



Molecular Diagnosis and Phylogenetic Analysis of the Orf Virus from An Outbreak of Contagious Ecthyma at Rewa in Madhya Pradesh

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10.18805/IJAR.B-5580

ABSTRACT

Background: Contagious ecthyma is a viral pustular dermatitis in small ruminants. The disease is of zoonotic importance. Contagious ecthyma usually resolves spontaneously and has low mortality. The Orf virus causes contagious ecthyma, a member of the genus *Parapoxvirus* in the subfamily *Chordopoxvirinae* and family *Poxviridae*.

Methods: The investigated goat farm experienced an outbreak of pustular dermatitis, especially in young goats. Scabs/tissue samples from lip commissures and over the lips of individual goats were collected in sterile eppendorf tubes, separately and stored in a frozen condition. Nucleic acid was extracted from these scabs with commercially available nucleic acid extraction kit. The extracted total deoxyribose nucleic acid was used in the Orf-specific B2L gene amplification in a molecular diagnosis method, polymerase chain reaction. The phylogenetic tree was constructed by using MEGA11 software tool.

Result: Young goats showing clinical signs and overt scabby lesions on the lips were used to collect scabs/tissue samples in sterile containers. Total deoxyribose nucleic acid was used in the polymerase chain reaction to amplify 594 bp product specific to the B2L gene (envelope gene) of Orf virus confirming the outbreak of Contagious ecthyma. The amplified product was confirmed by the sequencing method. Phylogenetic analysis was made using bioinformatic tools. The Orf viral strain in the first such study in this area showed similarity to that of the strain from Hisar at a national level and to that of China at the international level.

Key words: Contagious ecthyma, Goats, Orf.

INTRODUCTION

Orf also known as contagious ecthyma or contagious pustular dermatitis or sore mouth is one of the most common viral diseases of goats and sheep however it is more severe in goats (Nagarajan *et al.*, 2019). It is a highly infectious pox virus disease of goats and sheep manifested by the occurrence of pustular and scabby lesions on the lips, muzzle and udder (Herendra *et al.*, 2000). The contagious ecthyma in goats and sheep is reported across the country in Tamil Nadu, Haryana, Assam, Madhya Pradesh, Andaman and Nicobar islands, Uttar Pradesh (Kumar *et al.*, 2014; Nagarajan *et al.*, 2019; Sunder *et al.*, 2020; Kumar *et al.*, 2022; Sahu *et al.*, 2022; Nashiruddullah *et al.*, 2022; Manickam *et al.*, 2023) and also worldwide in Egypt, Nigeria, Argentina, Iraq (Peralta *et al.*, 2015; Zeedan *et al.*, 2015; Yuness and Abood, 2023; Onoja *et al.*, 2024). The disease is caused by an Orf virus classified in the genus *Parapoxvirus* of the subfamily *Chordopoxvirinae* in the *Poxviridae* family. The Orf virus infection is transmitted by direct and indirect contact. It is also having zoonotic importance (Karakas *et al.*, 2013; Zeedan *et al.*, 2015; Sleiy *et al.*, 2024; Salvi *et al.*, 2024).

India had a total goat population of 148.88 million in 2019. It has increased by more than 10% in the country compared to that of the previous livestock population census in 2012 (Anonymous, 2025a). Goats contributed about 27.8% of the total Indian livestock population (535.78 million). As per the 20th livestock census in 2019, Madhya Pradesh stands fifth in the country with 11.06 million goats

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How to cite this article: Audarya, S.D., Chatur, Y., Shrivastava, N., Niranjana, A. and Mishra, A.K. (2025). Molecular Diagnosis and Phylogenetic Analysis of the Orf Virus from An Outbreak of Contagious Ecthyma at Rewa in Madhya Pradesh. *Indian Journal of Animal Research*. 1-7. doi: 10.18805/IJAR.B-5580.

Submitted: 05-03-2025 **Accepted:** 03-07-2025 **Online:** 29-07-2025

counting more than 38% change in goat population from the previous census, with 8.01 million in 2012. The increased state goat population indicates the interest of

the state farming communities in goat farming and government policies in encouraging goat farming. In Madhya Pradesh, Dhar district recorded the highest goat population, followed by Alirajpur and Badwani, according to the census previously mentioned. Livestock from Madhya Pradesh contributes 179.99 lakh tonnes of milk production, 26,515.66 lakh eggs, 116.34 thousand tonnes of meat production and 431.07 thousand kilograms of wool production (Anonymous, 2023; Anonymous, 2025b).

