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Original Article

Seroprevalence Study of Peste Des Petits Ruminants in Sheeps of Shabestar, Iran

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Abstract

Background and Aims: Peste des petits ruminants (PPR) disease is one of the most important viral infections in sheep and goats that is caused by a *morbillivirus* from the *paramixoviridae* family, causing lesions in the gastrointestinal and respiratory tract.

Materials and Methods: In the present study, 250 blood samples were taken from the jugular vein of the apparently healthy and diseased sheep with common symptoms of PPR in Shabestar Region, Iran. Samples were randomly divided into different age groups (under 6, 6 to 12, and 12 to 24 and over 24 months). Serum samples were tested using PPR kit by ELISA antibody method to determine the prevalence.

Results: The overall rate of PPR seroprevalence in Shabestar Region sheep was 28%, which was 20% in the age groups under 6 months, 37% in the 6-12 months, 26% in the 12-24 months and 17% in the above 24 months.

Conclusion: According to the results,Our results revealed that the PPR seroprevalence high in sheeps of shabestar region and preventive proceeding need to control and eradication of the disease in that region. The severity of the disease was also reported in the age group of 6 to 12 months, which can be adviced as a best time for vaccination in the region.

Keywords: ELISA; Peste des petits ruminants; Seroprevalence; Iran

Introduction

Peste des petits ruminants (PPR) is caused by the PPR virus, which is a *morbillivirus* and the *paramexovirus* family, causing an epidemy in sheep and goats. The virus enters the body through direct contact with infected livestock and excretory secretions, especially diarrhea, but the major route of entry is through respiration, causing high fever, excessive nose and eye discharge, and clear oral lesions that spread throughout the mouth and developsmucous or bloody diarrhea occurs three to four days after the fever, and dyspnea and cough appear after gastrointestinal symptoms (Radostits *et al*, 2007). Animals with severe illness die within 7 to 10 days, and those that survive have a long convalescence, but remain safe for the rest of their lives, and in pregnant sheep, they cause abortion. In endemic areas, the clinical manifestations of PPR are not common and are often in the form of mild lesions of the mouth, diarrhea, and mild respiratory involvement (Scott, 2013). PPR is heavily involved small ruminants in almost 70 countries in Africa, East Asia and Central Asia. The disease threatens the food security of small livestock

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and prevents livestock breeding areas from reaching their economic potential (Gitao *et al*., 2016). The mainly histopathologic findings of PPR are observed in the oral cavity, digestive, respiratory and lymphoid systems. The digestive system lesions involve erosive and ulcerative stomatitis and fibrinohaemorrhagic enteritis in the digestive system and haemorrhages in the abomasum mucosa. The pulmonar lesions involve bronchitis, bronchiolitis, interstitial pneumonia, syncytial cells and intracytoplasmic and intranuclear inclusion bodies in bronchiolar and alveoler epithelium (Aytekin *et al*,2011) Methods of detection, prevention, and control of PPRV largely depend on regional facilities, available techniques, and the provision of veterinary services and vaccines. Anti-PPRV antibodies were identified by ELISA. The World Organization for Animal Health (OIE) recommends a competitive ELISA based on PPRV-specific monoclonal antibodies and virus neutralization tests. However, there are several other options, such as indirect N-ELISA, immunofiltration, sandwich ELISA, hemagglutination tests, and latex agglutination tests. Detection of PPRV antigens can be performed by methods such as immunocapture ELISA (IC-ELISA), counter immune electro phoresis (CIEP) or agar gel immunodiffusion (AGID). CIEP and ICE can distinguish PPRV from RPV, but AGID cannot distinguish these two viruses. AGID is relatively insensitive and may not detect small amounts of viral antigens in early-stage farms. Immunofluorescence and immunochemistry can be performed on conjunctival smears and tissue samples collected at autopsy (Banyard *et al*, 2010).

Methods

Sample collection. For of the PPR disease this study a total of , 250 blood samples were taken from the jugular vein of apparently healthy and diseased sheep with common symptoms (pneumonia, diarrhea, oral lesions) in July to November of 2017 in Shabestar region , Before the blood sampling, questionnaire was completed for the each animal. Then blood samples were collected for the jugular vein using the sterilized Syringe into the clot activating tubes.

Serological analysis. The samples were transferred to the laboratory in accordance with the principles of the cold chain, and the samples were centrifuged for 20 minutes at 1800 rpm and after the serum isolation, they stored at -4°C, until used. And the antibody titer was tested using a specific PPR kit by ELISA (ID-VET France©) to determine the prevalence.

