Indian Journal of Animal Sciences **92** (10): 1149–1153, October 2022/Article
https://doi.org/10.56093/ijans.v92i10.110006

Pathological studies and ORF 103 gene based molecular characterization of sheep pox virus from outbreaks in Maharashtra, India

DHAYGUDE VITTHAL[1✉], KAMDI BHUPESH[1], BHOSALE SHRUTI[1], SABHARWAL DIVYA[1], KULKARNI TEJASHRI[1] and ABHIJIT BARATE[1]

Krantisinh Nana Patil College of Veterinary Science, Shirwal, Satara, Maharashtra 412 801 India

Received: 30 January 2021; Accepted: 28 August 2022

ABSTRACT

Sheep and goat pox are endemic in India and causes considerable losses to the animal husbandry sector every year. There are multiple reports of this disease from different states of India; however, information on the occurrence of this disease in Maharashtra state is very scanty. In this context, the present investigation reports the outbreaks of sheep pox in Satara and Solapur districts of Maharashtra state, India, from August 2019 to February 2020 based on clinical signs, gross and histopathological lesions, and molecular detection. Additionally, the ORF 103 gene sequence analysis from field samples revealed 100 to 99% identity with SPPV from Egypt, China, Pune, Ahmedabad, and Makhdoom. In phylogeny, sequences of the present study grouped into SPPVs clade. The finding of this study adds to the knowledge of the epidemiology of sheep pox in Maharashtra state and may help in planning effective prevention and control strategies.

Keywords: Lesions, ORF 103 gene, PCR, Phylogenetic analysis, Sheep pox

According to the 20th Livestock Census, India's total goat and sheep population is 148.88 and 74.26 million, respectively. It ranks second in the goat population and fourth in the sheep population worldwide, which draws attention to economic implications associated with outbreaks of diseases like sheep and goat pox, which have been reported to cause average morbidity and mortality of 63.5 and 49.5%, respectively (Madhavan *et al.* 2016).

Sheep pox is a highly contagious disease caused by the sheep pox virus (SPPV), which belongs to the genus *capripoxvirus* (CaPV) in the family poxviridae. The other two species in the genus are the lumpy skin disease virus (LSDV), which causes disease in cattle, and the goat pox virus (GTPV). Diseases caused by members of the genus *capripoxvirus* are considered notifiable diseases by World Animal Health Organization. Its genome is double-stranded DNA (nearly 150 kbp), having 149 putative genes encoding for various proteins involved in replication, structure, virulence, and host range functions. The coding region of the CaPV genome has 1-156 ORFs in which central ORFs (024-123) are conserved genes involved in replication and transcription mechanisms (Moss *et al.* 2001), whereas the terminal ORFs (001-023 and 124-156) are variable and are involved in host immune evasion and host-range functions (Tulman *et al.* 2001).

Sheep pox and goat pox are endemic in Africa north of the Equator and parts of the Middle East and Asia, including India, which is evident through the Annual Report of National Institute of Veterinary Epidemiology and Disease Informatics, Bengaluru, India (2014-2015). This report describes a total of 3,245 pox outbreaks among sheep and goats from 2005 to 2013 in different states of India. In India, the disease is endemic and inflicts severe economic losses due to mortality, abortions, mastitis, loss of wool, condemnation of skin, and loss of export. The losses due to capripox in Maharashtra state (India) alone are estimated at over ₹ 105 million (US$2.3 million), and it takes nearly six years for a flock to recover from the outbreak (Garner *et al.* 2000).

Capripox viruses are mainly host-specific and cause severe clinical disease in either sheep or goats, while some strains have equal virulence in both species (Bhanuprakash *et al.* 2010, OIE 2013). Available serological assays could not distinguish SPV and GPV due to the close antigenic and virulence relationship (Balinsky *et al.* 2008). However, in the recent past, RPO30, GPCR, P32, ORF 095, and ORF 103 gene sequencing and phylogenetic analysis have been employed for the differentiation of CaPVs (Yan *et al.* 2012, Zhu *et al.* 2013).

The present research paper reports clinicopathological findings, gross and microscopic lesions during sheep pox outbreaks, and ORF 103 gene based differentiation of CaPVs from outbreaks in the Maharashtra state of India.

