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Short Communication

MOLECULAR DIAGNOSIS AND THERAPEUTIC MANAGEMENT OF CONTAGIOUS ECTHYMA IN BEETLE GOAT

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SUMMARY

Contagious ecthyma an important contagious disease with zoonotic importance caused by Orf virus, an epitheliotropic virus of poxviridae family. It affects primarily sheep and goat and easily transmitted to man. This study investigated an outbreak of orf virus in a herd of 30 goats, its diagnosis through typical clinical signs, virus specific PCR as well as successful management and treatment. Affected animals showed signs of hypersalivation, pain and fever. Animal became anorectic and there was drastic reduction of milk yield in milking goats. There were typical lesions as in other pox. Affected animals were treated with antibiotics and supportive therapy. Managemental advice was also given to the farmer to segregate the infected animals and to avoid direct contact with animals. It is concluded that clinical lesions indicative of Contagious ecthyma or orf virus disease, which can be confirmed by PCR. Lesions should be cleaned by weak solution of potassium permanganate (0.1%) and a topical application of antiseptic fly repellent cream. All the animals were recovered within 7 days.

Keywords: Contagious, Ecthyma, Goat, Orf, Sheep

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The Orf virus (genus parapoxivirus, Poxviridae family) disease is of zoonotic importance which spread from sheep and goat to man (Murphy *et al*., 2012).The disease has been reported throughout all seasons and across the world (Nandi *et al*., 2011). However, the outbreaks of the disease are more prevalent in grassland system of grazing and are frequently noticed in late summer, autumn and winter. It has wide host range affecting wild animals along with natural host such as sheep and goat (Sharma *et al*., 2016). The affected animal manifests painful lesions around mouth, tongue oral cavity, muzzle and perineal area and results in anorexia (Nandi *et al*., 2011).The probability of occurrence of orf infection is more in new born lamb or within a period of 3 to 4 months after birth (Lovatt *et al*., 2012). Herd prevalence rates of this disease are usually higher in goats than that of sheep (Scagliarini *et al*., 2012). It is highly contagious and debilitating disease affecting the economy of many developing countries, including all the geographic regions of India (Gelaye *et al*., 2016).

History and clinical observations

The Contagious Ecthyma (CE) occurred in a herd of thirty (30) goats at Rewasa, Mahendergarh, Haryana, India. Out of thirty goats, 8 were affected with typical signs of disease. Seven animals were of age from 7 month to 1 year and one was of 1.5 year old. Out of 8 affected animals, 2 were male and 6 female. Owner had already applied mustard oil and turmeric to all affected animals and 4 animals showed recovery signs.

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Affected animals showed signs of anorexia, hypersalivation, pain and fever around 104 °F. There were typical skin lesions like erythema, papule, vesicle, pustule, and scabs on mouth, tongue, oral cavity, muzzle, lids of eye, perineum and scrotal area (Fig. 1). Lesions were highly vascular and bleed easily.

The clinical scab samples were collected from eight (n=8) affected goats exhibiting symptoms of CE and scratching superficial crust over the lesions and immediately transferred to cryogenic vials and stored at -20 °C till analysis.

Since all affected animals were from same flock hence, we pooled all the scabs into two groups during processing. The two pooled scab samples were initially triturated and homogenized in TEbuffer (pH 8.0). The suspension was spun for 30 sec in the micro centrifuge (10,000×g). The supernatant obtained after centrifugation was used for viral DNA extraction. The total DNA was extracted from homogenised pooled scabs by using commercially available DNA extraction kit (Invitrogen). The extracted DNA was stored at -20 °C until used.

The major envelope membrane glycoprotein (B2L) gene of the virus was used in the molecular diagnosis. PCR amplification of partial B2L gene was done by the standard protocol as described by Inoshima *et al*. (2000) with some modifications. This protocol amplified partial sequences of the B2L gene by using a set of 3 primer pairs in a semi-nested PCR format. During the first round of amplification, a set of pan-parapoxvirus primer-1 (PPP-1/B2L) and pan-

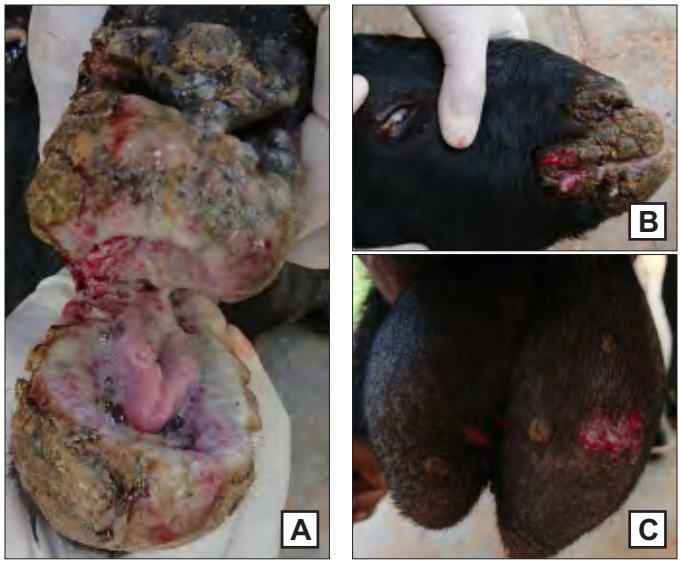

Fig. 1. A. Contagious ecthyma in beetle goat (*Capra aegagrus hircus*) having massive proliferative lesions in oral cavity, B: on nostrils, mouth and eye lids and C: on scrotum.

parapoxvirus primer-4 (PPP-4/B2L) pair was used to generate an amplicon of 594 bp (Table 1). The PCR thermal cycling parameters used were: initial denaturation at 95 °C for 3 min, 35 cycles of denaturation (94 °C, 1 min), annealing (55 °C, 45 sec) and extension (72 °C, 45 sec), final elongation (72 °C, 10 min) and the PCR products were kept at 4 °C until used.