Orf viruses have significant genetic diversity, with different lineages and subtypes identified. By combining phylogenetic analysis with other molecular techniques, researchers can gain a deeper understanding of Orf virus's biology, epidemiology and potential for future outbreaks. This knowledge can inform the development of effective prevention and control strategies for this important veterinary pathogen. As more Orf virus strains are sequenced, ongoing phylogenetic analysis will be important to monitor the evolution of Orf virus and the emergence of new variants. The present investigation was carried out for further confirmation of the Orf virus outbreak and phylogenetic analysis by using molecular methods. A polymerase chain reaction for specific amplification of B2L gene and appropriate bioinformatic tools were employed for phylogenetic analysis.

MATERIALS AND METHODS

Village

The farm in Kuthuliya (24.495065224288144, 81.290780 07232715) village is in Rewa district of Madhya Pradesh state in India. The village is situated 6 kilometers away from the district headquarters Rewa.

Livestock farm, collection and storage of clinical samples

26 Sirohi goats kept at the farm of the College were clinically exhibiting Orf lesions on lip commissures, ear tips in August 2021. There was a history of Orf virus infections in the herd. Clinical samples were collected in sterile containers from four Orf virus infected goats and stored in the deep freezer at -20°C in the Department of Veterinary Microbiology of the College.

Isolation of DNA

HiPurA® multi-sample DNA purification kit (MB554) recommended for the isolation of DNA from animal tissue was procured and used in the present study as per the manufacturer's protocol (HiMedia, India; <https://www.himedialabs.com/in/mb554-hipura-multi-sample-dna-purification-kit.html>).

Primers for PCR

F5'-GTCGTCCACGATGAGCAGCT-3', R5'-TACGTGGAA GCGCCTCGCT-3' were procured commercially (Kumar *et al.*, 2022).

PCR reaction

DNA extracted from the scab samples using the commercially available kit was used in PCR (Veriti 96 well

thermal cycler, Applied Biosystems) to amplify Orf virus-specific B2L gene (immunodominant envelope antigen). Initial denaturation at 95°C for 5 minutes was followed by 35 cycles comprising denaturation at 95°C for 45 seconds, annealing at 55°C for 45 seconds and extension at 72°C for 45 seconds. The final extension step was performed at 72°C for 7 minutes, thereafter the product was stored at 4°C. The 25 µl PCR reaction volume comprised of i) 2x Master mix (Promega) 12.5 µl ii) 1 µl of each of the primers, primer concentration of 10 pmole/µl iii) 3 µl of DNA template and 7.5 µl nuclease free water. The amplified 594 bp PCR product was visualized upon 1.5% agarose gel electrophoresis and documented using gel documentation machine (eGel imager, Life Technologies).

Nucleotide sequencing

The amplified PCR product (594 bp) was sequenced using a commercial facility at Barcode Biosciences, Bangalore. Both the strands of the PCR product sequenced using primer pair used in the PCR. The forward and reverse sequence was aligned by Geneious Prime® 2024.0.7 software. The sequences were aligned with previously published ORF011 putative EEV envelope phospholipase (genelid:2947733) NC_005336.1 ranging from 10857 to 11993 from GenBank (Table 1). The resulting partial nucleotide sequence of B2L gene (594 bp) was submitted to National Centre for Biotechnology Information's (NCBI) GenBank database and allotted accession number PP910121.

Phylogenetic analysis

The published parapox viral strains nucleotide sequence and nucleotide sequence data of B2L gene from Orf virus was downloaded from NCBI using BLAST tool and nucleotide similarity percentage was documented (Table 1). The partial nucleotide sequence of the B2L gene was aligned with 49 nucleotide sequences downloaded from the NCBI database by the Clustal W method using MEGA 11 software. A phylogenetic tree was constructed using the Kimura-2 parameters evolution model and the neighbor-joining method with 1,000 bootstrap replicates implemented by MEGA 11 software (Kimura, 1980; Felsenstein, 1985; Saitou and Nei, 1987; Tamura *et al.*, 2021).

RESULTS AND DISCUSSION

Orf virus belonged to the family *Poxviridae* is the original species classified in the genus *Parapoxvirus* and subfamily *Chordopoxvirinae* (Venkatesan and Kumar, 2018; Nair *et al.*, 2024). The disease caused by the Orf virus is responsible for economic losses in goat production (Dan *et al.*, 2023). Orf virus causes a skin infection in sheep, goats and humans. The Orf virus is highly contagious and can spread through direct contact with infected animals or their secretions. The Orf virus infection can be diagnosed conventionally, serologically and by molecular-based methods (Pang and Long, 2023). Besides causing production losses in Orf virus-infected animals such as

Table 1: Published parapox viral strains utilized from sheep and goats for phylogenetic analysis.