Present address: [1]Krantisinh Nana Patil College of Veterinary Science, Shirwal, District-Satara, Maharashtra (Maharashtra Animal and Fishery Sciences University, Nagpur, Maharashtra).
✉Corresponding author email: drvitthalp@gmail.com

MATERIALS AND METHODS

Gross and histopathological examination and sampling: Carcasses of sheep from 7 flocks from parts of Satara and Solapur districts of Maharashtra state were presented for necropsy at Department of Veterinary Pathology, KNP College of Veterinary Science, Shirwal, District-Satara, Maharashtra, India from August 2019 to February 2020. Thorough necropsies were conducted on two carcasses each from all seven flocks to note gross lesions (14 necropsies were carried out). The affected tissues viz. skin over teat, tongue, oesophagus, lungs, liver, heart muscles, kidneys and mediastinal lymph nodes from all seven flocks were collected in 10% neutral buffered formalin for histopathological examination. The tissues were processed by routine paraffin embedding technique and stained with haematoxylin and eosin method as suggested by Luna (1968).

After obtaining the history, visits were arranged at respective flocks, and the information on duration of illness, flock size, clinical signs, morbidity, mortality, history of vaccination and other managemental practices was recorded.

Molecular detection of SPPV: The parts of affected tissues, viz. skin over teat, lungs, and mediastinal lymph nodes, were collected in sterile containers following aseptic precautions and stored at -20°C until nucleic acid extraction. DNA was extracted from frozen pooled tissue samples from all seven flocks by proteinase K and phenol method with minor modifications (Sambrook and Russell 2001).

For confirmation of viral etiology, PCR targeting open reading frame 103 gene (ORF 103) of sheep pox virus was performed. The forward primer 5'-TGTCTGATAAAAATTATCTCG-3' and reverse primer: 5'-ATCCATACCATCGTCGATAG-3' reported by Zhu *et al.* (2013) were used. PCR was performed in 25 µl reaction volume comprising 12.5 µl master mix (EmeraldAmp GT 2× PCR Master Mix, Takara), 1 µl (10 pmol) each of forward and reverse primer, 3 µl template DNA, and 7.5 µl nuclease free water. PCR amplification proceeded with an initial denaturation step of 94°C for 5 min, followed by 35 cycles at 94°C for 1 min, 52°C for 45 sec, 72°C for 1 min, and a final extension of 72°C for 10 min. Amplicons were visualized via electrophoresis on a 2% agarose gel and documented with a gel documentation system.

Sequencing and phylogenetic analysis: The PCR products from one flock, each from Satara and Solapur districts located distantly, were subjected to sequencing. PCR products (570 bp) were gel purified using the PureLinkTM Quick Gel extraction kit (Invitrogen, Carlsbad, CA). The purified PCR products were sequenced in both directions using BigDye® Terminator v3.1 Cycle Sequencing Kit (NimaGen BV, The Netherlands). Nucleotide sequences were subjected to BLAST analysis (http:// blast.ncbi.nlm.nih.gov/) to confirm that the sequences represented the sheep pox virus. The sequences were compiled, aligned, and the phylogenetic tree was constructed using the neighbor joining method using MEGA 6 (Tamura *et al.* 2012).

RESULTS AND DISCUSSION

Clinical findings: Clinically, the disease was initially characterized by all or few signs like fever, depression, cutaneous lesions such as macules, papules and/or nodules, occulonasal discharge, and laboured. In later stages, before death, most of the animals showed noisy breathing. There was variation in severity of clinical signs from mild, moderate to severe. In many cases, tongue revealed very typical whitish raised lesions. Suman *et al.* (2020), Kamran *et al.* (2015) and Ramakrishnan *et al.* (2017) also reported similar clinical signs in sheep pox with little variability in frequency and severity.

The flock size ranged from 70 to 2,400. Morbidity in all seven affected flocks ranged between 31 to 70%, whereas mortality was between 6.5 to 43% over one month period. Sheep and goats were reared together in all seven affected flocks; however, only sheep were diseased, indicating the species specificity of the virus. None of the seven flocks affected with sheep pox were vaccinated against sheep pox. In the present investigation, variable morbidity and mortality was recorded in all seven affected flocks, wherein morbidity ranged between 31 to 70% and mortality was between 6.5 to 43% over one month period. Takele *et al.* (2018) also stated that in endemic areas, the morbidity rate reaches 70-90%, whereas the mortality rate is up to 5-10% and approaches 100% in newly imported animals. Madhavan *et al.* (2016) stated that, in a susceptible flock, morbidity is 75-100%, and mortality is 10-58% depending on the virulence of the virus and can reach up to 100% in naive animals. There seems to be variation in morbidity and mortality in sheep pox. The variable morbidity and mortality rates are mainly attributed to host species and virulence of the viruses (Bhanuprakash *et al.* 2006).