The Orf viral gene specific PCR amplicons were visualized in 1% agarose gel electrophoresis by ethidium bromide staining. The appropriate size or correct band was cut and gel purified using Qiaquick\gel extraction kit (Qiagen) as per manufacturer's protocol.

Affected animals were treated with enrofloxacin antibiotic (2.5 mg/kg) to combat secondary bacterial infections along with other supportive treatment of analgine and ascorbic acid. Enrofloxacin was used because it is broad-spectrum and cheap. Lesions were cleaned by potassium permanganate (0.1%) and topical antiseptic fly repellent cream was applied. It was also recommended to the animal owner to strictly follow the standard hygiene practices in order to control orf virus disease outbreak in flock. Every animal with sign of disease should be kept separately from the rest of animals. As diseases is zoonotic, it was advised that animal handler should wear protective gloves and mask. Infected area should be disinfected using 4% sodium hydroxide. All the affected goats recovered within 7 days.

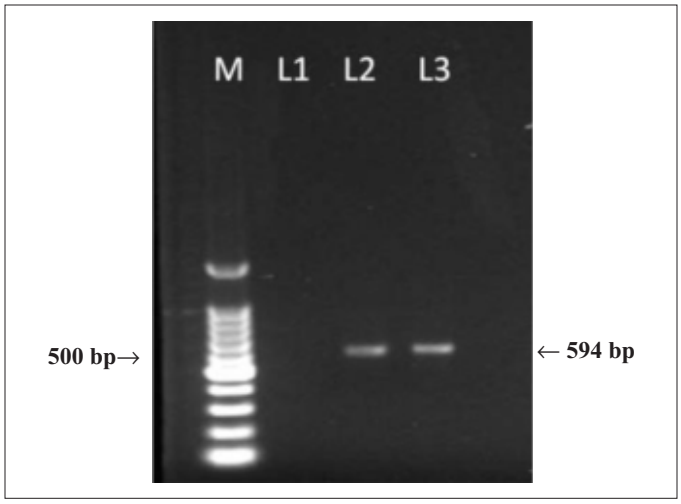

Fig. 2. Electropherogram showing expected size of amplicons of Orf specific. (594 bp product size). M: 100 bp DNA ladder, L1: non-template/negative control. L2-3: representative field samples

The partial B2L gene specific primer PPP1- PPP4 based PCR was successfully amplified and desirable DNA band of 594 bp was obtained in both the pooled scabs sample (Fig. 2).

Despite of great financial impact (Bennet and Ijpellar, 2005), Orf virus disease or CE does not seems to be a serious disease and is not notifiable disease. Many cases of this disease go unnoticed and not registered specially in developing countries. Orfvirus is epitheliotropic and cause vacuolization and hypertrophy in keratinocytes from the external spinous layer of the epidermis results in reticular degeneration with marked epidermal proliferation with intra-epidermal and intra-dermal micro-abscesses and crust formation on the surface (Fleming *et al*., 2015). The diagnosis of CE is based on the clinical signs with typical lesions. However, it can be confused with other vesicular diseases like bluetongue, foot and mouth disease, pox, papillomatosis, staphylococcal dermatitis and dermatophilosis. So, fast and accurate differential diagnosis is necessary which not only allows adopting control measures timely for animals but also prevents zoonotic infection. Molecular results based on PCR with viral specific gene primer confirm the disease and also differentiate from other similar diseases. Previously, different outbreaks of the disease have been reported. Maan *et al*. (2014) isolated and characterized orf virus

Table 1

List of different primer pairs used in PCR amplification of extracted viral nucleic acid

Sr. No.	Primer name	Primer sequence (5'–3')	Product size
Primer pair 1	PPP-1/B2L forward	GTCGTCCACGATGAGCAGCT	594 bp
	PPP-4/B2L reverse	TACGTGGGAAGCGCCTCGCT	

from an outbreak of Rajasthan. Another outbreak of caprine contagious ecthyma in a flock of Amritsari goats has been reported from Hisar district of Haryana (Kumar *et al*., 2016). Contagious ecthyma with similar lesions as we observed, has been reported in sheep and mountain goat and Sitka black-tailed deer (Tryland *et al*., 2018). It is an important zoonotic disease and there are several reports available of human infection from animals (Kitchen *et al*., 2014; Turk *et al*., 2014). So, its public health importance should not be ignored and extra care should be taken during handling of animals that are supposed to be infected with orf.

CONCLUSIONS

Orf virus is circulating among goat population in south Haryana. It can be easily diagnosed by clinical lesions which can extend to eyelids and scrotum beside mouth, muzzle, nostrils and tongue. Confirmatory diagnosis can be done using virus specific PCR and disease can be treated successfully with antibiotic and supportive therapy.

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