SERO-PREVALENCE OF PESTE DES PETITSRUMINANTS (PPR) VIRUS IN SHEEP AND GOATS IN NORTH PARS OF IRAN

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ABSTRACT
Peste des Petits Ruminants (PPR) is an acute febrile viral disease of and described by mucopurulentnasal and ocular discharges, necrotizing and erosive stomatitis, enteritis and pneumonia. The disease is endemic in Iran and causes large economic losses each year due to the high rates of mortality and morbidity in infected sheep and goats. The purpose of this study was to determine the prevalence of PPR by serology in small ruminants (sheep and goat) in Eghlid of Iran during the period early 2013tolate summer2014. In present study we selected 1800 small ruminants (900 sheep and 900 goats) which had clinically sings of PPROn the basis of sheep and goat distribution, this region divided into nine parts of Eghlid. Blood samples were aseptically collected from the neck veins using 10-ml sterile syringe, then serum samples were achieved and analyzed by using a competitive ELISAkite according to the instructions manufacturer. The results show that contamination rate in different regions, rearing systems and different seasons were significant ($p>0.01$). The results show that contamination rate between sheep and goat was not significant ($p>0.01$).

Keywords: *Peste des Petits Ruminants (PPR), Goat, Sheep, Sero-Prevalence*

INTRODUCTION
Peste des Petits Ruminants (PPR) is a disease of major economic importance and imposes a significant constraint upon sheep and goat production owing to its high mortality rate (Asim *et al.,* 2008; 2009). It is an acute, highly contagious and frequently fatal disease of sheep and goats caused by PPR virus (PPRV), a member of genus morbilli virus of family Paramyxoviridae. Therefore, it poses serious threat to the development of small Ruminants production in many countries where it is endemic and is a source of great financial loss to the farmers and livestock owners. It is a highly contagious, infectious and fatal viral disease of domestic and small ruminants (Ozmen *et al.,* 2009; Abdallal *et al.,* 2012).
Peste des petits Ruminants is highly contagious when it first occurs in a naive population. Periodic outbreaks may also be seen in endemic regions, particularly when animals are mixed or new animals are introduced into a herd. Some epizootics are associated with changes in weather, such as the beginning of the rainy season or a cold, dry period. In endemic regions, animals between three months and two years of age are most severely affected; young animals that are still nursing and older animals tend to be spared (Khan *et al.,* 2007).
Transmission of PPRV mainly occurs during close contact. Inhalation is thought to be an important route of spread. PPRV is shed in nasal and ocular secretions, saliva, urine and feces. It probably occurs in milk. Fomites such as water, feed troughs and bedding can probably transmit PPRV for a short time, but do not remain infectious for long periods.
Peste des petits ruminants occurs south of the Sahara desert and north of the equator in Africa, in most of the Middle East, and in parts of Asia including much of the Indian subcontinent. The four virus lineages are found in different geographic regions. Lineages 1 and 2 occur in parts of Africa, and lineage 3 has been reported from parts of Africa, the Middle East, and southern India. It is not certain whether lineage 3 has persisted in India; one study reports that there is no evidence for this virus after 1992. Lineage 4 has been found in the Middle East and the Indian sub-continent, but as of 2008, this virus has not been reported from Africa (Oie, 2008; Najes *et al.,* 2012).
The disease has been reported from many countries of the world including most parts of Africa, Middle East (Lefevre *et al.,* 1991), the Arabian Peninsula (Abu-Elzein *et al.,* 1990) and southern Asia (Shaila *et*

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al., 1996) and Europe. In Africa, the virus has been found circulating in Benin, Cameron, Central African Republic, Chad, Congo, Eritrea, Ethiopia, Gabon, Ghana Guinea, Ivory Coast, Mali, Mauritania, Niger, Nigeria, Senegal and Togo in addition to Kenya and Sudan (Dhar *et al.,* 2002). In Middle East and Arabian Peninsula; Iraq, Saudi Arabia, United Arab Emirates, Kuwait, Israel, Yemen and Oman are known to harbour infection. In Asia, the disease has been reported in India, Nepal, Bangladesh, Pakistan, Afghanistan and Iran, and in Europe. In Pakistan PPR had its beginning in 1988; which confirmed from 1998 onward. The disease now appears endemic in the region throughout south Asia and beyond including Iran and Afghanistan.

The present study was carried out to detect the PPRV antibodies in the serum samples of sheep and goats by using the monoclonal antibody based c-ELISA.