Gross and microscopic lesions: Postmortem examination of dead animals revealed cutaneous lesions either as small circumscribed hyperaemic areas, macules, papules, and or nodules distinctly visible on the sparsely wooled area of head, tail, perineum, udder, teat, medial thigh and other body parts. Tongue revealed very typical whitish raised lesions. In long standing cases, nodules turned to black scabs and then ulcers. Papules with occasional ulceration were also found on the abomasal mucosa, wall of the rumen and large intestine, hard and soft palate, trachea, and oesophagus. Diffuse to multifocal papular and nodular whitish gray lesions (gunshot lesions) were observed on all lobes of both lungs along with pneumonic consolidation in all animals that died of disease. Multifocally, pale areas of degeneration and necrosis were noted on the liver and kidneys in sheep from 5 flocks (Fig. 1).

Histopathological lesions corroborated gross findings. The skin over teat revealed hyperplasia of squamous epithelial cells leading to thickening of the epidermis. Hydropic degeneration and intracytoplasmic inclusion

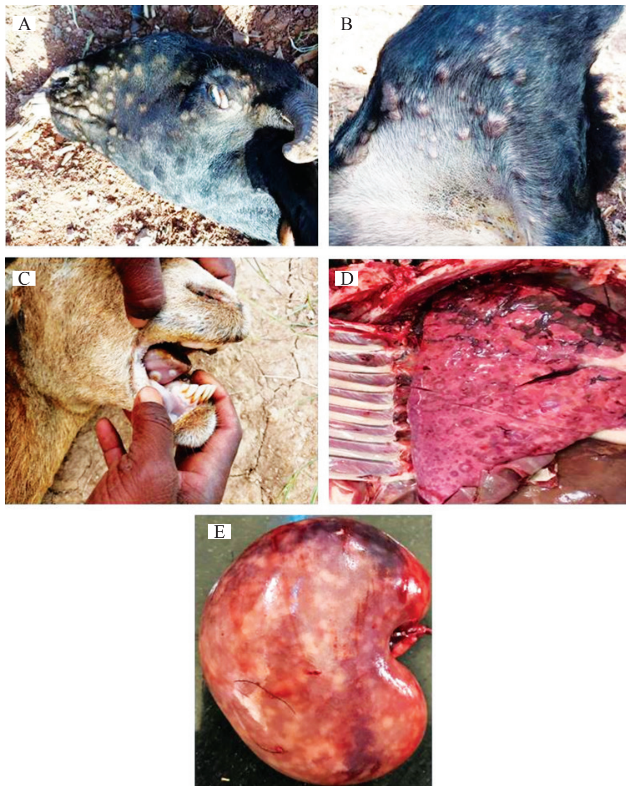

Fig. 1. **A**. Cutaneous lesions either as macules, papules and or nodules distinctly visible on head and mouth. **B**. Pox nodules on medial thigh. **C**. Tongue with very typical whitish raised pox nodules. **D.** Multifocal papular and nodular whitish gray (gunshot lesions) pox nodules on all lobes of both lungs along with pneumonic consolidation. **E**. Multifocal pale areas of degeneration and necrosis on kidney.

bodies in epithelial cells along with acanthosis, keratosis and parakeratosis were also the characteristic findings. The dermis showed focal areas of necrosis, vasculitis, and infiltration of macrophages and lymphocytes. Lungs revealed multifocal areas of proliferative alveolitis characterized by the presence of pox cells and debris in alveolar spaces. Multifocal areas of necrosis, pulmonary edema, and inflammation characterized by infiltration and congestion were also noted. The alveolar septa were thickened due to edema and infiltration of mononuclear cells. Bronchial hyperplasia and bronchiolar epithelium metaplasia into rounded cells was observed. Intracytoplasmic eosinophilic inclusions could be seen in the swollen bronchiolar epithelium and other cells in the alveolar areas. The bronchial lumen contained cell debris and inflammatory cells, predominantly PMNs (Fig. 2). Focal areas of degeneration and necrosis along with focal mononuclear cell aggregates were noted in liver. The kidney capsule appeared thickened, and beneath the capsule, there was hyalinization and proliferation of cells. Multifocal areas of interstitial infiltration of mononuclear cells were noted. Focal areas of tubular epithelial swelling and congestion were also noted in kidneys. The SPPV and GTPV have tropism for skin, lung and discrete sites within mucosal surfaces of oro-nasal tissues and gastrointestinal

