Iranian Journal of Ruminants Health Research (2018), 3(1): 11-18.
Doi: 10.22055/ijrhr.2020.30387.1026

Seroprevalence of pestivirus in small ruminants in Khorasan Razavi province, Iran

Kavoosy, M[1]., Taghavi Razavizadeh, S.A[2*]., Azizzadeh, M[2]., Ghaemi, M3., Rashtibaff, M4.

1- *Graduated of DVM, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.*
2- *Department of Clinical Sciences, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.*
3- *Pathobiology Department, School of Veterinary Medicine, Shiraz University, Shiraz, Iran.*
4- *Department of Survey and Surveillance, Khorasan-Razavi Province. Iranian Veterinary Organization. Iran.*

[*]***Corresponding author*:** razavizadeh@um.ac.ir

Abstract

Border disease, which is a congenital pestivirus disease, also known as "hairy Shaker or fuzzy lambs", is one of the causes of mortality and losses of lambs and kids in animal production. Due to the lack of new comprehensive information on the rate of infection with pestiviruses in sheep and goat population in Khorasan-Razavi province (in Iran), the present study was conducted to determine the prevalence of the above mentioned virus in 2017. After collecting the blood samples of 273 sheep and 179 goats, in 11 districts of the province by cluster sampling method, the prepared serum samples were tested for antibody against the pestiviruses by ELISA kit. The results showed that 217 sheep and 127 goats were serum positive. So, the seroprevalence of the virus in all the studied animals were estimated 75.9% (95% CI: 71.7- 80.1). At least one positive case was detected in all studied epidemiological units. By introducing the independent variables including species, age, sex and sampling district to the multivariable logistic regression model, it was found that, apart from gender (P> 0.05), the other variables had a significant relationship with seropositivity (P<0.05). The frequency of seropositivity was significantly higher in sheep than goat and those with age group of more than 3 years than under 2 years old (P<0.05). Since the seroprevalence of pestiviruses in small ruminants is higher in this province than the other parts of the country, therefore, appropriate strategies is essential to control it.

Keywords: Border disease, Khorasan-Razavi province, Pestivirus, Seroprevalence, Small ruminants

Introduction

Border disease (BD) which is associated with abortion, stillbirth or birth of lambs with congenital malformations and formation of PI (persistent infection) animals, is caused by a pestivirus within the family of *Flaviviridae* (Constable *et al*., 2017; Aitken, 2007). In the genus of pestivirus, four different species, including bovine viral diarrhea (BVDV) type 1 and 2, classical swine fever and border disease virus (BDV) have been identified. Cross-infection is possible between the four species (Mosaferi *et al*., 2010). On the basis of some studies, BVD virus was isolated from most of the

lambs with clinical signs of BD (Campbell *et al*., 1995; Fakur & Hematzadeh, 2007). Thirty - two isolates from small ruminants suffering from clinical symptoms of border disease were clustered into BVDV -1, BVDV - 2 and classical swine fever virus species and the tentative BDV-2 species (Giangaspero, 2011). Calves persistently infected with BVDV can infect sheep, and in countries where pregnant sheep and cattle are housed in close proximity during the winter this can be an important source of infection for outbreaks of border disease (Constable *et al*., 2017). So, the infection cycle between cattle and sheep population is one of the most important epidemiological aspects of the disease (Aitken, 2007). It can be concluded that BD may refer to a condition caused by either BDV or BVD virus (Pugh & Baird, 2012).

Multiple techniques, including virus isolation, antigen ELISAs, and RT-PCR are used for detection of the virus. Antibody can also be detected by Ab- ELISAs or serum neutralization (SN) tests . In studies conducted in Kurdistan and Chaharmahal-Bakhtiari, by SN test, the prevalence of pestiviruses in sheep were recorded as 29.28% and 21.2%, respectively (Fakur & Hematzadeh, 2007; Keyvanfar *et al*., 1999).

At present, a major problem in sheep breeding units of Khorasan Razavi province is the abortion of ewes. According to the available data, no study similar to the present research has been conducted in this area over the past 20 years (Keyvanfar *et al*., 1999); so, by determining the rate of infection with the virus, providing strategies for preventing its complications in northeastern of Iran will be possible in the future.

