SHORT COMMUNICATION

Goatpox outbreak at a high altitude goat farm of Mizoram: possibility of wild life spill over to domestic goat population

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Abstract In this study, pox-like outbreaks in goat population was investigated that occurred in a high altitude goat farm located in Mizoram, a hilly state of North eastern India. The outbreak initially involved the serows, an wild animal belonging to the family Bovidae, subfamily Caprinae and genus Capricornis, the state animal of Mizoram. Later, the disease affected the domestic goat population. The disease was diagnosed on the basis of gross lesions and PCR amplification of partial P32 gene of capripox virus. The virus was isolated in vero cells. The full length P32 gene was sequenced and phylogenetic tree was constructed. It was revealed that the capripox virus isolated from the outbreak was closely related to the Chinese strain of goatpox virus at both amino acid and nucleotide level. To the authors' knowledge, this is the first report on isolation and characterization of capripoxvirus from north eastern region of India.

Keywords Goatpox · P32 gene · Diagnosis · Isolation · Molecular characterization · Mizoram

Capripoxvirus infections comprising of sheepox and goatpox are the important OIE notifiable diseases of sheep and goats, respectively [14]. Clinically, both the diseases are characterised by fever and generalized pock lesions. These diseases are frequently associated with high morbidity, mortality and imposition of restrictions over sheep and goat including their by-products, and thus considered as economically important diseases of small ruminants [4, 19]. Goatpox virus (GTPV), a member of the *Capripoxvirus* genus of *Poxviridae* family is the etiological agent of Goatpox, which is closely related to sheepox virus (SPPV) and lumpy skin disease virus (LSDV) of cattle, the other members of the genus [7, 17]. Majority of SPPV and GTPV strains usually donot show a host preference, as a single strain may cause disease in both sheep and goats [19]. However, the out breaks of goatpox and sheepox in India are considered to be associated with two separate entities [10]. Sheepox and goatpox are enzootic in Africa, particularly to the north and west of the Sahara, in the Middle East and Far East, and on the Indian subcontinent [5]. Both the diseases are enzootic in India and regular outbreaks of goatpox and sheepox have been reported from different parts of the country incurring heavy economic losses to the sheep and goat industry [5, 12, 13, 16]. Mortality in young animals can exceed 50%. Indigenous sheep and goats exhibit some natural immunity, while the European breeds of sheep and goats are more susceptible to infection with these viruses [9], with occasional mortality up to 100% in naïve animals [6]. This article describes one goatpox outbreak occurred at Aizwal, Mizoram (India) and demonstrates the presence of pox virus antigen in suspected skin scab samples. Further, the causative agent was confirmed as GTPV using CIE, conventional PCR, isolation and sequence analysis of major immunodominant envelope protein gene (P32) of goatpoxvirus. To the

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authors' knowledge, this is the first report on confirmed detection, isolation and characterization of goatpox from north east India.

The outbreak occurred in two villages of Aizwal district, in the state of Mizoram (23.1645°N, 92.9376°E) India. The two villages affected were Sesawng under Thingsulthliah tehsil and Chhanchhuahna khawpui under Darlawn tehsil of Aizwal district, Mizoram, India. The disease started with pox like eruptions along with high fever affecting goat population of these two places. As per information provided by the local veterinary officer, the disease was initially seen in serows, a wild goat, the state animals of Mizoram state, India. The serows being wild animals it was not possible to trace the diseased animals while only the carcasses of serows died out of the infection was found lying in the jungles. The disease was seen in the domestic goat population during July–August, 2015 in the two places viz Sesawng and Chhanchhuahna khawpui of Mizoram. The disease outbreak at Sesawng village occurred in an organized goat farm, having 40 numbers of goats established in the year 2014 under Rashtriya Krishi Vigyan Yojana (RKVY), Govt. of India by procuring goats from neighbouring markets. The disease later spread to the nearby village affecting the backyard goats of the area. In the second village (Chhanchhuahna khawpui) also the disease affected around 15 goats of different age group. In both the cases, the affected animals had no history of vaccination against goatpox. Initially, the outbreak was noticed in the first week of July, 2015 affecting few animals of all age groups. The course of the disease was later progressed and by August, 2015 all the animals of the farm were affected. The affected animals showed high fever (up to 105 °F), conjunctivitis, mucous discharge from nostrils and mouth with a loss of appetite, and development of skin nodules over the hairless parts of body with severe ulcerative lesions over eyes and ears in some affected animals (Fig. 1a–f). The disease was found to be more severe in young ones in comparison to the adult. In the first outbreak (Sesawng village) a total of 10 (10 of 40, 25%) animals died out of the infection, mostly belonging to the young age group (> 2 years). No mortality however recorded in the second village. The affected animals were separated from the main stock and treated with antibiotics and supportive therapy. Clinical materials in the form of skin sacs (n = 15) and serum samples (n = 15) were collected from the affected animals and transferred to the laboratory in ice as soon as possible.

