



# Epidemiology, clinical features, and molecular detection of orf virus in Haryana (India) and its adjoining areas

Ramesh Kumar<sup>1</sup> · Pallavi Moudgil<sup>1</sup> · Kushal Grakh<sup>1</sup> · Naresh Jindal<sup>1</sup> · Maneesh Sharma<sup>2</sup> · Renu Gupta<sup>1</sup>

Received: 27 April 2022 / Accepted: 29 July 2022 / Published online: 18 August 2022  
© The Author(s), under exclusive licence to Springer Nature B.V. 2022

## Abstract

Orf is an acute, highly contagious, and economically important viral disease of small ruminants. In this study, six orf suspected outbreaks among goats and sheep were investigated from Haryana state and adjoining areas of Rajasthan state during the year 2021. The disease was diagnosed on the basis of clinical signs and molecular identification. The causative agent of the disease, orf virus (ORFV), was confirmed using polymerase chain reaction (PCR) targeting immunodominant envelope antigen (B2L) gene and confirmed by sequencing. The morbidity in goats ranged from 8.75 to 100%, whereas in sheep, it ranged from 0 to 8%. The higher mortality was observed among flocks with mixed infections of orf and peste des petits (PPR) or orf and haemonchosis as compared to other outbreaks. The phylogenetic analysis of sequenced PCR products clustered the current study strains in the same clad with Indian as well as strains from other countries with nucleotide identity more than 99%, signifying a close genetic relationship. The study highlighted the circulation of strains of a single cluster among sheep and goats in Haryana and adjoining areas. Prompt diagnosis of the disease is highly important for facilitating the implementation of control measures to minimize the losses suffered by small and marginal farmers in this region. Further detailed studies are required to delineate the molecular details of ORFV for better understanding the dynamics and molecular epidemiology of strains circulating in the country and for designing the effective vaccines against the disease which are currently lacking in the country.

**Keywords** Immunodominant envelope antigen · Orf · Outbreaks · Small ruminants

## Introduction

Orf also known as sore mouth, contagious ecthyma (CE) or contagious pustular dermatitis is an acute, highly contagious, and economically important viral disease of small ruminants (Gelaye et al. 2016). The disease is caused by orf virus (ORFV), a member of genus *Parapoxvirus*, family *Poxviridae* (Fleming et al. 2015). The ORFV is epitheliotropic and hardy in nature and remains viable for months or even years when environment is favorable and the transmission

of the virus generally happens through direct contact from infected to susceptible animals (Nandi et al. 2011). The lesions in the form of abrasions develop on or around the buccal cavity due to feeding on dry hay or other hard fodder might allow ORFV to enter and initiate the infection, when comes in contact with infected animals (Bala et al. 2019). In young animals, there might be other possible routes of transmission such as saliva or milk which remained unexplored in the literature (Ma et al. 2022). The disease has zoonotic implications, causing localized ulcerative lesions or nodules on the hands of high-risk individuals such as veterinarians, farmers, animal handlers/caretakers, and slaughterhouse workers (Bayindir et al. 2011; Bergqvist et al. 2017). The infection in humans in the regions with small ruminant population has been reported worldwide (CDC 2006). Animals infected with ORFV develop lesions including sores or erythematous lesions around lips, muzzle, and in buccal cavity, which progresses to develop into scabs (Zhang et al. 2010). Occasionally, the lesions can be found on the teats and rarely on other organs (Vikoren et al. 2008). The orf outbreaks

✉ Renu Gupta  
renu.gupta@luvas.edu.in

<sup>1</sup> Department of Veterinary Public Health and Epidemiology, College of Veterinary Sciences, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar 125004, Haryana, India

<sup>2</sup> Department of Veterinary Clinical Complex, College of Veterinary Sciences, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar 125004, India

were reported across various Indian states, viz., Uttarakhand (Venkatesan et al. 2011), Assam, Meghalaya (Bora et al. 2012), Haryana (Kumar et al. 2013), Uttar Pradesh (Kumar et al. 2014), Rajasthan (Maan et al. 2014), Tripura (Venkatesan et al. 2018), Kashmir (Ahanger et al., 2018), Tamil Nadu (Nagarajan et al. 2019), and Odisha (Sahu et al. 2022). The ORFV circulating in India had been clustered with Indian and Chinese isolates using phylogenetic analysis previously (Maan et al. 2014; Sahu et al. 2022). Usually, CE is self-limiting in sheep and goats, but long-standing cases may provide opportunity for bacterial pathogens to invade the lesions leading to complications (Haig and Mercer, 1998). The genome of ORFV is ~ 138,000 bp long, rich in GC content, and encodes 132 genes. Various virulence factors have been identified for ORFV including IL-10-like gene, vascular endothelial growth factor (VEGF), GIF, apoptosis inhibitor, and IFN resistance gene (Fleming et al. 2015). The envelope gene (B2L) encodes for highly immunogenic envelope protein and has been used previously for molecular diagnosis, characterization, and phylogenetic studies (Oem et al. 2013; Maan et al. 2014). The previous studies in the region were restricted to single outbreak, which might limit the epidemiological understanding and genetic diversity of ORFV.