Competition percentage (S / N percent) was calculated for each sample:

$$S/N\% = \frac{OD_{sample}}{OD_{NC}} \times 100$$

Statistical analysis. The statically analysis of data was performed by one-way ANOVA method using the SPSS software version 22.0. The significant difference between age groups was the determined the Duncan test. Significant difference was at less 5% level and the data were given as mean ± standard deviation.

Result

In this study, 56 specimens were obtained from the age group under 6 months, out of 32 apparently healthy sample, four sample (7.1%) and 1.6% in total samples were positive, and out of 24 patients, 10 (17.8%) and in all samples, four percent were positive. In the age group of 6-12 months, 60 samples were obtained. Seven (11.6%) out of 27 apparently healthy samples and 2.8% of all samples were positive and of the 33 patient samples, 19 (31.6%) and among all samples 7.6% were positive. In the age group of 12-24 months, 64 samples were obtained, out of 30 apparently healthy sample, six case (9%) and 2.4% of all samples were positive, and among 34 patients, 12 (18.7%) and of all samples, 4.8% were positive. Also in the age group above two years in total 70 samples were obtained, out of 37 apparently healthy sample, four (5.7%) and among all samples 1.6% were positive, and eight (11.4%) out of 33 patients and among all samples, 3.2% were positive.

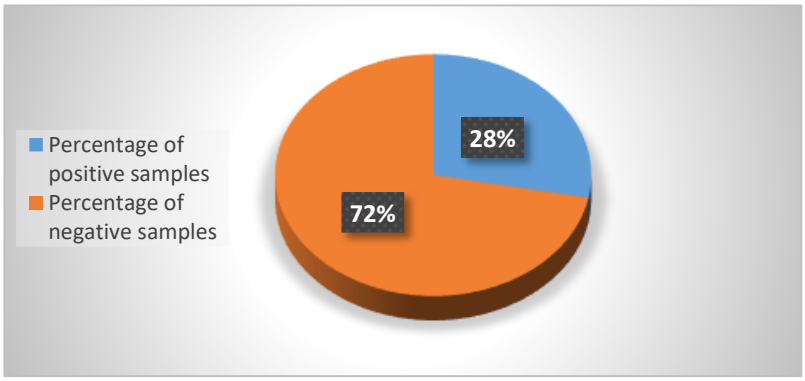


Fig. 1. Total percentage of PPR Positive and Negative Samples.

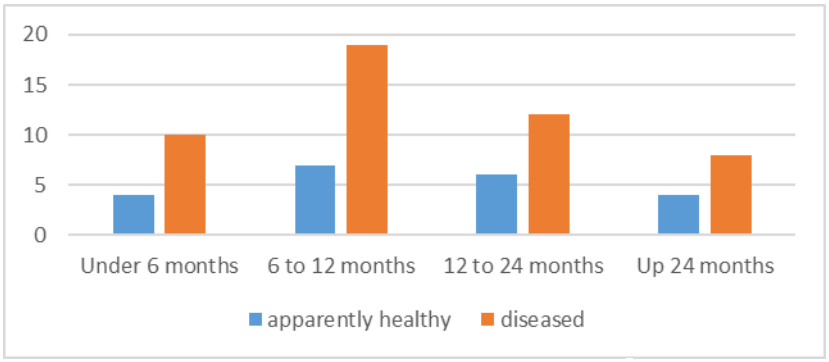


Fig. 2. Total number of positive samples in different age groups and health status.

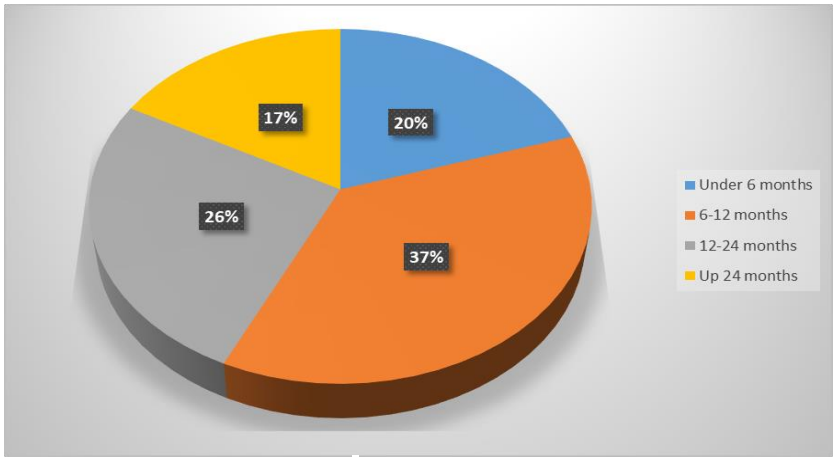


Fig. 3. Total prevalence percent in different age groups.