Materials and Methods

This study was carried out on the small ruminants population of Khorasan Razavi province (in Iran). In 2017, 10 ml of blood were taken from 452 sheep and goats in 28 flocks and 11 districts of the province and placed in an anticoagulant test tube. Sampling was performed by using multi-stage random cluster. In the first stage, 11 districts from all cities of the province (28), in the second stage, 28 flocks from 40 epidemiologic units (Sample districts) registered in selected districts and then 425 heads of sheep and goat from the flocks were selected randomly. The production systems were traditional and semi-industrial. After isolation of the serums and installation, an identification of the number on the microtubes including some information about the sampling site, gender and age of the studied animals, samples were frozen at -20 ° C until the ELISA test by using the IDVet kit (ID Screen BVD P80 Antibody Competition, made in France). After the wells were covered with antigen p80, the serum samples were incubated in them. Following the formation of the Ag- Ab complex, other steps including washing, adding conjugate, substrate and stop solutions were carried out. After reading the optical density with an ELISA reader in a 450 nm wave length for each sample, the S/N percentage (optical density /OD of the serum sample to OD of negative

control) was calculated using the following formula: S/N% = $\frac{OD_{Sample}}{OD_{NC}} \times 100$

Samples were considered positive, doubtful and negative for (S/N%) ≤40, 40<…….≤ 50, and > 50%, respectively.

According to the instructions of the kit, its sensitivity and specificity for detecting the antibody against the pestiviruses is high (100%). Although it cannot differentiate between BVD and BDV antibodies, because of some explanations in introduction, it seems that the term "antibody against BD virus" can be used for interpretation of the results in the small ruminants.

All statistical analysis was done by SPSS software version 21. P <0.05 was considered as the significant level. The seroprevalence of the disease was reported at 95% confidence interval. The relationship between the species, sampling district, age and sex with seroprevalence was analyzed by Chi-square or Fischer exact test. Independent variables that showed a correlation with seropositivity in univariate analysis with p <0.2 entered the multivariable model. To select those explanatory variables that are significantly related with seropositivity, a backward stepwise approach was used. Explanatory variables that were not related with seropositivity were removed from the model until the estimated regression coefficients, for all retained variables, were significant at an alpha level of =0.05.

Results

Out of the 273 sheep and 179 goats which were tested, 217 and 127 were seropositive, respectively. So, for all the studied animals, the seroprevalence was estimated 75.9% (95%CI: 71.7 – 80.1%). The infection of sheep and goats with the virus was detected in all studied districts. Out of the 28 studied epidemiologic units, at least one positive case was found in all units. The lowest and highest infection rates were 28.6 and 100 percent in the units. The lowest seroprevalence was in Kashmar and the highest was in Quchan areas. Other districts showed more than 63.6% of seropositivity (Table 1).

The multivariable logistic regression model showed no significant relationship between sex and seropositiveity (P> 0.05), but a significant relationship was observed between seropositiveity and species and age of the studied animals and the sampling area (P<0.05). The frequency of seropositivity was significantly higher in sheep than goats and those with the age group of more than 3 years than under 2 years old (P<0.05). Also, the chances of seropositivity in Kalat, Zaveh, Quchan, Neishabour, Fariman, Roshtkhar, Sabzevar and Joghatie were significantly higher than Kashmar (Table 1).

Table 1- Ratio of serum positive for different levels of independent variables and the results of logistic regression model in small ruminants

Variable	Level	No. of animals tested	Seropositive, N(%)	Odds ratio (OR)	95%CI for OR		P- value
Species	Goat	179	126(70.4%)				
	Sheep	273	217(79.5%)	1.86	1.07	3.23	0.027
Age	2<	121	83(68.6%)				
	2-3	181	139(76.8%)	1.588	0.843	2.991	0.152
	3>	150	121(80.7%)	1.987	1.017	3.883	0.045
District	Kashmar	30	13(43.3%)				
	Kalat	47	37 (78.7%)	3.20	1.05	9.76	0.041
	Zaveh	35	25(71.4%)	3.35	1.07	10.53	0.038
	Ghochan	32	29 (90.6%)	12.08	2.96	49.35	0.001
	Neishabour	92	67(72.8%)	2.93	1.16	7.41	0.023
	Fariman	43	35(81.4%)	4.91	1.67	14.47	0.004
	Roshtkhar	38	30(78.9%)	4.83	1.51	15.46	0.008
	Sabzevar	37	32(86.5%)	8.55	2.32	31.48	0.001
	Khooshab	22	14(63.6%)	2.83	0.80	10.10	0.108
	Joghatie	44	37(84.1%)	5.04	1.57	16.10	0.006
	Bakharz	32	24(75%)	2.31	0.72	7.39	0.159

P<0.05 is statistically significant. OR: Odds Ratio, and CI: Confidence interval.

