Identification of the emergent pestivirus infections of small ruminants in Tajikistan

A M Anoyatbekova, S V Alexeenkova and K P Yurov
Federal State Budget Institution “Federal Scientific Center VIEV” Moscow, Russia

E-mail: aafshona@mail.ru

Abstract. The efficacy of measures against peste des petits ruminants in Tajikistan possibly reduced through spreading of other viral infectious diseases. Pestiviruses are among these infections. Information on pestiviruses distribution in sheep and goats farms in Tajikistan is extremely limited. The obtained results of our studies showed that the border disease virus (Pestivirus D) is participating in the infectious pathology of small ruminants’ herds in Tajikistan. Pestivirus D was detected by serology, virological and molecular-genetic methods. In addition, the contamination of a series of peste des petits ruminants virus vaccine with Hobi-like pestivirus (Pestivirus H) was established. We hope that the findings of our research will improve the implementation of the International Program for the control and eradication of peste des petits ruminants.

1. Introduction
It is known that the spread of various infectious diseases among farm animals leads to tremendous economic damages. For the eradication of these diseases, various strategies and programs have been developed. However, the re-emergence or emergence of new viral infections in previously uninfected areas is not ruled out. In recent years, the so-called emergent infections of animals are spread. These infections occur as the results of the sudden appearance of newly virulent isolates of causative agents of infectious diseases, the intensive progress in animal husbandry and the method of its keeping, changes in climate and transportation and trade of animals [1]. In most cases, the importance of these infections is the severity of the manifestations of the disease, the absence of specific clinical signs, which complicates the differential diagnosis so as the whole diagnosis.

For livestock, particularly for sheep and goats farming, the greatest threat is caused by the peste des petits ruminants (PPR), which was determined as a transboundary and highly contagious disease of small ruminants by FAO and OIE [2-5]. In this regard, in 2013, these two international organizations have jointly launched a global program to eradicate PPR in many countries of Africa, Asia and the Middle East [4].

In almost all livestock farms in Tajikistan, diseases of the respiratory organs and the reproductive system of various etiologies are widely diagnosed. The peste des petits ruminants is not an exception. This disease in Tajikistan was first discovered in 2004 by common FAO/OIE and local veterinarians’ conducted investigations [6].

Nowadays, according to some researches, PPR is endemic in Tajikistan and young animals are more susceptible [7, 8]. The program for PPR eradication is also functioning in the country and is based on the vaccination of livestock with high-quality imported vaccines. However, the experience of using
some commercial biological products has shown their low efficiency, which can be explained by a number of reasons, including the reactogenicity of the products. It has been already confirmed almost all around the world that pestiviruses are frequent contaminants of live modified vaccines and fetal serum, which induce infection in the organism of animals [9]. The mixed infection of pestiviruses and PPR has also been reported [10, 11].

Among viral diseases, pestiviruses are very important pathogens having the ability to cause persistent infection [12]. Pestiviruses are the viruses of the genus Pestivirus in the family Flaviviridae. Currently, eleven members of this genus are known. All viruses are designated in a new format (Pestivirus A to Pestivirus K) [13]. The typical pestivirus infection of small ruminants was considered the border disease (BD), the causative agent of which is now referred to as Petivirus D. Currently, eight genetic groups of BDV is known apart isolates from Tunisia and Turkey that probably form another group of the virus [14-16]. The seroprevalence from BD was estimated from 5 to 50% in some countries [17,18]. Today, there are a lot of studies on natural and experimental infection of sheep and goats, not only with Pestivirus D but also with Pestivirus A (BVDV-1), Pestivirus B (BVDV-2) and Pestivirus H (BVDV-3, Hobi-like pestivirus) [19, 20].

In Tajikistan, data on pestiviruses are quite fragmented, despite the spread of respiratory, intestinal and reproductive diseases. There are known only the researches of Amirbekov M and Kurbonbekova Z on the mucosal disease – bovine viral diarrhea in cattle and small ruminants [21, 22].

The identification and typing of viruses during mass outbreaks of diseases and the study of their etiology particularly in the sheep and goats’ populations in Tajikistan is a relevant task of scientific research. The above-mentioned facts determined the purpose of our research as identification and study of the pestiviruses that caused the respiratory and reproductive diseases in small ruminants in Tajikistan.

2. Materials and methods

2.1. Sample collection
Samples of serum and parenchymal organs of slaughtered animals and aborted fetuses were sent to our laboratory. Totally 234 samples of serum and 32 post-mortem samples from thirteen districts of Tajikistan were examined. The material was stored at –70 °C and then examined for the presence of BD virus, BVDV and PPRV.

2.2. Virus isolation
The BDV isolation was carried out in accordance with the recommendations of the OIE [23]. The virus was isolated in the porcine kidney (PK-15) and primary culture of the lamb kidney cells (LK). Cell cultures were grown on EGLA MEM medium (PanEco, Russia) with the addition of L-glutamine (OOO BioloT), 10% of fetal serum (Biosera, France) and antibiotic: amikacin 1 mg/ml (ОАО Synthesis, Russia). The virus was cultivated for 3-7 days. During daily microscopy, no changes were observed in monolayers. The non-cytopathogenic (NCP) biotype of the virus was identified by RT-PCR as well as AGID with positive sheep sera.