Strain	Year of isolation	Nucleotide accession number	Length of nucleotide	Nt (%) similarity
Rewa	2021	PP910121	594	-
GPPV_Shwk1	2016	MN701773.1	921	98.15
ORF0903GAG	2003	KJ137682.1	975	98.64
gdzc011	2014	KP336704.1	1137	98.65
30092021	2021	OM174304.1	594	98.82
13092021	2021	OM174303.1	594	98.82
MK83A	2018	MG757233.1	1137	98.48
AH1610	2016	MF489111.1	1137	98.48
YNSLi	2013	MF172954.1	1137	98.48
Hub13	2013	KJ139994.1	1137	98.48
Chitradurgar/Goat-20	2013	KU597734.1	1106	98.48
ORF1111GRG1	2011	KJ137693.1	975	98.47
PPV-1/22	2021	OP651984.1	579	98.62
Guizhou	2010	KP994595.1	1137	98.48
rc24	2019	OK340643.1	1050	98.48
Hubei	2010	KF913722.1	1137	98.48
NE2	1993	JN088051.1	1137	98.48
Goat_PDS_2018	2018	MN316586.1	585	98.29
ORFV-Gujarat	2006	MH790952.1	1137	98.32
Jaganathpara/Tripura/14	2014	KT935588.1	1137	97.47
ORF14CNG	2014	MF497431.1	975	98.30
ORF1209MSG	2009	KJ137711.1	975	98.30
JXxy2022	2022	OQ686993.1	1137	98.32
JK-11	2018	MH998254.1	1137	97.14
Mukteswar_vaccine_passage50	2005	ON380500.1	139966	98.32
SJ1	2012	KP010356.1	139112	98.32
SDLC	2013	KP339952.1	1137	98.32
CIRG/2014	2014	KP745467.1	1038	98.32
rc20	2019	OK340639.1	1032	98.31
FJ-ZX ORFV011	2012	KC568400.1	1137	98.15
rc24	2011	JX968991.1	1137	98.32
ORFV/GD-JY4/2019/China	2019	MN862672.1	1137	98.32
WBPURLIA-7	2015	MH756171.1	1137	98.15
JS103A	2018	MG757234.1	1137	98.15
OV-SA00	2003	NC_005336.1	139962	98.15
PPV-2/22	2021	OP651985.1	579	98.27
CPD/IND2013/01	2013	KF441713.1	651	98.15
ORFV/Mon 1/2018/JVPA/Mongolia	2018	LC516489.1	1090	96.46
ORFV/Goat YNTJe/China	2023	PP733998.1	1137	98.32
Pondicherry 04/PDY/India 2024	2024	PP478095.1	1210	97.81
TR-ORF-2020-1-E1	2020	OQ455250.1	1137	97.64
Ind/MP/Dh/2017	2017	MT350784	780	97.09
Od/Kh/01/2016	2016	MG365656.1	780	97.09
Orf virus isolate 11082021	2021	OM174301	594	96.97
Orf virus ORFV Bangalore	2005	MH790951.1	1137	97.32
ORFV-Ludhiana_50/06	2006	MH790947.1	1137	97.14
ORFV-Palampur_20/06	2006	MH790948.1	1137	96.13
ORFV-Hyderabad	2006	MH790949.1	1137	97.47
Bovine Papular Stomatitis	2024	PP565904.1	132932	84.54
Virus, Munich, Germany				
Pseudocowpox virus isolate, Cameroon	2024	PP565900.1	136858	93.94



Fig 1: Orf virus outbreak in Sirohi breed of goats at Kuthuliya in Rewa district of Madhya Pradesh.

sheep and goats (Orta *et al.*, 2023), the infection is also zoonotic in nature meaning it can be transmitted between animals and humans. In human beings, in the case of adults or young ones Orf virus infections have been reported (Flores *et al.*, 2017; Karakas *et al.*, 2013; Rossi *et al.*, 2023; Salvi *et al.*, 2024; Sleiy *et al.*, 2024).

