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Full Length Research Paper

Seromonitoring of especially dangerous diseases in small ruminants in the Republic of Tajikistan

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Seromonitoring for antibodies against especially dangerous viral infections in small ruminants in the Republic of Tajikistan was conducted. Antibodies to sheep pox and PPR viruses were detected in blood sera of small ruminants.

Key words: Virus, antigen, enzyme-linked immunosorbent assay, sheep pox, peste des petits ruminants.

INTRODUCTION

Global epizootic situation on especially dangerous viral diseases of animals demonstrates the area of their spr-ead to expand steadily. It also concerns such a danger-ous disease of small cattle as peste des petits ruminants.

The disease is caused by an RNA-containing virus of *Paramyxoviridae* family, *Morbillivirus* genus. It is charac-terized by affection of the gastrointestinal and respiratory tracts in the form of diarrhea and mucous nasal and ocu-lar discharges. Small ruminants, goats and sheep, as well as some species of wild animals (saigaks, deer, gazelles, ibexes and oryxes) are susceptible to the disease. The rate of morbidity among them is 100%; the mortality rate reaches 90%. The disease occurs in Africa, on the Ara-bian peninsula, in most countries of the Middle East and in India causing great economic losses especially in those countries where small cattle is mainly grown (Perl et al., (1994); Shaila et al., 1989)

Findings of the current epizootological surveys evi-dence that PPR spreads to more and more territories. For instance, in years 1995-2005 an epizootic focus was de-tected among small ruminants in the Republic of Tajiki-stan (Bakulov, 2002). According to Bakulov (2002) the infection is registered in Europe and in Afghanistan contiguous to the southern regions of the former Soviet Union (Shaila et al., 1989). Annual migrations of wild animals and birds, ever-growing commodity circulation and refugee flow cre-ate the real threat of its introduction onto the territory of Kazakhstan. Moreover the ever increasing number of outbreaks of unknown infections or infections that have been eradicated previously is the key argument in favor of studying this disease.

Prompt diagnosis of peste des petits ruminants (PPR) is an important problem because success of prophylactic measures depends on its solution (Bakulov, 2002).

The objective of the study was to investigate possible usage of ELISA in serological monitoring on the territory of Tajikistan for control of PPR spread among small ru-minants.

Key achievements in this area are development of prin-ciples for solid-phase immunologic assay and application of an enzyme label that allows registering formation of antigen-antibody complex by up-to-date physical meth-ods (Bakulov IA, 2000; 2002; Barrett T, 1995; Forsyth MA and Stepanov AV, 2000; Lefevre PC et al., 1991; Orynbayev MB t al., 2005; Perl S et al., 1994; Shaila MS et al., 1989).

MATERIALS AND EQUIPMENT

Blood sera of convalescent sheep (78 samples) from different farms of Tajikistan.
Blood sera of convalescent goats (87 samples) from different farms of Tajikistan
ELISA kit for PPR.
ELISA kit for sheep pox.

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Abbreviations: PPR – peste des petits ruminants; ELISA – enzyme-linked immunosorbent assay; CAFMDI – Central Asian Foot-and-Mouth Disease Institute; RK – Republic of Kazakh-stan; CES – contagious ecthyma of sheep; SP – sheep pox; RIBSP – Research Institute for Biological Safety Problems; ABTS – 2,2-azino-di-(3-ethyl)benzotiazoline sulfonic acid; r/n – rayon (district).

Table 1. Detection of antibodies to PPR, sheep pox and CES viruses in blood sera of animals by ELISA

Tested Sera		ELISA Results		
		for PPR		
Blood serum from a sheep of karakul breed	10	1:3840	-	1:460
Blood serum from a goat of local breed	10	1:1080	-	1:80
Blood serum from a ewe of ghissar cross-breed	3	1:3200	-	-

Note: " – " – negative result

ELISA kit for contagious ecthyma of sheep.

METHODS

ELISA for PPR, sheep pox and contagious sheep ecthyma was performed by the method that had been optimized in the SRAI and included the following steps:

Sensibilization of wells in polysterene plates by specific antigens and interaction at 37ºC for 3 h or at 4ºC for 18 h; Application of tested and control sera in dilutions starting from 1:50 to plate wells and interaction at 37ºC for 1.5 h; Interaction of antispecific immunoperoxidase conjugate with antigen-antibody complex in a thermostat at 37ºC for an hour; Interaction with ABTS substrate at room temperature for 15-45 min.

