AGRICULTURAL BIOLOGY,
2019, V. 54, № 6, pp. 1225-1235
(SEL'SKOKHOZYAISTVENNAYA BIOLOGIYA)

ISSN 2412-0324 (English ed. Online)
ISSN 0131-6397 (Russian ed. Print)
ISSN 2313-4836 (Russian ed. Online)

Veterinary virology, microbiology

UDC 636.3:619:616.98:578:577.2

doi: 10.15389/agrobiology.2019.6.1225eng
doi: 10.15389/agrobiology.2019.6.1225rus

DEVELOPMENT OF TEST SYSTEMS USING A RECOMBINANT NUCLEOCAPSID VIRAL PROTEIN FOR SERODIAGNOSIS OF PESTE DES PETITS RUMINANTS

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The authors declare no conflict of interests
Acknowledgements:
Supported financially by a subsidy of the Ministry of Science and Higher Education of the Russian Federation No. 0451-2019-0005
Received July 2, 2019

Abstract

Peste des petits ruminants (PPR) is an acute febrile viral disease of small ruminants. In severe cases of PPR, when animals manifest clinical signs, virus-specific antigens can be detected in blood and/or tissue samples. In subclinically infected animals, PPR can only be diagnosed using serological testing. Taking into account their simplicity, high sensitivity and cost-effectiveness, the test systems for carrying out indirect or competitive enzyme-linked immunosorbent assay (c-ELISA) are considered most suitable to be used both for the disease diagnosis and seroepidemiological surveillance. The up-to-date techniques for PPR serodiagnosis are developed on the basis of a recombinant nucleocapsid (N) protein. Various options are being worked out to create test systems for PPR serodiagnosis, in particular, using polyclonal sera against N-protein for c-ELISA or some modifications of indirect ELISA. This work was aimed at studying the characteristics of the components of some ELISA experimental test systems for PPR diagnosis using a recombinant N-protein in indirect ELISA with a protein A peroxidase conjugate or in competitive ELISA using a peroxidase IgG conjugate obtained from polyclonal rabbit sera against the nucleocapsid protein. Owing to immunization of rabbits with the purified recombinant N-protein, sera with titers of 1:512 to 1:1024 in c-ELISA were obtained. The potential of constructing a test system for PPR diagnosis through indirect ELISA, in which the peroxidase protein A conjugate was used to identify the PPR-specific antibodies bound to the antigen, has been demonstrated in experiments with sera from convalescent goats, as well as from rabbits immunized with the N-protein. It is important that the protein A peroxidase conjugate reacts with goat sera antibodies in immune complexes. The antibodies obtained from the blood serum of a rabbit immunized with the purified recombinant N-protein have been shown to react with the same epitopes as the positive goat serum antibodies. To construct c-ELISA test system for PPR serodiagnostics, a peroxidase conjugate was prepared using the IgG isolated from an N-protein specific rabbit serum. The sera from pigs immunized with the purified PPR virus and vaccinated against caprine PPR with the titers ≥ 1:64 as observed in the neutralization test (NT) were positive in c-ELISA in which the components of the experimental test system were used. The obtained results make it possible to positively evaluate the prospect of the developed test systems for PPR diagnostics.

Keywords: peste des petits ruminants, serodiagnosis, ELISA, recombinant nucleocapsid protein

Peste des petits ruminants (PPR) is an acute, contagious and economically significant viral infection of small ruminants (goats, sheep, gazelles, oryxes, white-tailed deer) manifesting the morbidity and mortality rate respectively 100

and 90% [1]. Clinically, the disease resembles the cattle plague and is characterized by severe hyperthermia, necrotic and erosive stomatitis, enteritis and pneumonia [2]. The transmission of the virus occurs through the secretions and excretions of infected animals when the close contacting to them of susceptible animals. Cattle, buffalos and pigs may be infected with the PPR virus through the natural way or experimentally, but in both cases, they become dead-end hosts because the virus cannot transmit from them to other animals [3, 4]. The immune defense of young animals for the period up to 4 months is acquired with the intake of colostral milk [5].

The PPR virus belongs to the *Morbillivirus* genus of the *Paramyxoviridae* family [6-8]. The spread of one (Line 4) of the four known PPR virus lines is limited to Asia, and the other ones are spread in Africa (9].

In the laboratory diagnosing of the disease, the virus neutralization tests (NT) [10], the diffusion precipitation [11], counter immunoelectroosmophoresis, indirect immunofluorescence [12, 13] reactions, direct and indirect enzyme-linked immunosorbent assay (ELISA) [14] as well as the competitive ELISA (c-ELISA) based on monoclonal antibodies [15-18] are used for the detection of virus-specific antigens and antibodies.

In severe cases of PPR lesion, when the clinical signs appear in animals, the viral antigens are detected in blood and tissue samples. In subclinically infected animals, the PPR can only be diagnosed by serological testing. The neutralization test for detecting the antibodies to the PPR virus is laborious, expensive and requires using the infectious virus. It implies working with the cell cultures and availability of qualified personnel. For these reasons, using NT for large-scale routine investigations is problematic. The alternative to NT is quick, inexpensive and sensitive serological tests based on various ELISA variants which are successfully used in the diagnosing of many diseases.

