Biological Forum – An International Journal **16(11): 168-173(2024)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Molecular Detection of Sheep Pox Virus and its Differentiation from Goat Pox Virus

Manickam R.[1]*, Puvarajan B.[2], Balasubramaniam A.[3], Balakrishnan S.[4] and Selvaraj J.[5]
[1]Assistant Professor, Department of Veterinary Microbiology,
Veterinary College and Research Institute, Orathanadu, Thanjavur (Tamil Nadu), India.
[2]Professor and Head, Regional Research and Educational Centre (TANUVAS), Pudukottai (Tamil Nadu), India.
[3]Professor, Department of Veterinary Microbiology,
Veterinary College and Research Institute, Namakkal (Tamil Nadu), India.
[4]Professor and Head, Department of Veterinary Public Health and Epidemiology,
Veterinary College and Research Institute, Orathanadu (Tamil Nadu), India.
[5]Professor and Head, Department of Veterinary Pathology,
Veterinary College and Research Institute, Orathanadu (Tamil Nadu), India.

(Corresponding author: Manickam R.)*
(Received: 20 August 2024; Revised: 23 September 2024; Accepted: 21 October 2024; Published: 14 November 2024)
(Published by Research Trend)

ABSTRACT: Sheep pox is a viral disease affecting sheep, characterized by fever, papules or nodules, vesicles (rarely), lesions in internal organs, and mortality. The causative agent of sheep pox is the Sheep pox virus (SPPV), which, along with the Goat pox virus (GPV) and Lumpy skin disease virus (LSDV), constitutes the genus Capri poxvirus within the family Poxviridae. The objective of the present study is to detect the presence of the Sheep pox virus and to assess the current status of Sheep pox (SPPV) in the Cauvery Delta Zone and adjacent areas of Tamil Nadu. A total of sixty-two samples, comprising scabs and necropsies from internal organs, were collected from sheep suspected of having sheep pox across various regions. Molecular detection was conducted using polymerase chain reaction (PCR) to identify the Sheep pox virus through the amplification of the ORF103 gene. Additionally, the differentiation of Sheep pox virus isolated from sheep from the Goat pox virus was achieved by amplifying the RPO30 gene using specific primers. Our results indicated that forty-six samples (46/62; 74.19%) tested positive based on PCR analysis. In conclusion, our findings demonstrate the circulation of SPPV in the field. Further studies and surveillance are necessary to monitor the virus's evolution and transmission pathways, which will enhance the understanding of its pathobiology and aid in the control of SPPV.

Keywords: Capri poxvirus, Sheep pox virus, Polymerase chain reaction (PCR).

INTRODUCTION

Sheep pox viruses are classified within the Poxviridae family, Chordopoxvirinae sub-family, and Capripox virus genus (Buller *et al.*, 2005). These viruses are characterized by their large size (170–260 nm by 300-450 nm), double-stranded DNA, and enveloped structure (Tulman *et al.*, 2002; Biswas *et al.*, 2020). Capripox virus infections in small ruminants represent severe pox diseases affecting domestic animals. In susceptible herds, morbidity rates range from 75-100%, while case fatality rates, contingent upon the virus's virulence, vary between 10-85% during outbreaks. Mortality in older animals can escalate to 90% when capripox is compounded with *peste des petits ruminants* (PPR). Sheep and goat breeds originating from Europe exhibit high susceptibility to capripox, with mortality potentially reaching 100% (Kitching, 1986; Radostits *et al.*, 2006).

The Rambouillet sheep breed demonstrates heightened susceptibility to infection compared to Australian cross and American Merino breeds (Mondal *et al.*, 2004). The observed seasonality of sheep pox virus (SGP) may be attributed to the virus's ability to persist for extended periods in wet and cold conditions, its association with the lambing season, or the poor physiological state of flocks in autumn (Bhanuprakash, 2005; Yeruham *et al.*, 2007; Zangana, 2013). The primary vector for SGP virus dissemination is the movement of infected animals (Kitching, 2004; Radostits *et al.*, 2006). The peak shedding of infectious virus and viral DNA in the secretions of infected animals occurs approximately 1-2 weeks post-inoculation, with secretion persisting for an additional 3-6 weeks (Kitching, 1989; Bowden *et al.*, 2008). Stomoxys calcitrans, once infected, may transmit the pox virus to susceptible goats (Bhanuprakash *et al.*, 2006). The presence of antibodies against a virus in animal species indicates susceptibility; however, animals with such antibodies may not develop the infection or transmit the virus (Tuppurainen and Oura 2012).

