RESEARCH ARTICLE

Molecular Detection and Evolutionary Insights of Contagious Ecthyma (Orf) Virus in Goat in Andhra Pradesh, India

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Abstract

Contagious ecthyma or Orf is a zoonotic, transboundary disease affecting both domestic and wild ruminants. The present study investigated the incidence of severe Orf outbreaks in goats in Kurnool and Anantapur districts of Andhra Pradesh, a southern state of India during 2024. The presence of the Orf virus in the clinical samples was confirmed by PCR targeting the GM-CSF/IL-2 inhibitor (*GIF*) gene. Among the 11 samples tested, four skin scab samples yielded a positive result, showing a specific 408 bp PCR amplicon. Further testing using a capripox genus-specific PCR assay confirmed the absence of sheep pox and goat pox viruses in the samples. This is the first study on comprehensive genetic characterization of circulating ORFV strains in Andhra Pradesh. Phylogenetic analysis revealed two ORFV isolates (ATP 2833 and KRNL 4410) belong to distinct groups, sharing 97.46% nucleotide identity with each other. ATP 2833 isolate showed highest homology with Tamil Nadu field isolates and the TANUVAS P15 vaccine strain suggesting southern Indian ORFV circulation patterns. In contrast, KRNL 4410 strain demonstrated close phylogenetic proximity to the IVRI Mukteshwar P50 vaccine strain and Ludhiana 2006 strain, suggesting a possible association with vaccine-related lineages circulating in northern India. The divergence of both strains from the first-generation NZ2 isolate (1982) and the Japan (1985) isolate highlights significant evolutionary changes over time. These alterations are likely driven by regional transmission dynamics such as climate, host and breed characteristics. These results indicate the potential for regional variations in ORFV strains, which could have important implications for vaccine development and the optimization of disease control strategies.

Key words: Capripox viruses, Contagious ecthyma, GM-CSF/IL2 inhibitor gene, Orf virus (ORFV), Phylogenetic analysis.

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Introduction

Orf, also known as contagious ecthyma, contagious pustular dermatitis, or ovine pustular dermatitis, is a viral skin infection that predominantly affects sheep and goats, with reported outbreaks across various regions worldwide (Gelaye *et al.,* 2016). The disease is caused by the Orf virus (ORFV), which belongs to the genus *Parapoxvirus*, a prototypical member of the subfamily *Chordopoxvirinae* within the family *Poxviridae* (Nandi *et al.,* 2011). ORFV is known for its wide host range, infecting various wild species in addition to its primary hosts, sheep and goats. The clinical presentation of Orf includes cutaneous lesions that are predominantly located around the perioral area. In rare cases, a generalized form of the infection has been documented (Zhang *et al.,* 2019). The disease typically begins with erythematous patches or swelling around the mouth, followed by the development of papules and pustules with a characteristic yellowish or creamy appearance. These pustules eventually mature into scabs (Managga *et al.,* 2022). ORFV typically infects the host through dermal lesions or abrasions, where it replicates in proliferating keratinocytes. In some instances, infection may extend to the oral mucosa, leading to papulo vesicular stomatitis that affects the gums, palate, and tongue. Both mild and severe outbreaks of Orf have been documented worldwide, including in various regions of India (Kumar *et al.,* 2022). Orf virus is a zoonotic pathogen classified as an occupational zoonosis, posing a risk to individuals with close contact to sheep and goats, such as farmers, veterinarians, and wool shearers (Hosamani *et al.,* 2009).

The ORFV genome consists of a linear, double-stranded DNA molecule, approximately 135 kilobases (kb) with high G+C content, and encodes a total of 132 genes. The central portion houses highly conserved genes critical for viral replication and structural assembly, whereas the terminal regions exhibit higher variability and harbour genes linked to

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pathogenicity and immune escape (Coradduzza *et al.,* 2024). The genes involved in virulence encode multiple factors, including *VEGF, GIF, vIL10, VIR*, and inhibitors of apoptosis, all of which influence the host's immune system (Karki *et al.,* 2019). All these immune-modulating proteins allow ORFV to create a localized, immunosuppressive environment that supports viral replication, delays immune clearance, and ultimately results in only short-lived immunity, even after infection or vaccination.

