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Research Article

DETECTION OF RESPIRATORY VIRAL INFECTIONS IN SHEEP AND GOATS OF NORTH INDIA BY ENZYME LINKED IMMUNO-SORBENT ASSAYS

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ABSTRACT: Pneumonia is a leading cause of mortality in Indian sheep and goats. Viral pneumonia is the principal concern when talking about pneumonia in small ruminants because they are initiating causes of immune suppression which leads to other secondary bacterial or fungal pneumonia. SRLVs (MVV and CAEV), JSRV, Parainfluenza-3 virus, Respiratory syncytial viruses, Adenoviruses and Herpes viruses are primarily responsible etiologies for viral pneumonia. The present study was conducted for the states and UT of Northern India mainly- Jammu and Kashmir, Uttar Pradesh, Uttarakhand, Northern region of Madhya Pradesh and Delhi respectively. 367 serum samples (199 sheep and 168 goats) and 187 lung tissues were collected (after screening 1550 lung samples- 1043 sheep and 507 goats) from different parts of Northern India. c-ELISA and Ag-ELISA were subjected to collected serum and tissue samples for SRLVs and other viral etiologies (BoHV, BRSV, BPI-3 and BVDV) respectively. A seropositivity of 9.53% was recorded for SRLVs (35/367) which was higher in organized farms 29.63% than in unorganized farms. One case each was positive for BoHV, BRSV and BPI-3 while 3 cases came out to be positive for BVDV which shows cross species transmission of these bovine viruses in small ruminants. SRLVs in the J&K region could be the focus of persistence which needs further investigation using a larger number of samples from the same area. Testing of colostrum and milk samples can also be done in future from endemically infected sheep and goats.

Keywords: Virus, SRLV, Pneumonia, ELISA, Sheep, Goat, Seroprevalence.

INTRODUCTION

The total livestock population in India is 536.76 million showing an overall increase of 4.8% from the previous census, out of which the Sheep and Goat populations are about 74.26 million and 148.89 million, respectively [1]. Out of all possible causes of mortality in sheep and goats, pneumonia is one of the major causes of mortality in them [2]. Viral pneumonia is of principal concern as it is the initiating cause in most of the cases and is mainly caused by Parainfluenza type - 3 (PI-3), Herpes virus, Adenovirus, Respiratory syncytial virus, Jaagsiekte sheep Retrovirus (JSRV), Maedi-Visna virus (MVV) and Caprine Arthritis Encephalitis virus (CAEV).

PI-3 infection goes undetected in the field in most cases with signs of a mild infection of URT, nasal discharge, coughing, transient pyrexia and other respiratory signs [3, 4]. The damage caused by PI-3 allows other bacterial organisms to invade the lower respiratory tract and produce severe pneumonia. The Herpesvirus of Infectious Bovine Rhino tracheitis (IBR) appears to be capable of infecting goats and sheep causing severe respiratory diseases while Respiratory Syncytial Virus (RSV) leads to significant economic losses by causing respiratory system infections in cattle and sheep [5, 6]. The RSV could also be clinically isolated from goats that did not exhibit respiratory system infection [7].

The pestiviruses usually cause infection in cattle, but sheep and goats are also susceptible to BVDV-1 and BVDV-2 and show similar clinical signs including reproductive failure, abortion, stillbirth, respiratory disease, poor growth rate, diarrhoea, nervous signs and muscular tremor [8, 9, 10]. Small ruminant lentiviruses (SRLVs) which include MVV and CAEV lead to

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progressive interstitial pneumonia in sheep and goats along with the involvement of other organs [11]. The present investigation was carried out to study the occurrence of different viral pathogens associated with pneumonic changes in sheep and goats.

MATERIALS AND METHODS

Serum samples

A total of 367 serum samples (sheep-199; goat-168) were collected from different private and government slaughter houses in Uttar Pradesh, Delhi, Jammu and Kashmir (J&K), Uttarakhand and Madhya Pradesh (Table 1) and stored at -20°C until testing.