There is limited information regarding the epidemiology and economic impact of sheep pox (SP) in dairy sheep flocks. This disease results in significant economic losses due to morbidity, mortality, decreased meat and milk production, abortion, and the depreciation of wool and skin quality, as well as trade restrictions (Yeruham *et al.,* 2007). Economic analyses have identified several variables, such as the number of adult animals affected, the duration of illness, and flock size, as significantly influencing the economic losses associated with sheep pox (Garner *et al.,* 2000; Senthilkumar and Thirunavukkarasu 2010). The incubation period of sheep and goat pox (SGP) ranges from 4 to 14 days; however, the OIE has recorded a maximum incubation period of 21 days (OIE, 2010). Typically, the initial symptoms of the disease include swelling of the nostrils, followed by thick nasal discharges and watery ocular discharges. Infected animals exhibit elevated body temperatures (41-42°C), and keratitis may develop (Daoud, 1997).

In the malignant form of sheep pox, symptoms such as high fever, marked depression, ocular and nasal discharges, and pock lesions on hairless areas of the skin, as well as on the mucosa of the respiratory, digestive, and urogenital tracts, have been observed. Pock lesions on the skin initially present as papules, which then progress to nodular, pustular, and finally scab stages. Pock lesions have also been observed in the heart muscles, although this manifestation of the disease is rare. In the benign form, more common in adult animals, only skin lesions occur, particularly under the tail, with no systemic reaction, and animals typically recover within 3-4 weeks. Abortion and secondary pneumonia have been reported as complications of sheep pox (Radostits *et al.,* 2006). Animals that recover from sheep pox infection acquire lifelong immunity. The virus persists through continuous transmission from infected to susceptible animals, necessitating a minimum size of the susceptible population (Kitching, 2004). The size of this population is influenced by the virus strain, host population susceptibility, and the number of infected animals (Panchanathan *et al.*, 2008; Sadri and Fallahi 2010).

Active mass vaccination against sheep pox can induce strong herd immunity, effectively controlling the disease. A single vaccination has been reported to provide lifelong strong immunity (Bhanuprakash *et al.,* 2011). At least two different vaccines containing isolates of the SP virus are available to protect small ruminants against sheep pox (Carn, 1993; Rao and Bandyopadhyay 2000; Abu-Elzein *et al.,* 2003; Radostits *et al.,* 2006; OIE, 2013; Iran Veterinary Organization, 2014). The Kenyan vaccine strain (O-240), not considered an SP virus, has been associated with clinical disease when used as a vaccine (Tuppurainen *et al.,* 2014). In India, the occurrence of sheep pox in both sheep and goat flocks within the same geographical location has been reported (Bhanuprakash *et al.,* 2010).

Capripox viruses exhibit a high degree of host specificity, with most isolates predominantly affecting either sheep or goats (Bhanuprakash *et al.,* 2010). According to Kenubih *et al*. (2021), GTPV has been accountable for the occurrence of CaPV diseases in small ruminants. Due to the close antigenic and virulence relationship between sheep pox virus (SPV) and goat pox virus (GPV), serological assays are unable to differentiate between the two (Balinsky *et al.,* 2008). However, phylogenetically distinct serotypes can be identified through molecular characterization targeting specific Capripox virus genes, such as RPO30, GPCR, and P32 (Bhanuprakash *et al.,* 2006; Yan *et al.,* 2012; Biswas *et al.,* 2020). Some sheep strains cause mild disease in goats and severe disease in sheep, whereas virulent goat strains can infect sheep (Bhanuprakash *et al*., 2010; He *et al*., 2020).