MATERIALS AND METHODS

Our study was conducted during the period early 2013tolate summer 2014 with 1800 sheep and goats in rural of Eghlid (a city in south of Iran). On the basis of sheep and goat distribution, this region divided into nine parts of Eghlid: (for Sheep: Haji- Abad, Hossein-Abad1, Hossein Abad, Sharmiyn, Shahrak-Kiyn, Abbarik, Jadval- Now, Cheshmeh-Rahna & Soghad, forgoat; Bazbacheh, Emam Zadeh-Esmaeil, Sofiyn, Ghenat-Agmas, Bidsobhan, Sharak-Eigler, Abbarik, Haji Abad and Menj-Gholo). From each area for each 200 head in herds were randomly selected13-15 head. We selected 1800 small ruminants (900 Sheep and 900 goats) which had sings of PPR clinically. Blood samples were collected by jugular-vein puncture using 10-ml sterile syringe then serum samples were achieved and analyzed by using a competitive ELISA kit (collectively produced by Biological Diagnostic Supplies Ltd, Flow Laboratories and Institute for Animal Health Pirbright, Surrey, England) according to the instructions of the manufacturer. However, in order to avoid confusion, before use, the tubes are numbered. To avoid corruption of samples, all samples were maintained in the refrigerator and transported to laboratory within for the 48hrs. If a delay in sample transportation was expected, samples were centrifuged and frozen at -20^{oc} before being submitted to the laboratory.

The kit is based on a standard competitive enzyme linked immunosorbant assay (cELISA) to determine the presence of anti-PPR antibodies in serum, as described by Singh *et al.,* (2004). The test is based on the competition between the anti-H protein of PPR virus monoclonal antibodies and the serum samples for binding the PPR antigen (Libeau *et al.,* 1992).

The presence of antibodies to PPR virus in the serum samples blocks reactivity of the monoclonal antibodies which causes reduction in the expected color following the addition of enzyme labeled anti-mouseconjugate and chromogen solution. The negative and positive cut-off values were used from the controls of the test procedure. The ELISA micro-plates were read using an immunosunkan reader with an inference filter of 492 nm. The reader was connected to a computer loaded with ELISA Data interchange (EDI) software, which was used to automate the reading and calculation of percentage of inhibition (PI) values. The optical density (OD) values were converted to percentage inhibition by using the following formula:

PI = 100 – (OD control/test serum) x 100(OD monoclonal control)

The samples with PI >50% were considered as positive.

Statistical Analysis

Data on based area, Genus, rearing systems and year ware categorized. The data using software SAS 9.2, in a logistic model were analyzed statistically:

$$\eta_i = \log\left[\frac{p_i}{1 - p_i}\right] = m + \tau_i$$

RESULTS AND DISCUSSION

Results outbreak of disease in flocks of sheep and goats for the nine areas is showed in the table 1 and 2. Prevalence of disease in different regions was significant. The highest contamination rate in the flock of sheep was in the Haji-Abad area (25%) and lowest contamination rate was in Jadval-Now area (3.45%).

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High density of the flocks in this region, the neglect of hygiene conditions and quarantine are causes of contamination more in sheep in Haji Abad region.

In flocks of goats, the highest contamination rate was in Bidsobhan area (45%) and lowest contamination rate was in Sharad-Eigder area (2.5%). High density of the flocks in this region, the neglect of hygiene and quarantine, and migratory herds (herds of nomads) in these areas are causes of contamination more in goat in Bidsbhan region. Usually in mid-spring, Migratory herds migrate from south to this area of the country. In recent years, this patient has been reported in the countries around the Persian Gulf (Al-Dubaib *et al.,* 2008; Dhar *et al.,* 2002; Shaila *et al.,* 1996; Abu-Elzein *et al.,* 1990). Although the clinical and postmortem findings may be sufficient for the diagnosis of PPR in the endemic areas, yet labaratory confirmation is essential for definitive diagnosis. The contamination rate in the study is not in agreement with the results reported by others (Narjesi *et al.,* 2012; Abdollahpour *et al.,* 2006).

Detection of PPRV antibodies can confirm the diagnosis of PPR, however, in areas where specific vaccination against PPR is practiced, detection of PPRV antibodies may yield false picture of the prevalence of infection. Presence of maternal antibodies may further contribute to this problem. Thus, in such cases, detection of PPR virus in clinical samples becomes essential. Virus isolation is considered as gold standard test for confirmation of PPR virus but is laborious and requires high technical expertise as well as sterile condition of the samples. Monoclonal antibodies have often been used to develop ic-ELISA, which is rapid and highly sensitive (Libeau *et al.,* 1995; Saliki *et al.,* 1994).

