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Mixed infection of sheeppox and ORF in an organised farm of hilly region of the Nilgiris, Tamil Nadu, India

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Abstract
Sheeppox and ORF are two important viral diseases of small ruminants causing significant economic loss. Sheeppox is an OIE notifiable, systemic disease whereas ORF is a local proliferative skin infection of the sheep and goats. This report describes a dual infection of *Sheeppox virus* (genus *Capri poxvirus*) and ORF *virus* (genus *Parapoxvirus*) in an organised farm of Sheep Breeding Research Station, Sandynallah located at high altitude of the temperate terrain region of Nilgiris, Tamil Nadu. The outbreak was recorded in the farm consisting of 1499 sheep of varied age group during March- May 2016. On clinical examination, the affected adult animals (n=46) showed typical Sheeppox lesions confined to ventral abdomen and udder. While, the suckling lambs (n=90) showed proliferative lesions mainly on oral commissure and lips. A mortality of 12.22% was recorded only in lambs of preweaning age, which was mainly ascribed to isolation from mothers, debility and weakness. On histopathology, two animals showed typical intracytoplasmic inclusions suggestive of Sheeppox in the lung. The mixed infection was further confirmed with *P32* gene and *B2L* gene-based PCRs respectively to identify Sheeppox and ORF infections. Of 45 scab samples tested, 12 scabs were found positive for Sheeppox, 2 samples for ORF and 21 samples (11 lambs and 10 ewes) for both the infections. The flock was managed symptomatically with antibiotics and antipyretics and the outbreak was controlled by strict isolation, disinfection and disease manage mental practices along with proper nutrition.

Keywords: Sheep, mixed infection, sheeppox, ORF, Tamil Nadu, India

Introduction
Sheeppox (Syn. *Variola ovina*) is a trans boundary pox viral disease (Babiuk *et al*., 2008) [1] of sheep and goats and is notifiable to World Organisation for Animal Health (OIE, 2008) [2]. The causative agent is Sheeppox virus (SPPV) under genus *Capri poxvirus.* It is economically significant in the enzootic regions of Africa, Middle East countries, Asia, Indian subcontinent (Yogisharadhya *et al*., 2011) [3] and of late, in southern Europe. In naïve animals, the morbidity and mortality due to SPPV may reach as high as 100 percent (Bhanuprakash *et al*., 2006) [4].
ORF (Syn. contagious ecthyma; contagious pustular dermatitis) is usually a local proliferative skin disease of sheep and goats caused by the ORF *virus* (ORFV) belonging to the member of the genus *Parapoxvirus*. The disease is zoonotic, occurs globally and is regarded as enzootic in sheep and goats in India (Venkatesan *et al*., 2011; Bora *et al*., 2011) [5, 6]. It is also considered an economically significant disease as it results in high morbidity, able to cross infect other species and produce recurrent infections in susceptible host by subverting the immunity of host animal (Hosamani *et al*., 2009) [7]. Though the disease is most common in young ones (3-6 months of age), adult animals may also be affected (Lewis, 1996) [8]. It is often self-limiting disease, causing proliferative lesions observed as papules, vesicle and rapid scabs on the areas of skin around lips, buccal mucosa and around nostrils. The udder, eye or vulva may also show these characteristic lesions in severe cases that may become complicated by secondary bacterial infections and/or myasis (Housawi and Abu Elzein, 2000) [9].
Both SPPV and ORFV are DNA viruses classified under the subfamily *Chordopoxvirinae* of the family *Poxviridae*. Though these diseases have been reported commonly from enzootic regions, mixed infection of these two diseases is uncommon in sheep (Yeruham *et al*., 1998; Selim *et al*., 2016) [10, 11]. The present investigation deals with outbreak and management of mixed infections of Sheeppox and ORF diseases in an organised farm of Tamil Nadu.

Materials and Methods

Location of the investigation and details of the farm

The current investigation was carried during March-May' 2016 in an organized sheep farm located at Sandynallah which is ~12 kilometre away from Uthagamandalam (Ooty) town of the Nilgiris District, Tamil Nadu, a southern state of India.The farm is located at latitude of 11° 26' 15.25" north and longitude of 76° 38' 27.34" east in the Nilgiri Biosphere Reserve. The farm is situated at 2090 – 2235 m above mean sea level (MSL) in undulating hill range with common features a subtropical highland climate of cold weather. The annual mean temperature ranges from 0-24 °C with annual rainfall ~ 840 – 3000 mm (Prabhu *et al*., 2019) [12].