The diagnosis of ORFV is primarily based on clinical assessment, molecular methods, and virus isolation. While conventional symptomatic approach can be inaccurate, virus isolation remains the definitive method, though it is labour-intensive and time-consuming (Tedla *et al.,* 2018). Molecular techniques offer a more rapid means of confirming ORFV and differentiating it from goat pox and sheep pox viruses in cases of co-infection. Outbreaks of Orf have been documented in several regions across India (Kumar *et al.,* 2014; Bora *et al.,* 2015; Ahanger *et al.,* 2018; Nagarajan *et al.,* 2019; Sahu *et al.,* 2019; Rana *et al.,* 2022). Nevertheless, molecular confirmation of ORFV in Andhra Pradesh remains limited (Vimala Devi *et al.,* 2024), and no studies have been reported on the genetic characterization of circulating ORFV strains in this region. Elucidating the genetic composition of these strains is critical for the accurate identification of variants of this region, implementation of targeted outbreak control measures, and the development of region-specific vaccines. Therefore, the objective of this study was to perform molecular confirmation, sequencing, and phylogenetic analysis of the ORFV strains circulating in Andhra Pradesh.

Materials and Methods

Sample Collection and Genomic DNA Extraction

Over the period spanning July to December 2024, incidence of pustular dermatitis in goats was reported in Kurnool and Anantapur districts of Andhra Pradesh (India). All the affected animals exhibited pyrexia, marked nodular proliferations around the mouth and also on the face. A total of eleven representative samples (5 blood and 6 nodular skin scabs) were collected from two districts of the state.

Skin scab samples were homogenized to prepare a 10% suspension in sterile phosphate-buffered saline (PBS). The suspension was subjected to three freeze-thaw cycles, followed by centrifugation at 2300 g for 10 min. Viral genomic DNA was extracted from both blood and skin scab samples using the DNeasy® Blood & Tissue Kit (Cat. No. 69504, Qiagen, Germany) following the manufacturer's protocol.

PCR Targeting *GIF* Gene of ORFV

The extracted DNA was subjected to polymerase chain reaction (PCR) using primers specific to the *GIF* gene (Table 1). PCR amplification was performed according to the protocol described by Maan *et al.* (2014). A commercially available PCR master mix (Takara, Japan) was used for amplification of the target gene. The reaction mixture consisted of 25 µL, including 12.5 µL of master mix, 20 pmol of each forward and reverse primer, 5 µL of template DNA, and nuclease-free water to adjust the final volume. The thermal cycling conditions were: Initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 57°C for 1 min, extension at 72°C for 1 min, with a final extension at 72°C for 10 min.

Capripox Genus Specific PCR

To rule out the presence of sheep pox and goat pox virus, the extracted DNA was additionally subjected to a capripox genus-specific PCR targeting the *P32* gene (WOAH Terrestrial Manual, 2024) using primers outlined in Table 2. The preparation of the PCR master mix followed the same procedure as described for the Orf virus. The thermal cycling conditions for capripox PCR were: Initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 50°C for 30 sec, extension at 72°C for 1 min, with a final extension at 72°C for 5 min. Goat pox vaccine (Uttara kashi strain) was used as positive control. Amplified PCR products were gel electrophoresed on 1.5% agarose gel with ethidium bromide (0.5 µg/mL) along with 100 bp molecular weight marker. The PCR amplified products were visualized using gel documentation system (Biorad).

Sequencing and Phylogenetic Analysis

Two positive *GIF* gene PCR products representing the two districts were purified, and DNA sequencing was performed in both directions using the Sanger method (BioServe Pvt. Ltd., India). The resulting sequences were aligned and

Table 1: ORFV *GIF* gene specific primers nucleotide sequence

Sl. No	Name of the primer	Nucleotide sequence	Product size
1	Orf GIF/IL2 Forward	5'-GCTCTAGGAAAGATGGCGTG -3'	408 bp
2	Orf GIF/IL2Reverse	5'-GTACTCCTGGCTGAAGAGCG -3'	

Table 2: Capripox *P32* gene specific primers nucleotide sequence

Primers	Gene	Primer sequence	Product size
Capripox	*P32*	F: 5'-TCCGAGCTCTTTCCTGATTTTTCTTACTAT -3' R: 5'-TATGGTACCTAAATTATATACGTAAATAAC -3'	192 bp

compared with reference ORFV strains in the NCBI database (BLAST) to confirm the identity of ORFV and the *GIF* gene. The sequences generated were subsequently deposited in the NCBI Gen Bank for publication. The obtained *GIF* gene sequences were aligned with reference sequences from India and other countries available in the NCBI database using Clustal W in MEGA software version 11. A phylogenetic tree was constructed using the Maximum Likelihood (ML) method with the Tamura-Nei model with 1000 bootstrap replicates in MEGA 11 software to assess the evolutionary relationships.