Tissue samples

A total of 1550 lung tissues including 1043 from sheep and 507 from goats were screened randomly between August 2016 to May 2017 (Table 2), and among them, samples were collected from 187 lungs which showed lesions of pneumonia and stored at -20°.

Competitive ELISA (c-ELISA)

The serum samples were screened for the presence of anti-SRLV antibodies using a commercially available cELISA kit (VMRD, Inc, USA). All test procedures were applied according to the instructions of the manufacturer and the observance optical density (OD) of the ELISA plate was taken in an ELISA plate reader (BIO-RAD) at 630 nm wavelength. Percent inhibition (% I) was calculated by using the formula: %I = 100* [1-(Sample OD ÷ Mean Negative Control OD)]. The test samples produced >35% inhibition, were considered as positive and <35% inhibition, were considered as negative.

Antigen ELISA (ELISA)

Lung tissue samples were homogenized and tissue suspensions were prepared. These tissue suspensions were tested for the presence of antigens of BoHV-1, BVDV, BRSV and BPI-3 using commercially available Pulmotest Tetra Antigen ELISA Kit (Bio X, Belgium). The procedure was followed as per the manufacturer's instructions and absorbance was taken in ELISA plate reader (BIO-RAD) at 450 nm. Briefly, the validity of the test was checked as defined by the manufacturer. The test could be valid only if the positive reference give 10 minimum OD differences, that were greater than the values for the quality control appended to the package insert. The values were determined by dividing each resulting value by the corresponding value obtained for the corresponding positive control and multiplying this result by 100 to express it as a percentage.

Table 1. Area/location wise distribution of positive cases of SRLV along with number of samples tested using c ELISA kit (VMRD, Inc., USA).

		Sheep		Goat	
		Total samples	c ELISA Positive	Total samples	c ELISA Positive
Jammu and Kashmir	Organized farms , Srinagar J & K	66	20	15	4
	Ganderbal	34	6	-	-
Uttarakhand	Pantnagar	-	-	64	-
(Northern) Madhya Pradesh	Satna	-	-	4	-
Bareilly Slaughter house		-	-	85	4
Delhi Slaughter house		99	1	-	-
Total (367)		199	27	168	8

Table 2. Screening of lung specimens for selecting pathological lungs from sheep and goats.

Name of place	Number of lungs examined		Number of lungs sampled	
	Sheep	Goat	Sheep	Goat
Delhi Abattoir	930	0	83	0
Bareilly Abattoir	14	430	3	60
Srinagar, J and K	83	0	7	0
Post mortem facility Bareilly, IVRI	16	77	2	32
Total	1043	507	95	92

RESULT AND DISCUSSION

The serum samples of sheep and goats from the different North Indian states were tested with cELISA for detection of SRLV antibodies and it showed overall seropositivity of 9.53% (35/367; 27 sheep; 8 goats) with seropositivity in Sheep being 13.56% (27/199) and in Goats about 4.76% (8/168) Figure 1. The seropositivity was higher in an organized farm (29.63%, 24/81 cases) of Srinagar and stray cases of Ganderbal

Table 3. Species wise positivity for various viral etiologies in sheep and goats using multiscreen Pulmotest Tetra Antigen ELISA Kit (Bio X, Belgium)

Species of animal	Sheep	Goat
Etiological agent	Samples positive (from 88 tested samples)	Samples positive (from 99 tested samples)
BoHV	0	1
BRSV	0	1
BPI-3	1	0
BVDV	1	2
Total	2	4

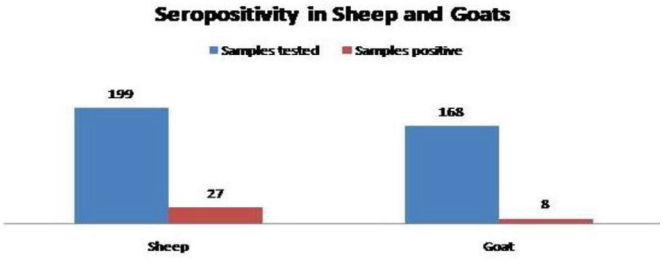

Fig. 1. Seropositivity in sheep and goats (comparing total number of samples positive from tested samples).

