Peste des petits ruminants: Monitoring, diagnostic and spread on the territory of the Central Asia

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Abstract: The monitoring researches and laboratory confirmation of peste des petits ruminants on the territory of the Central Asia is carried out. As a result of the conducted laboratory researches of the delivered biomaterials from sick, died goats and sheep from various regions of the Republic of Kazakhstan, Tajikistan and Kyrgyzstan it has been demonstrated that virus of this disease is present. The map of spread of peste des petits ruminants on the territory of the Central Asia is made.

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Keywords: Peste des petits ruminants; morbillivirus; RT-PCR - polymerase chain reaction with reverse transcription; RNA – ribonucleic acid; cDNA - complementary DNA; TCD50/ml - designation of the infectious activity of the virus in tissue cytopathic dose.

1. Introduction

Peste des petits ruminants (PPR) – la peste des petits ruminants is a viral disease characterized by necrotic stomatitis, diarrhea and bronchial pneumonia. The disease is caused by an RNA-containing virus of Paramyxoviridae family, Morbillivirus genus. Early from 1942 to 1982 years the disease occurred on the territory of Africa (Dahomey, Togo, Nigeria, Sudan, Cot Divuar, Senegal), where Nigeria, Sudan and Ethiopia are the most unfavorable (Taylor, Abegunde, 1979; Taylor et al., 1988; Abraham, 2005).

The disease was called PPR because of its clinical, pathological and immunological similarity to rinderpest. The incubatory period of disease lasts 4-5 days then there comes the fever lasting of 6-8 days and then goats comes death at the last stages of disease (goats are more sensitive than sheep) (P.Mornet et al., 1956).

PPR causes great economic losses, especially in the countries where mainly breed small ruminants. For example, annual in Nigeria losses make 1,5 million dollars. Therate of morbidity among susceptible animals is 100%; the mortality rate reaches 90%. The small ruminants of the small sizes belonging to breed of so-called "lagunes" or "guinean", which live in the Western African countries are especially sensitive to PPR (Mornet et al., 1956; Mahapatra M. et al., 2006; Mahdy M.M. et al., 1988).

In the general list of especially dangerous diseases of Morbillivirus genus it replaced nosoarea of the causative agent of rinderpest. Till 2005 year the disease is registered on the territory of the African continent and in the countries of Asia having the general intercontinental borders of contact and was considered that the northern border of localization of PPR are in latitude 40 (forty degrees) North-Turkey, the southern border is the equator (Bakulov I.A., 2000; Bakulov I.A. et al., 2002).

According to OIE for 2004 in the world 34 countries were unsuccessful on PPR among sheep and goats. In wild fauna of PPR it is noted only in Kuwait (Taylor W.P. et al., 1990; Taylor W.P., 1979).

Tension of situation and development of epizootic process of PPR is characterized by incidence and mortality coefficient. The coefficient of mortality was made from 1,6 to 74% in the countries of Asia and from 22 to 90,1% in the countries of Africathat notes to different degree of pathogenicity of virus. It is expressed among goats (Taylor W.P. et al., 1979; Taylor W. P., 1984; Taylor W.P., 1979).

During 1989-2003 years the disease was eradicated in the sixth African countries (Egypt, Jordan, Kuwait, Lebanon, Nigeria, Sudan) as a result of the carriedout the veterinary and sanitary measures. Nevertheless, in a number of the states with the preventive purpose continue vaccination against PPR.

PPR in the countries of the former Soviet Union is insufficiently studied.

In the Central Asia, including in the Republic of Kazakhstan, Tajikistan and Kyrgyzstan until recently there were no messages on presence of PPR virus and

epizootological monitoring of contamination degree of PPR was carried out only thanks to huge efforts of Services of the state veterinary supervision of these states, employees of FAO of the UNO and the Research Institute for Biological Safety Problems (RIBSP) of the Republic of Kazakhstan in sheep-breeding and goat-breeding farms of these republics.

