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Sneh Lata Chauhan
Scientist, Department of Veterinary Clinical Complex, LUVAS, Hisar, Haryana, India

Divya Agnihotri
Assistant Professor, Department of Veterinary Clinical Complex, LUVAS, Hisar, Haryana, India

Kanisht Batra
Assistant Professor, Department of Animal Biotechnology, LUVAS, Hisar, Haryana, India

Maneesh Sharma
Assistant Professor, Department of Veterinary Clinical Complex, LUVAS, Hisar, Haryana, India

Tarun Kumar
Assistant Professor, Department of Veterinary Clinical Complex, LUVAS, Hisar, Haryana, India

Neelesh Sindhu
Assistant Professor, Department of Veterinary Clinical Complex, LUVAS, Hisar, Haryana, India

Vinod Kumar Jain
Professor and Head, Department of Veterinary Clinical Complex, LUVAS, Hisar, Haryana, India

Corresponding Author:
Sneh Lata Chauhan
Scientist, Department of Veterinary Clinical Complex, LUVAS, Hisar, Haryana, India

Detection and therapeutic management of contagious ecthyma (ORF) in a kid

Sneh Lata Chauhan, Divya Agnihotri, Kanisht Batra, Maneesh Sharma, Tarun Kumar, Neelesh Sindhu and Vinod Kumar Jain

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Abstract
A kid of age two and a half months was presented to Veterinary Clinical Complex (VCC), of Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana with history of skin lesions on fore head and nasal planum, along with alopecia and pruritis since one month. On physical examination, the lesion was observed to be dry and scabby in appearance. History from the animal owner revealed more number of similar cases of skin lesions in same age group of kids, suspecting it to be contagious. Skin scabs on molecular investigation revealed an Orf infection to the kid. The PCR product of 594bp size was generated. The kid was treated with broad spectrum antibiotic along with symptomatic and supportive treatment till complete clinical recovery. The animal owner was briefed about the zoonotic implications of the disease and was advised to take proper hygienic measures while handling the kids.

Keywords: contagious ecthyma, kid, Orf, PCR, zoonotic

Introduction
Contagious pustular dermatitis (CPD) also known as contagious pustular stomatitis, contagious ecthyma, ecthyma contagiosum, infectious pustular dermatitis, infectious labial dermatitis, sore mouth, scabby mouth and orf (Murie 2011) [15] is a severe exanthematic viral disease of small domestic ruminants caused by a Orf virus (OV), a member of parapoxvirus genus belonging to family Poxviridae (Mohammad *et al*., 2016) [13].

Virions are cocoon shaped 160nm in width and 260nm in length and covered with long thread like surface tubules resembling a ball of yarn. The viral genome, one of the smallest in Poxviridae family, is composed of 140 kbp linear ds DNA with closed hairpin loop ends and genes located on both strands with a bidirectional orientation (Nandi *et al*., 2011, McInnes *et al*., 2001, Robinson *et al*., 1982) [18, 10, 20]. Parapoxviruses are distinguished from other poxvirus genera by crisscross pattern on the particle surface, the relatively small size, their ovoid shape and the high G+C content (approximately 64%) of the genome (Kottaridi *et al*., 2006) [9]. Parapoxviruses are sensitive to ether, chloroform, benzene and toluene and are resistant to glycerol and survives for several months in dry and cool environment but is destroyed by high and very low temperatures, wetting and UV light. The virus resists physical damage and persists through the winter months on hedges, feeding troughs and barns. It can be inactivated in 30 minutes at 60◦C. Orf virus on shaded ground retains infectivity for years, whereas sunlight exposed scab remains infective for longer period. The virus can retain infectivity about 15 years, at room temperature (Nandi *et al*., 2011, McKeever and Reid 1986, McKeever *et al*., 1988, Buxton A and Fraser G. 1977) [18, 11, 12, 2].