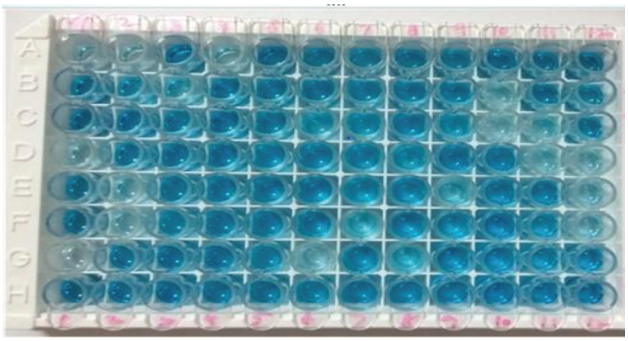

Fig. 2. SRLVs antibody detection in serum of sheep and goat by cELISA kit. The plate wells coated with antigen were charged with serum followed by antibody-peroxidase conjugate, TMB substrate and finally stop solution. The yellow color developed was read on a ELISA plate reader at 650nm. A1&A2 positive controls, H11 & H12 negative controls. The kit showed 24 positive test samples.

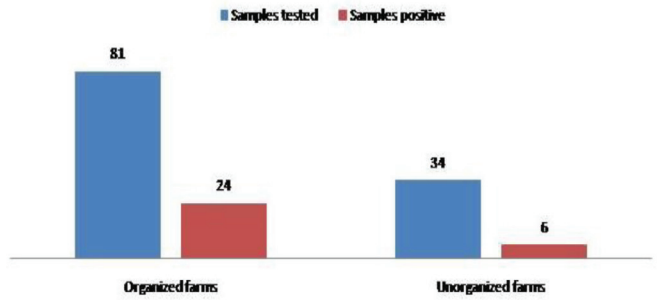

Fig. 3. Seroprevalence in organized and unorganized farms of Jammu and Kashmir.

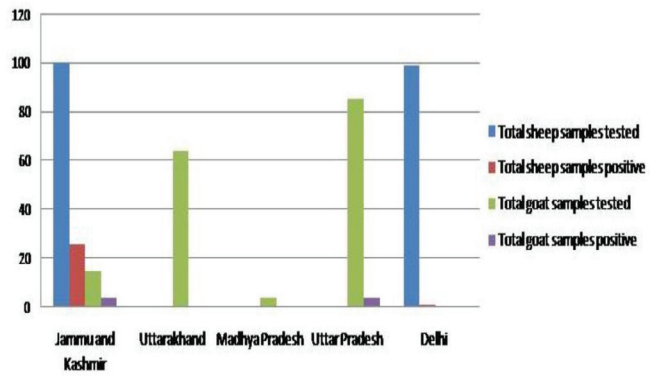

Fig. 4. Statewise seroprevalence of SRLV antibodies in sheep and goat population.

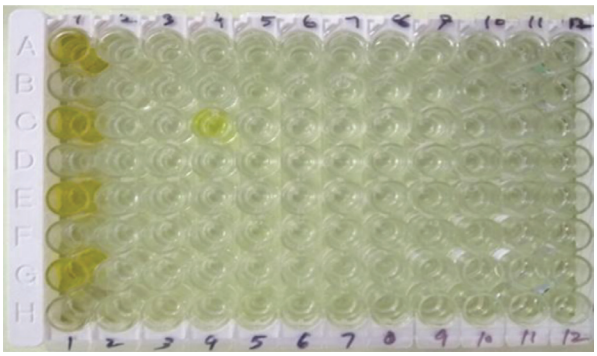

Fig. 5. Multiscreen pulmotest sandwich ELISA test kit showing positive result for BVDV (row3, column 4). Row A is specific for BoHV, Row B Negative control; Row C specific for BVDv, Row D negative control; Row E is BRSV specific, Row F negative control; Row G is specific for BPI3, Row H negative control.