In terms of simplicity, high sensitivity and cost-effectiveness, the test systems for indirect and competitive ELISA are considered the most suitable for diagnosing and seroepidemiological surveillance of PPR. They can be oriented for detecting the presence of hemagglutinin (H) [15, 16, 19] or nucleocapsid (N) protein [20-25].

It should be noted that in most of single-stranded RNA viruses including the PPR virus, the N protein is a highly conservative and the most immunogenic protein. This is conditioned by the fact that the N proteingene is located close to the 3′-end of the virus genome and therefore is translated in the amounts exceeding any other structural proteins of the PPR virus [26]. The antibodies to N-protein do not protect animals from the disease, but, given its immunogenicity and high production level, the N protein is considered the most acceptable antigen for the development of the means for diagnosing PPR [27]. In addition, the N-protein apparently has both type-specific and cross-reactive epitopes which are preserved in the PPR virus from the lines originated from different geographical regions. Given these, modern means of the PPR serodiagnostics are developed basing on the recombinant N protein [28]. The peroxidase conjugates for the test systems based on various ELISA variants are prepared using the IgG of anti-species sera or monoclonal antibodies (MABs) to animal immunoglobulins, as well as the MABs to N protein. However, the accidental loss of MAB-producing hybrid clones due to a laboratory accident or inappropriate storage conditions may cause problems in the test systems production [29]. Therefore, different test systems for the PPR serodiagnosis, in particular, those with using the polyclonal sera to N protein for c-ELISA or the modifications of indirect ELISA are studied.

In this paper, we present the results of study of two modifications of the

test systems for PPR serodiagnosis, which we have created based on the recombinant N protein in comparison with the commercial ID Screen® PPR Competition kit (IDvet, France). The verification of the components of these test systems for the indirect and competitive ELISA based on the PPR-positive antisera of goats and pigs allow us to positively assess the perspective of using the test systems recommended by us for the PPR serodiagnosis.

The objective of this research was the construction of test systems for diagnosing PPR basing on the recombinant N protein in the indirect ELISA with the peroxidase conjugate of protein A and in the competitive ELISA with the peroxidase conjugate of IgG of the polyclonal rabbit serum to nucleocapsid protein.

Techniques. The animals (goats, pigs, rabbits) taken from the animal preparation sector (Federal Research Center for Virology and Microbiology, FRCVM) were kept under standard conditions and used in accordance with the requirements of GOST R 53434-2009 dated 02.12.2009 "Principles of Good Laboratory Practice (GLP)". During the period of acclimatization and experiment, the animals were placed in individual quarters and cages in accordance with the GLP requirements [30]. The briquetted compound feed and purified water were given ad libitum into feeders and drinking bowls.

The goats were vaccinated with the dry cell-derived virus-vaccine against the Peste des petits ruminants (FRCVM) according to the vaccine administration manual.

The vaccine strain 45G37/35-K of the PPR virus was taken from the FRCVM State Collection of Microorganisms. PPR virus proliferation and the determination of its infectious activity were performed respectively in polystyrene mattresses and 48-well plates (Costar, France). We used the Vero cell culture (collection of the Federal Research Center of Virology and Microbiology) in the Eagle MEM maintenance medium (PanEco, Russia) with 2.5% fetal bovine serum. To assess titers, virus-containing material was sequentially diluted 10-fold in 4 replicates. The infected and control Vero cells cultures were kept at the temperature of 37 °C in an air atmosphere with 5% CO_2 with the replacement of the maintenance medium every 2-3 days. The results were estimated by the cytopathic effect during 10 days. The titer of the virus was calculated according to the Kerber's method in the modification of Ashmarin and expressed in lg TCD_{50}/cm^3 [31].

Two female goats (Nos. 1 and 2) of the Russian White breed were vaccinated 1 time subcutaneously at the age of 1 year. The blood was sampled from the goatlet (No. 3) born from the vaccinated female goat No. 1 at the age of 1 month. The blood samples for investigation were collected from the adult female goats on the day 28 after the vaccination.

Four pigs (Nos. 1-4) of the Large White breed weighing 25-30 kg were immunized 1 time with the purified concentrated PPR virus (strain 45G37/35-K) by 2.5 cm^3 injections intramuscularly and intranasally, the titer 10^5 TCD_{50}/cm^3. The virions were purified by differential centrifugation method. For this, the virus cultural 30 cm^3 suspension with the titer of 10^5 TCD_{50}/cm^3 was centrifuged at 5,000 g for 40 minutes (J68, Beckman Coulter, USA) to remove cell debris, the supernatant fraction was re-centrifuged through the sucrose cushion (20%, weight/volume) at 45,000 g for 4.5 hours (Avanti JXN-30, Beckman Coulter, USA). The supernatant fraction was decanted, and the precipitate was resuspended in 20 cm^3 phosphate buffer (PBS, pH 7.2). The serum samples were obtained from the blood taken from the pigs on days 0 and 28.