Haryana is a northern state of India spanning between 27° 39' and 30° 35' N latitude and 74° 28' and 77° 36' E longitude. The state houses ~ 625,100 sheep and goats (DADF 2019). Some parts of the state share boundary with Rajasthan, state that houses maximum number of goats in India, and total sheep and goat population stands at ~ 28,744,000 (DADF 2019). There is a regular movement of animals across the states through nomads and/or through purchase of animals. In India, the close proximity of animal caretakers and animals leads to frequent contacts

and thus increases the risk of transmission of infection to humans.

In this study, the authors present the epidemiological and clinical features of outbreaks of CE investigated at Disease Investigation (DI) Laboratory, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana, India, during the year 2021 along with its molecular detection and confirmation.

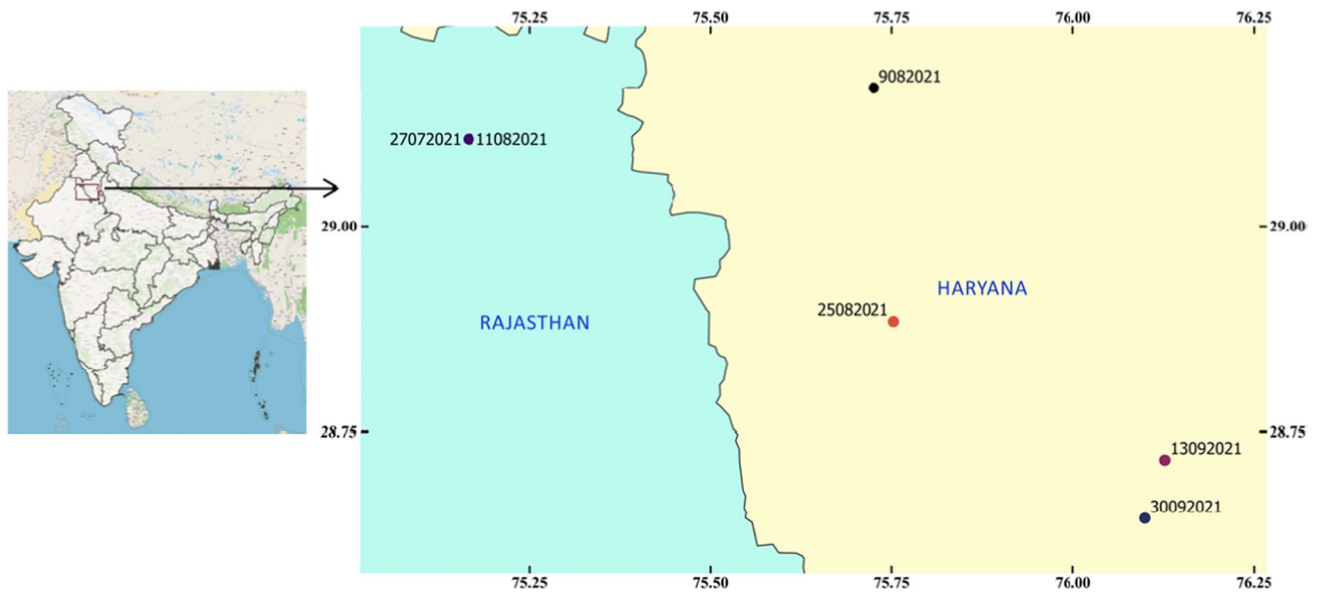
## Materials and methods

### Epidemiological data collection

Six outbreaks of CE were investigated by the laboratory during the year 2021 (Table 1, Fig. 1). The outbreaks were reported by the government-appointed veterinarians (State Animal Husbandry Department) to the DI laboratory of the LUVAS, Hisar. The existence of the outbreak was ascertained by the history of cases in previous 6 months in the herd. Each outbreak included one herd. An outbreak ID was provided to each suspected orf outbreak which corresponds to the reporting of outbreak and date of collection of samples at the laboratory. The farmers were regularly contacted for collection of information related to any new cases and mortality. The data pertaining to the location of farmed animals, total animal population, animals affected and died, history of the cases observed, possible source of infection, any new introduction to the farm/purchase of animals, management, and environment factors was collected through a questionnaire pre-designed for such events. Clinical signs as reported by the owner and directly observed by the investigators were also recorded.

**Table 1** Details of orf outbreaks investigated

Outbreak ID	Location	Date of start of event	New entrance in animal population	Remarks
27072021	Bhadra, Hanumangarh, Rajasthan	10/7/21	Yes (1/7/21) All from Ajmer, Rajasthan (the supplier purchased from different sources)	Peste des petits (PPR) and foot and mouth disease (FMD): negative
9082021	Shastri Nagar, Hisar, Haryana	25/7/21	No	-
11082021	Bhadra, Hanumangarh, Rajasthan	1/8/21	Yes (~4 months back)	PPR: negative
25082021	Dariyapur, Bhiwani, Haryana	15/8/21	No; but animals in the neighboring farms had similar problem	PPR, <i>Pasteurella multocida</i> (B2) and <i>Mycoplasma</i> spp. negative Fecal test: positive for haemonchosis
13092021	Roopgarh, Bhiwani, Haryana	29/8/21	Yes (~1 month back); 57 in no	PPR: positive Fecal test: presence of coccidia oocyst
30092021	Sarangpur, Bhiwani, Haryana	30/8/21	Yes (~4 months back) from Dadri (Haryana), Ajmer (Rajasthan)	PPR: positive



**Fig. 1** Map showing locations of the outbreaks (dots) investigated

### Sample collection and analysis

The clinical samples including skin scabs, blood in EDTA and blood without anticoagulant were collected from the animals and brought to DI laboratory. The collected samples were processed for the detection of ORFV. The blood samples were also tested for hematological parameters. Fecal samples were tested for the presence of any parasitic egg or oocyst.

