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Foot-and-mouth disease attributed to serotype A in sheep flocks of Jammu and Kashmir, India

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Small ruminants being a source of earning for two-third of rural community play a pivotal role in their livelihood security. Sheep constitutes an integral component of rural economy especially in the arid, semi-arid and mountainous areas of the country providing a stable source of income to the farmers during the crisis due to crop failure. Therefore, sheep is considered as the 'finance elevator' for the poor farming community. India with 74.26 million sheep stands second (6.4%) in the world, while Jammu and Kashmir with 3.2 million sheep population stands at sixth position in the country, where sheep and goat rearing has been the core activity of rural masses playing a vital role in socio-economic upliftment of the weaker sections of the society, viz. Chopans, Gaddies, Gujjars, Bakerwals and Changpas.

FMD is a highly infectious and contagious disease of cloven-hoofed animals caused by foot-and-mouth disease virus (FMDV). The clinical symptoms of FMD though quite obvious in cattle and swine, are generally mild or subclinical in sheep and goats (Kitching and Hughes 2002). However, the clinical signs of FMD may be influenced by the virus strain and the breed of sheep and goats. While sheep may not manifest clear clinical signs of FMD, they reportedly secrete and excrete considerable amounts of FMDV (Bravo de Rueda *et al*. 2014) and therefore, may play a significant role in virus transmission. Under Livestock Health and Disease Control Programme (LHDCP), FMD vaccination is being practiced only in cattle and buffalo, not in small ruminants. With this backdrop considering the importance of small ruminants to the farming community as well as in FMD epidemiology, the present study aimed at investigating the FMD outbreak in sheep flocks of Jammu and Kashmir Union Territory of India during the year 2021.

During the early part of May 2021, suspected FMD outbreak was reported in sheep flock at many places of Jammu and Kashmir. In this epidemic in Kashmir valley, nearly 144 outbreaks were reported with 20,503 affected sheep, where 218 animals succumbed among 19 lakh susceptible sheep population due to FMD or compound/secondary infection or other ailments caused by miscellaneous agents. Erosive lesions in lower lip mucosa (Fig. 1), upper dental pad (Fig. 2 and 3) were observed in the affected animals. A total of 13 sheep sera from Beerwah Budgam, 41 sera from Poshnar sheep farm, and 15 sera from Gundipora/Shalkani Budgam and Dara Srinagar were collected. Besides, 360 serum samples were also collected from districts of Pulwama, Ananthnag, Shopian, Kupwara, Baramulla and Bandipora. Clinical samples (1 saliva from Beerwah Budgam and 6 swabs from oral lesions of sheep from Poshnar sheep farm) were collected from symptomatic animals in phosphate buffered saline (*p*H 7.5) on cold chain.

Fig. 1. Erosive lesions in mucosal surface of lower lip.

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Fig. 2. Blisters and erosions in upper dental pad surface indicated by red arrow mark.

The serum samples were subjected to an in-house 3AB3 NSP ELISA as described previously (Rout *et al.* 2014). Test serum samples and anti-species HRP conjugated antibodies were diluted at the rate of 1:50 and 1:12000, respectively. For result interpretation, 3AB NSP-antibody seropositivity was considered for the samples producing corrected optical density values of ≥55% compared to that of the infected positive serum control. Clinical samples were tested in a serotype discriminating antigen detection ELISA as described by Bhattacharya *et al.* (1996). Samples found negative herein were further subjected to serotype differentiating reverse transcription multiplex polymerase chain reaction (RT-mPCR) as per Giridharan *et al.* (2005). The total RNA was extracted from the samples using RNeasy Mini Kit (Qiagen, Germany). Reverse transcription was performed using M-MLV reverse transcriptase (Promega, USA) and reverse primer NK61 (Knowles and Samuel 1995) followed by mPCR using three serotype-specific positive sense primers, e.g. DHP13, DHP15 and DHP9 for serotype O, A and Asia 1, respectively with the reverse primer NK61 using Hotstar Taq DNA polymerase (Qiagen, Germany). The PCR amplicons were analyzed by electrophoresis on 2% agarose gel stained with ethidium bromide.