The prompt detection of SPV is crucial for effective control and mitigation of the significant economic losses associated with outbreaks (Mangana-Vougiouka *et al.,* 2000). Diagnosis typically relies on clinical signs followed by laboratory confirmation, which primarily involves virus isolation and electron microscopy (EM) (Oguzoglu *et al.,* 2006). Conventional seromonitoring techniques, including immunofluorescence, immunoprecipitation, virus neutralization, and ELISA, are widely employed for routine diagnosis. Previously, a polymerase chain reaction (PCR) method based on fusion and attachment protein genes has been reported to offer greater sensitivity than antigen trapping ELISA (Ireland and Binepal 1998). In the face of adversity, inactivated Capripox vaccines are safe and efficacious against CaPV (Wolff *et al.,* 2021; Es-sadeqy *et al*., 2021).

MATERIALS AND METHODS

Sample Collection: Sheep pox virus (SPV) infection is characterized by symptoms such as pyrexia, oculo-nasal discharge, and pock lesions that range from erythema to scabs on the body, with occasional pulmonary nodules (Rao and Bandyopadhyay 2000), as illustrated in Photos (1, 2, and 3). In this study, sixty-two clinical samples, including skin nodules, lung tissues, and crusted scabs, were collected from unvaccinated sheep exhibiting SPV symptoms. The samples were transported to the laboratory in a transport medium (PBS) containing antibiotics, maintained under chilled conditions, and stored at -40ºC until further analysis.

Polymerase Chain Reaction: DNA Extraction: DNA extraction from the samples was conducted using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH), with modifications to the manufacturer's protocol. Specifically, 200 µl of the sample suspension was incubated with 10 µl of proteinase K and 200 µl of lysis buffer at 56°C for 10 minutes. Following incubation, 200 µl of 100% ethanol was added to the lysate.

The sample was subsequently washed and centrifuged according to the manufacturer's instructions. Nucleic acid was eluted with 200 µl of elution buffer provided in the kit (Yousif *et al.,* 2010). The primers used are listed in Table (1), and the cycling conditions for the ORF103 and RPO30 genes are depicted in Table 2. Primers were utilized in a 25-µl reaction containing 10 µl of PCR Master Mix, 1 µl of each primer at 20 pmol

concentrations, 3 µl of water, and 5 µl of DNA template. The reaction was performed in a thermal cycler (Lamien *et al.,* 2011).

The PCR products were separated by electrophoresis on a 1.5% agarose gel in 1x TBE buffer at room temperature, using a gradient of 5V/cm. For gel analysis, 5 µl of the products was loaded into each gel slot. A 100 bp DNA Ladder (Qiagen, Germany, GmbH) was used to determine the fragment sizes. The gel was photographed using a gel documentation system (Bio-Rad), and the data were analyzed using computer software.

RESULTS

The application of conventional polymerase chain reaction (PCR) for nucleic acid detection yielded positive results across all prepared samples, as evidenced by the amplification of characteristic 151 base pair (bp) fragments of sheep pox viral DNA, clearly depicted in Fig. 1. The agarose gel, stained with Ethidium Bromide, exhibited prominent bands, thereby visually confirming the presence of viral DNA in the chorioallantoic membrane (CAM) suspensions. This initial screening method demonstrated efficacy in identifying potential sheep pox virus infections within the sample set.

To further substantiate the findings, an additional amplification step targeting a 570 bp fragment from the ORF 103 gene was conducted on three selected positive samples (Biswas *et al.*, 2020). This secondary confirmation enhanced the reliability of the results. The study's overall outcome indicated a significant prevalence of sheep pox virus, with 46 out of 62 samples (74.19%) testing positive based on the PCR analysis. The presence of strong bands in the Ethidium Bromide-stained agarose gel, as illustrated in Figure 2, provided clear visual evidence of viral DNA amplification, reinforcing the PCR results and underscoring the effectiveness of this diagnostic approach in detecting sheep pox virus infections.

