An outbreak of sheep pox in an organized farm of Tamil Nadu, India

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ABSTRACT
Outbreak of sheep pox was noticed in flocks of Khilakaraaisal in an organized government farm of Tirunelveli district during 2013. Mortality and morbidity rates were 4.17% and 80.00%, respectively. Both the sexes of sheep between 6 months to 2 years old were affected. DNA extracted from the scab specimens (n=5) were amplified using Capri pox specific p32 gene primers and yielded 192 bp product. In order to differentiate between sheep pox and goat pox PCR – RFLP was carried out. Digestion of PCR product with EcoRI enzyme yielded two fragments with a size of 123 bp and 63 bp which confirmed the presence of sheep pox virus. Histopathological examination of tissues revealed extensive hydropic degeneration of proliferating epidermal cells with presence of large number of intracytoplasmic eosinophilic inclusion bodies in the keratinocytes of epidermal cells in skin. Lung revealed large infiltration of lymphocytes and macrophages with presence of intracytoplasmic eosinophilic inclusions in the macrophages, proliferation of type II pneumocytes were prominent along with bronchiolar epithelial hyperplasia. The outbreak was controlled by effective preventive measures like vaccination of healthy animals, affected animals were treated with a course of antibiotic for 7 days, contaminated areas were disinfected and restricted the movement of animals from unaffected areas to infected areas.

Key words: Antibiotics, Clinical symptoms, PCR, RFLP, Sheep pox.

India has vast resource of sheep which play a vital role in improving the socio-economic conditions of small and marginal farmers and landless laborers in rural areas. The factor which hampers the growth of sheep industry is infectious diseases. Among the infectious diseases, sheep pox poses main threat to the small ruminant industry. Sheep pox is a malignant cutaneous disease of sheep caused by a virus, a member of the genus Capri pox virus (Murphy et al., 1995). The disease causes skin defects, abortion, mastitis in ewes and high mortality in lambs (Roy et al., 2008). The present paper describes natural outbreaks of sheep pox in Khilakaraaisal breed of sheep in an organized government farm of Tirunelveli district and identification of etiological agent by PCR-RFLP.

The disease outbreaks: An outbreak of sheep pox was noticed in Khilakaraaisal breed of sheep in an organized government farm of Tirunelveli district. Total population in the farm was 180 sheep during 2013. The mortality rate observed was 4.17% and morbidity was 80.00% in sheep. Both the sexes of sheep between 6 months to 2 years old were affected. The affected animals were segregated and treated with strepto-penicillin parentally for 7 days and neem oil was applied locally on skin lesions. Animals in nearby farms were vaccinated with sheep pox vaccine. Restricted the movement of animals from unaffected to infected areas. Outbreak was controlled after about one month of treatment.

Samples collected: Nasal swabs, conjunctival swabs and skin nodule biopsies were collected from live animals in ice and tissue samples from skin, lung, heart, lymph nodes, intestine, kidney and spleen were collected in ice and 10% formalin and routinely processed for sectioning and 4 micron thick sections were cut and stained with Haematoxylin and Eosin method for histopathological examination (Bancroft and Gamble, 2008).

Polymerase chain reaction (PCR): DNA from scab suspension was extracted using Qiagen DNA extraction kit (QIAmp DNA minikit®) as per manufacturer’s protocol. The extracted DNA was amplified using Capri pox specific p32 attachment gene primer (F- 5’- TTT CCT GA T TTT TCT TAC TA T-3’ and R- 5’- AAA TTA TA TAC GTAAATAAC-3’)(Ireland and Binepal, 1998). PCR reaction was carried out in 50µl volume containing 25 µl of 2X red dye master mix (Ampliqon®), 2 µl (10 picomoles/µl) of each primer, 13 µl of DEPC water and 8 µl of DNA. The reaction mix was placed in an Eppendorf® thermal cycler with the following condition 94°C for 1min 35 cycles of 94°C -1 min, 54°C-30-sec, 72°C-1 min; and 72°C for 5 min. The amplified product was electrophoresed on 1.5% agarose gel containing ethidium bromide at 90 V for 45min and then visualized using Bio-Rad Molecular imager Gel doc® XR4™.