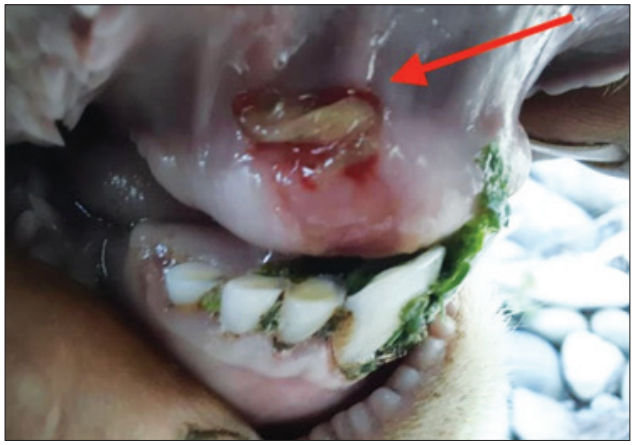

Fig. 3. Ruptured blister at the commissure of upper dental pad and mucosa of upper lip.

In NSP serology, 7 of 13 (53.8%) sera from Beerwah of Budgam, 11 of 41 (26.8%) sera from Poshnar farm, 5 of 15 (33.3%) sera from Gundipora/Shalkani of Budgam, 117 of 360 (32.5%) sera from districts Pulwama, Ananthnag, Shopian, Kupwara, Baramulla, Bandipora and Dara Srinagar were found positive for FMDV 3AB NSP antibodies indicating a high level of virus circulation. Further, such higher seropositivity observed in randomly collected samples from sheep population of different places is indicative of huge populations that have been exposed to the virus. At the same time, the possibility of lack of seroconversion against 3AB NSP could not be ruled out in some animals sampled at an early phase of the disease. All clinical samples were found negative in serotyping ELISA, while one clinical sample (saliva) from Gundipora/Shalkani of Budgam and Dara Srinagar was found positive for FMDV serotype A genome in RT-mPCR (Fig. 4). In this area, the first incidence was reported in cattle that got subsequently reported in sheep. During the particular year, relatively larger numbers of FMD outbreaks were documented in bovine in Jammu and Kashmir and also other parts of the country. More importantly, all three FMDV serotypes O, A and Asia 1 were reported in bovines of Jammu and Kashmir. Accordingly in the year 2021, the NSP antibody prevalence in bovine population of the region under random serosurveillance was recorded to be 26.37%, significantly higher than the overall apparent serorevalence of 16.63% in bovines in the country (Annual Report DFMD 2021).

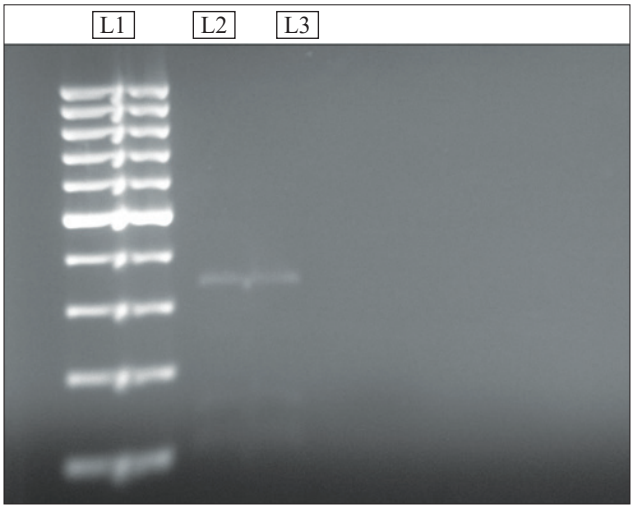

Fig. 4. FMDV serotype A specific PCR amplicon visualized on ethidium bromide stained 2% agarose gel. Here, Lane 1: 100 bp molecular weight DNA marker. The positive amplification of genomic target of FMDV serotype A (376 bp) in saliva sample from sheep is shown in Lane 2, Lane 3: no template control.