The results were readout visually or in the photometer at wavelength 405 nm starting with controls. At visual inspection wells with specific sera should be blue-colored, light-blue color diminishing in successive dilutions of sera is acceptable in wells with normal sera. No color should be observed in wells with solution for ELISA. The result is considered positive when wells with two or more successive dilutions of tested sera are blue-colored and color of corresponding dilutions of normal sera is faint.

In the photometer the reaction is assessed by difference in optical density of wells with specific and normal sera. Serum sample is considered to be positive if its optical density is twice and higher than of the corresponding two or more successive dilutions of the control normal serum and is not lower than 0.15.

Blood serum sampling: Sheep and goats were bled from jugular vein to tubes containing small amount (0.5-1.0 cm^3) of physiologic solution for moistening tube walls. For clotting the tubes with blood were placed into thermostat at (37 $\pm$ 1)ºC for 3 - 4 h and then into fridge at (4 $\pm$ 2)ºC for 15 h. Settled serum was poured out into sterile bottles, the blood clots were utilized. If regular blood elements were present in the serum it was centrifuged in sterile centrifuge tubes for 20 min at 2000 rpm.

The obtained animal blood sera were tested for antibodies to PPR, CES and sheep pox viruses.

RESULTS AND DISCUSSION

From 2003 the specialists of the Research Institute for Biological Safety Problems (Scientific Research Agricultural Institute till 2006), RK Ministry of Education and Science, perform serological monitoring for especially dangerous viral diseases among small ruminants in the Republic of Tajikistan pursuant to the request of the CAFMDI. In July of 2004 blood sera were sampled from sheep and goats of different breeds in Tajikistan. The specimens were tested in ELISA to detect antibodies to PPR, sheep pox and CES viruses. The results of the assays are shown in Table 1.

According to Table 1 data antibodies to PPRV with 1:1080 – 1:3840 activity in ELISA were detected in all tested blood serum samples as well as antibodies to sheep pox virus with 1:80 - 1:460 activity. Testing for antibodies to CES virus gave negative results.

In August of 2005 the staff members of the CAFMDI delivered 1350 blood serum samples of PPR reconvalescent sheep and goats from various farms to the RIBSP.

Blood sera from the delivered materials were randomly selected for determining the level of antibodies to PPRV in indirect ELISA. The results are presented in Table 2.

On the basis of Table 2 data it can be concluded that among sheep and goats in the farms of the Republic of Tajikistan PPR agent is circulating actively. The antibody activity in ELISA was mainly 1:50-1:5760. Reoccurrence of the infection among small ruminants especially among sheep and goats is possible because the level of antibodies in blood sera from sheep and goats taken in the farms of Faizabadskiy rayon, Pyandjskiy rayon, etc. is low. So, it is necessary to take preventive measures among farm animals to protect them against PPRV.

To study the dynamics of antibody accumulation blood sera of sheep taken after their vaccination with PPR vaccine, strain "G-45", were assayed by indirect ELISA in the SRAI. Ovine blood sera were gathered in the farms of Yuzhno-Kazakhstanaskaya and Zhambylskaya oblasts (Kazakhstan) in 7, 14 and 21 days after vaccination. The results of ELISA are shown in Table 3.

Table 3 data show that the indirect ELISA enables to detect virus-specific antibodies in sera samples from vaccinated animals in dilution range from 1:64 to 1:684. It has been found out that antibody accumulation in blood sera of vaccinated animals depends on duration of the period that has passed from the moment of vaccination. The sera samples from normal animals gave negative results in indirect ELISA and activity of the PPRV specific (control) sera was 1:6400.

