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ORIGINAL ARTICLE

Detection of peste des petits ruminants and concurrent secondary diseases in sheep and goats in Ngorongoro district, Tanzania

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Abstract

Small ruminants play an important role in the livelihoods of resource-constrained communities. This study was initiated because of a massive outbreak of a respiratory disease in sheep and goats in Loliondo area in Ngorongoro district of Arusha region in Tanzania in 2016. During flock examination, a total of 240 serum samples and 61 nasal swabs were collected. Antibodies to small ruminant morbillivirus, causative agent of peste des petits ruminants (PPR), were detected from sera using a competitive enzyme-linked immunosorbent assay. A multiplex reverse transcription real-time polymerase chain reaction assay was used to detect four pathogens: small ruminant morbillivirus, *Mycoplasma capricolum* subspecies *capripneumoniae*, *Pasteurella multocida*, and *Capripoxvirus* from the nasal swabs. Overall seroprevalence of PPR was 74.6%, with all four pathogens detected from nasal swabs. Co-infections of small ruminant morbillivirus and *Mycoplasma capricolum* subspecies *capripneumoniae*, small ruminant morbillivirus and *Capripoxvirus*, small ruminant morbillivirus and *Pasteurella multocida*, and *Mycoplasma capricolum* subspecies *capripneumoniae* and *Capripoxvirus* were also detected. Presence of PPR and the other diseases in this study provided insight into the severity of the outbreak in sheep and goats in Ngorongoro district. Thus, laboratory confirmation is critical for prompt and appropriate interventions to be made for control of diseases in sheep and goats with similar clinical signs. The findings also call for research into development of combined vaccines targeting common diseases of small ruminants in Tanzania.

Keywords PPR · CCPP · Goats · Sheep

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Introduction

Small ruminants contribute significantly to the economy of most rural communities in developing countries. Though small ruminants contribute towards alleviation of poverty, their productivity is hampered by, among other things, infectious diseases and poor husbandry practices (FAO and OIE 2016). Compared with cattle, there are limited studies on small ruminants' health and the information available is fragmented and sometimes incomplete (Farougou et al. 2013). One area which is poorly documented is the magnitude of multiple infections, by different types of pathogens such as viruses, bacteria, and parasites, in sheep and goats that result in respiratory diseases (Settypalli et al. 2016). Some clinical signs associated with respiratory diseases of small ruminants include ocular and nasal discharges, lesions in the oral and nasal mucus membranes, cough, pneumonia, diarrhea, and severe dehydration (Kul et al. 2015; Roeder and Obi 1999).

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One important respiratory disease affecting small ruminants currently worldwide is peste des petits ruminants, PPR. Peste des petits ruminants is a viral disease of small ruminants first reported in West Africa in the early 1940s and later recognized as endemic in both West and Central Africa (Parida et al. 2015; Gargadennec and Lalanne 1942). The disease is caused by small ruminant morbillivirus (SRMV), formerly peste des petits ruminants virus (PPRV), a member of the genus *Morbillivirus* of the Paramyxoviridae family (ICTV 2016). Currently, PPR is prevalent in Central, Eastern, and Western Africa, Asia, and the near and Middle East (Libeau et al. 2014). In East Africa, PPR was detected in Kenya and Uganda in 2007, while in Tanzania, it was officially confirmed in 2008 (Kivaria et al. 2009; Swai et al. 2009). The disease is considered endemic in Tanzanian domestic sheep and goat populations (Torsson et al. 2017; Kgotlele et al. 2016).

Other respiratory diseases of economic importance affecting small ruminants include contagious caprine pleuropneumonia (CCPP), pasturella, sheeppox, and goatpox (Kul et al. 2015; Emikpe et al. 2010; Brown et al. 1991; Ugochukwu and Agwu 1991). *Mycoplasma capricolum* subspecies *capripneumoniae* (Mccp) is the causative agent of CCPP, a highly contagious disease of goats first described in 1873 in Algeria (OIE 2014). The disease was later described in 1976 in Kenya by MacOwan and Minette (1976), followed by subsequent isolations in several African countries including Tanzania (Kusiluka et al. 2000a, b; Bölske et al. 1995). Sheep poxvirus and goat poxvirus, which belong to the *Capripoxvirus* genus, are responsible for pox diseases in sheep and goats. These occur in several parts of Africa, Asia, the Middle East, and India (Spickler 2015). *Pasteurella multocida* (*P. multocida*) is also isolated from cases of respiratory diseases in sheep, goats, pigs, and cattle, often causing pneumonia either alone or as an opportunist with other respiratory pathogens (Settypalli et al. 2016). Pneumonic pasteurellosis is one of the most economically important infectious diseases of ruminants with a wide prevalence throughout the continents (Mohammed and Abdelsalam 2008). The similarity in the signs caused by these pathogens, and their co-localization in nearly the same endemic areas, calls for appropriate differential diagnostic testing to accurately identify the responsible pathogen(s) (Settypalli et al. 2016). In this study, the aim was to investigate the etiological cause of a respiratory disease outbreak in sheep and goats in Loliondo area in Ngorongoro district of Arusha region in Tanzania.