(17.65%, 6/34) districts of J&K (Figure 2 and 3). On the contrary, only 2.72% (5/184) seropositivity could be detected in samples from the abattoirs of Bareilly and Delhi. Interestingly, none of the cases from Pantnagar, Uttarakhand and Satna, MP were found to be positive for SRLV antibodies (Fig. 4; Table 1).

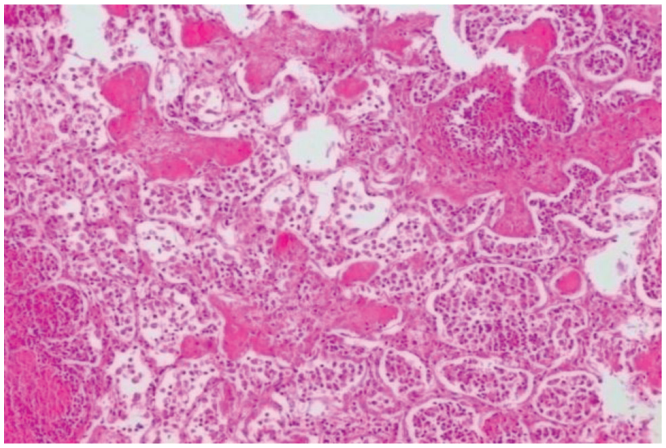

Fig. 6. Fibrinous Bronchopneumonia in goat (20X). [H & E stained sections showing necrotic areas admixed with fibrinous exudate and inflammatory cells predominantly neutrophils in the alveolar lumen].

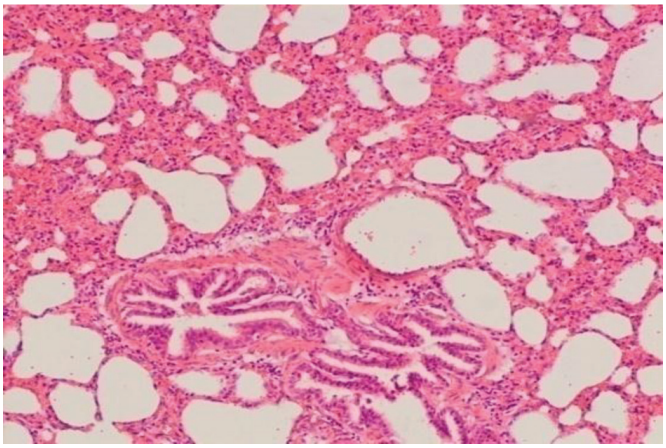

Fig. 7. Interstitial pneumonia in sheep (10 X). Thickening of interalveolar septae with cellular infiltrate. [H & E]
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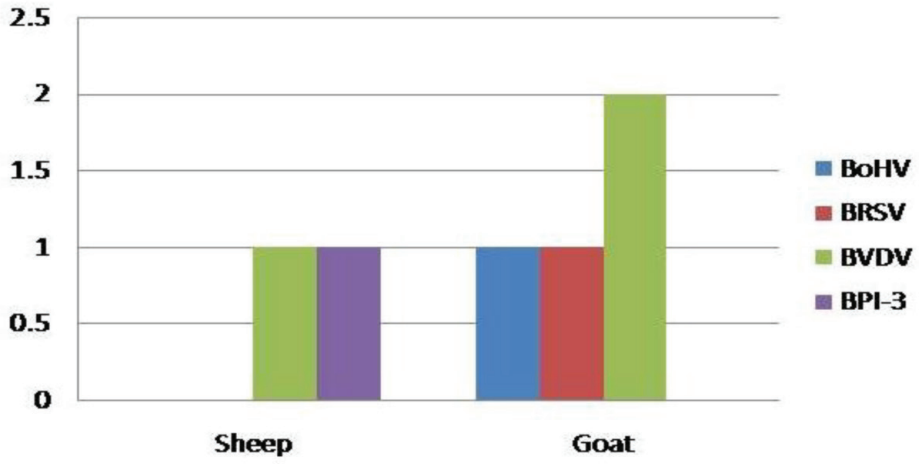

Fig. 8. Viral etiologies detected in sheep and goats using a multi-screen Pulmotest ELISA kit.