### DNA extraction and polymerase chain reaction (PCR)

DNA from scab samples was extracted using commercially available kit (DNeasy Blood & Tissue Kits, Qiagen, Germany) according to the manufacturer's instructions. The extracted DNA was subjected to PCR using previously published primers (Forward: 5'-GTCGTCCACGATGAGCAGCT-3' and Reverse: 5'-TACGTGGGAAGCGCCTCGCT-3') targeting immunodominant envelope antigen (B2L) (Gunther et al. 2017). PCR amplification of the target gene was carried out using commercial PCR kit (Takara, Japan) in 25  $\mu$ L reaction volume containing 12.5  $\mu$ L master mix, 5 pmol of each forward and reverse primer, and ~100 ng template DNA and adjusted to final volume with nuclease free water. PCR conditions were standardized as follows: initial denaturation at 95  $^{\circ}$ C for 5 min followed by 35 amplification cycles of denaturation at 94  $^{\circ}$ C for 1 min; 54  $^{\circ}$ C for 1 min, 72  $^{\circ}$ C for 1 min, with final extension at 72  $^{\circ}$ C for 7 min and hold at 4  $^{\circ}$ C. Amplified PCR products were gel electrophoresed in 1.5% agarose gel stained with ethidium bromide (0.5  $\mu$ g/ml) along with 100 bp molecular weight marker (Genei, India). The PCR amplified products were visualized using gel documentation system (Zenith, India).

### Sequencing and phylogenetic analysis

The PCR products were purified using Gel extraction kit (Qiagen, Germany) and then got custom sequenced in both directions (AgriGenome Labs Pvt. Ltd., India). The sequences obtained were subjected to BLAST analysis to confirm the presence of gene and virus identity and then submitted to GenBank for the designation of accession numbers. The nucleotide (nt) sequences of coding region of B2L gene were aligned with other sequences retrieved from NCBI database using Clustal W algorithm in MEGA 10 programme (Kumar et al. 2018). The evolutionary history was inferred by constructing a phylogenetic tree using the maximum likelihood method and Tamura-Nei model (Tamura and Nei 1993).

## Results

### Epidemiological features

Six outbreaks of CE were investigated by the laboratory during the year 2021 (Table 1, Fig. 1). The outbreaks reportedly started in the months of July and August. This period corresponds to rainy season. All the outbreaks were reported in goats except one (Outbreak ID 11082021), in which sheep were also affected, but there was no mortality in sheep. A higher mortality was observed in young ones (Table 2). The vital indices of the outbreaks investigated are presented in Table 2. Morbidity in goats ranged from 8.75 to 100%, consistently high among young animals. In sheep, morbidity was 0% in one outbreak and 8% in another as disease is

**Table 2** The vital indices during orf outbreaks investigated

Outbreak ID	Species	Total population		Animals affected (% morbidity)		Animals died (% mortality)	
		Adult	Young	Adult	Young	Adult	Young
27072021	G	30	30	30 (100)	30 (100)	-	17 (56.60)
09082021	G	-	2	-	2 (100)	-	-
11082021	S	60	15	6 (8.0)	-	-	-
	G	20	5	7 (28.0)	4 (25)	-	-
25082021	S	40	-	-	-	-	-
	G	40	24 (60)	17 (42.5)	-	-	-
13092021	G	50	75	10 (20.0)	20 (26.67)	4 (8.0)	8 (10.67)
30092021	G	80	20	7 (8.75)	15 (75)	2 (2.50)	12 (60.0)

reported to be more severe in goats than in sheep. The overall mortality rate among goats ranged from 2.5 to 60%.

**Clinical features**

Rectal temperature was below 104 °F in all the animals negative for PPR except two adult female animals with mixed infection of orf and haemonchosis (Table 3; outbreak ID 25082021). The consistent clinical signs observed were characteristic tightly packed scabby lesions around mouth, anorexia, and weakness (Fig. 2). The clinical signs divided the animals into two groups with blood oozing lesions on the hooves, scabby lesions around nostrils and ears, and peeling skin as the observed sign in one of the group (Outbreak ID 27072021, Outbreak ID 09082021, Outbreak ID 11082021).

The other group of animals showed nasal discharge, vesicles in oral cavity, diarrhea, and respiratory distress and glossitis in addition to the signs showed by first group (Outbreak ID 25082021, Outbreak ID 30092021, Outbreak ID 13092021).