According to the table 1, the overall prevalence in the age group under 6 months was 5.6% and in the age group of 6-12 months, 10.4%, which was the highest, and in the age group of 12-24 months, 7.2% and in the age group above two years was 4.8%, which was the least prevalent among the other age groups. According to Figure 1, the serum prevalence of PPR in sheep of Shabestar Region was 28%.

As shown in Figure 2, most of the positive cases were in the 6-12 month patient group and the least positive cases were in the age group below 6 months and over 24 months apparently healthy.

According to Table 2, there was a significant difference between the groups of apparently healthy and diseased animals ($P<0.05$) in the mean of antibody titers in the study groups, the highest was reported in the over 24 month age

Table 1: Number of specimens and Serum prevalence percentage of Peste des petits ruminants Based on age groups studied.

Age Group (months)	Number of samples	Health status	Number of seropositive than seronegative	Withing age group %	Total prevalence in age group
< 6	56	Apparently healthy	4/32	1/6	%5.6
		Patient	10/24	4	
6-12	60	Apparently healthy	7/27	2/8	%10.4
		Patient	19/33	7/6	
12-24	64	Apparently healthy	6/30	2/4	%7.2
		Patient	12/34	4/8	
> 24	70	Apparently healthy	4/37	1/6	%4.8
		Patient	8/33	3/2	

Age group (month)	Health status	Average antibody titer
< 6	**Apparently healthy**	44±20 [a]
	Patient	288±151 [b]
6 to 12	**Apparently healthy**	47±31 [a]
	Patient	311±187 [b]
12 to 24	**Apparently healthy**	32±28 [a]
	Patient	291±91 [b]
> 24	**Apparently healthy**	42±38 [a]
	Patient	328±101 [b]

There was a significant difference between the apparently healthy animals and the patient at the 5% level in each age group with a mismatched sign (a, b) ($P<0.05$).

patient group and the lowest in the 12-24 months age apparently healthy group.

Discussion

In a study comparing the sensitivity of two competitive ELISA and RT-PCR methods on small ruminants in Kermanshah province regarding PPR disease in the early stage before diarrhea, it was reported that in RT-PCR of 30 samples, 23 negative and Seven specimens were positive (23.33% positive), which two of the seven blood serum samples from these seven animals were negative in ELISA and the remaining five were positive in ELISA. Therefore, the overlap percentage of these two methods is 71.42% and PCR sensitivity is

70.6% higher than C-ELISA (Foroughi et al., 2013).

According to a study in the Arid region of the Republic of Niger, 519 serum samples were obtained from sheep and goats and the test was done with competitive ELISA, which in this study, the prevalence of serum was 45% in total, which the prevalence was 42% in sheep versus 47.9% in goats, and there was a significantly higher correlation (P = 0.04) in young sheep than in two year olds (51.8%) and in adults (37.6%). There was also no significant difference between male and female animals (Farougou et al., 2013).

According to a study of 431 sheep serum and 538 goat serum samples in Kenya by C-ELISA test and antibody observation in the samples, serum prevalence of goat was 40% and 32% in sheep. In addition, the presence of antibodies PPRV in the middle age group (six to 24 months) was observed in both species (Kihu et al., 2015).

A study in Punjab, Pakistan, found that PPR virus was detected in the serum of 10% of cows and 14.16% of buffaloes (Abubakr et al., 2015).

According to a study of 433 serum sample from six-month-old small ruminants, without a history of vaccination against PPR in Sokoto, Nigeria, and the samples analyzed by C-ELISA, the overall prevalence of PPR in small ruminants was reported to be 45.50%, and also the prevalence of sheep was significantly higher in sheep than in goats (Bashir, 2013).

In a study of 4407 serum sample from small ruminants in India using ELISA and antibody detection against PPR, they found that the prevalence of goat disease was higher than sheep and the prevalence of PPRV antibodies in small ruminants in India was reported at 33% (Singh et al., 2004).

Also, in a study of 280 samples of goats in the Karamoja region of Uganda by C-ELISA for the detection of antibodies against PPRV reported a prevalence of 57.6% (Mulindwa et al., 2011).

And based on a study of 391 goat serum samples (318 random and 73 suspicious) in northeast India, they reported a seroprevalence using competitive ELISA, with an overall prevalence of 17.90% in goats, which was 45.2% in suspected samples and 11.63% in northeast Indian random samples (Balamurugan et al., 2014).