The seropositivity detected in the present investigation was higher than some previous reports from Northern Somalia in goats, Pakistan in sheep and goats, and India in goats [12, 13, 14]. However, overall seropositivity was lower than detected by Mishra *et al.* i.e. 12.17% (460 total serum samples; sheep 312; goats 148) from North Indian Sheep (16.02%) and Goat (4.05%) population because of more serum samples screened [15]. However, lower seropositivity was reported initially by Singh *et al.* from India in goats, and Norouzi *et al*. from Iran in sheep [16, 17]. In addition, endemically infected flocks may also pass the virus via colostrum and milk [18].

Other infectious agents were detected in the pneumonic lungs of sheep and goats by using a multi-screen Pulmotest ELISA kit (BioX, Belgium) (Fig. 5). The lung tissues mainly showed lesions of bronchopneumonia (neutrophilic exudates in the alveolar lumen) and interstitial pneumonia (thickening of interstitial septa due to accumulation of inflammatory mononuclear cells) on histopathology there were no specific microscopic lesions noted. (Fig. 6 and 7). The lungs showing pneumonic lesions were randomly picked up to screen them for viral etiologies by using a commercially available multi-screen Pulmotest ELISA kit (BioX, Belgium) [19].

In the present study, only a few cases were found to show the presence of viral antigens which includes one case each positive for BoHV (goat), BRSV (goat) and BPI-3 (sheep), and three cases for BVDV (1 sheep and 2 goats). One case of parainfluenza-3 was from fallen sheep of IVRI, and the remaining positive

cases were from abattoirs (Fig. 8; Table 3). The bovine parainfluenza virus-3 causes an immunosuppressive effect to result in complications with co-infections in ruminants [20].

These viruses from cattle can infect small ruminants and due to common sharing of antigens between BoHV and CpHV-1, one case of CpHV-1 positive to bovine herpesvirus-1 antibodies was detected from a test kit in an adult goat in few studies [21, 22]. The seroprevalence of respiratory viruses in sheep and goats has been reported by many workers from Turkey and India. It was reported to the level of 32.1% (BVDV), 23% (BHV), 72.9% (BRSV) and 13.2% (PI-3) from Turkey and 23.4% (BVDV in sheep) and 16.9% (BVDV in goats) from India [21, 23].

CONCLUSION

The sheep and goat population of North India were screened for different viral etiologies SRLV, BoHV, BRSV and BPI-3 through enzyme linked immunosorbent assays. The seropositivity of SRLV antibodies was quite significant (9.53%) in the North Indian states, probably due to confined and closed housing systems to combat cold climatic conditions facilitating the spread of SRLVs, through exudate from concomitantly infected animals with bacteria or other viruses. These viral infections are prevalent in the sheep and goat population of Northern India and there is a need to systematically monitor these diseases and assess their economic impact. Although the cases of other viral etiologies screened from pulmotest antigen ELISA kits revealed one case each positive for BoHV (goat), BRSV (goat) and BPI-3 (sheep), and three cases for BVDV (1 sheep and 2 goats). This study demonstrates that BRSV, BoHV, BPI-3 and BVDV can infect small ruminants via cross species transmission and can be the reason of respiratory diseases in these species too. Further studies needs to be done to know about cross reactivity of cattle viruses with small ruminant specific viruses. The viral diseases cause immunosupression initially, due to which animal becomes susceptible for bacterial infections which can superimpose viral infection leading to lower detection levels. Sampling can be increased in terms of number of animals screened at early and later stages of life, so that prevalence of infection can be studied. The data generated can be correlated with the economic losses linked to these diseases in organized and unorganized setup, which will be fruitful for the sheep and goat rearing communities as well as to the commercial farms.

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