanguinous fluid, 2- unilateral inflamed lung lobe, 3C – 1-serofibrinous exudate on areas of lung and ribs attachment and 3D-1 serosanguinous fluid

3.2 Conventional PCR Results Analysis

Out of 205 small ruminants tested, 72.2% (148) and 20.8% (57) were from goats and sheep respectively, the overall prevalence (Fig. 5) revealed that, 16.1% (33) of the animals were positive for CCPP with a significant difference between species, goats being 7.3 more likely to be positive ($p = 0.003$). Significant difference ($p < 0.0001$) was also noted in detection of \textit{M. capricolum} and \textit{M. capri} pneumonia where goats were more likely to be positive than sheep, but in pneumonic manheimiosis, goats were less likely to be positive ($p = 0.047$). There were no statistical difference between species in pneumonic pasteurellosis and PPR (Table 2).

3.3 Conventional PCR Results Analysis in Goats

A total of 148 goats were examined, majority of them, 79.1% (117) had concurrent infections and these were detected from 70.9% (105) clinically examined animals and 29.1% (43) autopsied animals. Concurrent diseases detected in high proportions were \textit{M. capricolum} pneumonia and PPR, 11.1% (13), \textit{M. capricolum} pneumonia and pneumonic pasteurellosis, 9.4% (10) and pneumonic pasteurellosis and PPR, 9.4% (10). Detection of all diseases was significantly low in clinically examined animals ($p<0.05$), except in the detection of pneumonic pasteurellosis and PPR.
where the detection was high in clinically examined animals but with no statistical significance (p=0.056, 0.096) respectively (Table 3).

Table 2. Prevalence of diseases presenting with respiratory signs in relation to goats and sheep in Tanzania

<table>
<thead>
<tr>
<th>Variable</th>
<th>Category</th>
<th>Overall prevalence (n=205)</th>
<th>Total</th>
<th>Positives</th>
<th>OR (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contagious caprine pleuropneumonia (CCPP)</td>
<td>Goats</td>
<td>16.1</td>
<td>148</td>
<td>31 (20.9)</td>
<td>7.3 (2.3 - 23.5)</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td>Sheep</td>
<td></td>
<td>57</td>
<td>2 (3.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. capricolum pneumonia</td>
<td>Goats</td>
<td>4.4</td>
<td>148</td>
<td>9 (6.1)</td>
<td>0.1 (0.03 - 0.2)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Sheep</td>
<td></td>
<td>57</td>
<td>0 (0.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. capri pneumonia</td>
<td>Goats</td>
<td>6.8</td>
<td>148</td>
<td>14 (9.5)</td>
<td>0.1 (0.05 - 0.2)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Sheep</td>
<td></td>
<td>57</td>
<td>0 (0.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pneumonic pasteurellosis</td>
<td>Goats</td>
<td>42.4</td>
<td>148</td>
<td>59 (39.9)</td>
<td>0.7 (0.4 - 1.2)</td>
<td>0.234</td>
</tr>
<tr>
<td></td>
<td>Sheep</td>
<td></td>
<td>57</td>
<td>28 (49.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pneumonic mannheimiosis</td>
<td>Goats</td>
<td>9.3</td>
<td>148</td>
<td>10 (6.8)</td>
<td>0.38 (0.15 – 1)</td>
<td>0.047</td>
</tr>
<tr>
<td></td>
<td>Sheep</td>
<td></td>
<td>57</td>
<td>9 (15.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peste des petits ruminants (PPR)</td>
<td>Goats</td>
<td>25.9</td>
<td>148</td>
<td>38 (25.7)</td>
<td>1 (0.5 - 1.8)</td>
<td>0.928</td>
</tr>
<tr>
<td></td>
<td>Sheep</td>
<td></td>
<td>57</td>
<td>15 (26.3)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 4. Percentage proportions of postmortem lesions observed in examined cases in small ruminants
3.4 Conventional PCR Results Analysis in Sheep

In sheep, a total of 57 animals were examined and 28.1% (16) of them had concurrent infections, and, were detected from 68.4% (39) of clinically examined animals and 31.6% (18) of autopsied animals. Higher proportions of concurrent detected diseases was between pneumonic pasteurellosis and PPR 31.3% (5) and pneumonic pasteurellosis and pneumonic Mannheimiosis 25% (4). Only in CCPP, the difference in detection was significant, being more likely detected in clinically examined animals than in autopsied animals ($p=0.005$). In all other diseases, there were no statistical difference but the odds ratio in detection of pneumonic pasteurellosis and PPR were 1.79 and 1.38 more likely to occur in clinically examined animals compared to autopsied animals (Table 4).