**Hematological examination**

There were inconsistent findings in relation to hematological examination (Table 3). Hemoglobin concentration ranged from 3.0 to 9.6 g%, with lowest among animals affected concurrently with orf and haemonchosis. Leukocytosis was observed in almost all the cases of the outbreaks with count (TLC) ranging from 5580 to 24,390/mm<sup>3</sup>.

**Table 3** Hematological parameters during orf outbreaks investigated

Outbreak ID	Animal no	Age	Sex	Species/breed	Rectal temperature (°F)	Hematological parameters					
						Hb (g%)	TLC (10 <sup>3</sup> /mm <sup>3</sup> )	DLC (%)			
N	L	M	E								
27072021	1	14 M	Male	Goat/Sirohi	102	7.4	11	22	74	03	01
	2	6 M	Male	Goat/Sirohi	102	8.4	17.54	46	51	3	-
09082021	1	4 M	Female	Goat	-	-	-	-	-	-	-
11082021	1	2Y		Sheep	103	-	-	-	-	-	-
	2	2Y		Goat	103.5	-	-	-	-	-	-
25082021	1	3.5 M	Female	Goat	100.1	3.7	21.85	81	15	02	-
	2	1Y	Female	Goat	102.3	6.3	14.90	85	11	01	-
	3	3 M	Male	Goat	102	9.6	24.39	87	09	02	-
	4	3Y	Female	Goat/Sirohi	104.4	7.4	11.76	90	08	01	-
	5	2Y	Female	Goat	101.4	3.1	18.82	84	11	03	-
	6	3 M	Female	Goat	103.2	4.2	5.96	91	09	00	-
	7	2 M	Female	Goat	102.2	3.8	5.98	88	09	02	-
	8	2 M	Male	Goat	101.9	3.0	5.58	86	12	02	-
	9	4 M	Female	Goat	104.6	4.0	6.70	90	07	03	-
13092021	1	3 M	-	Goat	104	-	-	-	-	-	-
30092021	1	3 M	-	Goat	104	-	-	-	-	-	-



**Fig. 2** Clinical signs in goats with Orf: **a, b, c** thick scabby lesions on the muzzle and around nostrils, **d** shedding of wool of neck region due to dermatitis caused by ORFV, **e** lesions on foot, **f** lesion in buccal cavity

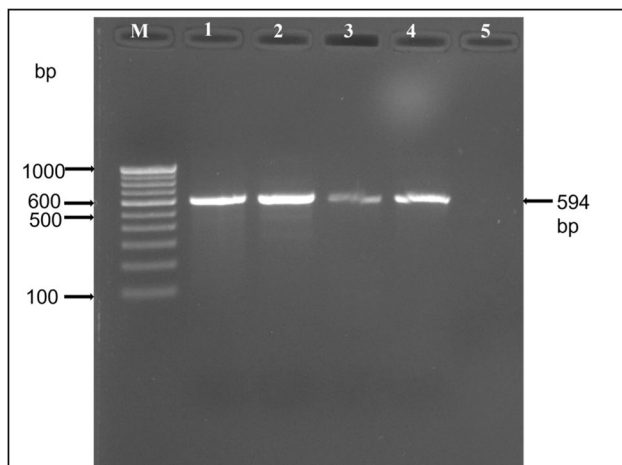


### PCR and phylogenetic analysis

The scab samples from all the six outbreaks were positive for ORFV by PCR assay confirming the presence of disease. All positive samples yielded a 594-bp amplicon specific to B2L gene (Fig. 3). The sequences submitted to GenBank database were assigned accession numbers as OM174299, OM174300, OM174301, OM174302, OM174303, and OM174304.

### Discussion

All the outbreaks investigated in the current study started in rainy season, during late summer. The researchers previously suggested that the virus mainly spreads and acts in late summer



**Fig. 3** PCR amplification of B2L gene of ORFV. M, 100 bp ladder; lanes 1–4, positive scab samples; lane 5, negative control