The economic loss from PPR in the Central Asia is significant. The average morbidity of sheep and goats was exceeded 40% in a number of farms according to data of the veterinary reporting of the above states.

The disease is a contagious among sheep and goats because the virus quickly spreads thanks to close contact of the infected animals. A zero grazing of sheep and goats is favorable to PPR epidemics.

The epizootology of PPR virus is similar to rinderpest virus in particular on clinical characteristics, pathological and immunological properties (Mornet et.al., 1956).

In most cases epidemics amplified at the close keeping of sheep and goats in the barrage and at addition of new herd to the former. Spreading disease in any zone can be determined by serological test of herd, using the method of neutralization of serum of PPR virus developed by Taylor (Taylor W.P., 1979). When using this method (Taylor and Abequnde, 1979) it has been detected that 30-40% of the small ruminants tested in Nigeria had contact with PPR virus judging by the immune level indicating the previous disease and the subsequent recovery from an infection (Durojaiye O.A. et al., 1983; Durojaiye O.A. et. Al., 1984; Dunn S.D., 1986; El Hag Ali B. and W. P. Taylor, 1984).

In the last 10 years with development of immunology and molecular biology, the polymerase chain reaction (PCR) and the enzyme-linked immunosorbent assay (ELISA) are even more often used for the laboratory diagnostics and carrying out monitoring on PPR. PCR of molecular and genetic method of diagnostics based on identification of specific fragments of nucleic acids of agent (DNA, RNA) by amplification process (increase in quantity) on the special primers (Couacy-Hymann E. et. al., 2002; Diallo A. et. al., Diallo A., Taylor W.P. et. al., 1989; Diallo A., T. Barrett M. et. al., 1989; Diallo A. et.al., 1987). Burabaevv A., Matveeva V., Koshemetov Z., Koryagina M., Yessirkepov M., Nurmashev B. 2013.

2. Material and Methods

At first PPR monitoring with using RDP, ELISA and PCR was provided on the territory of the Central Asia.

PPR tests in RDP, ELISA and PCR according to accepted in RIBSP ME&S RK on manual of statement and application of the specified tests.

Virus isolation had been provided in monolayer of green monkey kidney continuous cell line (Vero). Cell cultures were infected by 20% suspension of the pathological material, then after its treatment by antibiotics, 0,2 cm^3 on a test tube at 37°C for 1-1,5 hours. The supporting medium 1,0 cm^3 added in the infected test tubes and cultivated at 37°C for 7-8 hours with daily observation with using microscope. In case of isolation of the cytopathogenic agent carried out his identification in RDP, ELISA, PCR and electronic microscopy.

Monitoring researches on PPR were conducted on the territory of Zhambylskiy, Yuzhno-Kazakhstanskiy and Almatinskiy oblasts of the Republic of Kazakhstan, Khatlonskiy, Sogdiyskoy, Gorno-Badakhshanskiy oblasts, the Regions of Republican Submission (ARS) of the Republic of Tajikistan and Dzhal-Abadskiy, Oshskoy, Batkentskiy oblasts of the Republic of Kyrgyzstan.

3. Results

In 2003 the focus of disease with an unknown etiology was detected during epizootological survey of farms of Yuzhno-Kazakhstanskiy oblast where the outbreaks of an acute disease among small cattle were registered. It has been demonstrated that the disease was spread on 3 flocks with a total number of 1200 heads, mortality among young growth of goats and sheep and an adult livestock was made 100, 50 and 2%, respectively. Animals of nearby rural districts with a total number of 60000 heads were in the threatened zone. Also during carrying out of monitoring was detected mortality and disease among sheep and goats from an unknown infection in Dzhal-Abadskiy, Oshskiy, Batkentskiy oblasts of the Republic of Kyrgyzstan and in Khatlonskiy oblast of the Republic of Tajikistan. The unknown infection caused death of animals among young growth to 50% and in adults to 20%.