DISCUSSION

Sheep pox, induced by the sheep pox virus (SPV), represents a severe and highly contagious disease that poses a substantial threat to global sheep populations. The virus is classified within the Capripoxvirus genus, which also encompasses the goat pox virus (GPV) and the lumpy skin disease virus. Clinically, the disease is characterized by a spectrum of symptoms, including elevated fever, respiratory distress, and the development of distinctive pox lesions on the skin and internal organs. These manifestations can result in diminished productivity, economic losses, and, in severe instances, mortality among affected flocks (Garner *et al.,* 2000; Bhanuprakash *et al.,* 2006). The effective management of sheep pox outbreaks necessitates a comprehensive approach, integrating rapid and precise diagnostic methods with strategic vaccination initiatives. While traditional diagnostic techniques such as virus isolation and electron microscopy retain their value, molecular methods like PCR have significantly enhanced the speed and sensitivity of detection. Nevertheless, the close antigenic similarity between SPV and GPV complicates serological differentiation, necessitating the development of more specific diagnostic tools (Balinsky *et al.,* 2008). This highlights the importance of ongoing research to advance diagnostic capabilities and improve vaccine efficacy, ultimately contributing to the enhanced control and potential eradication of sheep pox in endemic regions (Bhanuprakash *et al.,* 2011).

Alternative molecular methods for identifying and distinguishing sheep pox virus (SPV) from goat pox virus (GPV) have been developed to augment diagnostic capabilities. These methods include restriction endonuclease analysis (REA), whole genome sequencing, P32 gene-based PCR-RFLP, PCR targeting the RPO30 gene, and GPCR gene-based quantitative PCR (Hosamani *et al.,* 2004; Lamien *et al.,* 2011). Among these techniques, PCR targeting the RPO30 gene has demonstrated particular efficacy in differentiating between SPV and GPV (Santhamani *et al.,* 2013). In this study, SPV was successfully identified in samples isolated and cultured in embryonated chicken eggs using the chorioallantoic membrane (CAM) route. The presence of SPV was confirmed through PCR analysis using primers specific to the RPO30 gene, resulting in the amplification of 151 bp fragments. These findings were further validated through gel electrophoresis (Fig. 1), consistent with previous research by Lamien *et al.* (2011); Yan *et al.* (2012) on Capripoxvirus (CaPV) species identification.

To further reinforce the identification of SPV, the study also targeted the ORF 103 gene, successfully amplifying a 570 bp fragment from samples suspected of sheep pox infection (Fig. 2). This multi-gene approach enhances the reliability of SPV detection and differentiation from other closely related poxviruses. The combination of virus isolation techniques, such as culturing in embryonated chicken eggs, with molecular methods like PCR and gel electrophoresis, provides a robust framework for accurate diagnosis and characterization of SPV. These findings contribute to the expanding body of knowledge on poxvirus diagnostics and underscore the importance of employing multiple molecular targets for comprehensive virus identification.

The successful isolation of a field strain of the sheep pox virus from infected sheep constitutes a significant advancement in the understanding and management of this infectious disease. Sheep pox, caused by the sheep pox virus (SPPV), is a highly contagious and potentially lethal disease affecting sheep populations globally. The isolation of the virus from infected specimens provides researchers with valuable live samples for further investigation, facilitating a more precise characterization of the pathogen and its behaviour within host organisms. The study's application of PCR assays targeting the RPO30 and ORF 103 genes, in conjunction with sequencing techniques, presents a robust method for identifying sheep pox virus infections. This approach not only enhances diagnostic capabilities but also lays the

groundwork for the development of more effective vaccination strategies. By accurately identifying the virus and its genetic composition, researchers can design vaccines that specifically target the most pertinent viral antigens, potentially enhancing immune responses and overall protection against sheep pox. Moreover, this methodology may contribute to epidemiological studies, enabling improved tracking of virus strains and their geographic distribution, which is essential for implementing targeted control measures and preventing outbreaks in susceptible sheep populations.

Photo (1): nodular lesions under the tail of suspected SPPV infected sheep

Photo (2): nodular and crusted lesions on the eyelid of suspected SPPV infected sheep

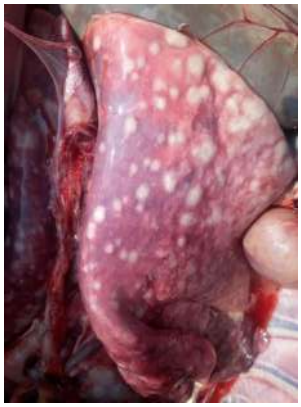

Photo (3): Pock lesions on the lung of suspected SPPV infected sheep

Table 1: Primers sequences, target genes, amplicon sizes.