Results and Discussion

Clinical examination revealed that all affected goats in the Kurnool district exhibited pyrexia, severe cutaneous lesions around the mouth, pustule formation, and scabbing, particularly on the lips, muzzle, oral commissures, nostrils, and gums (Fig. 1A). A slight variation in skin lesions was observed in the Anantapur district, where comparatively larger warts around the mouth and muzzle, as well as around the eyes and nose were observed (Fig. 1B). Higher incidence was reported in kids under 6 months of age and no mortality was recorded in either district. While the mortality rate for Orf is typically less than 1%, it can increase to 20-50% in cases with secondary complications, particularly in lambs and kids. In immune competent animals, lesions generally resolve within 6-8 weeks (Hosamani *et al.,* 2009).

Among the eleven samples analyzed using PCR, four skin scab samples tested positive for ORFV yielding a specific 408 bp PCR product (Fig. 2), while all blood samples were found negative. ORFV primarily replicates within dermis and epidermis of skin lesions, resulting in a higher viral load in skin samples. Conversely, blood samples usually exhibit lower positivity as the virus remains largely localized and is rapidly cleared from the blood stream. Given the potential for concurrent capripox infections in goat afflicted with Orf, it is essential to exclude the possibility of capripox virus presence. Mixed infections involving Orf and capripox viruses are frequently observed and can occur simultaneously (Venkatesan *et al.,* 2014). Therefore, distinguishing between these viruses is critical for implementing appropriate preventive and control measures, since effective vaccines are available for sheep pox and goat pox. In this regard, a capripox genus-specific PCR targeting the *P32* gene was utilized, and all tested samples were negative for capripox viruses (Fig. 3). These findings eliminated the presence of capripox viruses and confirmed that the infection was solely attributable to ORFV.

The obtained partial *GIF* gene sequences of the Ananthapur strain (ATP 2833) and the Kurnool strain (KRNL 4410) were deposited in NCBI gene pool and published with Accession numbers: PQ862856 & PQ862857, respectively. The two isolates exhibited a nucleotide sequence identity of 97.46%, forming a distinct cluster along with Tamil Nadu (2023) and Karnataka (2018) ORFV isolates in the Maximum Likelihood (ML) phylogenetic tree (Fig. 4). Anantapur isolate (ATP2833) demonstrated highest homology (98.42 % identity) with Tamil Nadu field isolates (2023) and also with TANUVAS Vaccine P15 strain, indicating a close evolutionary relationship

(1A) (1B)

Fig. 1: Clinical manifestation of goats suffering with Orf. **1(A):** Severe pustular dermatitis observed around mouth, oral commissures and also on the nose (Kurnool district). **1(B):** Crusty, scabby large wart like lesions prominent around the mouth and muzzle and extended slightly around the eyes and nose (Ananthapur district).

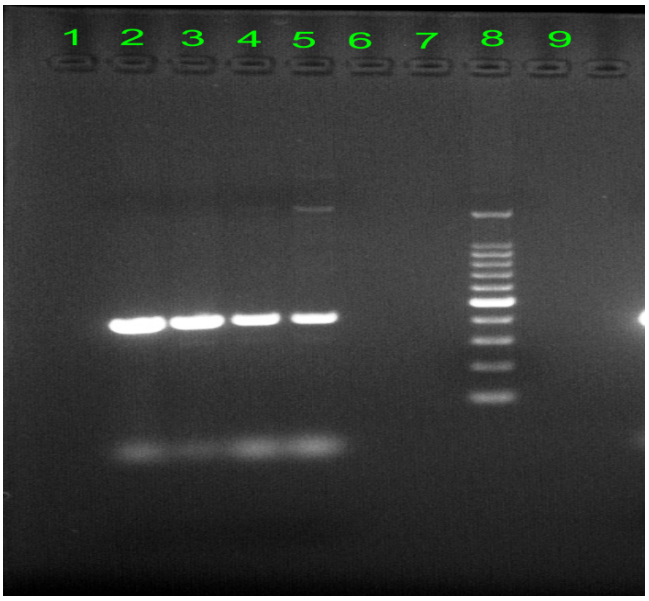

Fig. 2: Agarose gel image ORFV *GIF* PCR. Lane 2 & 3: Ananthapur district skin samples, Lane 4 & 5: Kurnool district skin samples, Lane 8: 100bp DNA ladder, Lane 1, 6, 7, 9: No sample