The disease is highly contagious, affects small domesticated and wild ruminants and is more severe in goats as compared to sheep (Nandi *et al*., 2011) [18]. As per World Organization for Animal Health, Orf is a notifiable and zoonotic disease transmitted from animals to humans Nadeem *et al*., 2010) [17]. It is known to cause a highly contagious and debilitating disease affecting the economy of many developing countries, including all the geographic regions of India (Guo *et al*., 2003, Mondol *et al*., 2006, Hosamani *et al*., 2007, Venkatesan *et al*., 2011, Gelaye *et al*., 2016) [5, 14, 7, 22, 3]. The causative Orf virus has been extensively investigated over recent years because of its growing host-range and zoonotic importance (Turrini *et al*., 2010) [21].

Material and Methods

A male kid of two and a half months of age was brought to the Veterinary Clinical Complex of Lala Lajpat Rai University of Veterinary and Animal Sciences, with the history of itching, appearance of small nodules on forehead and nasal planam, and alopecia since one month. Appetite and other physiological parameters of the affected kid were found to be normal. There was a history of contagious nature of the skin lesions as the owner reported that other kids were also affected, but were not brought to the clinics. Skin scrapings were collected in duplicate10% potassium hydroxide (KOH) mounted microscopic examination and second sample was used for suspecting it to be a case of CPD, the skin scrapping/scabs were aseptically collected with sterile scalpel blade in phosphate buffer saline (PBS, pH 7.2) from the lesion on the forehead and processed for conducting molecular investigations/PCR. Blood was also aseptically collected from the jugular vein in EDTA containing vial for performing CBC.

DNA Extraction: The viral DNA was extracted from scab samples collected in the PBS using PureLink™ Genomic DNA isolation Kit (Invitrogen) according to manufacturer's instruction. The extracted DNA was eluted in 30 µl of PureLink™ Genomic elution buffer provided with the kit. The DNA extracted was stored at -20 ^{0}C till further use.

PCR Diagnosis: The envelop membrane glycoprotein (B2L) gene of the ORF virus was used for the molecular diagnosis. For PCR amplification, the standard protocol as described by Inoshima *et al*. 2000 [8] was used. This protocol involves amplification of partial sequences of the B2L gene with two set of primers detecting pan-parapoxvirus such as primer 1 (PPP-1) and PPP-4. This primer pair generates an amplicon size of of 594 bp. For this PCR, following cycling parameters were used 95 ^{0}C for 3 min, 40 cycles of denaturation (94 ^{0}C, 30 sec), annealing (54 ^{0}C, 1 min) and extension (72 ^{0}C, 1 min) were used. The final elongation was done at 72 ^{0}C for 10 min.

Results and Discussion: Skin scraps in KOH mount smear were found negative for the presence of ectoparasites. The kid suspected of ORF viral infection was screened for the presence of ORF using published primers targeting B2L gene. The expected amplicon size of 594 bp was generated from the isolated DNA after PCR amplification as given in Fig.1

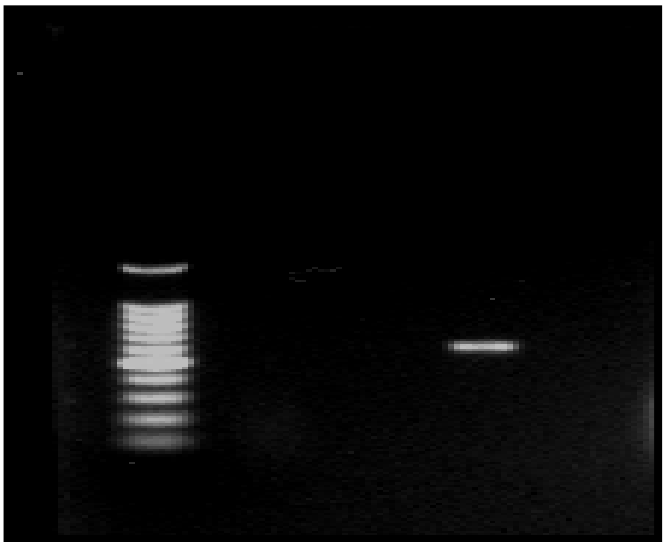

Fig 1: PCR amplification of B2L gene using PPP1 and PPP4 primer-pair (Pair 4) listed by Inoshima *et al*. 2000[8] showing amplification of 594 bp products

Result of the hematological parameters studied are depicted in the table 1 below. Blood picture revealed leucocytosis, neutrophilia and lymphopenia. Other parameters were found to be in the normal range.