3.5 Discussion

Contagious caprine pleuropneumonia (CCPP) and peste des petits ruminants (PPR) are diseases of high economic importance in the small ruminants industry especially in resource limited households in developing countries [26], including Tanzania. In the same way, pneumonic
pasteurellosis and pneumonic manheimiosis, independently or as secondary to virus and/or mycoplasma infections contributed significantly to socio-economic losses [27]. In the present study clinical and postmortem lesions, molecular prevalence and proportions of detection in species, clinically and postmortem examined animals for CCPP and other Mycoplasma mycoides “cluster” pneumonias, PPR, pneumonic manheimiosis and pneumonic pasteurellosis were assessed.

Our major findings of the clinical signs and postmortem lesions in the reported outbreaks in both goats and sheep were nasal discharges, anorexia, coughing, emaciation, difficult breathing, reluctance to move and diarrhea which were all non-specific to any disease, these findings are in agreement to the previous findings [1]. In the presence of such non-specific clinical signs presence of reliable laboratory tests is highly advocated. Major postmortem lesions revealed included, consolidated lungs, tracheal froth, hydrothorax and digestive lesions (vesicular lesions on the gums and intestinal hemorrhages) in both goats and sheep, these findings are similar to those reported in previous study [1] in PPR and concurrent infections and [28] in CCPP. Congested nasal discharge was highly observed in sheep, this could be associated with the fact that apparently healthy sheep infested with internal parasites such as Oestrus ovis may have congested nasal cavity [29]. Lung attachment to the chest wall and unilateral or asymmetric lung inflammation were observed more in goats and this could be due to the fact that, the lesions are more associated with CCPP which is primarily a disease of goats [9,28].

Table 3. Prevalence of diseases presenting with respiratory signs in goats with relation to the examination status

<table>
<thead>
<tr>
<th>Variable</th>
<th>Category</th>
<th>Overall prevalence (n=148)</th>
<th>Total</th>
<th>Positives</th>
<th>OR (95% ci)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contagious caprine pleuropneumonia (CCPP)</td>
<td>CSE</td>
<td>20.9</td>
<td>105</td>
<td>15 (14.3)</td>
<td>0.28 (0.14 -0.56)</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>PME</td>
<td></td>
<td>43</td>
<td>16 (37.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. capricolum pneumonia</td>
<td>CSE</td>
<td>6.1</td>
<td>105</td>
<td>1 (0.95)</td>
<td>0.04 (0.005 - 0.34)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>PME</td>
<td></td>
<td>43</td>
<td>8 (18.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. capri pneumonia</td>
<td>CSE</td>
<td>9.5</td>
<td>105</td>
<td>6 (5.7)</td>
<td>0.26 (0.10 -0.71)</td>
<td>0.015</td>
</tr>
<tr>
<td></td>
<td>PME</td>
<td></td>
<td>43</td>
<td>8 (18.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pneumonic pasteurellosis</td>
<td>CSE</td>
<td>39.9</td>
<td>105</td>
<td>47 (44.8)</td>
<td>2.1 (1.16 - 3.78)</td>
<td>0.056</td>
</tr>
<tr>
<td></td>
<td>PME</td>
<td></td>
<td>43</td>
<td>12 (27.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pneumonic manheimiosis</td>
<td>CSE</td>
<td>6.8</td>
<td>105</td>
<td>3 (2.9)</td>
<td>0.15 (0.04 -0.55)</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td>PME</td>
<td></td>
<td>43</td>
<td>7 (16.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peste des petits ruminants (PPR)</td>
<td>CSE</td>
<td>25.7</td>
<td>105</td>
<td>31 (29.5)</td>
<td>2.15 (1.09 - 4.25)</td>
<td>0.095</td>
</tr>
<tr>
<td></td>
<td>PME</td>
<td></td>
<td>43</td>
<td>7 (16.3)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Legend: CSE = Clinical signs examination, PME = Postmortem examination

Table 4. Prevalence of diseases presenting with respiratory signs in sheep with relation to the examination status

<table>
<thead>
<tr>
<th>Variable</th>
<th>Category</th>
<th>Prevalence (n=57)</th>
<th>Total</th>
<th>Positives</th>
<th>OR (95% ci) Z value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contagious caprine pleuropneumonia (CCPP)</td>
<td>CSE</td>
<td>3.5</td>
<td>39</td>
<td>2 (15.1)</td>
<td>0.17 (0.09 -0.35) -2.788</td>
<td>0.005</td>
</tr>
<tr>
<td></td>
<td>PME</td>
<td></td>
<td>18</td>
<td>0 (0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pneumonic pasteurellosis</td>
<td>CSE</td>
<td>49.1</td>
<td>39</td>
<td>21 (53.8)</td>
<td>1.79 (1.02 - 3.14) 1.012</td>
<td>0.331</td>
</tr>
<tr>
<td></td>
<td>PME</td>
<td></td>
<td>18</td>
<td>7 (39.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pneumonic manheimiosis</td>
<td>CSE</td>
<td>15.8</td>
<td>39</td>
<td>6 (15.4)</td>
<td>0.91 (0.42 - 1.93) 0.125</td>
<td>0.897</td>
</tr>
<tr>
<td></td>
<td>PME</td>
<td></td>
<td>18</td>
<td>3 (16.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peste des petits ruminants PPR</td>
<td>CSE</td>
<td>26.3</td>
<td>39</td>
<td>11 (28.2)</td>
<td>1.38 (0.72 - 2.62) 0.478</td>
<td>0.631</td>
</tr>
<tr>
<td></td>
<td>PME</td>
<td></td>
<td>18</td>
<td>4 (22.2)</td>
<td></td>
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</tr>
</tbody>
</table>