Four rabbits (Nos. 1-4) of the Chinchilla breed weighing 1.5-2.0 kg were immunized with the purified recombinant N protein according to the following

scheme: No. 1 — on day 0 by intracutaneous injection into the foot pads of all four legs, 200 μg of N protein with the Complete Freund's Adjuvant (CFA), and second time on day 25 by the intramuscular injection into the upper part of the thigh, then on days 50 and 57 by intravenous injection into the ear, 200 μg of N protein without adjuvant; No. 2 — injection of 40 μg of N protein to each leg similarly to No. 1; No. 3 − similarly to No. 1, but with the Incomplete Freund's Adjuvant (IFA) No. 4 — similarly to No. 2, but with IFA. On day 64, the rabbits were dehematized post mortem. The IgG from the antisera was purified according to the description [32]. The recombinant N protein of the PPR virus from the cell lysates of the pET32a/N/10 clone of *E. coli* obtained by us previously was purified by the metal chelate chromatography (Ni Sepharose HIS-Select Nickel Affinity Gel, Sigma-Aldrich, USA) under native conditions. The protein was eluted stepwise with the aqueous solutions of imidazole (50, 100, 250 and 500 mM). In the eluate fraction with 500 mM imidazole, the final concentration of the recombinant N protein after the dialysis against PBS was brought up to 2 mg/cm^3.

The neutralization test (NT) was performed with the 45G37/35-K strain of the PPR virus according to the recommendation of the OIE (World Organization for Animal Health), 2016 [2].

The peroxidase conjugates of horseradish (Sigma, USA) with IgG or A protein (Sigma, USA) for the developed ELISA-based test systems were prepared according to the description [33].

For the competitive or indirect ELISA on strips (Eppendorf, Germany) the purified recombinant N protein at the concentration of 0.25 μg/m^3 was immobilized in the carbonate-bicarbonate buffer (pH 9.6) (50 μl per well during 16 hours at 4 °C; 96-well plates, Corning, USA). Then, the wells were washed three times for 1.0 minute with 300 μl of PBS with 0.1% Tween 20 (PBS-t), then the active sites of polystyrene were blocked with PBS-t with 1% casein (blocking solution, 100 μl per well) during 1 hour at 37 °C. Then the wells were washed once with PBS-t and moisture was removed. The sera were diluted in the blocking solution, poured into the wells (50 μl per well) and kept for 2 hours at 37 °C. Then, after three-time washing with PBS-t, the conjugate from the commercial kit or the investigated conjugates diluted in the blocking solution were poured into the wells and incubated for 1.5 hours at 22 °C, the wells were washed 3 times with PBS-t and then 100 μl of the chromogenic substrate ABTS, the 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt, (Thermo Scientific, USA) was added into the wells with H_2O_2. After 15-20 minutes of incubation in the dark at 22 °C, the results were recorded using a Sunrise spectrophotometer (Tecan, Austria) at λ = 405 nm (OD_{405}).

The commercial ID Screen® PPR Competition kit (IDvet, France) designed for detecting the antibodies to the PPR virus nucleoprotein in blood sheep and goats was the control test system. The conjugate in this kit is the peroxidase-labeled monoclonal murine antibodies to the recombinant N protein of the PPR virus.

The statistical processing of the obtained data was carried out using the Microsoft Excel 2010 software. We determined the mean values (*M*) and standard errors of the mean (±SEM). The average values of the indices were compared according to the Student's *t*-test. The differences were considered statistically significant at $p < 0.05$.

Results. Initially, in order to obtain the rabbit polyclonal antibodies to the nucleocapsid protein of the PPR virus, four immunization schemes differing in the dose of the N protein administered to the animal (40 and 200 μg) and the Freund's adjuvants type (complete or incomplete) were tested. The activity of

blood antibodies to the N protein in rabbits was investigated by the c-ELISA method using the commercial ID Screen® PPR Competition kit (Fig. 1). All the studied sera had the titer values 1:512-1:1024. When the dilution in these ratios the OD_{405} values were more than 2.5 times lower than when the last dilution at 1:8192 or with the control sera obtained before the immunization. The serum after immunization of rabbit No. 1 with N protein at the dose of 200 μg with the complete Freund's adjuvant turned to be the most preferred. When its dilution at 1:1024 the $OD_{405} = 0.2$, whereas the similar optical density according to the interpolation results was achieved at the dilution of 1:172 for No. 2, 1:768 for No. 3, and 1:256 for No. 4.

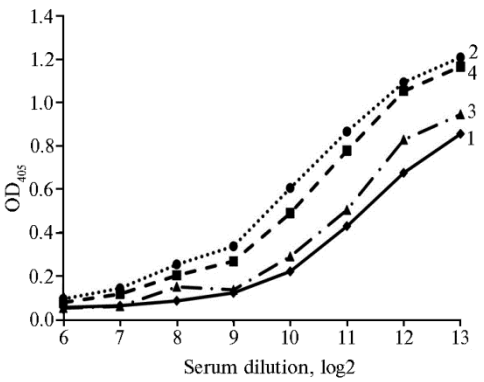

Fig. 1. Activity of blood antibody to the N protein in rabbits No. 1-4 (1-4) **immunized with the purified recombinant nucleocapsid protein of the peste des petits ruminants virus** (lab infection).