The study investigated the Sirohi breed of goats for Orf virus infection in age groups from 6 months to 4 years. The most common symptom is the development of one or more painful sores on the skin of the Orf virus-infected animal. Clinical signs noticed in the affected goats from the present study were high fever and scabs on and around the mouth, lips, muzzle and nostrils (Fig 1). A few years ago, goats from the present flock also experienced the Orf virus infection. The re-emergence of the Orf virus infection in the same flock could be due to short-term immunity after natural infection and also these goats were never vaccinated against Orf virus infection (Venkatesan and Kumar, 2018). All goats caught the infection and recovered spontaneously within a month, assisted by antibiotic treatment to check for secondary bacterial infection. There is no specific treatment for Orf virus infection. The present study recorded no mortality in the Orf virus-infected goat population. In Orf virus-infected human beings, sores start as small, red bumps that gradually grow larger and become filled with pus and can crust over to heal within a month. In a limited number of cases, Orf virus-infected people may also experience fever, chills, headache and muscle aches. The sores usually heal within a few weeks. Clinical signs on hands and feet were reported from a human case of Orf virus infection (Salvi *et al.*, 2024). Chronic papulo-vesicular cutaneous lesions on the left index finger in a man from Turkey were also reported (Karakas *et al.*, 2013). Orf lesion on the nose of a 4-month old male baby has been reported from Syria (Sleiy *et al.*, 2024). The best way to prevent the Orf virus infection is to avoid contact with infected animals. Washing hands thoroughly with soap and water, on contact with an infected animal is recommended. No in-contact human beings/animal attendants of the farm were reported to cause clinical signs such as sores on the hands, fingers, arms or any other parts of the body. This can be due to the

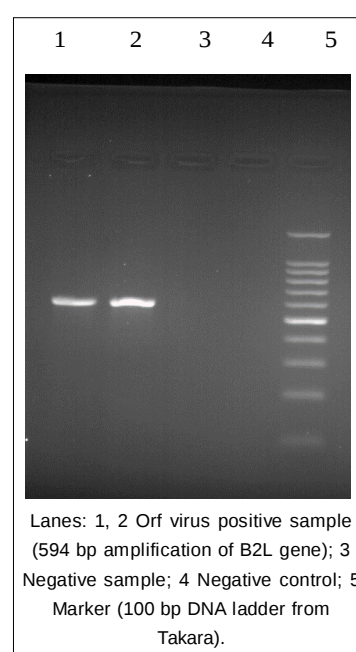


Fig 2: Amplification of Orf virus-specific B2L gene from Sirohi breed of goats at Kuthuliya of Rewa district in Madhya Pradesh by polymerase chain reaction and documentation of electrophoresed amplified products using gel documentation system.

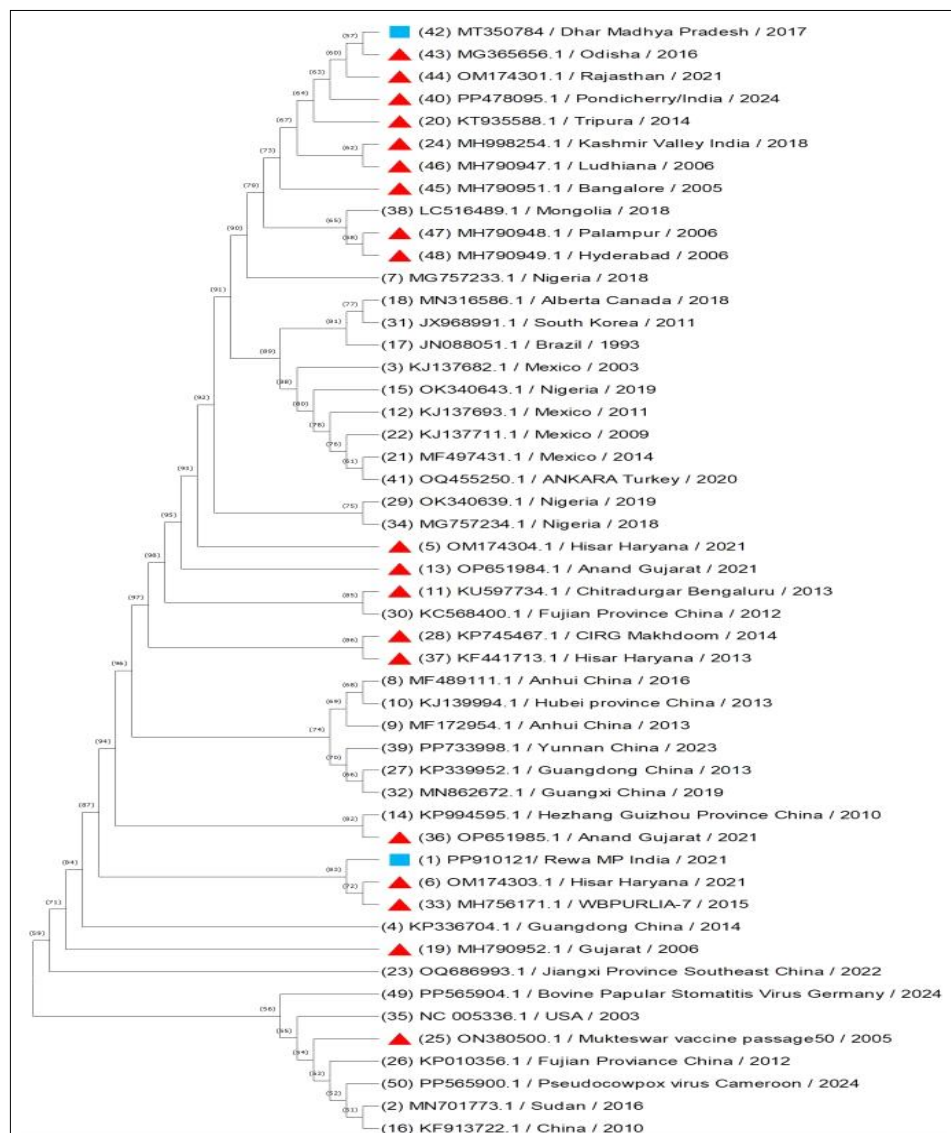
follow-up of hygienic practices at the livestock farm by animal handlers and/or better immunity.