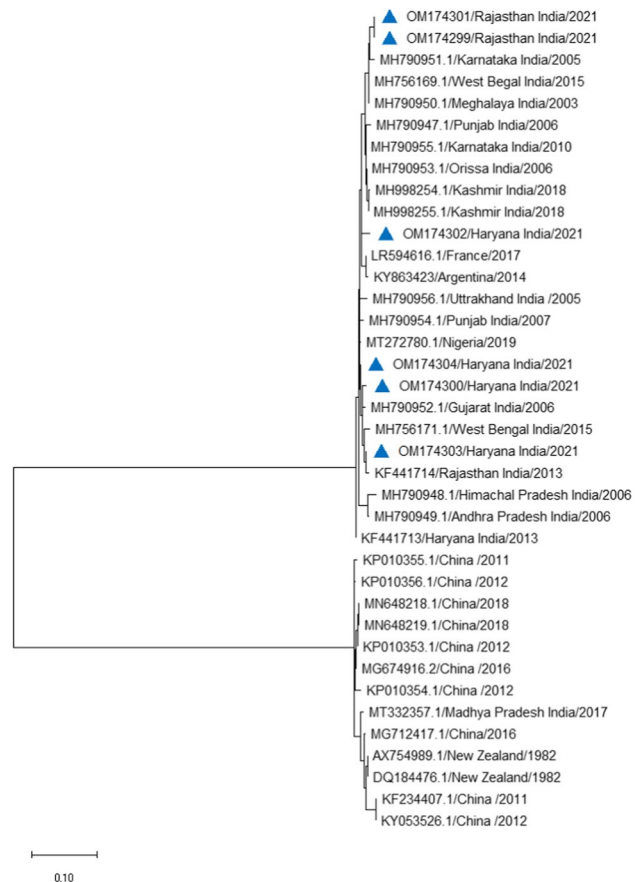
owing to hot, humid, and dry environments (Coradduzza et al. 2021). All the outbreaks were reported in goats except one (Outbreak ID 11082021), in which sheep were also affected. The morbidity among goats was far higher as compared to sheep and no mortality was observed among sheep flocks. However, due to smaller number of sheep involved, it could not be inferred from the current study, but the disease is believed to be more severe in goats as compared to sheep (Nagarajan et al. 2019). Morbidity figures observed in the present study corroborate with those reported by Housawi et al. (1993). Animals of all age groups were affected, although higher mortality was observed in young ones. The previous studies also reported higher mortality in young animals as compared to adults (Abdullah et al. 2015; Kumar et al. 2015). Although in uncomplicated cases, case fatality rate approaches to a maximum of 15%, it may be as high as 75%, where systemic invasions occur especially the respiratory tract (Radostits et al. 1994). In this study, the concurrent presence of haemonchosis and PPR seems to have aggravated the mortality among orf-affected animals as consistently higher mortality was observed in such cases. The viability of the virus in the wool and hides of animal remains for around 1 month after the healing of lesions (Spickler 2015). ORFV is also very resistant to inactivation in the pen environment (Kumar et al. 2015), enabling it to persist and reinfect the flock. This property makes the virus difficult to eliminate which might lead to public health significance among animals and humans in the future.

The typical clinical features of orf exhibited by the affected animals in this study were also reported by various researchers (Gelaye et al. 2016; Peralta et al. 2018; Tedla et al. 2018). Additionally, the clinical signs observed in mixed infection of ORFV and PPRV are also well described in the literature (Radostits et al. 1994; Saravanan et al. 2007). The lower-than-normal hemoglobin value in one of the outbreak can

be justified with the concurrent presence of *Haemonchus* spp. outbreak, as the parasite is responsible for severe anemia in sheep and goats (Soulsby 1982). The leukocytosis along with neutrophilia and lymphocytosis as observed in the current study has also been reported previously (Weiss and Wardrop 2010; Kazemi Asl et al. 2018). The reason for leukocytosis as well neutrophilia might be related to animals' response to inflammatory lesions of orf initially and to probable secondary bacterial infection later (Al Saad et al. 2017). Additionally, the deviation from normal values observed in hematological parameters of orf-affected animals could be related to weight loss, sub-nutrition, oxidative stress, pathological changes, and co-morbid conditions observed in two of the outbreaks (PPR in two and Haemonchosis in another outbreak) (Hayat et al. 1996). These findings could be useful for the management of cases of sheep and goats with orf.

Goats in two outbreaks were found to be positive for both ORF and PPR in the current study. Only a few mixed infections of PPR and ORF in goats were observed previously (Radostits et al. 1994; Martrenchar et al. 1997; Saravanan et al. 2007). The two outbreaks of mixed infection by ORFV and PPRV were observed in goats in the current study. Although the diseases were readily diagnosed by clinical signs, confirmation was based on various laboratory tests. The higher morbidity and mortality in both these outbreaks can be attributed to the mixed infection by these viruses. It is however difficult to assess which infection was established first on the basis of clinical signs, but previous reports stated that parapox viruses are opportunistic pathogens that may flare up when the host's immune responses are reduced by stress conditions (Tryland et al. 2005). In general, morbilliviruses induce the suppression of host immune responses but there is less information on PPRV's role in immune suppression (Wohlsein et al. 1995). However, a study on experimental PPRV infection in goats shows that it can lead to marked suppression of host immune responses accompanied by severe leucopenia (Rajak et al. 2005). More extensive studies on ORFV indicated that it can subvert the immune mechanism of the host to its advantage, to maintain itself in the host's system (Saravanan et al. 2007). Orf infection can precipitate whenever the host's immune system is weakened. The severity of the initial PPRV infection in two mixed infected outbreaks might be compounded by ORFV infection in the current study as also reported by Saravanan et al. (2007). In four of the reported outbreaks, introduction of new animals to the flock might be attributed as a factor for the outbreak and further spread of infection as the new animals might presumably had acquired the infection when purchased from the market. Based on the clinical signs, disease history, and laboratory tests, the etiology of the disease outbreak can be ascertained to both; ORF alone in four outbreaks and ORFV and PPRV in two outbreaks. Additionally, the close contact of the affected animals in a flock might have led to the spread to other animals including young ones.