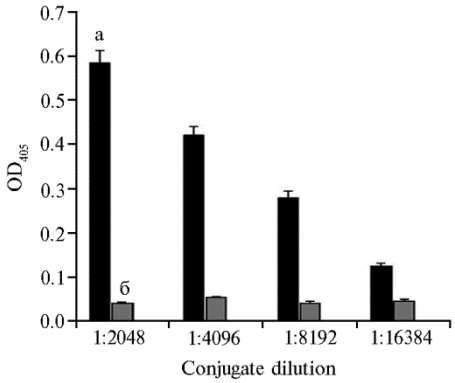

Fig. 2. Titration of protein A peroxidase conjugate using experimental strips with the negative (a) **and positive** (b) **blood sera of rabbit No. 1 diluted 1:1024** (indirect ELISA, $M \pm SEM$, $n = 4$, lab infection).

In the model experiment, the positive and negative blood sera of the rabbit No. 1 in different dilutions were added into the wells with the immobilized purified recombinant N protein. After the incubation and washing, protein A peroxidase conjugate in dilution of 1:500 was added. The results presented in Figure 2 confirm that the protein A peroxidase conjugate does not interact with the N protein directly and detects the rabbit antibodies positive to the N protein up to the serum dilution at 1:16384 that testifies about high analytical sensitivity of this indirect ELISA. The highest ratio of the OD_{405} for the positive and negative serum of the rabbit No. 1 was at their dilutions at 1:2048 (see Fig. 2).

In order to detect the antibodies to the PPR virus in goats, the strips were sensitized with the purified recombinant N protein. Then, the negative and positive goat sera taken from the commercial kit or from the rabbit No. 1 in the dilution of 1:2048 were separately added to the wells. After the incubation with protein A peroxidase conjugate, according to the reaction results, the ratio of the OD_{405} values with the positive and negative goat sera amounted to 5.54, and with the sera of the rabbit No. 1 to 7.99. It is important that in our experiments the protein A peroxidase conjugate reacted with the goat sera antibodies being a part of the immune complexes. Thus, the obtained results testify about the possibility to use the protein A peroxidase conjugate in the test systems for diagnosing PPR by the method of indirect ELISA (see Fig. 2).

When developing the c-ELISA, it was necessary to assess the correspondence of the antigenic determinants on the N protein, with which the antibodies from the goat sera and the antibodies from the serum of the rabbit No. 1 immunized with N protein interact. We found out, whether the antibodies of the positive goat serum from the commercial kit block the interaction of the anti-

bodies of the positive serum from the rabbit No. 1 with the antigenic determinants of the recombinant N protein. For this, the negative or positive goat sera were separately added into certain wells of strips with the immobilized N protein, and into other wells — first the negative or positive goat sera from the commercial kit were added, and then (after the incubation for 2 hours and three-time washing) the negative or positive serum of the rabbit No. 1 in dilution of 1:2048 was added. After washing, protein A peroxidase conjugate in dilution of 1:2000 was added into all wells. The results are presented in Figure 3.

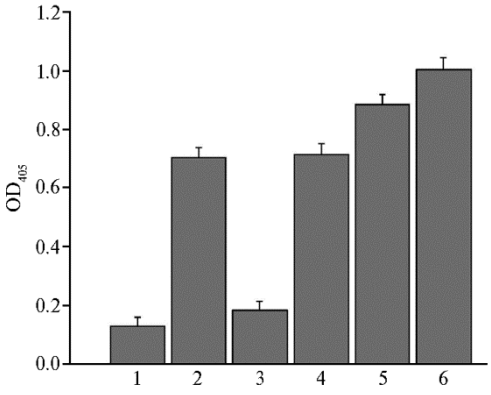

Fig. 3. Indirect ELISA with immobilized recombinant N protein, protein A peroxidase conjugate and different sera: 1 and 2 — the negative and positive goat sera, respectively, from the commercial ID Screen® PPR Competition kit (IDvet, France); 3 — adding the negative goat serum from the commercial kit followed by adding the negative serum of the rabbit No. 1; 4 — adding the positive goat serum from the commercial kit followed by the negative serum of the rabbit No. 1; 5 — adding the negative goat serum from the commercial kit followed by the positive serum of the rabbit No. 1; 6 — adding the positive goat serum from the commercial kit followed by the positive serum of the rabbit No. 1 (M±SEM, n = 4, lab infection).

The pre-incubation of the immobilized N protein with the negative or positive goat serum from the commercial kit followed by incubation with the negative blood serum of the rabbit No. 1 leads to an unreliable change ($p > 0.05$) of the OD_{405} indices. The successive incubation of the positive goat serum and the positive serum of the rabbit No. 1 led to the increase of OD_{405} from 0.70 to 1.00 ($p < 0.05$) (see Fig. 3). It follows from the aforesaid that in the rabbit immunized with the purified recombinant N protein the antibodies react mainly with the same antigenic determinants as the antibodies from the positive goat serum. At the same time, there is a probability that the antibodies from the serum of the rabbit № 1 react with the antigenic determinants which are inaccessible for the antibodies from the PPR-positive goat serum.