Initially, CIE was applied for screening viral antigens in clinical samples following standard protocols (12). Ten percent suspensions of infected tissue materials prepared in phosphate buffered saline (PBS) (pH 7.2) were used. GTPV hyperimmune serum (HIS, obtained from Division of Virology, ICAR-Indian Veterinary Research Institute,

Mukteswar campus, Nainital, India), raised in rabbits using GTPV (*Uttarkashi strain*) was used as positive control. The presence of GTPV in the scab materials were detected by partial amplification of P32 gene of CaPV using primers B68: 5'-CTAAAATTAGAGAGCTATACTTCTT-3' and B69: 5' CGATTTCATAACTAAAGTG-3' that amplify partial P32 gene of Capripoxvirus and yields an amplicon of 390 base pairs [11]. Briefly, the total genomic DNA was extracted either from scab material using DNAeasy Blood and Tissue Kit [Quiagen, Germany] as per manufacturer's protocol and finally DNA was eluted in 50 µl nuclease free water (NFW). DNA isolated was subjected for PCR assays for confirmation. The amplification was carried out using VETITI PCR system (Applied Biosystems, Rotkreuz, Switzerland) for 35 cycles of denaturation at 94 °C for 30 s, annealing at 47 °C for 1 min and extension at 72 °C for 1 min, the cycles were followed by a final extension at 72 °C for 5 min. An aliquot (5–10 µl) of PCR product was analyzed in 1.5% agarose gel electrophoresis to visualize the amplicons after staining with ethidium bromide. The scab/skin biopsy suspensions found to be positive in PCR for CaPV were processed for isolation of the virus in Vero cell lines. The suspension was initially subjected to three cycles of freezing and thawing. The homogenate was clarified at 1500 xg for 10 min. The antibiotics viz. penicillin (10,000 IU/ml), streptomycin (100 µg/ml) and antifungal agent, mycostatin (50 IU/ml) were added to the filtered supernatant and stored at – 80 °C until further use. A portion of the supernatant (500 µl) was used to infect 48 h old Vero cell confluent monolayer in 25 cm² flask as per standard protocol and cells were daily observed for the appearance of cytopathic effects (CPE). Full length amplification of P32 gene was performed from extracted viral DNA using capripox-specific primers [9] and the amplicon was purified using Mini Elute gel extraction kit (QIAGEN, Hilden, Germany) as per manufacturer's instructions. The amplicon was cloned to pGEMT-Easy vector (Promega, USA) and sequenced in an automated DNA sequencer (ABI PRISM 3100, Perkin Elmer). The generated sequence was aligned using Bioedit software [8].

The present study describes an outbreak of capripox in goat population of Mizoram, a north eastern state of India and characterization of isolated virus by sequence and phylogenetic analysis. The outbreak initially occurred in wild serows [2] and later spread as a spill over to the domestic goat population of non-descript local breed reared under semi intensive system. The overall morbidity and mortality were 100% and 40% respectively whereas it was severe in young kids as observed and reported in other parts of the country [18, 20]. Clinico-epidemiological findings, virus isolation, CIE, VNT and PCR initially confirmed that the outbreak was due to CaPV whereas PCR–RFLP and genetic analysis based on P32 gene [10] have confirmed etiology as GTPV. This is

Fig. 1 Typical pox lesions in different areas of the body of the affected goats

the first recorded report of goatpox virus infection in domestic goats as spill over infection and the outbreak highlights the disease as one of the emerging diseases in the North-eastern parts of India. Earlier reports show that CaPV isolates/strains exhibit host preference rather than host specificity [3]. In the Middle East, some isolates such as Yemen can cause disease in both sheep and goats even though they show a distinct host preference for goats compared with sheep [4]. In this outbreak involving only goats, causative agent was confirmed as GTPV by PCR–RFLP described earlier [10, 19].

