Incidence and identification of peste des petits ruminants virus in Tajikistan

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Abstract. The recent data on the epizootological situation, clinical manifestations, and molecular genetic characteristics of the peste des petits ruminants virus identified in Tajikistan are presented in this article. Animals from four administrative divisions were examined. When analyzing seroprevalence using a competitive ELISA test, it was noted that infection was heterogeneous between regions. In the Regions of republican subordination, it was composed 70%, in Khatlon regions 43%, in the districts of Sughd 50% and Gorno-Badakhshan Autonomous Region 40%. The obtained results of phylogenetic analyses determined the continuous extension of lineage IV in Tajikistan.

1. Introduction

Peste des petits ruminants (PPR) is a transboundary disease causing significant economic losses to sheep and goat farming. The economic damage from the disease consists of high morbidity (100%), mortality (90%) of suspected animals, costs of antiepizootic measures and elimination of social and financial consequences connected with the restrictions on further use of the territory by livestock and products obtained from them. The economic costs are also connected with the emergence of concomitant diseases. PPR predominantly infect both domestic and wild small ruminants [1]. Their infection mainly observed in acute and subacute form. From its first discovery (1942, Cote Divoire), the nosoareal of PPR is steadily widened and currently, in more than 70 countries the disease is reported [2-3]. Today, many governments are concerned about the control of PPR and its total eradication. In this regard, a global program of FAO and OIE for the disease' eradication is presently operating [3].

The PPRV is an RNA-containing virus of the family Paramyxoviridae, subfamily Paramyxovirinae and genus Morbillivirus, which contains 6 proteins (N-P(C/V)-M-F-H-L) in its structure [2] [4]. Following the analysis of PPRV proteins (F and N), four phylogenetic groups (lineages I, II, III, IV) of the virus that are distributed differently, were divided. In the territory of Africa, three of these lineages (I, II, III) can be found. The fourth group known as Asian is also present in the subcontinent of India, the Middle East and East Africa [5-7].

The Central Asian isolates and strains refer to lineage IV. In the CIS (Commonwealth of Independent States) countries, the epizootic situation is not certain. Thus, in Armenia, Azerbaijan and Turkmenistan, the PPRV outbreaks were not reported. However, considering the epizootic situation in neighbouring...
countries, the monitoring studies and preventive vaccination are carried out. In Uzbekistan and Kyrgyzstan, separate outbreaks of the disease were previously recorded, and active monitoring and preventive immunization are currently underway [8]. Mass outbreaks of PPR were noted in Kazakhstan and subsequently a virulent strain “Kentau” was isolated from infected sheep [9].

In Tajikistan, in the flocks of southern regions bordering with Afghanistan since the mid-90s, a disease resembled PPR was noticed. However, in that period it was mixed up with Pasteurellosis. Latterly of 2003, periodically in almost all areas of Tajikistan was noted disease. In 2004, for the first time, experts of the OIE reference laboratory detected the PPRV [10]. Today, PPR is considered endemic for the republic, young animals from 4-5 to 8-10 months of age get infected in most cases. Outbreaks occur annually and year-round [11]. In subsequent years, through the investigations of many researchers, the diagnostic methods and measures for reducing losses in sheep breeding were developed. However, the incidence of the disease is not decreased and annual monitoring is necessary. In this regard, the objective of our study was to investigate the current incidence of PPR in Tajikistan and the identification of new virus isolates.

2. Materials and methods

2.1. The design of the study and collection of the samples
During 2013 to 2018, animals from four administrative divisions of Tajikistan were examined. Based on random sampling design, fifty-eight districts (of which 169 suspected to areas PPR) have been selected. Participatory epidemiology tool in PPR surveillance and research in the examined area was carried out according to the FAO method “Participatory epidemiology”. While research, 1882 samples of blood serum and 133 samples after necropsy (parenchymal organs) and swabs of oculo-nasal were taken from dead, suspected and slaughtered animals.

2.2. Competitive ELISA test (c-ELISA)
The C-ELISA test was performed according to the OIE protocol (2004) for the detection of antibodies to the H and N proteins of PPRV. Reading was performed in a Multiskan (Titertek, Helsinki, Finland) ELISA reader at 492 nm.

2.3. Real-Time PCR
Tissues samples of parenchymal organs and oculonasal swabs were examined by Real-Time PCR. Total RNA was obtained by a modified method using guanidine isothiocyanate (GITC) followed by phenolic deproteinization [12]. The method of extraction technique is described in an earlier published work [9]. Reagents and primers for PCR and c-ELISA were kindly provided by the FAO in Tajikistan and the Swedish National Veterinary University, Uppsala.

2.4. Sequencing and genetic typing
Samples gave PCR amplicons were sequenced by Big Dye terminator cycle and run on an ABI Prism 3130 Genetic Analyser as the manufacturer’s protocol. Sequences were analyzed by BLAST and aligned with other PPRV strains available in GenBank by BioEdit (Ibis Biosciences). Phylogenetic analysis was performed in Mega 6.0 employing Neighbour-Joining methods for the construction of the tree. The “Bootstrap” values were calculated after 1000 replicates.

3. Results and discussion
Tajikistan is an agrarian country and the main feature of its livestock breeding is transhumance. The country has natural pastures which allow owners not to harvest hay but graze their animals all year round. However, while driving animals to mountain pastures, the flocks from different regions encountered and as a result, the risk of reinfection with various causative agents including PPRV appears. According to reporting of the National Veterinary Laboratory of Dushanbe PPRV has an important role in the infectious pathology of small ruminants and shares 65% (figure 1).
Figure 1. Percentage of PPR in the infectious pathology of small ruminants in Tajikistan.