During survey by specialists of RIBSP the clinical situation of disease is studied, the pathological and anatomical openings of died animals are carried out and the pathological material from sick and died animals for laboratory research is selected.

The clinical situation of disease and the results of the pathological and anatomical opening of died kid from an unknown infection on the territory of Khatlonskiy oblast of the Republic of Tajikistan is shown in Figure 1.

The clinical situation of disease and the results of the pathological and anatomical opening of the died sheep from an unknown infection on the

territory of Kentau village Yuzhno-Kazakhstanskiy oblast of the Republic of Tajikistan is shown in Figure 2.

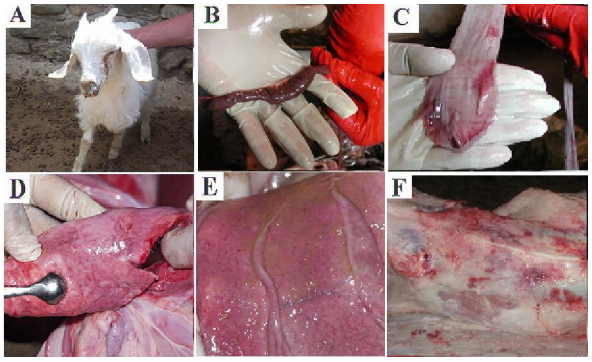

Figure 1. (A) The festering outflow from nose and eyes of sick kid. (B) Hemorrhages in the thin part of intestines of died kid. (C) Hemorrhages in the separate parts of a bladder of died kid. (D) Dotted hemorrhages in lungs of died kid. (E) Hemorrhages in cicatrix of died kid. (F) Hemorrhages on a body of died kid.

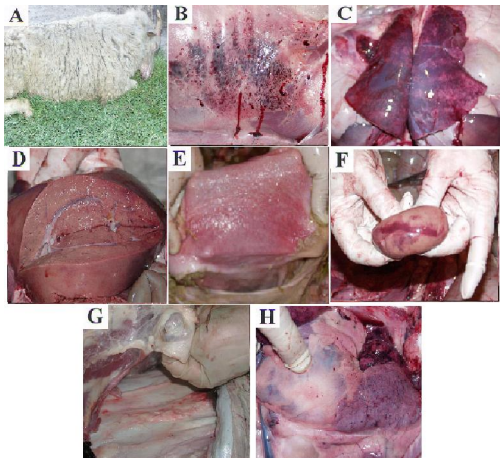

Figure 2. (A) Sick ewe, the outflow from mouth. (B) Hemorrhages on the separate parts of carcass of died ewe. (C) The extensive hemorrhages in lungs of died ewe. (D) Hemorrhages, increase in size and changes in structure of liver of died ewe. (E) Cicatrix of died ewe, hemorrhages. (F) Kidney of died ewe, hemorrhages. (G) The prescapular lymph node of died ewe. (H) The internals (heart, lungs) of died ewe.

On the basis of the clinical finding and the pathological and anatomical opening the provisional diagnosis is made: acute catarrhal enteritis; serous lymphadenitis of mesenteric; prescapular lymph nodes; dotted and spotty hemorrhages under serous cover of lungs, liver, in mucous membrane of blind gut; acute catarrhal pneumonia; serous lymphadenitis of bronchial lymph nodes; serous conjunctivitis; serous rhinitis; exicosis; emaciation and paralytic heart.

The injured organs of died animals (slices of lungs, liver, spleen, superficial lymph nodes, kidney, heart, intestines) were taken for virology researches.

In the conditions of RIBSP the pathological material was put to virology examination.

As the result of the researches of 83 samples of the pathological material was detected antigen to PPR virus in RDP in 20 samples, in ELISA –n 83 samples, these data of test systems are confirmed by the electronic microscopy. Besides, PPR virus was isolated in monolayer of cell culture Vero.