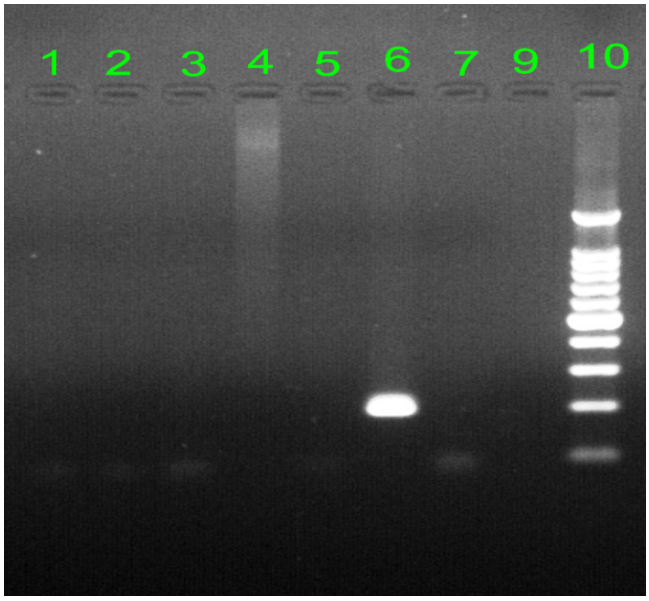

Fig. 3: Agarose gel image of Capripox P32 PCR. Lane 1, 2, 3: Skin samples, Lane 4 & 5: Blood samples, Lane 6: Positive control (Goat pox vaccine), Lane 7: Negative control, Lane 9: No sample, Lane 10 : 100 bp DNA marker

with neighbouring South Indian isolates. In contrast, it showed a lower sequence identity of 96.65% with the IVRI Mukteshwar P50 vaccine strain (2005) and Ludhiana field strain (2006), suggesting greater genetic divergence from Northern states isolates. Conversely, Kurnool isolate (KRNL 4410) showed greater homology with IVRI, Mukteshwar P50 vaccine strain (2005) and Ludhiana field strain (2006) with 98.48% sequence identity, which points to a strong genetic affiliation. Although the two districts are geographically proximate, the observed variation in the *GIF* gene of the ORFV strains responsible for the two outbreaks suggests that the infections may have originated from divergent sources. The viral strains in each outbreak could have evolved independently or been introduced through different transmission pathways, reflecting the complexity of the virus's spread and its ability to adapt to distinct host environments. Such findings highlight the need for further epidemiological investigation to track potential reservoirs and transmission dynamics within the region. However, complete genome sequencing is crucial to fully elucidate the genetic variability between the two strains and to understand their potential link to the observed differences in clinical presentations and disease severity. Similar findings on ORFV strain diversity was reported in previous studies from different geographical areas of the India (Venkatesan *et al.,* 2018) as well as worldwide (Bala *et al.,* 2019; Saeed *et al.,* 2020). Mutations in the virulence genes were found to correlate with high viral loads and persistent infections in animals and these mutations are influenced by the region's climate, livestock practices, host and breed variability (Abu Ghazaleh *et al.,* 2023; Coradduzza *et al.,* 2024).

Both isolates in this study were phylogenetically distinct from the first-generation New Zealand NZ2 isolate (1982) and the Japan (1985) isolates, indicating significant genetic differentiation from these historical reference strains. The percentage identity of both isolates with various reference ORFV strains published in NCBI site is provided in detail in Tables 3 and 4. To the best of the authors' knowledge, this report represents the first molecular characterization study of circulating ORFV strains in the state of Andhra Pradesh, India.