Materials and methods

Study area

The study was conducted in Ngorongoro district, one of the districts of Arusha region in northern Tanzania. The villages involved in the investigation were Sukenyan, Mondorosi, Ololosokwan, and Enguserosambu. The villages are inhabited by Maasai and Sonjo ethnic groups that are traditionally pastoralists and agro-pastoralists, respectively. The study area was chosen after a reported outbreak of a disease affecting sheep and goats with high mortality rates. The animals were said to present respiratory distress, diarrhea, and mucopurulent nasal discharges. The outbreak was reported to have been going on for 4 months before collection of samples for this study.

Study animals and samples

Sheep and goats were randomly examined for clinical signs from the four villages. Clinical samples collected were 240 sera (from 59 sheep and 181 goats) and 61 nasal swabs (from 37 sheep and 24 goats) randomly from the four villages. Age and sex of sampled animals were recorded during sample collection.

Serological assay

Serum samples were tested for presence of antibodies to SRMV using commercial kit from ID screen® PPR competition ELISA (IDVet, Grabels, France). The kit was used and interpreted according to manufacturer's instructions.

Detection of nucleic acids

Total nucleic acids were extracted from nasal swabs using QIAamp Viral RNA Mini extraction kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The extracted samples were tested for the presence of nucleic acid material of four pathogens using a multiplex RT-qPCR method developed by Settypalli et al. (2016). Briefly, a 20-μl reaction volume containing reagents from iScript™ Universal Probes One-Step Kit (Bio-Rad Laboratories, Hercules, USA), four pathogen-specific primer pairs (500 nM each) and probes (250 nM each) labeled at the 5′ends with different reporter dyes: *Capripoxvirus* with CY5, SRMV (formerly PPRV) with HEX, *P. multocida* with FAM, and Mccp with TEXAS RED. The assay was run using CFX 96™ real-time PCR machine (Bio-Rad) with the following cycling conditions: 50 °C for 20 min followed by 95 °C for 5 min and 40 cycles of denaturation at 94 °C for 10 s, annealing at 56 °C for 20 s, and extension at 62 °C for 20 s. The data acquisition was performed during the annealing step. An amplification peak and quantification cycle (Cq) value of less than 35 indicated presence of a pathogen.

Data analysis

Data generated were entered in Microsoft Excel 2016 and analyzed using descriptive statistics. Odds ratio (OR) was calculated according to Altman (1991) using serological data.

The OR assesses the association of being seropositive for PPR where p value < 0.05 was considered as significant.

Results

General observations of animals

Different clinical signs were observed from the sheep and goats examined randomly in the four villages. Generally, most clinical signs observed were suggestive of different diseases. Clinical signs observed in the examined flocks included nodules on skin of some animals, nasal discharges, loss of body condition, and diarrhea. A combination of nasal discharges and diarrhea was the most prevalent clinical sign in all herds examined with some diarrhea tinged with blood. Figure 1 shows some of the animals with nasal discharges and diarrhea.

Serological examination

Serological examination of samples collected from the sheep and goats indicates PPR occurrence with an overall seroprevalence of antibodies to SRMV at 74.6%. There was no statistical difference of seropositivity between species, sheep and goats, and in sex, being male or female, in this study as indicated by p value > 0.05 (Table 1). Significant differences ($p < 0.05$) were noted in age where animals less than 2 years were more likely to be seropositive for antibodies to SRMV than animals older than 2 years.

Multiplex RT-qPCR analysis

Of the 61 nasal swabs tested, 38 were positive for one or more of the four pathogens being analyzed (Table 2). Majority of the pathogens were detected from nasal swabs collected in goats than in sheep. The most detected pathogen was *P. multocida* while the least detected pathogen was Mccp. Co-infection of Mccp/CaPV was also observed to be common in nasal swabs from goats than in sheep.

Table 1 Seroprevalences of antibodies to SRMV in goats and sheep in Ngorongoro district, Tanzania

Variable	Category	Total	Positives (%)	OR (95%CI)	p value
Species	Goat	181	137 (75.7)	1.26 (0.65–2.43)	0.490
	Sheep	59	42 (71.2)		
Age	≤ 2 years	84	53 (63.1)	0.329 (0.19–0.6)	0.0002
	> 2 years	156	126 (80.8)		
Sex	Female	167	126 (75.4)	1.16 (0.62–2.16)	0.641
	Male	73	53 (72.6)		

Discussion

Clinical signs observed in the examined animals were nasal discharges and diarrhea. These clinical signs are expressed in many infectious diseases of small ruminants. Infectious diseases that present similar observed clinical signs include PPR, pneumonic pasteurellosis, and CCPP for nasal discharges; PPR, coccidiosis, or gastro-intestinal helminth infestations for diarrhea; and contagious ecthyma, sheep pox, and goat pox for nodular lesions (Zro et al. 2014; Munir et al. 2009; Diallo et al. 2007; Roeder and Obi 1999). In Tanzania, difficulty in differentiating clinical signs in sheep and goats has been mentioned as a major limiting factor in diagnosis especially for PPR and CCPP (Mbyuzi et al. 2014). In such cases where specific manifestations of the diseases are absent, reliable laboratory tests are needed.

