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Full Length Research Paper

Detection of serum antibodies to parainfluenza type 3 virus, respiratory syncytial virus, bovine viral diarrhea virus, and herpes virus type 1 in sheep in the Region of Botucatu, São Paulo - Brazil

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Viral respiratory infections are common in sheep, and losses related to the introduction and spread of viral agents in flocks are inevitable. In spite of the growth of sheep production in the State of Sao Paulo, little is known about the frequency and dissemination of these agents in this State. The objective of this study was to investigate the occurrence of antibodies to bovine virus parainfluenza type 3 virus (BPI3), respiratory syncytial virus (BRSV), bovine viral diarrhea virus (BVDV), and herpes virus type 1 (BoHV-1) in sheep flocks in Botucatu Region, São Paulo, Brazil. Blood samples were collected from 194 clinically healthy dams, one to three years of age and analyzed for neutralizing antibodies against the target viruses. Frequencies of antibodies were 82% (159/194) for BPI3; 58.8% (114/194) for BRSV; 0.5% (1/194) for BVDV, and no detection of BoHV-1. Titer of reactor samples ranged from 2 to 2048 for BPI3, from 2 to 64 for BRSV, and was 10 for the single reactor for BVDV. Results indicate that BPI3, BRSV, and BVDV occurr in sheep flocks of the Region of Botucatu, São Paulo, and that BPI3 is probably the main agent involved in viral pneumonia cases in the region.

Key words: Sheep, viral respiratory infections, serum, antibody titration.

INTRODUCTION

Respiratory disease is a major health concern in sheep production, leading to high morbidity and mortality rates (Martin, 1996; Cutlip et al., 1998). Respiratory disease is responsible for 10-40% of adult sheep mortality and for 17% of perinatal deaths (Rook et al., 1990; Vieira et al., 1993).

Viral respiratory infections, chiefly parainfluenza 3 virus (BPI-3) (Lehmkuhl and Cutlip, 1982) and respiratory syncytial virus (BRSV) play an important role in sheep production (Alley, 1975). Other viruses, such as ovine adenovirus type 6 (AVO-6), bovine viral diarrhea virus (BVDV) (Pommer and Schamber, 1991), herpes virus type 1 (BoHV-1), and ovine progressive pneumonia (MVV) caused by the lentivirus maedi-visna (Pugh, 2005) may occur less frequently.

During the past decade, sheep-rearing in the State of Sao Paulo has increased to become one of the main occupations on many farms. With the intensification of production systems, animals are kept in closer contact, and diseases, mainly viral respiratory infections, have become more frequent. It is important to know which

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agents may be found among these animals in order to determine adequate measures to prevent or to control and manage viral respiratory infections and to limit the losses they cause. Although Sao Paulo is an important Brazilian State in terms of sheep production, there are no reports on the occurrence of viral respiratory infections in this species. The objective of this study was to determine antibody levels to BPI3, BRSV, BVDV, and BoHV-1 in sheep in the region of Botucatu, São Paulo, Brazil.

MATERIALS AND METHODS

Study area

The Botucatu Region is located in South-central Sao Paulo, Brazil and comprises a 1,482 km^2 area at an average altitude of 873 m. Climate in the region is humid subtropical with characteristically dry winters and hot summers. Annual mean temperature is 18ºC.

Study animals

A total of 194 Santa Inês, 1 to 3 years old dams from five farms were used in the study. Animals were healthy at physical examination, had no history of recent respiratory disease and were not vaccinated against any of the viruses studied. They were kept in semi-intensive systems and had only incidental contact with other animal species, mainly bovines. The farms held a mean of 40 animals per hectare.

Study design

The study took place in 2008, between June and August, when a higher frequency of viral respiratory infections is expected (Radostits et al., 2007). Farms participating in the study were designated A, B, C, D, and E. Samples were collected randomly from at least 10% of the sheep in each flock. According to the Instituto Brasileiro de Geografia e Estatística (Igbe, 2010), there were 13,777 sheep in the region of Botucatu in 2008, amounting to 3% of the total number of sheep in the State of Sao Paulo (453,261 heads).

Selection criteria

The following criteria were used to select the farms for the study: they should have medical records in the Large Animal Practice of the Hospital Veterinário da Faculdade de Medicina Veterinária e Zootecnia de Botucatu - FMVZ, have a history of sheep with clinical signs of respiratory disease in the two years preceding the study, not have animals of any species immunized with live or dead vaccine against the respiratory viruses, have adequate conditions for the samples to be collected, and have permission of the owners for the activities of the study to be carried out. The number of animals that had these conditions was around 900. Based on these facts and assuming that the prevalence of at least one infection was between 75% and 85%, the minimum sample size was 194 animals in order to develop a cross-sectional study, with types I and II errors of 5 and 20%, respectively.