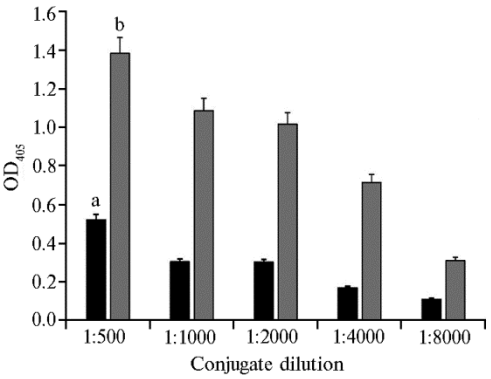

Fig. 4. Titration of the of rabbit IgG-based peroxidase conjugate on the experimental strips with the negative (a) **and positive** (b) **sera of the rabbit No. 1 in dilution of 1:1024** (competitive ELISA, M±SEM n = 4, lab infection).

For creating the test system for the PPR serodiagnosis in c-ELISA, the peroxidase conjugate based on the IgGs isolated from the positive serum of the rabbit No. 1 has been prepared. The prepared conjugate was titrated on the experimental strips with the purified recombinant N protein immobilized in wells in the dose of 0.0125 μg per well and with using the negative and positive sera of the rabbit No. 1 in dilution of 1:1024 (Fig. 4).

According to the results of c-ELISA, the ratio of OD_{405} with the negative and positive sera of the rabbit No. 1 with the conjugate amounted to 2.66 at 1:500 dilution, to 3.60in at dilution 1:1000 , 3.38 at 1:2000, 4.25 at 1:4000, and 1.59 at 1:8000. The 1:4000 dilution of the conjugate has been adopted as the operating dilution. The conjugate has not reacted with the control antigen pre-

pared from the untransformed 16-hour *E. coli* culture Rosetta 2(DE3)pLysS.

Then, in c-ELISA on the experimental strips with using the conjugate we prepared or the commercial conjugate, we compared the commercial goat sera with the sera of the rabbit No. 1 (Table 1). In case of both conjugates the results were positive, but the ratio of the OD_{405} values for the negative and positive goat sera with the commercial conjugate was higher than with the peroxidase conjugate of the IgGs of the rabbit No. 1.

1. Comparison of the negative and positive goat sera from the commercial ID Screen® PPR Competition kit (IDvet, France) and the sera of the rabbit No. 1 (competitive ELISA, $M \pm SEM$, $n = 3$, lab infection)

Serum	Experimental conjugate, 1:4000		Commercial conjugate	
	serum of the rabbit No. 1	commercial goat sera	serum of the rabbit No. 1	commercial goat sera
Negative (–)	0.63±0.04	0.46±0.03	0.56±0.05	0.59±0.03
Positive (+)	0.08±0.01	0.17±0.01	0.07±0.01	0.10±0.00
–/+	7.9	2.7	8.0	5.9

2. Comparison of neutralization test and competitive ELISA (experimental test system) for vaccinated goats and pigs inoculated with the peste des petits ruminants virus ($M \pm SEM$, $n = 3$, lab infection)

Serum	Animals						
	pigs				goats		
	No. 1	No. 2	No. 3	No. 4	No. 1	No. 2	No. 3
	Neutralization test						
Titer	1:64	1:128	1:64	1:128	1:32	1:128	1:4
	Concurrent ELISA test						
Negative (–)	1.21±0.13	1.17±0.09	0.89±0.07	0.90±0.08	0.30±0.02	0.32±0.02	0.33±0.04
Positive (+)	0.33±0.05	0.32±0.02	0.30±0.02	0.33±0.01	0.18±0.01	0.10±0.01	0.17±0.01
–/+	3.7	3.6	3.0	2.7	1.7	3.2	1.9

Using the components of the experimental test system, i.e. the strips with the immobilized purified recombinant N protein and IgG peroxidase conjugate of the rabbit No. 1 immunized with N protein, the sera of four pigs inoculated with the purified vaccine PPR virus (Nos. 1-4), two vaccinated female goats (Nos. 1 and 2) and a 1-month-old baby goat (No. 3) born from the vaccinated goat (No. 1) were investigated (Table 2). All sera of the pigs inoculated with the purified PPR virus turned out to be positive in the c-ELISA because the ratio of the OD_{405} values for the negative and positive sera was more than 2.5. Among the two vaccinated goats, the antiserum of the goat No. 2 turned out to be positive. According to NT data, all investigated sera were positive, however, the titer of the virus-neutralizing antibodies in goat No. 2 was higher than in goat No. 1. The obtained data testify that in the competitive variant of ELISA, the peroxidase conjugates obtained on the basis of IgGs of the rabbits immunized with the purified recombinant N protein of the PPR virus can be used for diagnosing the PPR virus.