Reference	Amplicon size	Primer sequence (5'-3')	Target gene
Zhu *et al*. (2013)	570 bp	5' - ATGTCTGATAAAAAATTATCTCG -3' 3' - ATCCATACCATCGTCGATAG - 5'	ORF103
Santhamani *et al*. (2013)	151bp	5' - TCTATGTCTTGATATGTGGTGGTAG - 3' 5' - AGTGATTAGGTGGTGTATTATTTTCC-3'	RPO30

Table 2: Cycling conditions – PCR (ORF103 & RPO30 genes).

Target gene	Primary denaturation	Amplification (35 cycles)			Final extension
		Secondary denaturation	Annealing	extension	
RPO30	94°C, 5min.	94°C, 30 sec.	55°C, 30 sec.	72°C, 30 sec.	72°C, 7 min.
ORF103	94°C, 5min.	94°C, 30 sec.	52°C, 30 sec.	72°C, 30 sec.	72°C, 7 min.

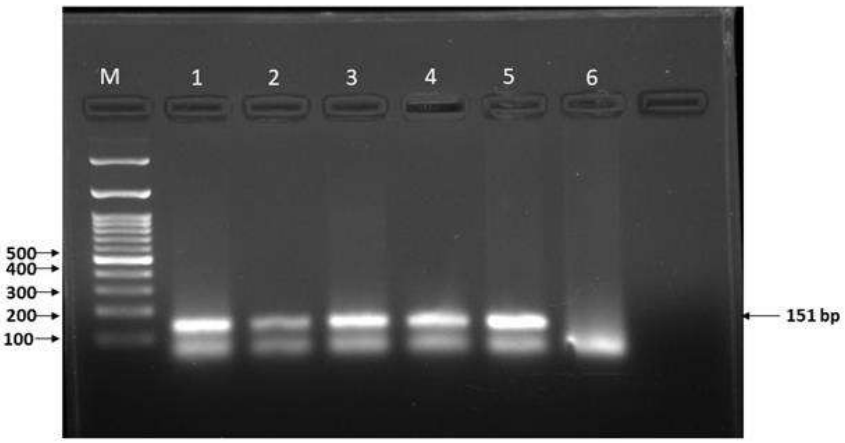

Fig. 1. Amplification of RPO30 gene of sheep pox virus by PCR. M: molecular marker. Lane (1): positive control Lane (2-5): positive PCR products from sheep pox virus isolate (CAM). Lanes (6): negative control

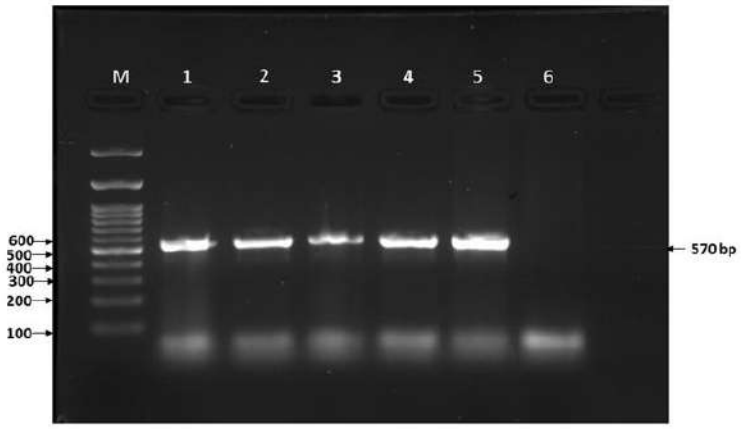

Fig. 2. Amplification of ORF 103 gene by PCR. M - DNA marker, lane (1): positive control; lane (2 - 5): positive isolate; lane (6): Negative control.