Table 2. Results of assaying blood sera from PPR reconvalescent sheep and goats delivered from various farms in the Republic of Tajikistan

Tested Samples	Farms	Number of Tested Samples	ELISA Results			
			Number of Positive Samples	Number of Negative Samples	% of Positive Samples	Antibody Activity
Caprine blood sera	Nurobodskiy rayon, farm "Aligalabon"	11	11	–	100	286
Ovine blood sera	Vosseiskiy rayon, farm "Shurchashma"	11	11	–	100	2472
Caprine blood sera	Tavildarinskiy rayon, farm "Shakhrinav"	6	6	–	100	567
Ovine blood sera	Tavildarinskiy rayon, farm "Shakhrinav"	5	5	–	100	5760
Caprine blood sera	Baljovunskiy rayon, farm Kairubak	6	6	–	100	1400
Ovine blood sera	Baljovunskiy rayon, farm Kairubak	5	5	–	100	5120
Caprine blood sera	Vosseiskiy rayon, farm "Gulgasht", team No. 7	6	6	–	100	292
Ovine blood sera	Vosseiskiy rayon, farm "Gulgasht", team No. 7	5	5	–	100	1050
Caprine blood sera	Baljovunskiy rayon, farm "Saroi-malik"	6	3	3	50	600
Ovine blood sera	Baljovunskiy rayon, farm "Saroi-malik"	5	5	–	100	500
Caprine blood sera	Khovalingskiy rayon, farm "Sangovak"	6	3	3	50	66
Ovine blood sera	Khovalingskiy rayon, farm "Sangovak"	5	5	–	100	2200
Caprine blood sera	Khovalingskiy rayon, farm "Safedshakhrak"	6	1	5	15	50
Ovine blood sera	Khovalingskiy rayon, farm "Safedshakhrak"	5	5	–	100	250
Caprine blood sera	Jirgitalskiy rayon, farm "Yarosh"	6	2	4	33	50
Ovine blood sera	Jirgitalskiy rayon, farm "Yarosh"	5	5	–	100	1200
Caprine blood sera	Nurekskiy rayon, farm "Kibil"	6	6	–	100	117
Ovine blood sera	Nurekskiy rayon, farm "Kibil"	5	5	–	100	1920
Caprine blood sera	Rashtskiy rayon, farm "Zarangak"	6	5	1	83	130
Ovine blood sera	Rashtskiy rayon, farm "Zarangak"	5	5	–	100	640
Caprine blood sera	Tajikabodskiy rayon, farm "Kalai labi ob"	6	4	2	67	50
Ovine blood sera	Tajikabodskiy rayon, farm "Kalai labi ob"	5	5	–	100	680
Caprine blood sera	Faizabadskiy rayon, farm "Doshmandi"	6	–	6	0	–
Ovine blood sera	Pyanjskiy rayon, farm "Dzerzhinskiy"	4	4	–	100	163
Caprine blood sera	Tajikabodskiy rayon, farm "Kalai labi ob"	6	6	–	100	208
Ovine blood sera	Tajikabodskiy rayon, farm "Kalai labi ob"	5	5	–	100	1840
Ovine blood sera	Khovalingskiy rayon, farm "Sangovak"	11	11	–	100	1563

Table 3. Assessment of antibody accumulation rate in blood sera of sheep after their vaccination in ELISA

Description of Sera	Farms	Indirect ELISA	
		Number of Tested Samples	Serum Activity, M±m
Blood sera of sheep prior to vaccination	Yuzhno-Kazakhstanskaya oblast, Sairamskiy rayon	15	–
Sera from vaccinated sheep in 7 days		15	64±7
Sera from vaccinated sheep in 14 days		15	315±11
Sera from vaccinated sheep in 21 days		15	633± 30
Blood sera of sheep prior to vaccination	Zhambylskaya oblast, Baizakskiy rayon, Assa village, farm "Pioneer"	30	–
Sera from vaccinated sheep in 7 days		30	72±9
Sera from vaccinated sheep in 14 days		30	376±17
Sera from vaccinated sheep in 21 days		30	684±38
Specific sera to PPR virus	Control	5	6400±75
Normal sera		5	–

Note: Antibody activity is shown in reverse values;
" – " negative result.

CONCLUSION

The obtained data evidence sufficiently high sensitivity and specificity of indirect ELISA in assaying blood sera samples from vaccinated and reconvalescent animals as well as its potential use in retrospective rapid PPR diagnosis and assessment of postvaccinal immunity stress.

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