Table 3: Nucleotide sequence percent identity of Anantapur isolate (ATP 2833) with different reference ORFV isolates

ORFV isolates (NCBI uploaded)	% identity of ATP2833
PP512717: Tamil Nadu, Virudhunagar/2023	98.42
PP512718: TANUVAS p15 vaccine strain/2021	98.42
OR452360: Bangladesh/2021	97.78
PQ862857: Kurnool 4410/ 2024	97.46
PP932669: China/ 2023	97.21
MF414637: Ludhiana, Punjab/2006	96.70
ON389500: IVRI vaccine strain P50/2005	96.65
DQ184476: New Zealand, NZ2/1982	94.42

Table 4: Nucleotide sequence percent identity of Kurnool isolate (KRNL 44100) with different reference ORFV isolates

ORFV isolates (NCBI uploaded)	% identity of KRNL4410
ON389500: IVRI vaccine strain P50/2005	98.48
MF414637: Ludhina, Punjab/2006	98.23
OR452360: Banglaesh/2021	98.33
PP932669: China/2023	97.98
PP512717: Tamil Nadu, Virudhunagar/2023	97.67
PP512718: TANUVAS p15 vaccine strain/2021	97.67
PQ862857: ATP 2833/2024	97.46
DQ184476: New Zealand, NZ2/1982	95.45

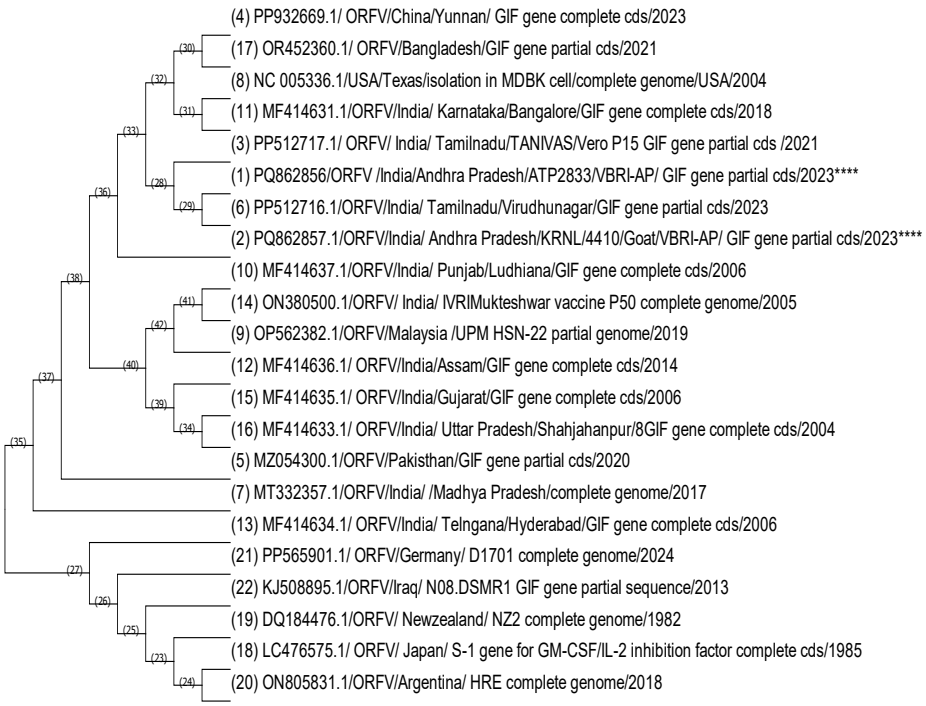

Fig. 4: Phylogenetic analysis based on partial sequence of GIF gene : The phylogenetic tree was constructed by maximum-likelihood algorithm using Mega 11 software and bootstrap analysis was performed with 1000 trials. All the reference sequences were retrieved from NCBI Genbank. *** indicates isolates of the present study.

Conclusion

The status of the molecular presence and genetic characterization of circulating ORFV strains in two districts of the state of Andhra Pradesh are reported. On genomic sequencing and phylogenetic analysis, the two isolates clustered in one group. Ananthapur isolate showed highest nucleotide homology with Tamilnadu field and vaccine strains. Conversely, Kurnool strain showed greater percent identity with IVRI, Mukteshwar P50 vaccine strain. The study highlights potential genetic variation in the *GIF* gene of ORFV isolates across different geographical regions, indicating significant evolutionary divergence from the first-generation strains. Hence continued surveillance and complete genomic characterization of these isolates are essential for strategic control of outbreaks and reducing the zoonotic transmission.

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References

Abu Ghazaleh, R., Al-Sawalhe, M., Abu Odeh, I. *et al.* (2023). Host range, severity and trans boundary transmission of Orf virus (ORFV). *Journal of Molecular Epidemiology and Evolutionary Genetics in Infectious Diseases, 112, 105448.*

Ahanger, S.A., Parveen, R., Nazki, S., Dar, Z., Dar, T. *et al.* (2018). Detection and phylogenetic analysis of Orf virus in Kashmir, Himalayas. *Virus Disease, 29*(3), 405-410.