CONCLUSIONS

Sheeppox and goatpox (SGP) is highly infectious disease in sheep and goats, which is distributed to different part of the world. It seriously affects exotic breeds. Mortality and morbidity rate get to 100%. The virus enters via the respiratory tract and transmission commonly is by aerosol and infection is usually associated with close contact with infected animals. This disease is more severe in young animals than adults; the affected lambs die without showing clinical signs. Sheeppox and Goatpox require an urgent and precise laboratory confirmation as the diseases are severe contagious. The RPO30 and ORF103 gene-based PCR assay in combination with sequencing can be used for identification sheep pox virus infection. Management strategies like clean water, well enclosure housing, balanced diet and minimizing stress during cold season and lambing stage should be provided. Since this disease has no effective treatment, it is essential to vaccinate sheep and goat flocks regularly on an annual basis with a safe and efficient vaccine for the control of this serious and economically important disease in endemic regions. In enzootic areas biosecurity measures should also be considered.

FUTURE SCOPE

Expanding on the epidemiological perspective of sheep pox virus, further investigation into the sequence analysis of immunomodulatory protein genes is crucial for developing effective control strategies. These genes play a vital role in the virus's ability to evade host immune responses and establish infection. By conducting comprehensive sequence analyses across different strains and isolates of sheeppox virus, researchers can identify conserved regions and potential targets for vaccine development or antiviral therapies. Moreover, understanding the genetic diversity and evolution of these immunomodulatory proteins can provide insights into the virus's adaptation mechanisms and potential cross-species transmission. This knowledge is essential for predicting and mitigating future outbreaks, as well as designing diagnostic tools with improved sensitivity and specificity. Additionally, comparative genomic studies with related poxviruses may reveal common immunomodulatory strategies, facilitating the development of broad-spectrum control measures that could be effective against multiple poxvirus infections in sheep and potentially other livestock species.

Acknowledgement. The authors would like to thank field veterinarian for facilitating the sample collection.
Conflict of Interest. None.

REFERENCES

Abu-Elzein, E., Housawi, F. and Ramadan, O. (2003). Observations on natural and experimental infection of sheep and goats with a virulent field Capripox virus with high affinity to goat. *Veterinarski Arhiv*, *73*, 119-131.

Balinsky, C., Delhon, G., Smoliga, G., Prarat, M., French, R., Geary, S., Rock, D. and Rodriguez, L. (2008): Rapid preclinical detection of Sheep pox virus by a real-time PCR assay. *J. Clin. Microbiol.*, *5*, 438-442.

Bhanuprakash, V. (2005). An epidemiological study of sheep pox infection in Karnataka state, India. *Revue scientifique et technique (International Office of Epizootics)*, *24*, 909-920.

Bhanuprakash, V., Hosamani, M. and Singh, R. (2011). Prospects of control and eradication of capripox from the Indian subcontinent: a perspective. *Antiviral Res*., *91*, 225-232.

Bhanuprakash, V., Venkatesan, G., Balamurugan, V., Hosamani, M., Yogisharadhya, R., Chauhan, R., Pande, A., Mondal, B. and Singh, R. (2010). Pox outbreaks in sheep and goats at Makhdoom (Uttar Pradesh). India: evidence of Sheep pox virus infection in goats. *Transboundary and Emerging Diseases*, **57**: 375-382.

Bhanuprakash, V., Indrani, B., Hosamania, M. and Singh, R. (2006). The current status of sheep pox disease. *Comparative Immunology, Microbiology and Infectious Diseases*, *29*(1), 27-60.

Biswas, S., Noyce, R. S., Babiuk, L. A., Lung, O., Bulach, D. M., Bowden, T. R., Boyle, D. B., Babiuk, S. and Evans, D. H. (2020). Extended sequencing of vaccine and wild-type capripoxvirus isolates provides insights into genes modulating virulence and host range. *Transboundary and Emerging Diseases*, *67*(1), 80–97.

Bowden, T. R., Babiuk, S. L. and Parkyn, G. R. (2008). Capripoxvirus tissue tropism and shedding: A quantitative study in experimentally infected sheep and goats. *Virology*, *371*, 380-393.

Buller, R. M., Arif, B. M. and Black, D. N. (2005). Poxviridae. In: Virus Taxonomy: Eight Report of the International Committee on the Taxonomy of Viruses. Elsevier Academic Press, Oxford; pp 117.

Carn, V. M. (1993). Control of capripoxvirus infections. *Vaccine*, *11*, 1275-1279.