Discussion

This study showed a lower seroprevalence of the virus in goats than sheep. In goats, fetal death is the major outcome of infection of the pregnant doe with both BDV and BVDV; moreover PI shaker or clinically normal kids are less common manifestation of the disease than sheep (Constable *et al*., 2017). Although, the difference in the rate of infection between males (65.09) and females (79.48%) was not significant, due to the

high economic losses caused by the reproductive failure, special attention to the ewes is needed (Constable *et al*., 2017). In a survey conducted around the Qom province, it was found that 19.1% of the sheep abortions were caused by border disease (Mosaferi *et al*., 2010).

High seroprevalence of the virus in animals older than 3 years old can be attributed to increasing chance of exposure with the virus in older animals in comparison with younger ones (Tolouei Kaleibar *et al*., 2014; Fakur & Hematzadeh, 2007; Nikbakht *et al*., 2015). In a study conducted in Ahwaz (Southwest of Iran) by the serum neutralization (SN) test, the prevalence of pestivirus in sheep and goats was 46.62% and 32.87%, respectively. The researchers found a significant difference between seropositivity of sheep <2 years (25%) and >4 years (53%), that is consistent with the results of this study. Seroprevalence in flocks with PI sheep has been high in all age groups but in flocks with past evidence of PI sheep, seroprevalence has been high in old animals and much lower in young sheep (Seyfiabad Shapouri *et al*., 2007).

In East Azerbaijan, like this study, a high frequency of infection with the border virus has been reported (75.6%) and the seropositivity in ewes and rams was almost the same. In the latter study, BDV is considered as a possible reason for the reduction of reproductive efficiency of sheep herds in that area (Mosaferi *et al*., 2010). In a study performed in algeria by the Ab- ELIZA test, the seroprevalence of the pestiviruses was estimated 68.20% and thus 98% of the flocks had at least one positive case. The researchers concluded that infection with the virus in the studied area was endemic (Feknous *et al*. 2018). In a study conducted in suburban villages of Sanandaj, the high levels of infection were related to three issues: importation and purchase of livestock from neighboring countries, keeping of cattle (as a source of transmission) along with sheep, presence of wild ruminants as carriers of the virus in Kurdistan province (Fakur & Hematzadeh, 2007). Similar situation may play a role in Khorasan Razavi province. For example, unlicensed entry of livestock from neighboring countries (especially Afghanistan) to some cities of studied province along with the presence of traditional and rural approaches to holding different livestocks along with one another and wildlife around the Quchan region may be contributed to increasing the amount of serum positive cases in this district.

In a study, by using ELISA test, the prevalence of border disease in large Khorasan province (including North, Razavi and South Khorasan provinces at present) was reported 3.2% (Keyvanfar *et al*., 1999), which was much lower than other parts of Iran and the present study. Different results can be due to the sampling time and size, and dispersion of the sampling site in that study. Also, it has been found out that small antigenic differences between BDV strains cause differences in serological test results. In that research, the similarity between the prevalence of BD and BVD was related to the same source of infection for sheep and cattle and

the reduction of maternal antibodies was mentioned as an effective factor in increasing the infection rate in sheep over 18 months (Keyvanfar *et al*., 1999).

In a study carried out in Iraq, the high rate of infection with pesiviruses in Baghdad (36.9%) compared to other governorates of that country (30.35%) was related to the closeness of Baghdad to Iran and high rate of infection with the BVDV in the bovine population (Al-Rubayie & Hasso, 2014). According to the results of several studies that indicate high seroprevalence of BVD in Iran, especially in Khorasan province (55.3% in 2015 and 72.25% in 2009) and the possibility of interspecies transmission of the virus, it seems that the high infection in this province may be partially related to the cycling of the pestiviruses between the population of cattle and sheep (Nikbakht *et al*., 2015).

In an investigation conducted in Turkey (the province of Kurs as a place of animal transit between Europe and Asia), it was found that 74.57% of the tested livestock were positive for border virus (Yilmaz *et al*., 2014) and in another study carried out in that country, decreasing of pestivirus seroprevalence in warm coastal compared with cold central regions has been associated with reduction in the integrity of the virus membrane and its natural resistance in hot weather; also, keeping different species of ruminants near each other with high density was considered as a cause of increasing seropositivity (Okur-Gumusova *et al*., 2006). If we accept the above theory, the increase in the seroprevalence of the disease in sheep in Quchan area may be due to its cold and relatively wet climate and the decreasing infection rate in Kashmar occurs due to its warm weather.

Except for the above mentioned cases, it seems that structure of sampled flock, individual and regional differences, type of test, the sample used for detecting of the virus (milk, blood, serum, tissues, body secretions), sample size and time of sampling (pestiviruses are usually released at the time of birth from embryonic fluids) are effective in seroprevalence rate obtained in different studies (Neill *et al*., 2004; Jenvey *et al*., 2015; Yilmaz et al., 2014).