The B2L gene used in the current study has been used for molecular characterization and phylogenetic analysis of ORFV previously (Guo et al. 2003; Hosamani et al. 2006; Zhang et al. 2010). The multiple sequence alignment and evolutionary analysis based on partial sequence of B2L gene showed that all the current study strains were clustered in the same clad. Both the strains from Rajasthan showed 100% nt identity with each other and the strains from Haryana revealed 99.98–99.99% nt identity among each other. The strains from outbreak previously reported from Rajasthan by Maan et al. (2014) revealed 99.97% nt identity with current study strains from Rajasthan, signifying the probability of the same strains causing the disease in the region. The strains from outbreak previously reported in Haryana by Kumar et al. (2014) showed 99.98–99.99% nt identity with current strains from the region, implying that the same or closely related strains are involved or circulating in the region. The overall maximum nt identity (99.99%) of current study strains was observed with strain from Rajasthan by Maan et al. (2014) and minimum identity (99.22%) with strains from Madhya Pradesh by Sahu et al. (2022), signifying a close genetic relationship and high conservation of B2L gene (Fig. 4) as also previously reported (Maan et al. 2014). The maximum



**Fig. 4** Phylogenetic analysis based on partial nucleotide sequence of B2L gene. The phylogenetic relationship was constructed by the maximum likelihood method using MEGA X software

nt identity was observed with strains from Nigeria (99.99%) and minimum with Chinese strains (99.20%). The B2L gene-based phylogenetic analysis of ORFV in China reported three different genotypes owing to clustering of the virus to different clusters, where Indian strains only clustered with Chinese strains in a single genotype (Zhang et al. 2014). The previous Indian studies however also reported such clustering and close relation with Chinese strains of ORFV (Maan et al. 2014; Kumar et al. 2014; Sahu et al. 2022) but the clustering of ORFV strains from Nigeria within the same clad with current study isolates provides useful and updated information for further understanding of the biology, epidemiology, and researches towards diagnosis and vaccine development.

## Conclusion

The present study presented the clinical and molecular identification and phylogenetic analysis of orf virus causing natural outbreaks among sheep and goats in Haryana and adjoining areas of Rajasthan, India. The initial infection with parasites or viruses in goats may be compounded by ORFV infection resulting in severe outcomes. The virus strains under study shared a close phylogenetic relationship with strains from India, New Zealand, China, France, and Nigeria. Prompt diagnosis of the disease is important for facilitating the implementation of control measures for minimizing the losses suffered by farmers. Further detailed studies are required to delineate the molecular details of ORFV for better understanding the dynamics and molecular epidemiology of strains circulating in the country and for designing the effective vaccines against the disease which are currently lacking in the country.

**Acknowledgements** The authors would like to acknowledge the financial assistance provided by Government of Haryana (India) for supporting the research work through state scheme “Epidemiological investigation of diseases in livestock and poultry in Haryana” [C(a)-VEPM-1-non-Development (09)].

**Author contribution** All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Renu Gupta, Pallavi Moudgil, Kushal Grakh, Ramesh Kumar, and Nareesh Jindal. Hematological analysis was performed by Maneesh Sharma. The first draft of the manuscript was written by Renu Gupta, Pallavi Moudgil, and Kushal Grakh and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

**Funding** The study was funded through state scheme “Epidemiological investigation of diseases in livestock and poultry in Haryana” [C(a)-VEPM-1-non-Development (09)] by Government of Haryana (India).

**Data availability** The data used to support the findings of this study is included in the article. The rest of the datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

## Declarations

**Ethics approval** The study was undertaken on the samples collected from affected animals brought for diagnosis and treatment at Disease Investigation laboratory at LUVAS, Hisar, and did not require ethical approval.