The advantage of serological analysis of PPR is that antibodies to the PPR virus can be detected in convalescence animals [34]. We compared the results of indirect ELISA obtained with the system developed by us and with the test system of Indian specialists, in which the purified virions of the attenuated PPR virus grown in the Vero cell culture were used as the antigen, and the peroxidase conjugate of the rabbit IgG was used against the goat IgG [17]. The test system included the standard controls (conjugate, negative goat and sheep serum with high and low antibodies' titer values). The average values of OD_{405} in goats in the case of negative sera and sera with high and low antibody titer values were respectively 0.19±0.07, 1.13±0.09 and 0.75±0.08. However, OD_{405} values with the samples of control panel of the sheep serum were 0.15±0.05, 0.95±0.08 and 0.49±0.07. In the opinion of the authors of that paper, this is due to the incom-

plete cross-reactivity of the anti-goat conjugate with the sheep antibodies. The average values of OD_{405} for the "zero" control reaction (the reaction between the convalescent goat antibodies to the PPR virus and the conjugate in the absence of the antigen) and for the control with the conjugate without antibodies were respectively 0.14±0.05 and 0.12±0.02 [17]. In our researches with the experimental test system for indirect ELISA with the negative and positive goat sera from the commercial kit, the OD_{405} value amounted to 0.13 and 0.70, and with the negative and positive rabbit sera No. 1 when the dilution in 1:1024 ratio, it was 0.09 and 0.74. It is known that goat immunoglobulins weakly bind to A protein [35]. It was also reported that binding of the antigen by the Fab fragments increases the affinity between the Fc fragment of IgG and A protein [36]. This expands the possibility of using A protein in ELISA because it makes it possible to use animal IgGs weakly interacting with it. Thus, the goat and sheep IgGs bound to the antigen interact with A protein 100 times more intensive than without the antigen. It is important to note that in our experiments, the A protein's peroxidase conjugate have reacted with the goat serum antibodies. So, the obtained results testify about the possibility of using of the A protein's peroxidase conjugate in the test systems for diagnosing PPR by the method of indirect ELISA.

In order to replace the neutralization test when wide-scale investigation of animal blood, it was previously proposed to use the c-ELISA based test system in which the lysate of the cell culture infected with the attenuated strain of the PPR virus and the monoclonal antibodies obtained for the neutralizing epitope of H protein were used as the antigen [37]. In the opinion of the cited publication's authors, the advantages of the said test system are the simplicity, quick obtaining of the result, less dependence on the serum samples' quality and convenience when investigating a large number of samples. Its sensitivity relative to the NT (neutralization test) was 92.2%, specificity – 98.4%. The authors note that usually in blood, the antibodies' titers in c-ELISA were by 1-3 $\log_2$ lower than in NT. Typically, the antibody titers in NT after the vaccination, especially in sheep, ranged from 1:8 to 1:16. The sera with low antibody titer values in NT were negative in c-ELISA. Therefore, for identifying positive samples it was proposed to choose the low cut-off (average negative value +2 standard deviations) [38].

So, it was found that in rabbits immunized with the purified recombinant N protein of the peste des petits ruminants (PPR) virus, the antibodies which react mainly with the same antigenic determinants of this protein as the antibodies of the PPR-positive goat serum are produced. We have created and tested the experimental test system for the PPR serodiagnostics by competitive ELISA (c-ELISA) based on the recombinant N protein and peroxidase conjugate based on IgGs from the N-protein-specific rabbit serum. When using the proposed test system, it was shown that the animal sera with titers of the antibodies to the PPR virus in the neutralization test of 1:64 and higher in c-ELISA were positive. In terms of the ratio of the OD_{405} values for negative and positive goat sera, the results obtained using the commercial ID Screen® PPR Competition test system (IDvet, France) were 2 times higher than with the experimental one. The obtained results allow us to positively evaluate the perspective of indirect and competitive ELISA-based test systems we created for the PPR serodiagnosis.

REFERENCES

1. Banyard A.C., Parida S., Batten C., Oura C., Kwiatek O., Libeau G. Global distribution of peste des petits ruminants virus and prospects for improved diagnosis and control. *Journal of General Virology*, 2010, 91(12): 2885-2897 (doi: 10.1099/vir.0.025841-0).