Sample collection

Blood samples were collected (8 mL per animal) by venipuncture of the jugular vein using sterile vacuum tubes containing a serum separator gel (BD, Vacutainer®, USA) . Nutritional status and signs of any disease were assessed when blood was collected. Tubes were labeled and taken to the Laboratório de Viroses de Bovídeos at Instituto Biológico de São Paulo in isothermal containers held at 4°C, where they were centrifuged at 900 *x g* for five minutes for serum separation (5804R, Eppendorf®, Germany).

Neutralization tests

Each serum sample was submitted for a virus neutralization test (VNT) against BoHV-1 and BVDV according to the guidelines of the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals of the World Organization for Animal Health (OIE, 2009) and the BPI3 according to recommendations of the Code of Federal Regulation (CFR, 2005). The quantification of antibodies was performed in 96 wells microtitre plates using eight serial dilutions starting from 1:2 to 1:256 in the test for BPI3 and BoHV-1 and starting from 1:10 to 1:1280 for BVDV. Then 100 $TCID_{50/50mL}$ was added to each virus strain (BPI3 – from the American Type Cell Collection; BoHV-1/Los Angeles and BVDV/NADL strains provided by the Institute of Virology in Hannover, Germany). The microtitre plates were incubated at 37°C with 5% CO_2, BPI-3 and BVDV for 1 h; while BoHV-1 for 18 h. Then 50 µl of a bovine kidney epithelial (MDBK) cell suspension for BVDV and BPI3 and 100 l of cell suspension for BoHV-1 were added at a concentration of $3x10^5$ cells/mL. The microplates were further incubated at 37°C with 5% CO_2 for 72 h, after which readings were made. The VNT was validated by back titration and control of viral dose (which should give a value of 100 $TCID_{50}$ with a permissible range of 20–300 $TCID_{50}$), control cells and using sera from sheep that were known to be positive and negative. The test serum results are expressed as the reciprocal of the dilution of serum that neutralized the virus in 50% of the wells. The titers were calculated according to the method of Reed and Muench. Animals were considered BVDV positive for those which had titers equal to or greater than 10. For BoHV-1 and BPI3 titers above, 2 were considered positive.

Statistical analysis

The results, expressed as the frequency of occurrence in carriers of antibodies to the viruses, were analyzed by Chi-square and Fisher's exact test (Snedeor and Cohran, 1980), using the algorithms developed for PASW Statistics, v. 17.0.2 according to the expected distribution obtained (Statistical Software PASW - version 17.0.2, Chicago, Illinois, USA). Significance level was set at 5%.

RESULTS

Frequencies of reactor animals among the 194 serum samples were: BPI3 = 82% (159/194) ($p < 0.001$); BRSV = 58.8% (114/194) ($p < 0.001$); and BVDV = 0.5% (1/194). No sample was positive for BoHV-1 (Table 1). Titers ranged from 2 to 2048 for BPI3, 2 to 64 for BRSV, and the only sample reactor to BVDV showed a titer of 10 (Table 2).

Only one sample showed antibodies to BPI3, BRSV, and BVDV (0.5%); 107 showed antibodies to BRSV and BVDV (55.1%). Fifty-seven samples were positive for only one type of virus (29.4%): 6 for BPI3 (3.1%), and 51 for BRSV (26.3%) ($p < 0.001$).

Table 1. Parainfluenza type 3 virus (BPI3), bovine respiratory syncytial virus (BRSV), and bovine diarrhea virus (BVDV) in sheep from farms in the Botucatu Region, São Paulo, Brazil.

Farm	No. of sample (n=194)	Parainfluenza-3 (BPI3)[a]			Respiratory syncytial virus (BRSV)[b]			Bovine viral diarrhea virus (BVDV)[c]		
		Positive samples	%	Titers * (min/max)	Positive samples	%	Titers (min/max)	Positive samples	%	Titers
A	55	52	94.5	2 – 2048	49	89	2–64	-	-	-
B	54	47	87	2 – 2048	27	51.8	2–16	1	0.5	10
C	19	19	100	32 – 2048	16	84.2	2 – 8	-	-	-
D	15	15	100	16 – 2048	8	53.3	2–32	-	-	-
E	51	26	51	4 – 2048	13	26	2–32	-	-	-

* Titer expressed as inverse of the dilutions; min – minimum; max - maximum; [a] PI-3: non-reactor < 2 (Fischer's exact test = 47.419, p < 0.001); [b] BRSV: non-reactor < 2 (Fischer's exact test = 53.464, p < 0.001); [c] BVDV: non-reactor < 10 (not performed).