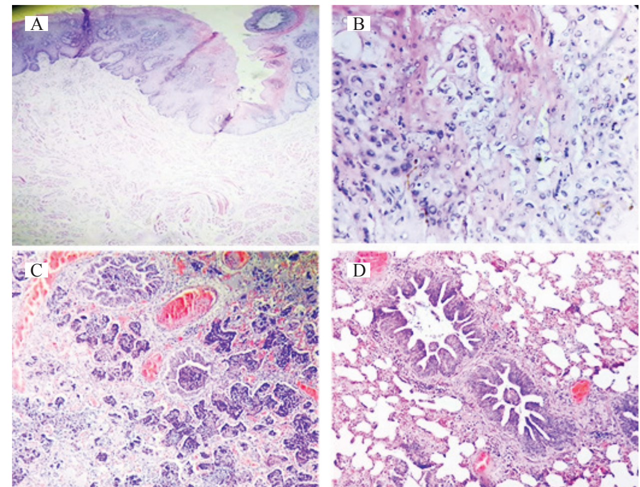

Fig. 2. **A**. Section of skin over teat revealing hyperplasia of squamous epithelial cells leading to thickening of epidermis along with acanthosis, keratosis and parakeratosis (H&E, 100×). **B**. Section of skin showing intracytoplasmic inclusion bodies in proliferating squamous. epithelial cells (H&E, 400×). **C**. Section of lung depicting multifocal areas of proliferative alveolitis characterized by presence of pox cells and debris in alveolar spaces along with areas of necrosis, pulmonary edema, congestion and infiltration of inflammatory cells (H&E, 100×). **D**. Section of lung showing proliferation of bronchial epithelium along with peribronchial infiltration, pulmonary edema, congestion and necrosis (H&E, 100×).

tract with lesser extent, lymphoid tissue. Tropism of SPPV and GTPV for the skin and minor involvement of the liver and spleen suggests that the pathogenesis of capripox resembles smallpox and monkeypox (Fenner 1988, Zaucha *et al.* 2001, Jahrling *et al.* 2004, Babiuk *et al.* 2008). In present investigation, the gross and microscopic lesions typical of pox on skin, tongue, esophagus, lung, kidney, liver etc were in accordance with descriptions by Rinku *et al.* (2013), Madhavan *et al.* (2016) and Takele *et al.* (2018).

PCR amplification of ORF 103 gene to detect SPPV: PCR amplification of ORF 103 gene to detect sheep pox virus genome from DNA extracted from tissue samples of all seven flocks revealed bands of expected 570 bp size, indicating a positive reaction (Fig. 3). This result is

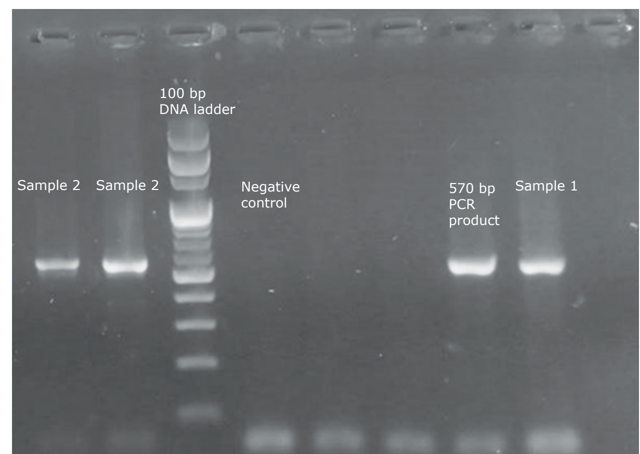

Fig. 3. PCR amplification of ORF 103 gene of pox virus (570 bp product).