2. World Organization for Animal Health (OIE). Peste des petits ruminants (infection with Peste des petits ruminants virus). Chapter 2.7.10. In: *Manual of diagnostic tests and vaccines for terrestrial animals*. Cambridge, 2016.
3. Baron M.D., Diop B., Njeumi F., Willett B.J., Bailey D. Future research to underpin successful peste des petits ruminants virus (PPRV) eradication. *Journal of General Virology*, 2017, 98(11): 2635-2644 (doi: 10.1099/jgv.0.000944).
4. Schulz C., Fast C., Schlottau K., Hoffmann B., Beer M. Neglected hosts of small ruminant morbillivirus. *Emerging Infectious Diseases*, 2018, 24(12): 2334-2337 (doi: 10.3201/eid2412.180507).
5. Mariner J.C., Jones B.A., Rich K.M., Thevasagayam S., Anderson J., Jeggo M., Cai Y., Andrew R., Peters A.R., Roeder P.L. The opportunity to eradicate Peste des petits ruminants. *The Journal of Immunology*, 2016, 196(9): 3498-3506 (doi: 10.4049/jimmunol.1502625).
6. Amarasinghe G.K., Aréchiga N.G., Ashley C., Christopher C.B. Taxonomy of the order *Mononegavirales*: update 2018. *Archives of Virology*, 2018, 163(8): 2283-2294 (doi: 10.1007/s00705-018-3814-x).
7. *Virus taxonomy: classification and nomenclature of viruses. Ninth report of the International Committee on Taxonomy of Viruses*. A.M.Q. King, M.J. Adams, E.B. Carstens, E.J. Lefkowitz (eds.). Elsevier, 2012.
8. Libeau G., Diallo A., Parida S. Evolutionary genetics underlying the spread of Peste des petits ruminants virus. *Animal Frontiers*, 2014, 4(1): 14-20 (doi: 10.2527/af.2014-0003).
9. Dhar P., Sreenivasa B.P., Barrett T., Corteyn M., Singh R.P., Bandyopadhyay S.K. Recent epidemiology of Peste-des-petits-ruminants virus (PPRV). *Veterinary Microbiology*, 2002, 88(2): 153-159 (doi: 10.1016/S0378-1135(02)00102-5).
10. Liu F., Wu X., Zou Y., Li L., Wang Z. Peste des petits ruminants virus-like particles induce both complete virus-specific antibodies and virus neutralizing antibodies in mice. *Journal of Virological Methods*, 2015, 213: 45-49 (doi: 10.1016/j.jviromet.2014.11.018).
11. Durojaiye O.A. Precipitating antibody in sera of goats naturally affected with Peste des petits ruminants. *Tropical Animal Health and Production*, 1982, 14(2): 98-100 (doi: 10.1007/BF02282591).
12. Kozat S., Sepehrizadeh E. Peste des petit ruminants. *Journal of Istanbul Veterinary Sciences*, 2017, 1(2): 47-56 (doi: 10.30704/http-www-jivs-net.319937).
13. Durojaiye O.A., Taylor W.P. Application of countercurrent immuno-electro-osmophoresis to the serology of Peste des petits ruminants. *Revue d'élevage et de médecine vétérinaire des pays tropicaux*, 1984, 37(3): 272-276 (doi: 10.19182/remvt.8396).
14. Obi T.U., McCullough K.C., Taylor W.P. The production of Peste des petits ruminants hyperimmune sera in rabbits and their application in virus diagnosis. *Journal of Veterinary Medicine, Series B*, 1990, 37(1-10): 345-352 (doi: 10.1111/j.1439-0450.1990.tb01068.x).
15. Anderson J., Mckay J.A., Butcher R.N. The use of monoclonal antibodies in competition ELISA for detection of antibodies to rinderpest and peste des petits ruminants viruses. In: *Final research co-ordination meeting on the sero-monitoring of rinderpest throughout Africa, Bingerville (Cote d'Ivoire), 19-23 Nov 1990*. International Atomic Energy Agency (IAEA), Vienna, 1991: 43-53.
16. Abubakar M., Mahapatra M., Muniraju M., Arshed M.J., Khan E.A., Banyard A.C., Ali Q., Parida S. Serological detection of antibodies to Peste des petits ruminants virus in large ruminants. *Transboundary and Emerging Diseases*, 2017, 64(2): 513-519 (doi: 10.1111/tbed.12392).
17. Balamurugan V., Singh R.P., Saravanan P., Sen A., Sarkar J., Sahay B., Rasool T.J., Singh R.K. Development of an indirect ELISA for the detection of antibodies against Peste-des-petits-ruminants virus in small ruminants. *Veterinary Research Communications*, 2007, 31(3): 355-364 (doi: 10.1007/s11259-006-3442-x).
18. Singh R.P., Sreenivasa B.P., Dhar P., Shah L.C., Bandyopadhyay S.K. Development of a monoclonal antibody based competitive ELISA for detection and titration of antibodies to peste des petits ruminants (PPR) virus. *Veterinary Microbiology*, 2004, 98(1): 3-15 (doi: 10.1016/j.vetmic.2003.07.007).
19. Singh R.P., Screenivasa B.P., Dhar P., Roy R.N., Brandyopadhyay S.K. Development and evaluation of a monoclonal antibody based competitive enzyme-linked immunosorbent assay for the detection of rinderpest virus antibodies. *Revue Scientifique et Technique*, 2000, 19(3): 754-763 (doi: 10.20506/rst.19.3.1243).
20. Choi K.S., Nah J.J., Choi C.U., Ko Y.J., Sohn H.J., Libeau G., Kang S.Y., Joo Y.S. Monoclonal antibody-based competitive ELISA for simultaneous detection of rinderpest virus and peste des petits ruminants virus antibodies. *Veterinary Microbiology*, 2003, 96(1): 1-16 (doi: 10.1016/S0378-1135(03)00201-3).
21. Basagoudanavar S.H., Hosamani M., Muthuchelvan D., Singh R.P., Santhamani R., Sreenivasa B.P., Saravanan P., Pandey A.B., Singh R.K., Venkataramanan R. Baculovirus expression and purification of peste-des-petits-ruminants virus nucleocapsid protein and its application in diagnostic assay. *Biologicals*, 2018, 55: 38-42 (doi: 10.1016/j.biologicals.2018.07.003).
22. Liu F., Wu X., Li L., Liu Z., Wang Z. Use of baculovirus expression system for generation of virus-like particles: successes and challenges. *Protein Expression and Purification*, 2013, 90(2): 104-116 (doi: 10.1016/j.pep.2013.05.009).
23. Liu F., Wu X., Li L., Liu Z., Wang Z. Formation of peste des petits ruminants spikeless virus-like particles by co-expression of M and N proteins in insect cells. *Research in Veterinary Science*, 2014, 96(1): 213-216 (doi: 10.1016/j.rvsc.2013.10.012).