Table 2. Number of samples for various titers of antibodies to parainfluenza type 3 virus (BPI3) and respiratory syncytial virus (BRSV) in sheep from farms in the Botucatu Region, São Paulo, Brazil.

Virus	Antibody titers *				
	Farm	2 - 8	16-64	128 - 512	1024
Parainfluenza type 3 (BPI3[a])	A	4	10	26	12
	B	6	17	18	6
	C	0	4	10	5
	D	0	4	7	4
	E	1	10	11	4
Respiratory syncytial virus (BRSV[b])	A	33	16	0	0
	B	26	2	0	0
	C	16	0	0	0
	D	7	1	0	0
	E	11	2	0	0

* Titer expressed as the inverse of the dilution; [a] BPI3: non-reactor < 2; [b] BRSV: non-reactor < 2.

DISCUSSION

The presence of BPI3, BRSV and BVDV in sheep herds of Botucatu São Paulo, Brazil was confirmed. Since there are no commercially available vaccines against these viruses in sheep, and that animals in the study were not immunized with vaccines specific for bovines, antibodies detected indicate that these animals were exposed to natural infection, providing evidence of virus activity in the area. It should also be emphasized that no live vaccines were used in the immunization of bovines that had possible contact with the sheep studied. The frequency of animals positive for BPI3 was greater than those reported

by Dal Pizzol et al. (1989), Manchego et al. (1998), and Cabello et al. (2006) suggesting that, among the respiratory viruses studied, BPI3 was the most widely spread in the flocks analyzed.

In countries such as the USA, where sheep are raised in intensive systems, BPI3 frequency in flocks may be over 70% (Lehmkuhl et al., 1985; Manchego et al., 1998), similar to the result observed in this study. Although rearing methods and management conditions of Brazilian flocks are very different from those of countries in the northern hemisphere, the frequency of BPI3 was extremely high. It is possible that close contact among animals, even for short periods of time, in the semi-intensive system used in the farms studied contributed to the high infection rate observed. Climatic conditions in the region during the study period, with mean temperature of 19ºC, relative humidity of 51.25%, and

Precipitation of 45 mm, may have contributed to maintaining BPI3 in the environment.

Many animals showed antibody titers to BPI3 higher than 128, which is considered to be high for unvaccinated flocks (Cabello et al., 2006), suggesting that animals may have been recently challenged by the virus.

Although the frequency of occurrence of BRSV was similar to previous reports (Lamontagne et al., 1985; Cabello et al., 2006; Ye ilba and Güngör, 2009), it was lower than that of BPI3. BRSV and BPI3 have a special tropism for the respiratory tract and are widely spread among sheep, primarily in young animals (Lehmkuhl et al., 1985; Van Der Poel et al., 1994). The lower frequency of BRSV in sheep when compared to BPI3 may be related to its longer incubation period and slower spread among susceptible animals (Collins et al., 1988).

The finding of only one animal reactor to BVDV is strong evidence that there is no sheep persistently infected in the herds, reducing the spread of the virus (Heckert et al., 1994). The presence of one persistently infected animal would have led to the identification of more infected animals due to the susceptibility of sheep to BVDV. Greater prevalence rates reported by other authors, such as 14% by Lees et al. (1991), are associated with flocks that have persistently infected animals. In addition, pestiviruses are easily inactivated and are not resistant in the environment. A short infectivity outside the host may have contributed to the low frequency observed (Duffel and Hakness, 1985). In spite of the infrequent occurrence of BVDV, the presence of the agent in the region should not be ruled out, especially in outbreaks of respiratory disease. More intense research efforts may clarify the role of BVDV in the etiology of respiratory diseases of sheep.

No animal sampled showed antibodies to BoHV. This finding corroborates data in the literature reporting generally low or absent antibody titers against this virus in sheep (Howe and Woods, 1966; Parks and England, 1974; Brako et al., 1984; Rosadio et al., 1984; Lehmkuhl et al., 1985).

Although it was not possible to determine the origin of the viral infections, it is likely that incidental contact between sheep and bovines may have led to infection and maintenance of the agents in these flocks. Most sheep that make up the flocks in the south-central region of São Paulo come from southern Brazil, chiefly from the State of Rio Grande do Sul, where sheep and bovines are commonly reared together.

Conclusion

BPI3, BRSV, and BVDV were found in sheep from flocks in the Region of Botucatu, São Paulo, Brazil. Due to its high frequency of occurrence, BPI3 is probably the main agent implicated in viral pneumonia cases in the region. However, a wider and more intense research effort is necessary to better understand the role of each of these agents in the occurrence of sheep respiratory diseases in the region.

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