As a result of the conducted researches it has been demonstrated that the disease of small cattle in the surveyed focus of the Yuzhno-Kazakhstanskiy oblast is caused by PPR virus.

Five foci of small cattle were detected on the territory of Zhambylskiy oblast and one focus – in Almatinskiy oblast. The laboratory researches of 85 samples of the pathological material delivered from Zhambylskiy oblast 21 samples of the pathological material from Almatinskiy oblast delivered from these foci are performed at the RIBSP.

Antigen to PPR virus in 60-100% samples were detected by direct sandwich ELISA method and in 20-40% samples were detected by RDP method in the tested of pathological material from died (lymph nodes, spleen, lungs). The percent of the positive samples in ELISA was varied from 33 to 55%, in RDP was showed the negative results in samples of liver, kidneys and abomasum.

As a result of the conducted researches it is defined that the morbidity of small cattle in the tested foci of Zhambylskiy and Almatinskiy oblasts is caused by PPR agent.

It should be noted that penetration on the territory of Yuzhno-Kazakhstanskiy and Zhambylskiy oblasts of PPR virus led to huge economic losses in animal husbandry on especially dangerous infectious diseases.

The monitoring surveys in various farms were conducted on the territory of the Republic of Kyrgyzstan for clarification of spread of PPR virus. Samples of organs from died and sick animals (14 samples) and blood serum of sheep and goats (245 samples) were selected from these focus.

It has been demonstrated that on the base of the conducted researches on the territory of the Republic of Kyrgyzstan circulates PPR virus. Antibodies to PPR virus with 1:800-1:6400 activity in ELISA were detected in all tested blood serum samples. Antigens of PPR in 12 samples from 14 tested were detected by ELISA.

Annually from 2005 to 2010 jointly with the specialists of the RIBSP was performed serological

monitoring for especially dangerous viral among small cattle in the Republic of Tajikistan pursuant to the request of the central Asian Foot-and-Mouth Disease Institute (CAFDI).

During this period researches of the pathological materials and blood sera the sick and died animals delivered of different farms of the Republic of Tajikistan were performed to detect antibodies to PPR virus.

The results of serological tests in ELISA testify to wide spread of PPR virus among sheep and goats. The high percent of positively reacting animals (to 100%) was noted among sheep and goats of Khatlonskiy oblast.

2728 blood serums samples of sheep and goats from 37 livestock farms of Khatlonskiy oblast were detected antibodies to PPR virus in RDP with 1:50-1:6400 activity.

The percent of positively reacting sera in ELISA to PPR virus in Khatlonskiy oblast was 100%, in RDP – from 30 to 50%. The greatest percent of animals having antibodies to PPR were registered in Baljovunskiy rayon, farm "Kairubak"; Tavildarinskiy rayon, farm "Shakhrinav"; Vakhshskiy rayon, farm "Chovodor"; Kumsangirskiy rayon, farm "Ozodi"; Shurabadskiy rayon, farm "Bobo"; Dangarinskiy rayon, farm "Bulyenipoyen"; Khusravskiy rayon, farm "Bakhor". 402 samples from died sheep and goats were tested to detect antigen of PPR virus.

Antigen of PPR with using ELISA virus was detected in 183 samples from 402 tested samples and has been demonstrated that PPR virus has wide spread in the sheep-breeding farms and is one of the etiological factors causing mass mortality and the compelled slaughter of sheep and goats in the republic. From 12 unsuccessful regions of the republic, PPR virus was detected in 11 regions.

Thus, researches the etiological importance of PPR virus among sheep and goats in the Republic of Tajikistan confirms by the conducted tests.

The obtained data has been demonstrated that PPR virus in Tajikistan has wide geographical spread and has enzootic character. In the epizootic process from 20 to 50% of young growth are involved generally 2-6 monthly ages.

According to epizootic surveys for 2005-2010 PPR virus in Tajikistan is registered almost all the year round, except for December and January. In April-May the quantity of sick sheep and goats sharply increases and epizootic process reaches the maximum intensity in June. From July to September it weakens, and in October-November meet the isolated cases of disease.