Clinically, the affected animals showed high fever (105°C), mucous discharge from the nostrils and in-appetence. The affected animals showed classical clinical signs and lesions of goatpox [15, 18]. Pock lesions, which were measuring ≈ 1 cm in diameter, were widely distributed throughout the body and especially in the hairless parts of the body such as around the mouth and eyes, ventral side of the abdomen, under the tail and around the inguinal region (Fig. 1a–f). The nodules progressed into pustules and scab in the later stages of the disease. In general, goatpox inflicts damage invariably in all age groups, either of sex of different breeds during any season. However, is more common in very young and old animals during winter and summer [18].

The tissue samples collected from the affected animals were subjected to CIE [6] and diagnostic PCR [11] where

all the samples and twelve ($n = 12$) out of 15 tissue samples collected were found positive respectively. The scab samples positive in CIE as well as PCR were pooled and processed for virus isolation in Vero cells. The CPE in infected cells was characterized by ballooning, increased refractility, formation of syncytia and detachment of the cells from surface at 6 dpi. The virus isolate was recovered after four blind passages in Vero cells were again confirmed by PCR as GTPV. The sequence analyses of full length P32 gene of GTPV isolated in the present study revealed a homology of 97.8–98.9 and 96.1–98.7% with other GTPV isolates, 94–96 and 96–97.7% with SPPVs, at nt and aa levels, respectively. Highest sequence homology (98.9%) of the present GTPV isolate was observed with the GTPV isolate from China (Strain Ly/Hn, Acc No. KJ026566). The same kind of genetic identity of Indian GTPV isolate with Chinese strains/isolates was reported elsewhere [18]. Presence of P32 gene ORF with a length of 969 bp and absence of aspartic acid residue at the position 55 of P32 protein confirmed the isolate as GTPV as reported earlier [10, 18]. The phylogenetic tree constructed based on aa sequence of P32 protein of virus isolated from present outbreak in comparison to other CaPV showed clustering of all GTPVs in one separate group and are branched separately from other members (Fig. 2). In the Indian subcontinent, the CaPV isolates prefer as sheep or goats to infect and are designated either as SPPV and

Fig. 2 Phylogenetic tree constructed based on the P32 gene sequences of Capripox virus. Red colour bullet indicate the virus isolated in the present study and Blue coloured bullets indicated the goatpox viruses reported from India earlier (colour figure online)

GTPV. Moreover, SPPV and GTPV partially cross protect other species which demands for development of a single vaccine using CaPV field isolate to protect both sheep and goats. Such a vaccine has been evaluated and reported [1] and will be of immense value to protect against both viruses.

In our study, Serows (wild goats) is suspected to be the source of infection for domestic population in Sesawng and Chhanchhuahna khawpui of Mizoram state, a North-eastern India. Capripox is one of the highly contagious infectious diseases which get spread to susceptible species by direct contact with infected hosts or indirect contact through environmental contamination by secretions and excretions from affected hosts [5]. Increased contact between susceptible and affected animals due to improper husbandry practices with high stocking densities of the animals in the endemic areas is responsible for quick spread of the infection [20]. First confirmatory evidence of goatpox infection in this region indicates that CaPV is an emerging entity in Mizoram state and can affect susceptible hosts including wild life species prevalent in other North-eastern state of India. This study on goatpox outbreak implicate that necessary steps is taken to curtail any further transmission and control the disease by undertaking systematic diagnostic investigation for mass vaccination and proper disposal of affected animals including wild animals. In addition to above control aspects, detailed epidemiological analysis on virus host range, host preferences, host specificity, carrier states in both domestic and wild animals and role of vectors in disease transmission have to be

carried out in North-eastern parts of India in order to control and ultimate eradicate the infection.

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Compliance with ethical standards

Conflict of interest All the authors declare that they have no conflict of interest.