Table 1: Percent of contamination ratein different areasinthe flocks of sheep

	Soghad	CHeshmehRana	Jadval Now	Abbarik	Areas Shahra kKiyan	Sharmiyan	Hossein Abad	Hossein Abad1	Hagi Abad
Sheep	10.2^{bc}	4.17^{d}	3.45^{de}	6.33^{c}	10.31^{bc}	5.63^{cd}	15.79^{b}	6.85^{c}	25.09^{a}

Numbers with different letters are significant at 5%

Table 2: Percent of contamination rate in different areas in the flocks of goats

Areas	Baz Bacheh	Emamza dehesmaeil	sofiyan	Ghenat Almas	Bid Sobhan	Shahra kEigder	Abbarik	Hagi Abad	Menjgholo
Goat	6.06^{fg}	7.69^{def}	23.08^{b}	6.10^{f}	45.00^{a}	2.51^{h}	10^{de}	10.81^{cd}	15.79^{bc}

Numbers with different letters are significant at 5%

The results show that between 2013 and 2014, the prevalence of the disease among the herds of sheep and goats there was a significant difference ($p> 0.05$). In 2013 and 2014, contamination rate in sheep were 13.7% and 8.3% respectively. That a reduction compared with the previous years hows. This reduction in the amount of contamination rate can because by lack of data during the autumn and winter of 2014 (Table3), hygienic conditions and less immigration of the flocks (because of the drought). In 2013 and 2014, contamination rate in flocks of goats were 12.94% and 8.22%, respectively (Table 3).

Table 3: Percent during the 2013-2014 outbreak in a flock of sheep and goats

Genus	Year 2013	2014
Sheep	13.74^{a}	8.26^{b}
Goat	12.20^{a}	8.26^{b}

In each row, numbers with different letters are significant at 5%

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The results show that rearing systems have a significant effect on the incidence of disease (Table4). In flocks of sheep, the highest contamination rate was related to semi-extensive rearing system (15.4%), and lowest contamination rate was related to extensive rearing system or pasture system (7.46%). More contamination rate in semi-extensive rearing system (rural system) could be due to the greater density of animals and the neglect of sanitary conditions as compared to the extensive rearing system. The lowest contamination rate was related to complex rearing system (kept cattle and sheep in one place). This decrease may be due to the lower density of animals in the house and better sanitary conditions for dairy cows.

In herds of goats, the highest contamination rate was related to extensive rearing system (15.67%), and lowest was related to semi-extensive rearing system (5.22%). Increasing contamination rate level in the extensive rearing system may be due to less maintenance herds of goats in semi-extensive rearing system in the area.

Table 4: Effect of rearing system on the incidence of disease in flocks of sheep and goats

	Rearing systems		
Genus	**Semi extensive**	**extensive**	**complex**
Sheep	15.04%[a]	7.46%[b]	8.26%[b]
Goat	5.22%[a]	15.65%[b]	-

In each row, numbers with different letters are significant at 5%

The results show that the seasons have a significant effect on the incidence of disease ($p> 0.01$). The results in Table 5 show that the highest contamination rate was related to spring (17.69%) and the lowest contamination was related to autumn (19.5%). Increasing contamination rate in spring can be due to migratory herds to the region of Eghlid, since, nomads migrate from south country to the region of Eghlid in mid-spring.

Table 5: Effect of season on the prevalence of disease in flocks of sheep and goats

Seasons	Spring	Summer	Fall	Winter
Prevalence %	17.69[a]	8.4[b]	5.19[c]	7.55[bc]

Numbers with different letters are significant at 5%

Climatic factors favourable for the survival and spread of the virus may also contribute to the seasonal distribution of PPR outbreaks. With the start of the rainy season (between June/July and August/September) the migratory activity of animals is reduced due to the increased availability of local fodder.

The nutritional status of the animals also improves, resulting in an increased resistance to infection. These factors may play a key role in limiting the transmission of PPRV and hence reducing the frequency of PPR outbreaks during this period. Similar observations were also made during a five year study of PPRV in the tropical humid zone of southern Nigeria (Wosu *et al.,* 2004).

The results show that species (sheep and goats) had no significant effect on the incidence of disease ($p> 0.05$). Average of contamination rate for herds of goats and sheep were 13% and11.67% respectively. The results in not in agreement with the results reported by Khan (2007), Gurcay(2013),that greater sensitivity of goats than sheep have reported.

Conclusion
Given that, the disease has a high prevalence and mortality among small ruminants. On the other hand, in parts of the region, contamination rate is relatively high. Prevention and control actions are needed by the district Veterinary Office.

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