In a study done in Bangladesh's Patuakhali region, out of 183 goat samples, 92 had PPR infection, which was 50.27%, and the prevalence of PPR in the age group of seven to 12 months was maximum (63.33%) which was higher in compare to the under 6-month age group (44.68%), 18-13 months (41.87%) and more than 19 months (45.45%) (Islam et al., 2012).

In a study in Egypt, the rate of contagion was 26.1%, mass mortality was 10.5%, and case fatality 40.2%, and was higher in younger animals and of the 243 sera studied, 154 (4.63%) contained PPR antibodies (Abdul Rahim et al., 2010).

In a study of 150 swabs, tissues, and blood samples from unvaccinated goats in a outbreak of Peste des petits ruminants or capripox in the Democratic Republic of the Congo, they used conventional PCR and RT-PCR, the results were as follows: of the 150 animals tested, 64.7% (n = 97) were positive for PPRV, 52.7% (n = 79) were positive for capripox, and 38.7% (n = 58) for both PPRV and capripox were positive (Birindwa et al., 2017).

A study conducted in Sudan showed a seroprevalence of 61.8% for PPR, which according to ranchers in the study area, was one of the most important diseases in the country (Abdullah et al., 2012).

According to a study in Libya, 721 serum samples were collected from unvaccinated animals (601 sheep and 120 goats) and tested using the commercial C-ELAISA kit, which determined a 46.7% overall seroprevalence. Meanwhile, the prevalence among species in imported animals illegally was 69.5% (228/328), while in native species was 27.7% (109/393) (Almeshay et al., 2017).

In a study conducted on a flock of sheep with symptoms of fever, lameness, diarrhea, stomatitis, respiratory distress and high mortality in Saudi Arabia, 50 serum and 50 buffy coat samples were obtained, Using FMDV NS commercial ELISA kit, 38/50 (76%) of serum samples were positive for the presence of FMD

NS viral proteins. Evaluation of serum samples for the detection of PPR antibodies by cELISA PPR, led to positive results in 32/50 (64%). While Ic ELISA identified 32 (64%) positive for PPR antigen (Mahmoud & Galbat, 2017).

Also, a study was performed on 700 serum samples of sheep and goats to detect PPR virus antibody using C-ELAISA in Ethiopia. It has been reported that the serum prevalence percentage is 48.43% and there is no difference in serum prevalence between sheep and goats (50.85% and 46.68%). However, there was a significant statistical difference ($p < 0.05$) in the serum prevalence of the disease in the young (33.9%) and adult (55.8%) age groups. The serum prevalence of males and females was 42.07% and 50.09%, respectively, with no significant difference ($p > 0.05$) (Gari et al., 2017).

And in a study to determine the seroprevalence of Peste des petits ruminants by competitive ELISA on blood samples of 7096 sheep and goats in Pakistan, the results showed that the seroprevalence was different in different age groups, and in the three age groups, namely, less than one year, 1-2 years and more than two years, were 33.41%, 33.34% and 39.15%, respectively. Prevalence was higher in males (35.94%) than males (31.23%) (Nizamani et al., 2015).

According to a study done in Turkey on 1607 goat and sheep samples collected from 18 different locations in Turkey to investigate the presence of PPRV and RPV antibodies, prevalence for PPRV infection was different (range 82.6-87%) and was higher in sheep (29.2%) than goats (20%). Overall antibody responses to PPRV and RPV were 22.4% and 6.28%, respectively. The two PPRVs were isolated from Turkish sheep in line with the many other PPRVs originating in the Middle East, the Arabian Peninsula, and South Asia (Ozkul et al., 2002).

Based on the results of previous studies (all references), the use of ELISA to detect the prevalence of the disease is more economical and applicable. Seroprevalence was also higher in Niger, Nigeria, Uganda, and Sudan than in Shabestar, Iran, and was similar to Turkey, Pakistan, India, and Kenya. In addition, the results regarding the age of conflict were similar to most of our studies, except for the study in Ethiopia, which was interesting. And in some cases, simultaneous occurrence of PPR was also reported in diseases such as Goat Pox and FMD. In general the native cattle of the region were more resistant than imported cattle.

Conclusion

Based on the results of our study, the serum prevalence of Peste des petits ruminants in Shabestar, Iran was 28%, indicating the presence of the disease in the region and measures should be taken to control and even eradicate it in the area, therefore, training and informing the farmers can be helpful. The severity of the disease was also reported in the age group of 6-12 months, which could be used for timely vaccination in the area.

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