The factor of using routes of driving of animals from winter pastures on summer, especially during following of flocks from unsuccessful farms has a certain impact on an epizootic situation on PPR.

The tests were carried out for virus isolation in monolayer of cell cultures. For this purpose objects of researches were lungs as 20% suspensions from died kid from the focus of epizootic on the territory of the Republic of Tajikistan and spleen of 20% suspension from died ewe from the focus of epizooty on the territory of the Republic of Kazakhstan Kentau City. Primary trypsin lamb kidney cell culture (LK) and green monkey continuous cell line (Vero) were used for experience as cell cultures. 5 sequences blind passages are performed on each cell cultures. PPR virus was isolated at the third passage level from lungs and spleen samples.

Cytopathic effect of PPR virus in monolayer Vero cell cultures were characterized by development of the rounded cells, appearance emergence multinuclear syncytiums and lysis of monolayer of culture on 7-9 days.

For sample differentiation cell cultures were tested with using RT-PCR. Besides for test in RT-PCR were taken organs from died kid and ewe. The results of PCR are presented in Figure 3.

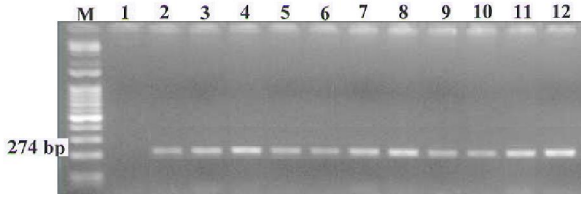

Figure 3 – Electrophoregram of RT-PCR for PPR. M – "DirectLoad™ Wide Range DNA Marker, 500-10000 bp" Sigma; 1- positive control; 2 – cultural sample infected by 20% of suspension lungs; 3 - cultural sample infected by 20% of suspension spleen; 4 – 20% of suspension lungs from died kid; 5 - 20% of suspension spleen from died kid; 6 - 20% of suspension liver from died kid; 7 - 20% of suspension thin part of intestine from died kid; 8 - 20% of suspension lungs from died ewe; 9 - 20% of suspension spleen from died ewe; 10 - 20% of suspension liver from died ewe; 11 - 20% of suspension thin part of intestine from died ewe; 12 – the negative control (water)

Figure 3 show that RNA PPR virus in RT-PCR is detected in all tested samples.

The results of the pathological and anatomical openings with further research of the selected samples of pathological material in RDP, ELISA and PCR was confirmed PPR.

At further researches of these viruses containing materials with using method of electronic microscopy was virion of PPR virus. Results are presented in Figure 4.

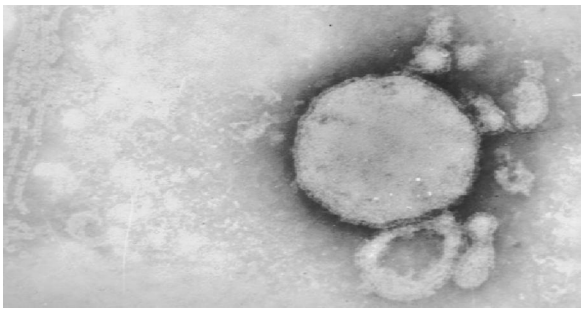

Figure 4. Virion morphology and structure of the purified PPR virus

The size of virion of the isolated PPR virus made 170 nm. The surface of supercapsid capsules contains of the 9 nm of spinuliferous. Thickness of supercapsid capsule is about 12 nm.

On the basis of the obtained data may conclude that the conducted complex laboratory tests prove that PPR virus actively circulates among small cattle in farms of the Republic of Tajikistan, Kyrgyzstan and Kazakhstan.

4. Discussions

Thus, up-to-date PPR presents a great threat for economy of the states of the Central Asia and has to remain constant object of attention for the veterinary experts.