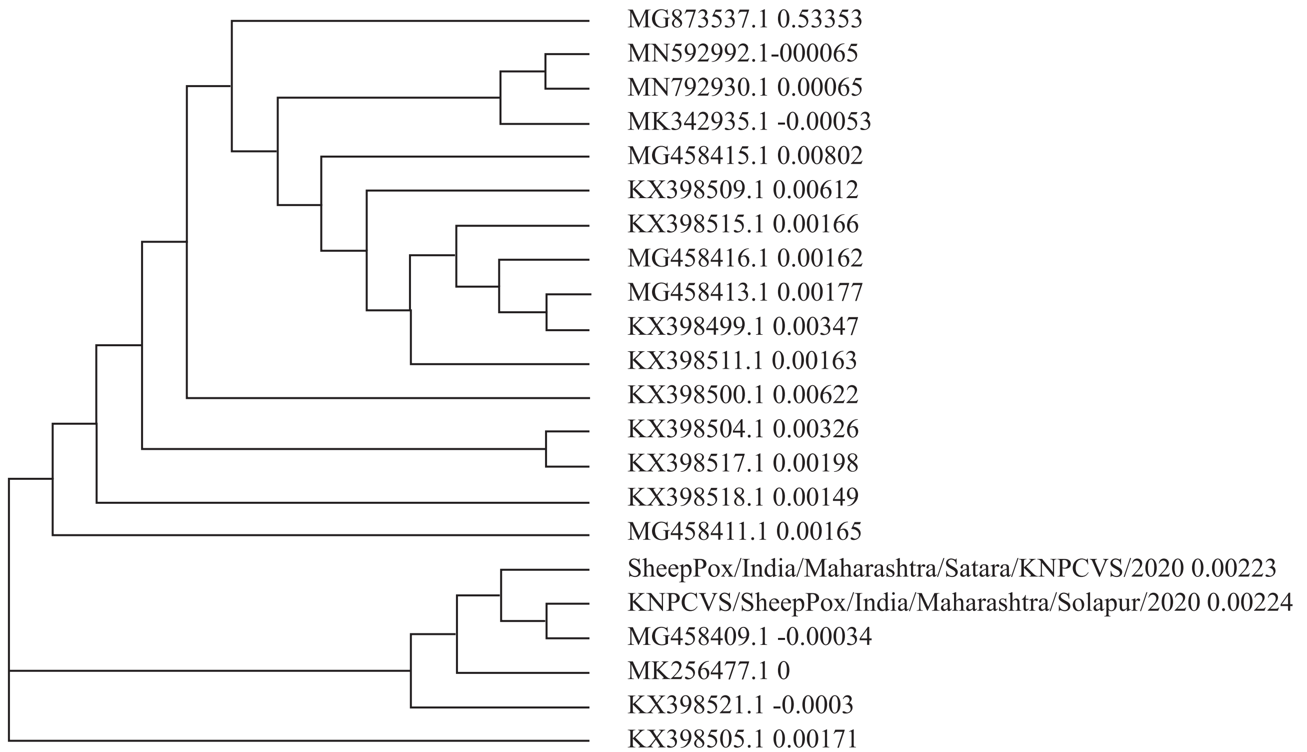

Fig. 4. Phylogenetic analysis showing both the sequences from present outbreaks grouped into SPPVs clade compared to other poxviruses.

in agreement with previous investigations that used the specific primers and observed a PCR amplification of 570 bp size (Zhu *et al.* 2013, Hassanien *et al.* 2021, Mikhael and Ali 2022).

Sequencing and phylogenetic analysis: Sequence analysis of ORF 103 gene of SPPV from one affected flock each from Satara and Solapur districts of Maharashtra revealed 100 to 99% identity with SPPV from Egypt (MG873537.1); China (MG458409.1); Pune, India (KX398518.1); Ahmedabad, India (KX 398517.1) and Makhdoom, India (KX 398504.1). The sequence analysis revealed that two viruses belonged to SPPV with SPPV-specific nucleotides as compared to GTPV and LSDV. The phylogenetic analysis showed that the sequences from present outbreaks' grouped into SPPVs clade compared to other poxviruses (Fig. 4).