Bala, J.A., Balakrishnan, K.N., Jesse, F.F.A. *et al.* (2019). Identification of strain diversity and phylogenetic analysis based on two major essential proteins of Orf viruses isolated from several clinical cases reported in Malaysia, Infection, *Genetics and Evolution, 77,*104076.

Bora, M.D.P., Barman, N.N., Borah, B. *et al.* (2015). Isolation and molecular characterization of Orf virus from natural outbreaks in goats of Assam*., Virus Disease, 26*(1-2), 82-88.

Coradduzza, E., Scarpa, F., Rocchigiani, A.M., Cacciotto, C. *et al.* (2024). The global evolutionary history of orf virus in sheep and goats revealed by whole genomes data. *Viruses*, *16,* 158.

Gelaye, E., Achenbach, J.E., Jenberie, S., Ayelet, G. *et al.* (2016). Molecular characterization of orf virus from sheep and goats in Ethiopia, 2008-2013. *Virology Journal, 13,* 34.

Hosamani, M., Scagliarini, A., Bhanuprakash, V., McInnes, C.J., & Singh, R.K. (2009). Orf: An update on current research and future perspectives. *Expert Review of Anti-Infective Therapy, 7*(7), 879-889.

Karki, M., Venkatesan, G., Kumar, A., Kumar, S., & Bora, D.P. (2019). Contagious ecthyma of sheep and goats: A comprehensive review on epidemiology, immunity, diagnostics and control measures. *Veterinarski Archiv*, *89*(3), 393-423.

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., Moudgil, P., Grakh, K., Jindal, N., Shrama, M., & Gupta, R. (2022). Epidemiology, clinical features and molecular detection

of Orf virus in Haryana (India) and its adjoining areas. *Tropical Animal Health and Production, 54,* 268.

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. *Virus Disease*, *25*(3), 376-380.

Managga, H., Bala, J.A., Balakrishnan, K.N., Bukaretal *et al.* (2022). Genome-wide analysis and molecular characterization of Orf virus strain UPM/HSN-20 isolated from goat in Malaysia. *Frontiers in Microbiology, 2022*(3), 877149

Nagarajan, G., Pourouchottamane, R., Reddy, G.B.M. *et al.* (2019). Molecular characterization of Orf virus isolates from Kodai hills, Tamil Nadu, India. *Veterinary World, 12*(7), 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.

Rana, K., Bhanderi, B., & Gadhavi, S. (2022). Detection of Orf virus causing contagious ecthyma in goats by PCR. *The Pharma Innovation Journal*, *SP-11*(11), 2571-2574.

Saeed, N., Sajad Pezeshki, N., Homayoon, M., Elaheh, A., Arezou K. *et al.* (2020). A Comparative study on global genetic diversity and population genetic analysis of Orf virus isolates from outbreaks and it's implications for the vaccine development. *American Journal of Biomedical Science & Research, 8*(4), 235-245.

Sahu, B., Majee, P., Sahoo, A., Nayak, D. *et al.* (2019). Molecular characterization, comparative and evolutionary analysis of the recent Orf outbreaks among goats in the Eastern part of India (Odisha). *Agricultural Genetics*, *12,* 198712.

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.

Venkatesan, G., Balamurugan, V., & Bhanuprakash, V. (2014). Multiplex PCR for simultaneous detection and differentiation of sheep pox, goat pox and Orf viruses from clinical samples of sheep and goat. *Journal of Virological Methods, 195*, 1-8.

Venkatesan, G., De, A., Arya, S., Kumar, A., Muthuchelvan, D., Debnath, B.C., Dutta, T.K., Hemadri, P.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.

Vimala Devi, B., Sireesha, G., Neeraja, D., Ravibabu, G., & Karthika, I. (2024). Molecular identification and differential diagnosis of contagious ecthyma (ORF) virus in sheep in Andhra Pradesh. *The AP Veterinarian e-Magazine, 1,* 1-10.

WOAH Terrestrial Manual (2024). Chapter 3.4.12. Lumpy skin disease 30.

Zhang, K., Lu, Z., Shang, Y., Zheng, H., Jin, Y., & Liu, J.H.X. (2019). Diagnosis and phylogenetic analysis of Orf virus from goat in China: A case report. *Virology Journal*, *7*, 78.