PPR is economically important disease and is subject to eradication of global programme of vaccination (Rweyemamu M.M. et.al., 1995).

The effective, sensitive diagnostical express-tests as ELISA and PCR are needed in strategy of eradication of PPR in the world.

In connection with test-systems of ELISA and PCR were used for monitoring of PPR on the territories of the Republic of Kazakhstan, Tajikistan and Kyrgyzstan.

Similar to seroepidemiological tests for PPR was conducted in all countries of Asia and Africa. So, for example, (Kamol Kashire Daset et. al., 2007) PPR outbreak among black goats in Bangladesh was confirmed with using ELISA. They were tested discharges from nose at an early stage of disease, dyspepsical excrements and lungs, and also postmortal samples of organs. All these materials were sufficient source of the antigen detected in this reaction. It had been shown that ELISA method is suited for sensitive and specific confirmation of PPR in the field and the laboratory conditions, especially in the developing countries.

Such researches were performed with the pathological materials and blood serum from the sick and died animals delivered from various farms of the republics of Kyrgyzstan and Tajikistan for detection PPR agent and antibodies to PPR. In addition to direct ELISA and RDP for detection of PPR antigen, high sensitive RT-PCR with using primers of F-gen of PPR virus and indirect ELISA foe detection of antibodies were used in these tests. During 2005-2010 402 samples of the pathological materials from died and the sick animal at the serological tests were tested on the territory of the Republic of Tajikistan. It has been demonstrated that antigen of PPR virus was detected in 45,5% of the tested samples of the pathological materials in ELISA. 100% of the tested samples of lymph nodes, spleen and lungs in ELISA were shown the positive result.

Similar researches on studying of seroprevalence and monitoring of PPR in Pakistan were carried out by Haider AliKhan et al. 2011. The authors were tested 151 clinical samples (tissue and discharges) from the sick animals (sheep and goats) with the characterized symptoms for PPR, which were delivered from various disease foci in the province of Punjab and tested with using IcELISA kit (CIRAD/EMVT France) and RDP (AGID test) ***for detection PPR antigens. The total percentage of the positive samples in IcELISA was 63,58%. Higher percentage*** of the positive samples was tested in lymph nodes (86,36%) and smaller percentage in discharges was 43,90%. The total percentage of the positive samples in AGID was 49,01%. In these researches were noted that RT-PCR was shown equally good results with cELISA. All samples were positive in cELISA and in RT-PCR. But using of RT-PCR for diagnostic in tissue samples is more successful for the molecular characteristic of PPR.

In total 933 of sera samples of sheep and goats from 25 various geographic regions of Punjab (Northern, Southern, Western, Eastern and Central regions) were tested for presence of specific antibodies against PPR with using cELISA. The total percentage of the positive serum samples to PPR virus of small ruminants was 51,34% from the general livestock of the province of Punjab. As for various species the total prevalence for sheep was 56,80% and for goats – 48,24%.

Haque M.E. et al., 2004 with co-authors were provided the seromonitoring of PPR in sera of 750 goats and 500 cattle in 15 various regions of Bangladesh. In the most regions the antibodies level against PPR virus for goats were колебался from 4 to 98% and in average was 49,33% and only 3-10% of cattle were positive against rinderpest in C-ELISA.

Conclusion.

This work is devoted to monitoring and laboratory confirmation of PPR in the Central Asia. Many PPR outbreaks were diagnosed on the base of clinical and pathological and anatomical

characteristics of disease. So, up-to-date with developing of immunological and molecular methods of diagnosis the test-systems of ELISA and PCR are used for PPR monitoring and laboratory diagnosis in the world. Applying of these diagnostical tests help in tracking of outbreaks and serological monitoring of PPR in various geographical areas, estimation of the economic losses of infection and in studying of epidemiology of PPR for susceptible animals (DeNardi M. et.al., 2012; Forsyth M.A. et.al., 1995; Balamurugan V. et.al., 2012).

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