Nowadays, the epizootic situation of PPR is becoming more complicated and needed constant monitoring because the infection is expanding its areas and new epizootic foci are occurring. Thereby, investigation of the epizootic situation and the influence of geological objects and processes on animal health, the implementation of geographic information systems (GIS) as the optimal assessment tool is essential [13].

Figure 2. Clinical manifestation of PPR in sheep and goats: (a) conjunctivitis; (b) erosive and ulcerative lesions of the lips; (c) purulent discharges of the eyes and nose; (d) oral mucosa damages; (e) necrotic stomatitis; (f) diarrhoea.
The results of the examination described in this paper were conducted from 2013 to 2018 in under the strategic plan of the OIE and FAO. In this period herds of sheep and goat from districts of four administrative divisions of Tajikistan were examined. From fifty-eight districts where outbreaks resemble PPR was noticed the samples were obtained. Among lambs of 20-45 days old the symptoms were mainly observed. In adult sheep and goats clinical signs were manifested after 2-6 days of infection. After the beginning of fever (40.5-41.5 °C), hyperaemia of mucosal, discharges of ocular nasal which first were mucoid then purulent were discovered. The epithelial necrosis of lips, soft palate surface of tongue was obvious. Diarrhoea was observed in some animals within 2-3 days after pyrexia. The post mortem examination showed absence of obvious changes including only haemorrhages in lower lungs lobes, spleen, mesenteric lymph nodes and jejunum.

During the molecular-genetic study of parenchymal organs by Real-time PCR, PPRV was identified in 36 cases which composed 27% of the total studied samples. The results are presented in table 1.

**Table 1. Identification of PPRV in the territory of Tajikistan by Real-time PCR.**

| Administrative divisions | 2013 | 2014 | 2015 | 2016 | 2017 |
|--------------------------|------|------|------|------|------|
|                          | Total| Positive | Total| Positive | Total| Positive | Total| Positive | Total| Positive |
| Khatlon                  | 9    | 3     | 9    | 4     | 8    | 2     | 5    | 1     | 19   | 0     |
| Sughd                    | 5    | 3     | 11   | 5     | 5    | 2     | 5    | 1     | 0    | 0     |
| GBAO                     | 3    | 0     | 0    | 0     | 2    | 0     | 9    | 0     | 0    | 0     |
| RRS                      | 8    | 4     | 3    | 1     | 13   | 4     | 3    | 1     | 16   | 5     |
| Total                    | 25   | 10    | 23   | 10    | 28   | 8     | 22   | 3     | 35   | 5     |

The largest number of positive samples was found in the districts of RRS, Khatlon and Sughd where more than 90% of stocks are concentrated. Samples from GBAO districts were negative. The incidence of PPRV is decreased in 2015, 2016 and 2017 years as it is seen from table 1. This is probably related to the mass PPRV vaccination. It is supposed that outbreaks of PPR occurred while using a common route during movement of the animals to pastures.

While phylogeny, the detected nucleotide sequences were grouped with the isolates and strains of PPRV lineage IV found in India, Iran, Turkey, Saudi Arabia, Kazakhstan, Pakistan and Afghanistan, thereby confirming the continuous circulation of PPRV lineage IV in Tajikistan, as the first isolated virus in 2004 in Tajikistan has also belonged to this lineage. The main vehicle of transmission of PPRV between countries is probably by the widespread trade of animals and transhumance practice [15].

In serology 1882 serum of unvaccinated animals were tested by c-ELISA. The results demonstrated positive seroprevalence in different areas of the country.

**Table 2. Seroprevalence of sampled animals to PPR.**

| Administrative divisions | Sheep | Goats | Sheep and goats |
|--------------------------|-------|-------|------------------|
|                          | Total | % sero-prevalence | Total | % sero-prevalence | Total | % sero-prevalence |
| RRS                      | 184   | 65     | 156   | 76    | 340   | 70    |
| Khatlon                  | 451   | 53     | 480   | 33    | 931   | 43    |
| Sughd                    | 418   | 50     | 69    | 46    | 487   | 50    |
| GBAO                     | 51    | 31     | 73    | 45    | 124   | 49    |
It was amounted 70% in RRS, 43% in Khatlon, 50% in Sughd and 40% respectively in GBAO. The rates of PPRV were higher in goats than in sheep while examining animals from RRS and GBAO. Of 156 goats examined in RRS regions, 118 (76%) were seropositive. The results of previous investigations of Tajik scientists also emphasized that in the condition of Tajikistan the susceptibility to PPRV is more common in goats than in sheep [16]. However, during researching samples from Khatlon and Sughd districts, the sheep were more often infected than goats. These findings indicate that PPRV equally affects sheep and goats, as it was previously described by other authors [17-19].

4. Conclusion
The results of our conducted studies have found that the seroprevalence of PPR was heterogeneous throughout of administrative divisions. Internal correlation of seropositive samples was not heterogeneous across divisions but also across species within divisions.

The lineage IV is still continuing its circulation in the territory of Tajikistan. Both sheep and goats were equally suspected to PPR.

For establishing the link between the disease pattern and factors that could influence the disease dynamics such as trade and transhumance practice further studied are needed.

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