On an average 1499 animals were maintained at the farm during the period of investigation and Nilagiri, Dorset x Nilagiri cross and Sandyno were the three breeds maintained. Though the animals were vaccinated against enterotoxaemia and bluetongue, the vaccination against sheeppox or ORF has not been carried out as routine. Yet, other manage mental practices such as periodical deworming, disinfection of sheds was adopted as per the Animal Disease Review Committee of the University with semi-intensive system of management. The animals were fed with concentrate feed in the shelter and after 3 months of age were allowed to open pasture for about ~ 8 hours for grazing.

Clinical appraisal of the animals and sample collection

During March 2016, few animals started showing the lesions suggestive of pox viral disease. The clinical signs observed include initial pyrexia followed by characteristic skin lesions mainly in the areas of udder, ventral abdomen, buccal mucosa, oral commissures and perineum. Some young animals exhibited anorexia, rhinitis, dypnoea and terminally laboured breathing. Many suckling lambs showed the proliferative skin lesions over the lips and oral commissure and were suggestive of ORF infection. Since, the mixed infection with SPPV and/or ORFV was suspected the scab samples were collected from the animals. A total of 136 animals were suspected to be affected with either of the two diseases. Yet, a sum of 45 scab or skin lesions have been collected from the animals with the evident pox lesions and samples were stored at -20 °C till use. Further, organ samples (lung, intestine, lymphnode etc.,) from dead animals with evident clinical lesions (n=12) were send for histopathological examination at Central University Laboratory, a constituent of Tamil Nadu Veterinary and Animal Sciences University, India.

Sample processing and molecular detection of sheeppox and ORF

Collected scab tissues were triturated and homogenized in sterile PBS, pH 7.4 to make 10% w/v suspension. The tissue homogenates were freeze-thawed thrice and centrifuged at 3000 rpm for 10 min to get rid of gross debris. The supernatant containing the virus was used for genomic DNA isolation by using DNeasy® Blood & Tissue Kit (Cat. No. 69504; M/s Qiagen, Germany), according to the manufacturer's instructions. Briefly, 100 µl of skin tissue homogenate was subjected to 3-8 h lysis (56 °C) and eluted in 30 µl elution buffer and stored at -20 °C. The isolated DNA from a total of 45 scab/skin tissue samples were subject to PCR for both sheeppox (*p32* gene based) and ORF (*B2L* gene based). The details of primer sequences and amplification condition used were described in Table 1. The reaction volume of 25 µl included Taq DNA polymerase 2x Master Mix RED (Ampliqon, Denmark) with 1.5mM $MgCl_2$ (12.5 µl), forward and reverse primers (each 10 pmol), template DNA (2µl) and nuclease-free water (8.5 µl). The amplification was carried out in a thermal cycler (Eppendorf Mastercycler® nexus GX2, Hamburg, Germany) and the PCR products were analyzed by agarose gel electrophoresis (1.5% gel containing 1 µg/ml of ethidium bromide).

Table 1: Details of Primers and PCR cycling conditions used in the study

Gene Target	Orientation	Primer Sequence (5'-……-3')	Cycling conditions	Amplicons Size	Reference
CaPV Viral attachment protein (*p32*)	Forward	TCCGAGCTCTTTCCTGATTTTTCTTACTAT	95 °C, 3 min; 34 cycles of 95 °C, 45 sec, 50 °C for 50 sec, 72 °C for 1 min; followed by 72 °C, for 10 min	192 bp	Ireland and Binepal, 1998 [13]
	Reverse	TATGGTACCTAAATTATATACGTAAATAAC			
ORF virus Major envelope protein (*B2L*)	Forward	TCCCTGAAGCCCTATTATTTTTGTG	94 °C, 3 min; 29 cycles of 94 °C, 1 min, 52 °C for 1 min, 72 °C for 1 min; followed by 72 °C, for 7 min	1206 bp	Hosamani *et al.*, 2006 [14]
	Reverse	GCTTGCGGGCGTTCGGACCTTC			

Results and Discussion

Sheeppox is a devastating, highly contagious disease of sheep causing severe economic loss due to huge mortality mainly in lambs, condemnation of skin, loss of wool and mutton, abortions and mastitis in ewes and trade restrictions (Bhanuprakash *et al*., 2006) [4]. Despite of the availability of many live vaccines, India is considered to be enzootic to sheeppox. The disease is characterised by pyrexia, generalised/circumscribed papules or nodules, vesicles (infrequently), lesions in internal organs (frequently in lungs), and death (OIE, 2008) [2]. Garner *et al*. (2000) [15] reported severe outbreaks of sheeppox in Maharasthra state (India) with 63.5 and 49.5% of morbidity and mortality rate, respectively during an outbreak with the total loss of INR 107.5 million.

ORF is one of the most prevalent, contagious pox viral disease of small ruminants with zoonotic potential. Yet, it is not a devastating disease. In general produce non-systemic, proliferative skin lesions most often around the muzzle and oral cavity. Though morbidity is very high, mortality is generally low to moderate. Gumbrell and McGregor (1997) [16] reported a mortality of 10 percent during an outbreak in lambs. This could have been attributed to inability to take feed due to oral lesions and resulting pain, anorexia and secondary bacterial infections.