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PCR-RFLP: PCR-RFLP was carried out as per the procedure described by Fulzele et al. 2006. Briefly 15 µl of PCR product was mixed with 9 µl of 1X NE buffer and 1 µl (10U) of EcoRI-HF enzyme (New England Biolabs® inc). Incubated at 37°C for 1hr and inactivated at 65°C for 20min. The digested product was eletrophorosed on 2% agarose gel containing ethidium bromide at 90V for 45min and then visualized using Bio-Rad Molecular imager Gel doc® XR+.™

Clinical signs and necropsy findings: The clinical signs observed were cough, sneezing, bilateral mucopurulent nasal discharge with high body temperature, hair loss, generalized raised skin nodules on body and anorexia. Cutaneous lesions were mainly observed around nostrils, eyes, lips, ears and on the abdomen. Most of the lesions were covered with purulent material and on cleaning with sterile swabs, fresh wounds were exposed. Dry scabs were also observed over the oral commissures and extensive lesions resulted in tissue sloughing off and wounds around nostrils. Both the sexes of Khilagaraisal breeds of sheep between 6 months to 2 years old were affected. Necropsy lesions in Khilagaraisal breeds of sheep was nodular lesions in lungs.

The mortality rate in the present outbreaks was in accordance with Australian cross (4.5%) recorded in India (Mondal et al., 2004), Roy et al., (2008) observed in Madras Red and Mechery breeds of Indigenous sheep in Tamil Nadu. The cutaneous lesions were found extensively and were often associated with wounds. The nodular lesions were not only restricted to the cutaneous surface but were also seen in the lungs which has resulted in pneumonia. The outbreaks were controlled by about 30 days after segregation of the animals, application of neem oil locally to skin lesions and supportive therapy.

Polymerase Chain reaction (PCR) and Restriction Fragment Length Polymorphism (RFLP): The DNA obtained from all the five scab specimens were amplified by capripox specific p32 attachment gene primer and yielded 192 bp product (Fig.1). Restriction enzyme digestion of field sample and sheep pox control with Eco-RI enzyme yielded 129 bp and 63 bp (Fig.2) for field samples and sheep pox positive control. Earlier, the diagnosis of goat pox can be made mainly based on clinical signs and serological tests. But, it is difficult to differentiate sheep pox and goat pox based on the conventional methods and differentiation is highly essential for effective control of sheep pox especially in the areas where the sheep and goat are reared together. Hence, PCR –RFLP was carried out for the differentiation of sheep pox and goat pox. PCR –RFLP results confirmed that the present outbreak was due to sheep pox virus which corroborates with the findings of Ireland and Binepal, (1998).

Histopathological examination revealed hyperplasia of epithelium of skin with ballooning degeneration, vesicle formation and necrosis with intracytoplasmic eosinophilic inclusion bodies in the epidermal cells (Fig.3). The dermis revealed fibroplasia with a moderate infiltration of lymphocytes, plasma cells, histiocyte and a few neutrophils. The bronchi and bronchioles showed varying degrees of epithelial hyperplasia. Alveolar septa was thickened, septal pneumocytes contained intracytoplasmic eosinophilic inclusions, alveola lumen were filled with numerous viable and degenerate neutrophils and moderate numbers of...
Fig 3: Sheep-Skin-Hydropic degeneration and presence of intracytoplasmic eosinophilic inclusion bodies in the epidermal cells HE x 400 X

Fig 4: Sheep-Lung - Intracytoplasmic eosinophilic inclusion bodies in pneumocyte

macrophages that had large nuclei and abundant, palely stained, foamy cytoplasm containing eosinophilic intracytoplasmic inclusion bodies (Fig.4). Lumen was filled with numerous neutrophils and mucinous exudates. The gross and histopathological findings were in concurrence to those reported earlier (OIE,2008; Zangana and Abdullah,2013 ; Chanie,2011). In conclusion, the outbreak was controlled by effective preventive measures like ring vaccination of susceptible animals, affected animals were treated with a course of antibiotic for 7 days, contaminated areas were disinfected and restricted the movement of animals from unaffected areas to infected areas

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REFERENCES