**Conflict of interest** The authors declare no competing interests.

## References

- Abdullah, A.A., Ismail, M.F., Balakrishnan, K.N., Bala, J.A., Hani, H., Abba, Y., Awang Isa, M.K., Abdullah, F.F., Arshad, S.S., Nazariah, Z.A., Abdullah, R., Mustapha, N.M., Mohd-Lila, M.A., 2015. Isolation and phylogenetic analysis of caprine Orf virus in Malaysia. *Virusdisease*, 26(4), 255–259.
- Ahanger, S.A., Parveen, R., Nazki, S., Dar, Z., Dar, T., Dar, K.H., Dar, P., 2018. Detection and phylogenetic analysis of Orf virus in Kashmir Himalayas. *Virusdisease*, 29(3), 405–410.
- Al Saad, K.M., Thweni, H.T., Abdali, D.A., Tarik, A.S., 2017. Clinical and diagnostic studies of contagious ecthyma (ORF) in sheep. *IOSR Journal of Agriculture and Veterinary Science*, 10, 64–9.
- Bala, J.A., Balakrishnan, K.N., Abdullah, A.A., Adamu, L., bin Noorzahari, M.S., May, L.K., Lila, M.A.M. 2019. An association of Orf virus infection among sheep and goats with herd health programme in Terengganu state, eastern region of the peninsular Malaysia. *BMC Veterinary Research*, 15(1), 1–15.
- Bayindir, Y., Bayraktar, M., Karadag, N., 2011. Investigation and analysis of a human orf outbreak among people living on the same farm. *New Microbiologica*, 34(1), 37–43.
- Bergqvist, C., Kurban, M., Abbas, O., 2017. Orf virus infection. *Review in Medical Virology*, 27, e1932.
- Bora, D.P., Barman, N.N., Das, S.K., Bhanuprakash, V., Yogisharadhy, R., Venkatesan, G., Chakraborty, A., 2012. Identification and phylogenetic analysis of orf viruses isolated from outbreaks in goats of Assam, a northeastern state of India. *Virus Genes*, 45(1), 98–104.
- CDC., 2006. Orf Virus Infection in Humans --- New York, Illinois, California, and Tennessee, 2004—2005. *Morbidity and Mortality Weekly Report*, 55(03), 65–68. Accessed at <https://www.cdc.gov/poxvirus/orf-virus/index.html>.
- Coradduzza, E., Sanna, D., Rocchigiani, A.M., Pintus, D., Scarpa, F., Scivoli, R., Bechere, R., Dettori, M.A., Montesu, M.A., Marras, V., Lofrano, R., 2021. Molecular insights into the genetic variability of ORF Virus in a Mediterranean region (Sardinia, Italy). *Life*, 11(5), 416.
- DADF., 2019. *Basic Animal Husbandry Statistics*. Department of Animal Husbandry, Dairying & Fisheries, Ministry of Agriculture, Government of India.
- Fleming, S.B., Wise, L.M., and Mercer, A.A., 2015. Molecular genetic analysis of orf virus: a poxvirus that has adapted to skin. *Viruses*, 7(3), 1505–1539.
- Gelaye, E., Achenbach, J.E., Jenberie, S., Ayelet, G., Belay, A., Yami, M., Loitsch, A., Grabherr, R., Diallo, A., Lamien, C.E., 2016. Molecular characterization of orf virus from sheep and goats in Ethiopia, 2008–2013. *Virology Journal*, 13, 34.
- Gunther, T., Haas, L., Alawi, M., Wohlsein, P., Marks, J., Grundhoff, A., Becher, P., Fischer, N., 2017. Recovery of the first full-length genome sequence of a parapoxvirus directly from a clinical sample. *Scientific Reports*, 7(1), 3734.
- Guo, J., Zhang, Z., Edwards, J.F., Ermel, R.W., Taylor, C.Jr., Concha-Bermejillo, A., 2003. Characterization of a North American orf virus isolated from a goat with persistent, proliferative dermatitis. *Virus Research*, 93, 169–179.