In India, both sheeppox (Bhanuprakash *et al*., 2006; Manimaran *et al*., 2017) [4, 17] and ORF (Bora *et al*., 2011; Venkatesan *et al*., 2011; 2012) [6, 5, 18] were frequently reported. The current study describes the natural occurrence of dual infection of sheeppox and ORF in sheep. This type of mixed infection was reported earlier by few authors (Yeruham *et al*., 1998; Selim *et al*., 2016) [10, 11]. Earlier, the ORF

infection in goats was reported in high altitude from a hamlet nearby this organised farm (Balakrishnan *et al*., 2017) [19]. In the present investigation, clinical manifestations such initial pyrexia, edema of eyelids, discharge from nostrils, inappetence/anorexia, lacrimation, dyspnoea/ coughing were observed. Further, the affected animals showed characteristic pox lesions suggestive of both sheeppox and ORF. Initial papules progressed to vesicle and pustule which later ulcerated and resulted in scab formation.

The affected adult animals (n=46) showed typical sheeppox lesions confined to ventral abdomen, perineum, groin and udder (Fig. 1). Whereas, the affected suckling lambs (n=90) showed proliferative lesions only in the oral commissure and lips (Fig. 2). However, in the present investigation though total morbidity was reported to be 9.07%, in lambs less than 3 months old the morbidity was found to be 19.60%. A mortality of 12.22% was recorded only in lambs of preweaning age, which was mainly attributed to isolation from mothers, debility and weakness. Whereas, no mortality was reported among adult sheep.

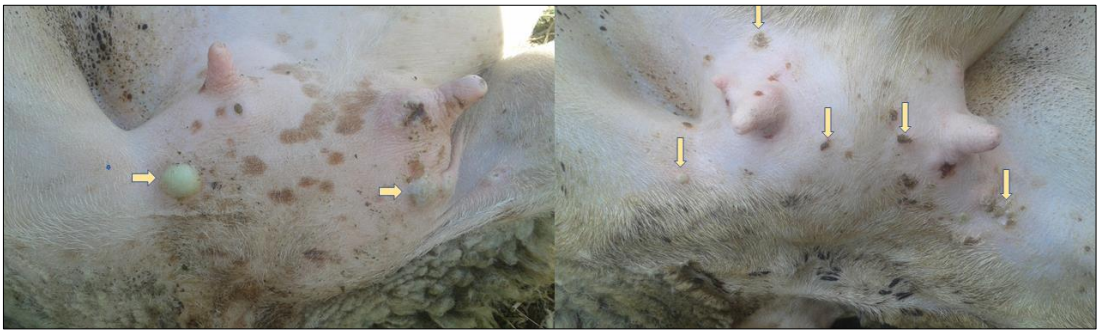


Fig 1: Typical pox lesions noticed on the udder and ventral aspect of the body of sheep

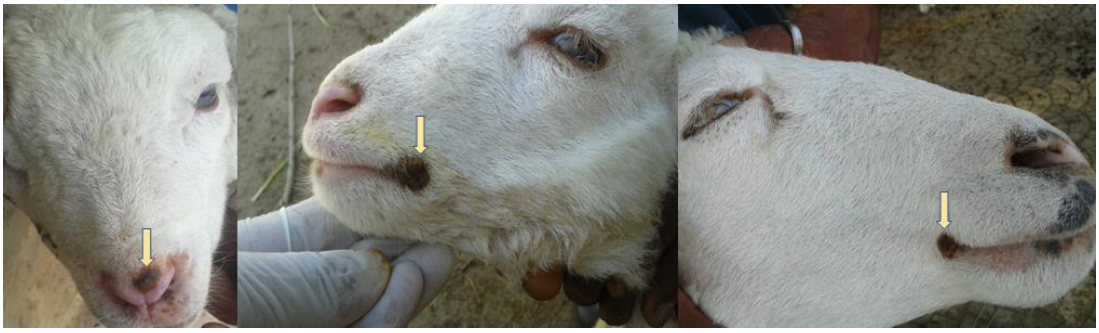
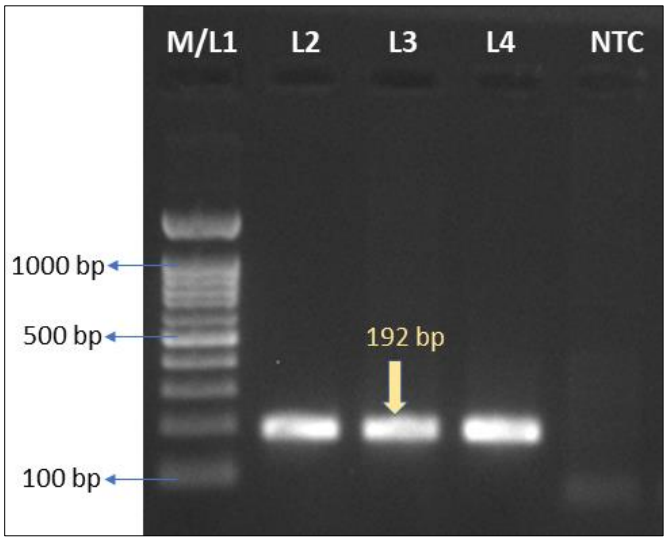