The disease directly impacts the feed intake of goats (Sunder *et al.*, 2020). The seasonal disease is spread across the country and the globe. This Orf virus outbreak was noticed in August 2021 (Sunder *et al.*, 2020). Various serological and molecular methods are available to achieve a laboratory diagnosis of Orf virus infection. PCR a molecular method shows higher sensitivity and specificity than the gold standard virus isolation method in diagnosing Orf virus infection (Pang and Long, 2023). Hence, in the present study, successful amplification of 594 bp Orf virus-specific partial B2L gene sequence in polymerase chain reaction from two samples further confirmed the outbreak

in Sirohi goats of Kuthuliya in Rewa district of India (Nagarajan *et al.*, 2019; Kumar *et al.*, 2022; Orta *et al.*, 2023) (Fig 2). Very few reports were available on Orf virus outbreak in the state. However, in one of the Western districts, Dhar district of the state in 2017 an outbreak of Orf virus was recorded in black Bengal goat breed (Sahu *et al.*, 2022).

Phylogenetic studies based on partial gene sequences, such as commonly used B2L and the F1L genes, or on complete genome sequences of orf virus conducted to understand the evolutionary history, genetic diversity and geographical distribution of this virus (Sahu *et al.*, 2022). The present study constructed a B2L gene-specific phylogenetic tree using appropriate bioinformatics tools as previously reported. The amplified PCR product of 594 bp was outsourced for sequencing to a private service centre. The Orf viral strain sequence generated in the

present study was deposited in the NCBI database. It has received accession number PP910121. In a search using BLAST tools, the highest nucleotide sequence similarity of 98.82% was observed at the national level with strains identified from Hisar, Haryana (OM174303.1, OM174304.1) in 2021, the same year this Orf virus outbreak was identified in the present investigation. A nucleotide sequence similarity of 98.65% was observed to the viral strain from China (OQ686993.1). A nucleotide sequence similarity of 97.09% was observed for the Orf virus strain from goats in Dhar district, Madhya Pradesh; however, it is distantly placed among the strains (Fig 3). The B2L and F1L genes are highly conserved (Zhang *et al.*, 2024); hence, phylogenetic analysis based on these genes may not always accurately reflect the true genetic diversity of Orf viruses. Phylogenetic analysis of B2L region indicated an American cluster (formed by the Brazilian, US American, Argentinian Orf



viruses and South American vaccine strains) in the first molecular characterization of Argentinian Orf viruses. The great level of conservation of B2L gene among Orf viruses from the globe was proved by nucleotide sequence analysis (Peralta *et al.*, 2015). Hence, for a more detailed understanding of Orf virus genetic diversity and evolutionary history, whole genome sequencing is essential for the identification of genetic variations across the entire Orf viral genome, for a more precise picture of the virus's genetic landscape (Sahu *et al.*, 2022).

CONCLUSION

The present study indicates Orf virus detection at the molecular level in Sirohi goats at Kuthuliya in the Rewa district of Madhya Pradesh in India. Phylogenetically, the Orf virus is closely aligned with the strains from Hisar, Haryana. Further, it is hoped that, in the future, full viral genome sequencing of Orf viruses will not only reveal any nucleotide sequence changes among Orf viruses but also be helpful in understanding the evolution of Orf viruses.

ACKNOWLEDGEMENT

The authors express thanks to the staff at the livestock farm and undergraduate students for their help during sample collection.

Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

All animal procedures for experiments were approved by the Committee of Experimental Animal care and handling techniques were approved by the University of Animal Care Committee. However, no experimental animals were involved in the study.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish or preparation of the manuscript.

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