Sequence analysis of the ORF 103 gene could differentiate SPPV from GTPV and LSDV. Sequences from Satara and Solapur had 96.03 and 96.29% identity with GTPV from China and Palampur, India (MG458415.1 and KX398509.1), respectively. Whereas both the sequences of SPPV from Satara and Solapur districts of Maharashtra had 96.56 and 96.82% identity with LSDV from Egypt (MN592992.1, MN 792930.1, and MK342935.1) respectively showing distant genotypic relation. In both flocks from Satara and Solapur districts, sheep and goats were reared together and only sheep showed clinical disease indicating species specificity of virus, which supports ORF 103 gene based differentiation of SPPV, GTPV, and LSDV.

Balinsky *et al.* (2008) stated that serological assays could not distinguish SPPV and GTPV due to the close antigenic and virulence relationship. However, in the recent past, RPO30, GPCR, P32, ORF 095, and ORF 103 gene sequencing and phylogenetic analysis have been employed for the differentiation CaPVs (Yan *et al.* 2012, Zhu *et al.* 2013). The coding region of CaPV genome has 1-156 ORFs in which central ORFs (024-123) are conserved genes involved in replication and transcription mechanisms (Moss *et al.* 2001), whereas the terminal ORFs (001-023 and 124-156) are variable in nature involved in host immune evasion and host-range functions (Tulman *et al.* 2001). In the present investigation, sequence analysis of ORF 103 gene could differentiate SPPV from GTPV and LSDV. For the first time, Zhu *et al.* (2013) described the ORF 095 and ORF 103 gene amplification and sequencing for detection and distinguishing GTPV and SSPV. It was suggested that the ORF 95 and ORF 103 genes might be recognized as new markers to distinguish GTPV and SSPV. Similarly, Abd-Elfatah *et al.* (2018) and Khameis *et al.* (2018) also successfully used ORF 103 gene sequencing and phylogenetic analysis for molecular characterization and differentiation of CaPVs. In the present investigation, sheep and goats were reared together in all the seven affected flocks, however, only sheep were affected, indicating specificity of virus to this species. Results of molecular characterization in the present investigation also proves host specificity of CaPVs. Zro *et al.* (2014) have also reported similar host specificity previously.

There are multiple reports of SPPV and GTPV from different states of India; however, information on disease

occurrence in Maharashtra state is very scanty. Most recently, Chahota *et al.* (2022) reported the outbreak of sheep pox among native sheep breeds in the Western Himalayas of India. Sumana *et al.* (2020) reported outbreaks of sheep pox in the Karnataka state of India. In this context, the present investigation reports the outbreak of SPPV in Satara and Solapur districts of Maharashtra state, India based on clinical signs, gross and histopathological lesions, and molecular detection. Results of this investigation adds to the information on the epidemiology of this disease in Maharashtra state and are also crucial regarding the prevention and control of disease through effective mass vaccination with homologous strains.

ACKNOWLEDGEMENT

The authors would like to acknowledge the financial support extended by the Hon'ble Vice Chancellor, MAFSU, Nagpur and Associate Dean, KNP College of Veterinary Science, Shirwal, Satara, Maharashtra, India.

REFERENCES

Babiuk S, Parkyn G, Copps J, Larence J E, Sabara M I, Bowden T R, Boyle D B and Kitching R P. 2007. Evaluation of an ovine testis cell line (OA3.Ts) for propagation of capripoxvirus isolates and development of an immunostaining technique for viral plaque visualization. *Journal of Veterinary Diagnostics and Investigation* **19**: 486–91.

Balinsky C A, Delhon G, Smoliga G, Prarat M, French R A, Geary S J, Rock D L and Rodriguez L L. 2008. Rapid preclinical detection of sheep pox virus by a real-time PCR assay. *Journal of Clinical Microbiology* **46**(2): 438–42.

Bhanuprakash V, Venkatesan G, Balamurugan V and Hosamani M. 2010. Pox outbreaks in sheep and goats at Makhdoom (Uttar Pradesh), India: evidence of Sheep pox virus infection in goats. *Transboundary Emerging Diseases* **57**: 375–82.

Bhanuprakash V, Indrani B K, Hosamani M and Singh R K. 2006. The current status of sheep pox disease. *Comparative Immunology, Microbiology and Infectious Diseases* **29:** 27–60.

Chahota R, Sharma P, Kumar R, Gupta T and Sharma M. 2022. Investigation of an outbreak of sheeppox among native sheep breeds in the Western Himalayas of India. *Veterinary Research Communications* **46**(1): 101–07.