Daoud, J. A. H. (1997). Sheep pox among australian sheep in Jordan. Tropical Animal Health and Production, 29: 251-252.

Es-sadeqy, Y., Bamouh, Z., Ennahli, A., Safini, N., El Mejdoub, S., Omari Tadlaoui, K., Gavrilov, B. and El Harrak, M. (2021). Development of an inactivated combined vaccine for protection of cattle against lumpy skin disease and bluetongue viruses. *Veterinary Microbiology, 256*, 109046.

Garner, M. G., Sawarkar, S. D., Brett, E. K., Edwards, J. R., Kulkarni, V. B., Boylem, D. B. and Singh, S. N. (2000). The Extent and Impact of Sheep Pox and Goat Pox in the State of Maharashtra, India. *Tropical Animal Health and Production*, *32*, 205-223.

He, C., Tong, J., Zhang, X., Tuohetiniyazi, M., Zhang, Y. and Li, Y. (2020). Comparative analysis of ankyrin (ANK) genes of five capripoxviruses isolate strains from Xinjiang province in China. *Virology Journal*, *17*(1), 1–18.

Hosamani, M., Mondal, B., Tembhurne, P. A., Bandyo, S. K., Singh, R. K. and Rasool, T. J. (2004). Differentiation of sheep pox and goat poxviruses by sequence analysis and PCRRFLP of P32 gene. *Virus Genes*, *29*, 73-80.

Iran Veterinary Organization (2014). survey analysis on sheep pox and goat pox in IRAN during 2010-2014. Pox National Commitee Bultin of Iran Veterinary Organization, Tehran, Iran.

Ireland, D. C. and Binepal, Y. S. (1998): Improved detection of capripoxvirus in biopsy samples by PCR. *Journal of Virological Methods*, *74*, 1-7.

Kenubih, A., Mammo,B., Terefe, G. and Fentahun, T. (2021). Assessment of the impact of sheep and goat pox lesions on skin quality in selected warehouses of central Ethiopia. *Veterinary Medicine: Research and Reports*, *12*, 199–204.

Kitching, R. P. (1986). The control of sheep and goat pox. Revue Scientifique et Technique de l'OIE (France), *5*, 503-511.

Kitching, R.P. (1989). The characterization of African strains of capropoxviruses. *Epidemiology and Infection*, *102*, 335-343.

Kitching, R.P. (2004). Sheep pox and goat pox. In: Infectious Diseases of Livestock, (Coetzer JAW, 2nd Edn). Capetown: Oxford University Press Southern Africa; pp. 1277-1281.

Lamien, C. E., Le Goff, C. and Silber, R. (2011). Use of the Capripoxvirus homologue of Vaccinia virus 30 kDa RNA polymerase subunit (RPO30) gene as a novel diagnostic and genotyping target: development of a classical PCR method to differentiate Goat poxvirus from Sheep poxvirus. *Vet. Microbiol*., *149*, 30-39.

Mangana-Vougiouka, O., Markoulatos, P., Koptopoulos, G., Nomikou, K., Bakandritsos, N. and Papadopoulos, P. (2000). Sheep poxvirus identification from clinical specimens by PCR, cell culture, immuno-fluorescence and agar gel immuno-precipitation assay. *Mol Cell Probe*, *14*, 305-310.

Mondal, B., Hosamani, M. and Dutta, T. K. (2004). An outbreak of sheep pox on a sheep breeding farm in Jammu, India. *Revue scientifique et technique-Office international des épizooties*, *23*, 943-949.

Oguzoglu, T. C, Alkan, F., Ozkul, A., Atalay Vural, S., Gungor, A. B. and Burgu, I. (2006). A Sheep pox Virus Outbreak in Central Turkey in 2003: Isolation and Identification of Capripoxvirus Ovis. *Veterinary Research Communications*, *30*, 965-971.

OIE (Office International des Epizooties) (2010). Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, Chapter 2.4.14, Lumpy Skin Disease, Paris: OIE.