Introduction of infected animals and intermixing of animals in common animal sheds by the farmers, access to common pasture land for community grazing followed by their unrestricted movement through migratory routes might have been the most important factors behind the

origin and spread of the outbreak. Mixing of diseased and healthy animals (both sheep and cattle) while grazing at common pasture land enhances the probability of exposure to circulating viruses favoring its spread (Paton *et al.* 2018). Because of the prevailing mixed farming practices along with common grazing practice of different susceptible animal species in our country, FMDV easily spreads between different species. It has been studied that during mixing of different species, the rate of spread of infection not only depends on the occurrence of intraspecies transmission but also on the interspecies transmission (Bravo de Rueda *et al.* 2014). Such circumstances might have favored FMDV transmission from sheep to cattle during the historical 1994 serotype O epidemic in Greece (Donaldson 2000), 1999 type O epidemics in Morocco (Blanco *et al.* 2002) and 2001 type O epidemics in UK (Gibbens *et al.* 2001). Similarly, uncontrolled animal movements have been exemplified to be one of the most common ways of transmission of FMD (Paton *et al.* 2018).

The timing of the widespread FMD outbreaks observed in sheep in the region overlapped with the disease outbreaks in bovine population as well as the migratory movement of sheep flocks at the beginning of the summer. Thus, it is most likely that sheep might have been exposed to infectious virus from infected bovines and subsequently their mass movements in flocks might have triggered the virus spread to other animals. Besides, small ruminants play a crucial role in the FMD epidemiology because of their subdued symptoms and lesions for which they are largely ignored during the vaccination or surveillance programmes (Rout *et al.* 2014). Furthermore, sheep and goats become carriers for up to 9 months and 6 months, respectively (Stenfeldt *et al.* 2014) and may therefore become potential reservoirs of the contagion. During the year 2021, FMD outbreaks turned into a disaster among cattle and sheep farmers. Lack of vaccination during the year might have facilitated the outbreak due to waning of immunity. Hence, preventive vaccination practice should be carried out regularly. In order to mitigate the risk of contracting FMD and its silent spread through diseased sheep population, strategic vaccination needs to be implemented in the country at least in high sheep density/flux areas.

The study concluded the involvement of FMDV serotype A in the outbreak in sheep population in Jammu and Kashmir UT of India during 2021. Infection-specific 3AB NSP antibodies have been demonstrated in the affected and in-contact animals. Introduction of infected animals and sharing of common pasture land followed by their unrestricted movement through the migratory tracts might have been the probable reasons behind the origin and rapid spread of the outbreak.

SUMMARY

The present study investigates the suspected FMD outbreak in sheep flock in Jammu and Kashmir Union Territory of India during May 2021. Swab materials, saliva and serum samples from symptomatic and in-contact apparently healthy animals were collected. A total of 13, 41 and 15 serum samples from Beerwah Budgam, Poshnar sheep farm, and Gundipora/Shalkani Budgam and Dara Srinagar, respectively were collected. Also 360 serum samples were collected from the districts of Pulwama, Ananthnag, Shopian, Kupwara, Baramulla and Bandipora. The serum samples were subjected to the in-house indirect 3AB3 NSP ELISA, while the swab and saliva samples were processed and subjected to serotyping ELISA and multiplex RT-PCR. One sample from Gundipora/Shalkani Budgam and Dara Srinagar was found positive for FMDV serotype A in multiplex RT-PCR. FMD virus serotype A was last documented in Jammu and Kashmir pretty long years ago. In NSP serology, 53.8%, 26.8% and 33.3% serum samples from Beerwah Budgam, Poshnar farm and Gundipora/Shalkani Budgam and Dara Srinagar, respectively were found positive for 3AB NSP antibodies of FMDV. Further, 119 out of 360 (32.5%) serum samples collected from affected flock of different districts were also found positive for 3AB NSP antibodies suggesting a high level of virus circulation. Introduction of infected animals and intermixing of animals while sharing common pasture land followed by the unrestricted movement of diseased/subclinically infected animals might have been the probable reason behind the origin and spread of the outbreak.

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