Fenner F. 1988. The pathogenesis, pathology and immunology of smallpox and vaccinia. (Eds) Fenner F, Henderson D A, Arita I, Jezek Z and Ladnyi I D. *Smallpox and its Eradication*. World Health Organization, Geneva, pp. 121–168.

Garner M G, Sawarkar S D, Brett E K, Edwards J R, Kulkarni V B, Boyle 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**: 205–23.

Hassanien R T, Afify A F, Abdelmegeed H K and Danial N M. 2021. Isolation, antigenic and molecular characterization of sheep pox virus from clinical cases in Egypt. *Advances in Animal and Veterinary Sciences* **9**(3): 416–21.

https://www.oie.int/fileadmin/Home/eng/Animal_Health_in_the_World/docs/pdf/Disease_cards/SHEEP_GOAT_POX.pdf

Jahrling P B, Hensley L E, Martinez M J, Leduc J W, Rubins K H, Relman D A and Huggins J W. 2004. Exploring the potential of variola virus infection of cynomolgus macaques as a model for human smallpox. *Proceedings of the National Academy of Sciences USA* **101**: 15196–15200.

Kamran M, Seyed M B and Saied B. 2015. A review of sheep pox and goat pox: perspective of their control and eradication in Iran. *Journal of Advanced Veterinary Animal Research* **2**: 373–81.

Luna L G. 1968. Manual of histologic staining methods of the Armed Forces Institute of Pathology, 3rd edn. McGrawHill, New York.

Madhavan A, Venkatesan G and Kumar A. 2016. Capri poxviruses of small ruminants: Current updates and future perspectives. *Asian Journal of Animal and Veterinary Advances* **11**(12): 757–70.

Mikhael C A and Ali A M. 2022. Molecular differentiation between field lumpy skin disease isolate and capripoxvirus vaccinal strains in Egypt 2018. *Veterinarska stanica* **53**(1): 91–104.

Moss B and Shisler J L. 2001. Immunology 101 at poxvirus U: Immune evasion genes. *Seminars in Immunology* **13**: 59–66.

Ramakrishnan M A, Santhamani R and Pandey A B. 2017. Capripox outbreak in a mixed flock of sheep and goats in India. *Transboundary and Emerging Diseases* **64**(1): 27–30.

Sharma R, Patil R D, Parimoo H A, Thakur D and Katoch V C. 2013. Clinico-pathology of sheep pox disease in Himachal Pradesh, India. *Ruminant* **2**(2): 127–30.

Sambrook J and Russell D W. 2001. *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Sumana K, Revanaiah Y, Apsana R, Roy P and Reddy G B M. 2020. Molecular characterization of sheep pox virus from outbreaks in Karnataka, India. *Veterinary World* **13**(2): 386–91.

Takele H T, Jing Z, Jia H, Chen G and He X B. 2018. A review on sheeppox and goatpox: Insight of epidemiology, diagnosis, treatment and control measures in Ethiopia. *Journal of Infectious Diseases and Epidemiology* **4**: 057.

Tamura K, Battistuzzi F U, Billing-Ross P, Murillo O, Filipski A and Kumar S. 2012. Estimating divergence times in large molecular phylogenies. *Proceedings of the National Academy of Science USA* **109**: 19333–38.

Tulman E R, Afonso C L, Lu Z L, Sak Z and Sur J H. 2002. The genomes of sheeppox and goatpox viruses. *Journal of Virology* **76**: 6054–61.

Tulman E R, Afonso CL, Lu Z, Zsak L, Kutish G F and Rock D L. 2001. Genome of lumpy skin disease virus. *Journal of Virology* **75**: 7122–30.

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. *Veterinary Microbiology* **156**(3-4): 425–28.

Zaucha G M, Jahrling P B, Geisbert T W, Swearengen J R and Hensley L. 2001. The pathology of experimental aerosolized monkeypox virus infection in cynomolgus monkeys (*Macaca fascicularis*). *Laboratory Investigation* **81**: 1581–1600.

Zhu X L, Yang F, Li H X and Dou Y X. 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): 1670–78.

Zro K, Zakham F, Melloul M, El Fahime E and Ennaji M M. 2014. A sheep pox outbreak in Morocco: isolation and identification of virus responsible for the new clinical form of disease. *BMC Veterinary Research* **10:** 31.