The overall seroprevalence of PPR in this study was 74.6%. This seroprevalence is in line with findings from a recent study that analyzed samples from 14 regions of Tanzania, with Arusha region having an overall seroprevalence of 71.1% (Kgotlele et al. 2016). Other studies done in northern Tanzania have found PPR seroprevalence of 45.5% in 2008 (Swai et al. 2009) and 22.1% in 2008–2009 (Kivaria

Fig. 1 A goat with mucopurulent nasal discharge (**a**) and a goat showing soiled behind with diarrhea (**b**)

Table 2 Detected pathogens that cause similar respiratory syndromes in sheep and goats in Ngorongoro district using one-step multiplex RT-qPCR test

Pathogen(s) detected	Sheep (%) $n = 37$	Goats (%) $n = 24$	Total (%) $n = 61$
[a]SRMV	2 (5)	3 (13)	5 (8)
[b]Mccp	1 (3)	2 (8)	3 (5)
[c]*P. multocida*	12 (32)	7 (29)	19 (31)
[d]CaPV	2 (5)	2 (8)	4 (7)
[a]SRMV/[b]Mccp	1 (3)	0	1 (2)
[a]SRMV/[d]CaPV	1 (3)	0	1 (2)
[a]SRMV/[c]*P. multocida*	1 (3)	0	1 (2)
[b]Mccp/[d]CaPV	1 (3)	3 (13)	4 (7)
Total positive	21 (57)	17 (71)	38 (62)

[a] Small ruminant morbillivirus, [b] *Mycoplasma capricolum* subspecies *capripneumoniae*, [c] *Pasteurella multocida*, [d] *Capripoxvirus*

et al. 2013). This variation over the years may be due to uncontrolled animal movement that spread PPR in the sheep and goat population reared without proper vaccination. In this study, presence of antibodies to SRMV was most likely caused by natural exposure as none of the animals were vaccinated according to records from the District Veterinary Office. The animals in this study were grazed in communal pastures, which could be where they encounter infected animals. Intermingling of animals in communal grazing lands has been found to facilitate spread of infectious diseases such as PPR, CCPP, contagious ecthyma, and goat and sheep pox (Kusiluka and Kambarage 1996).

There was statistical diference between animals under 2 years more likely to be seropositive than those above 2 years. The differences noted between the age groups are most likely due to younger animals loosing their aquired passive immunity after 3 months and the older ones having life long immunity after survival of infection (Kul et al. 2015). In this study, there was no statistical difference between males and females. This finding is different from another study done in Tanzania where females were more likely to be seropositive than males (Torsson et al. 2017). It is believed that females are used in reproduction hence kept longer in the herd than males, therefore, have a longer risk period for exposure in the herd. Stress associated with pregnancy and milk production could also predispose them to infection (Aziz-ul- et al., 2016).

All four pathogens investigated in this study were detected from nasal swabs, including co-infections of SRMV and Mccp, SRMV and CaPV, SRMV and *P. multocida*, and Mccp and CaPV. Co-association of SRMV with other viral and bacterial infections in small ruminants has been demonstrated before in other studies in different parts of the world (Kul et al. 2015). Viral diseases with co-association with SRMV include sheep and goat pox virus, while co-associations with bacterial pathogens include *Pasteurella* spp., Mccp, and *Mannheimia haemolytica* (Malik et al. 2011; Emikpe et al. 2010; Brown et al. 1991; Ugochukwu and Agwu 1991). The co-infections detected in this study may have provided complications during field diagnosis that resulted in the persistence and severity in the affected flock. Thus, laboratory confirmation is critical for appropriate interventions to be made for the different diseases of sheep and goats especially regarding PPR. Development and use of specific diagnostic tests that can distinguish PPR from diseases with similar signs has helped unquestionably to improve knowledge and understanding in geographical distribution and spread of the disease in specific areas (Libeau 2015). This is critical as PPR has been identified as the next animal disease to be eradicated after rinderpest. One of the factors identified in the PPR Global Control and Eradication Strategy (PPR GCES) that make eradication possible is availability of appropriate diagnostic tests and protocols for surveillance (FAO and OIE 2016). Thus, help attain one of the objectives to progressively reduce the incidence and spread of PPR and ultimately eradicate.

In conclusion, SRMV, Mccp, CaPV, and *P. multocida* were detected in sheep and goats in Ngorongoro district. The study also confirmed occurrence of co-infection of pathogens that are associated with respiratory distress in sheep and goats in Tanzania, an important factor for consideration in small ruminant disease diagnosis and control strategies especially for PPR.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with animals performed by any of the authors.

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