OIE (Office International des Epizooties) (2013). Sheep Pox and Goat Pox. http://www.oie.int/ fileadmin / Home / eng / Animal _ Health_in_the_World /docs/pdf/Disease_cards/sheep_goat_pox.pdf. (Accessed on November 1, 2015)

Panchanathan, V., Chaudhri, G. and Karupiah, G. (2008). Correlates of protective immunity in poxvirus infection: where does antibody stand? *Immunology and Cell Biology*, *86*, 80-86.

Radostits, O. M., Gay, C. C. and Hinchcliff, K. W. (2006). Veterinary Medicine. 10th Edn. Saunders; pp 1430-1431.

Rao, T. V. S. and Bandyopadhyay, S. K. (2000). A comprehensive review of goat pox and sheep pox and their diagnos. *Animal Health Research Reviews*, *1*, 127-136.

Sadri, R. and Fallahi, R. (2010). A new approach to develop a vaccine against capropox infection in sheep and goats using a new strain of sheep pox virus in Iran. *Iranian Journal of Veterinary Medicine*, *4*, 221-224.

Santhamani, R, Yogisharadhya, R., Venkatesan, G., Shivachandra, S. B., Pandey A. B. and Ramakrishnan, M. A. (2013). Detection and differentiation of sheeppox virus and goat pox virus from clinical samples using 30 kDa RNA polymerase subunit (RPO30) gene based PCR. *Veterinary World*.

Senthilkumar, V. and Thirunavukkarasu, M. (2010). Economic losses due to sheep pox in sheep farms in Tamil Nadu. Tamil Nadu Journal of Veterinary and Animal Sciences, *6*, 88-94.

Tulman, E. R., Afonso, C. L., Lu, Z., Zsak, L., Sur, J. H., Sandybaev, N. T., Kerembekova, U. Z., Zaitsev, V. L., Kutish, G. F. and Rock. D. L. (2002). The genomes of sheep pox and goat pox viruses. *Journal of Virology*, *76*, 6054-6061.

Tuppurainen, E. S. M. and Oura, C. A. L. (2012). Review: Lumpy Skin Disease: An Emerging Threat to Europe, the Middle East and Asia. *Transboundary and Emerging Diseases*, *59*, 40-48.

Tuppurainen, E. S. M., Pearson, C. R. and Bankowska, K. B. (2014). Characterization of sheep pox virus vaccine for cattle against lumpy skin diesase virus. *Antiviral Research*, *109*, 1-6.

Yan, X. M., Chu, Y. F., Wu, G. H., Zhao, Z. X., Li, J., Zhu, H. X. and Zhang, Q. (2012). An outbreak of sheep pox associated with goat poxvirus in Gansu Province of China. *Vet Microbiol*, *156*, 425-428.

Yeruham, I., Yadin, H. and Van Ham, M. (2007). Economic and epidemiological aspects of an outbreak of sheeppox in a dairy sheep flock. *The Veterinary Record*, *160*, 236-237.

Yousif, A. A., Al-Naeem, A. A. and Al-Ali, M. A. (2010). Rapid non-enzymatic extraction method for isolating PCR-quality camel pox virus DNA from skin. *J. Virol. Meth*., *169*, 138-142.

Wolff, J., Moritz, T., Schlottau, K., Hoffmann, D., Beer, M and Hoffmann, B. (2021). Development of a safe and highly efficient inactivated vaccine candidate against lumpy skin disease virus. *Vaccines*, *9*(1), 1–30.

Zangana (2013). Epidemiological, clinical and histopathological studies of lamb and kid pox in Duhok, Iraq. *Bulgarian Journal of Veterinary Medicine*, *16*, 133-138.

Zhu, X. L., Yang, F, Li. H. X., Dou, Y. X., Meng, X. L., Li, H., Luo, X. N. and Cai, X. P. (2013). Identification and phylogenetic analysis of a sheep pox virus isolated from the Ningxia Hui Autonomous Region of China. *Genetics and Molecular Research*, *12*(2): 1670-1678.

How to cite this article: Manickam R., Puvarajan B., Balasubramaniam A., Balakrishnan S. and Selvaraj J. (2024). Molecular Detection of Sheep Pox Virus and its Differentiation from Goat Pox Virus. *Biological Forum – An International Journal, 16*(11): 168-173.