References

1. Abu Elzein EME, Housawi FMT, Al-Afaleq AI, Ibrahim AO. Protection of goats, with a sheepox vaccine, against a virulent field capripoxvirus with high affinity to goats. *Sci J King Faisal Univ (Basic Appl Sci)*. 2004;5:263.
2. Anonymous. Goat pox in Mizoram, 102 serows killed. Staff reporter, *The Times of India*, Sept. 7; 2016.
3. Babiuk S, Bowden TR, Boyle DB, Wallace DB, Kitching RP. Capripoxviruses: an emerging worldwide threat to sheep, goats and cattle. *Transbound Emerg Dis*. 2008;55:263–72.
4. Babiuk S, Bowden TR, Parkyn G, Dalman B, Hoa DM, Long NT, Vu PP, Bieu do X, Copps J, Boyle DB. Yemen and Vietnam capripoxviruses demonstrate a distinct host preference for goats compared with sheep. *J Gen Virol*. 2009;90:105–14.
5. Bhanuprakash V, Hosamani M, Juneja S, Kumar N, Singh RK. Detection of goat pox antibodies: comparative efficacy of indirect ELISA and counterimmunoelectrophoresis. *J Appl Anim Res*. 2006;30:177–80.
6. Bhanuprakash V, Indrani BK, Hosamani M, Singh RK. The current status of sheepox disease. *Comp Immunol Microbiol Infect Dis*. 2006;29:27–60.
7. Diallo A, Viljoen GJ. Genus capripoxvirus. In: Mercer AA, Schmidt A, Weber O, editors. *Poxviruses*. Basel: Birkhäuser; 2007. p. 167–81.
8. Hall TA. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucl Acids Symp Ser*. 1999;41:95–8.
9. Heine HG, Stevens MP, Foord AJ, Boyle DB. A capripoxvirus detection PCR and antibody ELISA based on the major antigen P32, the homolog of the vaccinia virus H3L gene. *J Immunol Methods*. 1999;227(1/2):187–96.
10. Hosamani M, Mondal B, Tembhurne PA, Bandyopadhyay SK, Singh RK, Rasool TJ. Differentiation of sheep pox and goat poxviruses by sequence analysis and PCR-RFLP of P32 gene. *Virus Genes*. 2004;29:73–80.
11. Ireland DC, Binopal YC. Improved detection of capripoxvirus in biopsy samples by PCR. *J Virol Methods*. 1998;74:1–7.
12. Karim A, Bhattacharjee U, Puro K, Shakuntala I, Sanjukta R, Das S, Ghatak S, Sen A. Detection of Peste des petits ruminants virus and goatpox virus from an outbreak in goats with high mortality in Meghalaya state, India. *Vet World*. 2016;9(9):1025–7.
13. Mondal M, Hosamani TK, Dutta VS, Senthilkumar R, Rathore R, Singh RK. An outbreak of sheep pox on a sheep-breeding farm in Jammu, India. *Rev Sci Tech OIE*. 2004;23:943–9.

14. OIE. Office internationale des epizooties (World Health Organization for animals), 12, Rue de prony, 75017. Paris: OIE; 2008.
15. Rao TVS, Bandyopadhyay SK. A comprehensive review of goatpox and sheeppox and their diagnosis. *Animal Health Res Rev.* 2000;1(2):127–36.
16. Roy P, Purushothaman V, Sreekumar C, Tamizharasan S, Chandramohan A. Sheeppox disease outbreaks in Madras Red and Mechery breeds of indigenous sheep in Tamilnadu, India. *Res Vet Sci.* 2008;85:617–21.
17. Van Regenmortel MHV, Fauquet CM, Bishop DHL. Virus taxonomy. Seventh report of the international committee on taxonomy of viruses. San Diego: Academic Press; 2000.
18. Venkatesan G, Balamurugan V, Singh RK, Bhanuprakash V. Goatpox virus isolated from an outbreak at Akola, Maharashtra (India) phylogenetically related to Chinese strain. *Trop Anim Health Prod.* 2010;42(6):1053–6.
19. Venkatesan G, Balamurugan V, Yogisharadhya R, Kumar A, Bhanuprakash V. Differentiation of sheeppox and goatpox viruses by polymerase chain reaction-restriction fragment length polymorphism. *Viro. Sin.* 2012;27:352–8.
20. Verma S, Verma LK, Gupta VK, Katoch VC, Dogra V, Pal B, Sharma M. Emerging Capripoxvirus disease outbreaks in Himachal Pradesh, a northern state of India. *Transbound. Emerg. Dis.* 2010;58:79–85.