- Haig, D.M., Mercer, A.A., 1998. Ovine diseases Orf. *Veterinary Research*, 29(3–4), 311–26.
- Hayat, C.S., Hussain, S.M., Iqbal, Z., Hayat, B., Akhtar, M., 1996. Effect of parasitic nematodes on haematology and productivity of sheep, *Pakistan Veterinary Journal*, 16(2), 81–83.
- Hosamani, M., Bhanuprakash, V., Scagliarini, A., Singh, R.K., 2006. Comparative sequence analysis of major envelope protein gene (B2L) of Indian orf viruses isolated from sheep and goats, *Veterinary Microbiology*, 116, 317–324.
- Housawi, F.M., Abu Elzein, E.M., Gameel, A.A., Alafaleq, A.I., 1993. A close comparative study on the response of sheep and goats to experimental orf infection, *Zentralbl Veterinarmed B*, 40(4), 272–82.
- Kazemi Asl, S.A., Aslani, M.R., Mohebbi, A., Mokhtari, A., 2018. Hematological and biochemical evaluation of goats naturally infected with contagious ecthyma, *Iranian Journal of Veterinary Science and Technology*, 10(2), 43–7.
- Kumar, A., Maan, S., Ghosh, A., Batra, K., Sharma, H., Mahajan, N.K., Maan, N.S., 2013. Identification and Characterization of orf virus from goats, *Indian Journal of Comparative Microbiology, Immunology and Infectious Diseases*, 34(2), 10–14.
- Kumar, N., Wadhwa, A., Chaubey, K.K., Singh, S.V., Gupta, S., Sharma, S., Mishra, A.K., 2014. Isolation and phylogenetic analysis of an orf virus from sheep in Makhdoom, India, *Virus Genes*, 48(2), 312–319.
- Kumar, R., Trivedi, R.N., Bhatt, P., 2015. Contagious Pustular Dermatitis (Orf Disease) - Epidemiology, diagnosis, control and public health concerns, *Advances in Animal and Veterinary Sciences*, 3(12), 649–676.
- Kumar, S., Stecher, G., Li, M., Knyaz, C., and Tamura, K., 2018. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms, *Molecular Biology and Evolution*, 35, 1547–1549.
- Ma, W., Pang, M., Lei, X., Wang, Z., Feng, H., Li, S., Chen, D., 2022. Orf Virus Detection in the Saliva and Milk of Dairy Goats, *Frontiers in Microbiology*, 1073.
- Maan, S., Kumar, A., Batra, K., Singh, M., Nanda, T., Ghosh, A., Maan, N.S., 2014. Isolation and molecular characterization of contagious pustular dermatitis virus from Rajasthan, India, *Virusdisease*, 25(3), 376–80.
- Martrenchar, A., Zoyem, N., Diallo, A., 1997. Experimental study of a mixed vaccine against peste des petits ruminants and capripox infection in goats in northern Cameroon, *Small Ruminant Research*, 26, 39–44.
- Nagarajan, G., Pourouchottamane, R., Reddy, G.B., Yogisharadhya, R., Sumana, K.R., Rajapandi, S., Murali, G., Thirumaran, S.M., Mallick, P.K., Rajendiran, A.S., 2019. Molecular characterization of Orf virus isolates from Kodai hills, Tamil Nadu, India, *Veterinary World*, 12, 1022–1027.
- Nandi, S., De, U.K., Chowdhury, S., 2011. Current status of contagious ecthyma or orf disease in goat and sheep—a global perspective, *Small ruminant research*, 96(2–3), 73–82.
- Oem, J.K., Chung, J.Y., Kim, Y.J., Lee, K.K., Kim, S.H., Jung, B.Y., 2013. Isolation and characterization of orf viruses from Korean black goats, *Journal of Veterinary Science*, 14(2), 227–30.
- Peralta, A., Robles, C.A., Micheluod, J.F., Rossanigo, C.E., Martinez, A., Carosio, A., König, G.A., 2018. Phylogenetic analysis of ORF viruses from five contagious ecthyma outbreaks in Argentinian goats, *Frontiers in Veterinary Science*, 5, 134.
- Radostits, O.M., Blood, D.C., and Gay, C.C., 1994. Peste des petits ruminants, In *Veterinary Medicine*, 8th edn. Bath Press, pp 986–988.
- Rajak, K.K., Sreenivasa, B.P., Hosamani, M., Singh, R.P., Singh, S.K., Singh, R.K., Bandyopadhyay, S.K., 2005. Experimental studies on immunosuppressive effects of Peste des petits ruminants (PPR) virus in goats, *Comparative Immunology, Microbiology and Infectious Diseases*, 28, 287–296.
- Sahu, B.P., Majee, P., Singh, R.R., Sahoo, N., Nayak, D., 2022. Recombination drives the emergence of orf virus diversity: evidence from the first complete genome sequence of an Indian orf virus isolate and comparative genomic analysis, *Archives of Virology*, 167(7):1571–1576.
- Saravanan, P., Balamurugan, V., Sen, A., Sarkar, J., Sahay, B., Rajak, K.K., Hosamani, M., Yadav, M.P., Singh, R.K., 2007. Mixed infection of peste des petits ruminants and orf on a goat farm in Shahjahanpur, India, *Veterinary Record*, 160(12), 410.
- Soulsby, E.J.L., 1982. Helminths, arthropods and protozoa of domesticated animals, 7<sup>th</sup> Edn. Tindall: The English Language Book Society and Bailliere.
- Spickler, A.R., 2015. Contagious Ecthyma. Accessed at <http://www.cfsph.ias.tate.edu/DiseaseInfo/factsheets.php>
- Tamura, K., and Nei, M., 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees, *Molecular Biology and Evolution*, 10, 512–526.
- Tedla, M., Berhan, N., Molla, W., 2018. Molecular identification and investigations of contagious ecthyma (Orf virus) in small ruminants, North west Ethiopia, *BMC Veterinary Research*, 14, 13.
- Tryland, M., Klein, J., Nordoy, E.S. and Blix, A.S., 2005. Isolation and partial characterization of parapoxvirus isolated from skin lesion of Weddell seal, *Virus Research*, 108, 83–87.
- Venkatesan, G., Balamurugan, V., Bora, D.P., Yogisharadhya, R., Prabhu, M., Bhanuprakash, V., 2011. Sequence and phylogenetic analyses of an Indian isolate of orf virus from sheep, *Veterinaria Italiana*, 47(3), 323–332.
- Venkatesan, G., De, A., Arya, S., Kumar, A., Muthuchelvan, D., Debnath, B.C., Dutta, T.K., Hemadri, D., Pandey, A.B., 2018. Molecular evidence and phylogenetic analysis of orf virus isolates from outbreaks in Tripura state of North-East India, *Virus-disease*, 29(2), 216–220.
- Vikoren, T., Lillehaug, A., Akerstedt, J., Bretten, T., Haugum, M., Tryland, M., 2008. A severe outbreak of contagious ecthyma (orf) in a free-ranging musk ox (*Ovibos moschatus*) population in Norway, *Veterinary Microbiology*, 127, 10–20.
- Weiss, D.J., Wardrop, K.J., 2010. *Schalm's Veterinary Hematology*, Ames Wiley-182 Blackwell.
- Wohlsein, P., Wamwayi, H.M., Troutwein, G., Pohlenz, J., Liess B., Barrett, T., 1995. Pathomorphological and immunohistological findings in cattle experimentally infected with rinderpest virus isolates of different pathogenicity, *Veterinary Microbiology*, 44, 141–147.
- Zhang, K., Shang, Y., Jin, Y., Wang, G., Zheng, H., He, J., Lu, Z., Liu, X., 2010. Diagnosis and phylogenetic analysis of Orf virus from goats in China: a case report, *Virology Journal*, 7, 78.
- Zhang, K., Liu, Y., Kong, H., Shang, Y., Liu, X., 2014. Comparison and phylogenetic analysis based on the B2L gene of orf virus from goats and sheep in China during 2009–2011, *Archives of Virology*, 159(6), 1475–1479.

**Publisher's Note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Springer Nature or its licensor holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.