2.3. Agar-Gel Immunodiffusion Test (AGID)
The AGID test was performed according to generally accepted methods. As antigen, the vaccine strain of PPR virus, a clarified lysate culture of cells infected with referent strain OregonC24V of BVDV-1a were used. The test was performed in Petri dishes in 1% layer of Difco Agar in phosphate-buffered saline (PBS), pH 7.2-7.4. To study sera, an antigen that was clarified by centrifugation was dropped in the central well and serum samples in the peripheral wells. For the antigen detection, control serum was dropped in the central well and samples of infected cell culture in the peripheral wells. The Petri dishes were then stored in the room temperature and after 24 hours were examined for the precipitin lines.

2.4. RT-PCR and phylogenetic analysis
Isolation of total RNA of viruses was carried out by the standard method using a commercial RNA-Extran kit manufactured by Syntol (Moscow, Russia) with accordance to protocol. The RT-PCR was conducted by the instruction of a commercial kit “OT-1” company Syntol. Primers were chosen for a partial gene encoding NS3 of BVDV; 5'UTR mRNA region of BVDV, Npro gene of BDV and partial N and F genes of PPRV according to OIE recommendations [23, 24]. Phylogenetic analysis was performed in Mega 7.0 by the Neighbour-Joining method with Bootstrap values based on 1000 replicates.

3. Results and discussion
In Tajikistan, goat and sheep farming are traditional livestock industries. The breeding of sheep and goats are more profitable due to the fact that they are pasture animals and unpretentious to the conditions of feeding and keeping. Various breeds of these animals are bred in different regions. Among sheep, the Karakul, Hissar and small Darvaz breed are predominating. Tajik woolly goat breed is very common among goats. The main part of small ruminants of the republic is focused in state and private farms. Livestock farms annually use the local and interstate pastures which have a certain effect on the epizootic situation of the country [22]. In animals’ herds, respiratory and gastrointestinal diseases are widespread. According to the veterinary reporting in the period from 1997 to 2004, in Tajikistan, the mortality rate from these diseases ranged from 12.3% to 16.7%, respectively [22]. Due to the active economic and trade relations between countries and common pastures using during animal movements, there is a high probability of pestiviruses distribution in the territories free from these infections.

In our study, flocks of small ruminants from several districts of Tajikistan with symptoms of gastrointestinal, reproductive and respiratory failures were examined aiming to detect the pestivirus infections. In the flocks, mass abortion and stillbirth were noticed. The bronchopneumonia, nose and eyes discharges, oedema of the mucous membranes of the nose and mouth cavity, stomatitis and diarrhea were observed during a clinical examination. A decrease in weight, growth retardation and inability to stand independently were noted in lambs and kids. In the second trimester, the pregnant ewes were aborted. The fetuses were poorly developed. At necropsy, an increase in lymph nodes, liver, spleen, and haemorrhagic pneumonia was detected. The prevalence of pestiviruses was evaluated by AGID which is recommended in the OIE manual for the BDV diagnosis. The strain OregonC24V can be used as antigen [23].

The results of the retrospective study in AGID had revealed 27% positive samples with BVDV antigen and 12% with PPRV antigen. A detailed analysis of the results determined that some samples were positive with both BVDV and PPRV antigens which composed 5% of the studied serum. In subsequent studies, by the isolation of a virus from pathological material was found that the virus does not produce a cytopathic effect. Therefore, a non-cytopathogenic virus was identified by AGID and RT-PCR.

In AGID we used the positive precipitating ovine serum. The results had demonstrated that the epizootic NCP isolates substantially differed in the ability to reproduce in vitro, which is explained by the sensitivity of cell cultures. The most demonstrative results were obtained while using lungs and liver as a source of virus isolation. In cases when we changed the reaction method we had observed crossed precipitation lines. In this regard, further viral agent identification was applied by molecular-genetic methods.

The BD virus was identified by RT-PCR. Phylogenetic analysis was determined that the border disease virus (Pestivirus D) supposedly belongs to the 3rd genetic group of BDV and in 90-91% was similar to the available isolates of GenBank. The sequences of the studied virus were submitted to GenBank and assigned the accession number KX900608. Accordingly, when conducting differential diagnostics, the infection with PPRV and BVDV was excluded in samples.

During an experimental study in AGID, when a PPR vaccine strain was used as a control positive antigen, its contamination was detected. The results were confirmed by RT-PCR and phylogenetic methods, which established that a series of a vaccine against PPRV was contaminated by the pestivirus H the so-called causative agent of Hobi-like pestivirus (BVDV-3). The nucleotide sequences were
recorded to GenBank. The accession number of the sequences is KX900607. It is known that Hobi-like pestivirus is a frequent contaminant of fetal serum which is an important component of many antiviral vaccines [25, 26].