24. Berguido F.J., Bodjo S.C., Loitsch A., Diallo A. Specific detection of peste des petits ruminants virus antibodies in sheep and goat sera by the luciferase immunoprecipitation system. *Journal of Virological Methods*, 2016, 227: 40-46 (doi: 10.1016/j.jviromet.2015.10.008).
25. Bodjo S.C., Baziki J.D., Nwankpa N., Chitsungo E., Koffi Y.M., Couacy-Hymann E., Diop M., Gizaw D., Tajelser I.B.A., Lelenta M., Diallo A., Tounkara K. Development and validation of an epitope-blocking ELISA using an anti-haemagglutinin monoclonal antibody for specific detection of antibodies in sheep and goat sera directed against peste des petits ruminants virus. *Archives of Virology*, 2018, 163(7): 1745-1756 (doi: 10.1007/s00705-018-3782-1).
26. Lefevre P.C., Diallo A., Schenkel F., Hussein S., Staak G. Serological evidence of Peste des petits ruminants in Jordan. *Veterinary Record*, 1991, 128(5): 110 (doi: 10.1136/vr.128.5.110).
27. Cosby S.L., Weir L. Measles vaccination: threat from related veterinary viruses and need for continued vaccination post measles eradication. *Human Vaccines & Immunotherapeutics*, 2018, 14(1): 229-233 (doi: 10.1080/21645515.2017.1403677).
28. Munir M., Zohari S., Berg M. Current advances in molecular diagnosis and vaccines for peste des petits ruminants. In: *Molecular Biology and Pathogenesis of Peste des Petits Ruminants Virus. SpringerBriefs in Animal Sciences*. Springer, Berlin, Heidelberg, 2013: 105-133 (doi: 10.1007/978-3-642-31451-3_6).
29. Getachew B., Upmanyu V., Haq A.A., Santhamani R., Rajak K.K., Muthuchelvan D., Gupta S.K., Yousuf R.W., Mahapatra M., Parida S., Sharma B., Singh R.P. Monoclonal antibody resistant mutant of Peste des petits ruminants vaccine virus. *VirusDisease*, 2018, 29(4): 520-530 (doi: 10.1007/s13337-018-0483-z).
30. Clark J.D., Gebhart G.F., Gonder J.C., Keeling M.E., Kohn D.F. The 1996 guide for the care and use of laboratory animals. *ILAR Journal*, 1997, 38(1): 41-48 (doi: 10.1093/ilar.38.1.41).
31. Ashmarin I.P., Vasil'ev N.N., Ambrosov V.A. *Bystrye metody statisticheskoi obrabotki i planirovanie eksperimentov*. L., 1974.
32. Berzina A.G., Gamaleya N.B., Kapanadze G.D. *Biomeditsina*, 2013, 2: 95-102 (in Russ.).
33. Berg E.A., Fishman J.B. Conjugation of antibodies to horseradish peroxidase. *Cold Spring Harbor Protocols*, 2019, 9: pdb.prot099309 (doi: 10.1101/pdb.prot099309).
34. Wohlsein P., Singh R.P. Peste des petits ruminants in unusual hosts: epidemiology, disease, and impact on eradication. In: *Peste des Petits Ruminants Virus*. M. Munir (ed.). Springer, Berlin, Heidelberg, 2015: 95-118 (doi: 10.1007/978-3-662-45165-6_6).
35. *Antibodies: a laboratory manual*. E.A. Greenfield (ed.). Cold Spring Harbor Laboratory Press, 2013.
36. Verbov V.N. *Trudy instituta imeni Pastera*, 1998, 64: 3-27 (in Russ.).
37. Mapaco L., Monjane I., Fafetine J., Arone D., Caron A., Chilundo A., Quembo C., Carrilho M.D.C., Nhabomba V., Zohari S., Achá S. Peste des petits ruminants virus surveillance in domestic small ruminants, Mozambique (2015 and 2017). *Frontiers in Veterenary Science*, 2019, 6: 370 (doi: 10.3389/fvets.2019.00370).
38. Sreenivasa B. P., Dhar P., Singh R.P., Bandyopadhyay S.K. Development of peste des petits ruminants (PPR) challenge virus from a field isolate. In: *XIV annual conference and national seminar on management of viral diseases with emphasis on global trade and WTO regime of Indian Virological Society*. Pantnagar, India, 2002.