Fig 2: Proliferative lesions noticed on the lips and oral commissures of affected lambs and sheep

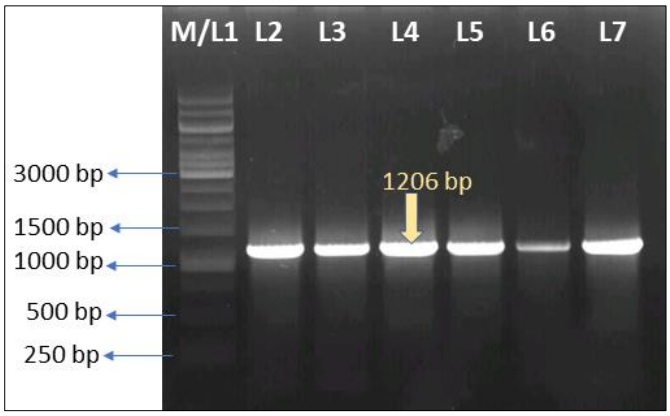
Though sheeppox is reported throughout the year, the outbreaks peak during winter and summer months in India (Bhanuprakash *et al*., 2006) [4]. Here also we have reported the outbreak during summer months of March-May. During histopathological examination, the typical intracytoplasmic inclusion bodies suggestive of sheeppox were observed in bronchiolar epithelium (n=2). Further, congestion of liver, lung, lymphnodes and pericardium, haemorrhages in the alveoli, and bronchopneumonia were also noticed.

Though both the diseases are readily diagnosed based on clinical signs and lesions, confirmation is based on laboratory tests. Sheeppox must be ruled out from bluetongue (BT), Peste des petits ruminants (PPR), ORF, photosensitization, mange infection and insect bites (Bhanuprakash *et al*., 2006) [4]. While, ORF must be differentiated from BT, foot and mouth disease, capripox and staphylococcal infection and hence warrants laboratory diagnosis (Hosamani *et al*., 2009; Bora *et al*., 2011) [7, 6]. In the present study, the conventional gel based PCRs confirmed the presence of both SPPV and ORFV, respectively. Capri poxvirus *p32* gene specific (Fig. 3) and ORF specific *B2L* gene based (Fig. 4) PCR assays yielded an amplicon size of 192 bp and 1206 bp, respectively. Out of 45 samples tested, 12 scabs were found positive for Sheeppox, 2 samples for ORF and 21 samples (11 lambs and 10 ewes) positive for dual infections.



Lanes L1/M: 100 bp DNA ladder; L2-L4: Positive samples NTC: Non template control

Fig 3: Capri poxvirus specific p32 gene based PCR



Lanes L1/M: 1 kbp DNA ladder; L2-L7: Positive samples

Fig 4: ORF virus specific *B2L* gene based PCR

Sheeppox is major hazzard for sheep husbandry and can best be controlled by live attenuated sheeppox vaccines than inactivated vaccines (Yogisharadhya *et al*., 2011) [3]. Hence, in our study too, the unaffected sheep were vaccinated against sheeppox to control the same. However, the animals without any clinical signs and lesions only were vaccinated after thorough clinical examination. While, the animals with fever, mild clinical signs and suspected to have contracted the viruses were omitted from vaccination and were kept isolated. ORF can also be controlled by vaccination as practiced in some parts of the world. However, in India though an indigenous live attenuated ORF vaccine was developed (Bora *et al*., 2015) [20], it is not available for use in the field. Further, symptomatic treatment of animals with antibiotics and antipyretics also provided little relief. The infections were well controlled by strict isolation, disinfection and disease manage mental practices along with proper nutrition.

In conclusion, the present study deals with the dual infection of sheeppox and ORF in an organised farm of the hilly region in the Nilgiris district of Tamil Nadu, a southern state in India. The infections were confirmed based on clinical signs and lesions, histopathology and PCR. The morbidity and mortality rates were reported to be 9.07% and 12.22% (in lambs), respectively. The flock was managed with symptomatic therapy and other control measures. Thus, proper vaccination and other disease manage mental practices are warranted in future to control these viral infections.

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