Thus, the factors mentioned above can significantly complicate the implementation of the planned measures of PPR eradication in Tajikistan in the scheduled time.

4. Conclusion
The emergent pestivirus D (BDV) for the first time was identified and isolated in small ruminants in Tajikistan. Based on phylogenetic analysis, it was shown that the virus can be assigned to the 3-genetic group of BDV. The nucleotide sequences of a typical virus isolate are submitted to the GenBank. The contamination of a commercial vaccine against PPRV with a new, atypical Hobi-like pestivirus has been established. The dissemination of these pestivirus infections in the Central Asian region can significantly reduce the effectiveness of anti-epizootic measures against PPR.

Acknowledgements
The authors would like to acknowledge the staff of the National Center of Veterinary Diagnostics of Dushanbe for their direct and indirect help.

References
[1] Borisevich S V, Sizikova T E, Siromyatnikova S I, Pantyukhov V I and Lebedev V N 2018 Journal of NBC Protection corps 2(2) 61-9
[2] Kumar N, Mahendran S, Kashyap S K et al. 2014 Viruses 6 2287-327
[3] Parida S, Muniraju M, Mahapatra M, Muthuchelvan D, Buczkowski H and Banyard A C 2015 Veterinary Microbiology 181 90-06
[4] OIE and FAO Global Strategy for the control and eradication of PPR 2015 83
[5] Anoyatbekova A M, Dias Himenes K A, Alexeyenkovna S V and Yurov K P 2015 Rus. Vet. J. 4 36-8
[6] Kwiatek O, Minet C, Grillet C, Hurardy C, Carlsson E, Karimov B, Albina E, Dialloyand A and Libeau G 2007 Journal of Comparative Pathology 136(2-3) 111-19
[7] Murvatulloev S A, Anoyatbekov M, Shonaza J M and Amirbekov M 2011 Kishovarz 2 23-5
[8] Abdulloyev A O, Zhbanova S Y, Belemenko V V, Anoyatbekov M and Gavrilov V A 2018 Collection of scientific papers on materials IV Int. Sci. Conf. Int. Acad. of Sci. 62-6
[9] Giangaspero M 2013 Products Trop Med Surg. 153 2-4
[10] Kul O, Kabakci N, Ozkul A, Kalender H and Atmaca T 2008 Vet Pathol. 45 191-6
[11] Toplu N, Oguzoglu T C and Albayrak H 2012 J. Comp. Path. 146 289-97
[12] Yurov K P, Anoyatbekov A M, Dias Himenes K A and Alexeyenkovna S V 2015 Veterinary medicine 9 3-8
[13] Smith D B, Meyers G, Bukh J, Gould E A, Monath T, Muerhoff A S, Pletnev A, Rico-Hesse R, Stapleton J T, Simmonds P and Becher P 2017 Journal of General Virology 98 2106-112
[14] Peletto S, Caruso C, Cerutti F, Modesto P, Zoppi S, Dondo A, Acutis P L, Masoero L 2015 Arch Virol. 161 471-77
[15] Ciulli S, Purpari G, Agnello S, Di Marco P, Di Bella S, Volpe E, Mira F, De Aguiar Saldanha Pinheiro AC, Vullo S and Guercio A 2016 Transboundary and Emerging Diseases 64 1243-253
[16] Thabti F, Letellier C, Hammami S, Pepin M, Ribiere M, Mesplede A, Kerkhofs P and Russo P 2005 ArchVirol. 150 215-29
[17] Oguzoglu T C 2012 Animal Health, Production and Hygiene 1 1-9
[18] Naouel F, Jean-Baptiste H, Marylène T, Hamza K, Abdallah B and Brigitte C 2018 BMC Veterinary Research 14:339 2-11
[19] Passler T and Walz P H 2009 Animal Health Research Reviews 11(2) 191-5
[20] Shi H, Kan Y, Yao L, Leng C, Tang Q, Ji J and Sun S 2016 Transboundary and Emerging
Diseases 63 480-84

[21] Amirbekov M 2000 Respiratory diseases of cattle in the conditions of industrial and pastoral livestock in Tajikistan: etiology, prevention and treatment 388

[22] Kurbonbekova Z D 2007 Epizootic situation of the respiratory and intestinal diseases of young cattle and small ruminants in the central regions of Tajikistan 176

[23] OIE 2012 Manual of Diagnostic Tests and Vaccines for Terrestrial Animals

[24] OIE 2013 Manual of Diagnostic Tests and Vaccines for Terrestrial Animals

[25] Decaro N, Mari V, Pinto P, Stella L M, Sciarretta R, Cirone F, Colaianni M L, Elia G, Thiel H J and Buanovoglia C 2012 Journal of General Virology 93 1976-983

[26] Dias R K, Cargnelutti J F, Weber M N, Canal C W, Bauermann F V, Ridpath J F, Weiblen R and Flores E F 2017 Veterinary Microbiology 203 221-28