Legend: CSE = Clinical signs examination, PME = Postmortem examination
The study revealed that, 20.9% of goats and 15.1% of sheep were positive for CCPP the findings which are in agreement with previous study which reported 31.2% of goats and 12.9% of sheep being positive to CCPP in Borana zone, Southern Oromia, Ethiopia [28]. Contrary to this study, low prevalence of CCPP in goats was reported in another study in Ethiopia [30]. Contagious caprine pleuropneumonia (CCPP) has been also reported in goats and sheep in Tanzania [9,31,32]. It was also revealed in this study that, 9.5% of goats had M. capri pneumonia whereas 6.8% had M. capricolum pneumonia, which together form a conglomerate of clinical signs hampering diagnosis [33,34]. Variations in the prevalence in those different studies may be attributed to differences in study areas, diagnostic techniques used, management and production systems and the status of the animals at the time of sampling.

In our study, PPR was detected in 25.7% and 26.3% of goats and sheep respectively, the findings which are in agreement with the previous report in Tanzania [35] who reported a true prevalence of 48.3% and 45.5% for goats and sheep, respectively. Our findings were however far below the findings reported in another study in Ngorongoro, where PPR seroprevalence was reported to be 74.6% [1]. The variations in the prevalence for PPR in both goats and sheep may be due to difference in the diagnostic tests used, status of the animals sampled and the geographical location and livestock keeping systems.

Based on molecular analysis, the current study confirmed the presence of pneumonic pasteurellosis, and pneumonic mannheimiosis for the first time in Tanzania. Pneumonic pasteurellosis was confirmed in 49.1% of sheep compared to 39.9% in goats whereas, mannheimiosis was 15.8% in sheep and 6.8% in goats. Higher detection of both pneumonic pasteurellosis and pneumonic mannheimiosis in sheep than in goats was also reported in the previous study [27], where sheep had higher prevalence (37.1%). Presence of pneumonic pasteurellosis and pneumonic mannheimiosis as secondary infecting diseases in small ruminants may further complicate the diagnosis of diseases presenting with respiratory signs.

Our study also reports statistical significance between species in detecting CCPP, goats were more likely to be positive than sheep (OR =7.3), this is due to the fact that sheep is not a natural host to M. capripneumonia. Similarly, sheep was a protective factor for M. capricolum and M. capri pneumonia (OR = 0.1) for both, this could be due to the fact that both are known to affect more goats [36,37]. Furthermore, in goats, all diseases except pneumonic pasteurellosis and PPR, were more likely to be diagnosed from the postmortem examined animals which is in agreement to the previous report [28] for CCPP. Pneumonic pasteurellosis and PPR were highly detected in clinically examined animals and this could be due to the fact that pneumonic pasteurellosis can easily be picked by swabs in the upper respiratory system and PPR can manifest and be detected from the nasal swabs samples [1]. On the other hand, CCPP in sheep was more likely to be detected in clinically examined sheep (OR=0.17) and this could be explained by the fact that, CCPP is not known to cause disease in sheep, but can harbour M. capripneumonia, the causative agent for CCPP [9].

4. CONCLUSION

In the outbreaks presenting with respiratory signs, the major endemic and transboundary diseases CCPP and PPR are involved. Furthermore, there is also high involvement of pneumonic pasteurellosis and pneumonic mannheimiosis which occur as secondary to CCPP or PPR infection or independently, also there is high proportions of concurrent occurring diseases in the same outbreaks. This results in complications in diagnosis, persistency of the outbreaks due to misdiagnosis, partial diagnosis and improper control strategies. It is advised to consider differential diagnoses in the interventions and control strategies including developing of simple multiple disease diagnostic kits and use of multivalent vaccines.

CONSENT

Animal sampling was done on consent of the livestock owners and all authors declare that written informed consent was obtained from the livestock owners for publication of the obtained information and accompanying images.

ETHICAL APPROVAL

Handling of the animals sampled during the course of this research work followed all the stipulated animal rights as per the Universal Declaration of the Animal Welfare and Tanzania Animal Welfare Act 2008.
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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

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