Although the detection of the antibody by serological tests indicate that the herd is exposed to the pestivirus and it can only be assumed that the virus is a possible factor in reducing of the reproductive performance in sheep flocks (Constable *et al*. 2017; Mosaferi *et al*., 2010), because of high seropositivity in this area, it is considered as an alarm for control of viral infection. Due to the relationship between the presence of PI animals in the herd with hjgh prevalence of the disease, it seems that identifying them and improving the management methods, including isolation of the aborted and defective ewes and following up their causative agent are beneficial (Constable *et al*., 2017; Seyfiabad Shapouri *et al*., 2007).

Acknowlegements

The authors would like to thank Dr. Nafiseh Kayvani Rad for her contribution in performing the laboratory tests.

References

Aitken, I (2007). Diseases of sheep. 4th Edn. Edinburgh: John Wiley & Sons. PP: 119-125.

Al-Rubayie, K.M.I and Hasso, S.A (2014). Detection of border disease in ovine using ELISA in Iraq. Int.J.Curr.Microbiol.App.Sci.3(3): 1051-1055

Campbell, JR; Radostits, OM; Wolfe, JT and Janzen, ED (1995). An outbreak of Border disease in a sheep flock. Can Vet J., 36, May: 307-309.

Constable, PD; Hinchcliff, KW; Done, SH and GrÜnberg, W (2017). Veterinary medicine. 11th Edn. Philadelphia, USA, Saunders Ltd. PP: 795-798, 1249.

Fakur, Sh and Hematzadeh, F (2007). A serological survey on the prevalence of border disease in sanandaj, Iran. Iran. Vet. Sci., 3(4): 661-666.

Feknous, N; Hanon, J-B; Tignon, M; Hamza Khaled,H; Bouyoucef, A and Cay, B (2018).

Seroprevalence of border disease virus and other pestiviruses in sheep in Algeria and associated risk factors. BMC Vet Res., 14 (339): 1- 11.

Giangaspero M. Genetic variation of border disease virus species strains. Veterinaria Italiana (2011); 47(4): 415-435.

Jenvey, CJ; Weir, AM; Reichel1, MP and Peter D. Cockcroft, PD (2015). Performance characteristics of ELISA to detect Bovine Viral Diarrhea Virus (BVDV) antibody using colostrum. Open J Vet Med., 5: 35-41

Keyvanfar, H; Hemmatzadeh, F and Kargar-Moakhar, RA (1999). Serological survey on prevalance of sheep border disease in Iran. Arch Razi Inst., 50: 1-6.

Mosaferi, S; Hassanpour, A and Foroohar, E (2010). Serological investigation of the prevalence Of border disease (BD) in sheep herds of tabriz area with a history in 2008. Vet. J. of Islamic Azad Uni. Tabriz Branch, 4,2(14): 811- 815.

Neill, RG; Connor, M and Reilly, PJ (2004). A survey of antibodies to pestivirus in sheep in the Republic of Ireland. Ir Vet J., 57(9): 525- 530.

Nikbakht, G; Tabatabaei, S; Lotfollahzadeh, S; Nayeri Fasaei, B; Bahonar, A and Khormali, M (2015). Seroprevalence of bovine viral diarrhoea virus, bovine herpesvirus 1 and bovine leukaemia virus in Iranian cattle and associations among studied agents. J. Appl. Anim. Res., 43(1): 22-25.

Okur-Gumusova, S; Yazici, Z and Albayrak, H (2006). Pestivirus seroprevalence in sheep populations from inland and

coastal zones of Turkey. Revue Méd. Vét., 157(12): 595-98.

Pugh, D and Baird, A (2012). Sheep and Goat Medicine. 2nd Edn. America: Elsevier Health Sciences. PP: 501, 596-600.

Seyfiabad Shapouri, M.R; Hajikolaei, M.R; Lotfi, M; Rasouli, A and Karimi, A (2007). Serological survey of pestivirus infection of small ruminants in Ahvaz, Iran. Arch.Razi Inst., 62(2): 105-108.

Tolouei Kaleibar, M; Madadgar, O; Jalilvand, A and Mohammadpour, H (2014). A survey on the status of the border disease virus infection in sheep with reproductive failure using cell culture and polymerase chain reaction (PCR) methods in Tabriz, Iran. Comp Clin Path., 23(5): 1429-1434.

Yilmaz V, Yildirim Y, Coskun N. Molecular and serological investigation of border disease virus infection in sheep in the kars district of Turkey. Acta Vet Brno 2014; 83(3): 175-179.