Table 1: Blood picture of the kid affected with contagious ecthyma

Parameters	Diseased goat	Reference(Merck's Veterinary Manual 10th edition)
HB (g/dl)	9.1	8-12
PCV (%)	25	22-38
TLC × 10^3/mm^3	21.44	4-13
DLC (%)		
N	65	30-48
L	32	50-70
M	1	0-4
B	2	0-1
E	0	1-8

The Orf virus is epitheliotropic and has an affinity for the skin. The disease is manifested by proliferative lesions on the muzzle and mouth that usually resolve in 1–2 months. Primary lesions are more severe with a clinical progression of erythematous macule, vesicle, papule, pustule and scab formation in 4–6 weeks and in young animals in their post weaning period are at greater risk than others (Bharathy and Akila. 2015) [16]. Different degrees of proliferative lesions are found in different parts of the body of affected animals. Lesions usually crust over, rapidly become growing scabs, and heal spontaneously within 4 weeks (McKeever *et al*., 1988) [12]. Re infection lesions progress through the same clinical stages, but are not proliferative and are generally smaller, usually resolving rapidly within 2–3 weeks (Nandi *et al*., 2011) [18]. Orf frequently affects young ones during post weaning period. Lesions of the disease are most commonly seen on the lips and mouth of infected animals, but lesions may also occur on the udder and between the toes. In the present case the lesions were observed on forehead and nose (Fig.2 and Fig.3). The disease is not normally fatal but is a debilitating disease but under circumstances when the lambs and kids are deprived from suckling or succumb to secondary bacterial or fungal infections the disease can be fatal (Mohammad. *et al*., 2016) [13]. Extensive foot lesions may lead to lameness. Acquired immunity is reported in most of the animals after contracting the disease (Nandi *et al*., 2011) [18]. Animals that contact the disease usually develop a strong immunity and will not be re-infected for at least one year (Alam *et al*., 2016) [1]. The infection occurs by direct contact and indirect contact from infected animals or by contact with infected saliva or tissue containing virus. Zoonoses occur most frequently during lambing, docking, shear-ing, drenching or slaughtering of affected animals. Financial losses are related to decline in production devaluation of meat, leather and wool in national and international markets, as well as costs related to being zoonotic (Nandi *et al*., 2011, Haig *et al*., 2002 Gumbrell *et al*., 1997) [18, 6, 4].

It has been reported that mortality from this disease may reach 10% and 93% in kids and newborn lambs, respectively that are complicated by secondary infection (Alam *et al*., 2016) [1]. The disease starts with water filled blebs that eventually become pustules. When the pustules break, raised brown scabs form over the resulting wounds. The treatment was administered aiming to reduce the severity of the lesions and for early recovery without any secondary complication (Nandi *et al*., 2011, Radostits *et al*., 2007) [18, 19]. The supportive

treatment included the topical application of ointments and systemic antimicrobial administration against secondary bacterial complications. The owner of the affected kid was advised to keep the kid and other affected animals in isolation and to take proper precautions while handling the animals as this could be a zoonotic infection. The kid was treated with antibiotic (Cephalosporin) to prevent secondary bacterial infection, anti-inflammatory drugs (Meloxicam) and antihistamine (Pheniramine maleate), Vitamins B complex and Vitamin C as supportive therapy. Broad spectrum topical ointment was prescribed for the skin lesions. Complete clinical recovery was observed after 2 weeks of treatment.

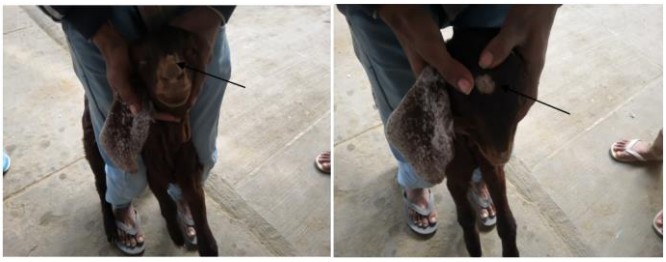

Fig 2-3: Kid showing lesions on the nasal planum and forehead (arrow) respectively

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