CHAPTER «VETERINARY SCIENCES»

MICROBIOCENOSIS OF THE INTESTINES OF PIGLETS IN THE CLINICAL COURSE OF PIG EPIDEMIC DIARRHEA

Dmitry Masiuk[1]
Andrey Kokarev[2]
Tatiana Vasylenko[3]

DOI: https://doi.org/10.30525/978-9934-588-15-0-75

Abstract. Porcine epidemic diarrhea virus (PEDV) has been circulating in Ukraine since 2014 and induces an especially dangerous viral infection with a lethal diarrheal syndrome in newborn piglets, with the primary occurrence of a foci of infection. The number of infected and lethality among diseased pigs 1-5 days of age can reach 100%, which together with the forced anti-epizootic measures brings significant economic losses. PED can spread to all pigs, but the emergent qualities of infectious pathology shows on newborn piglets. Effective and biosafety means of specific antiviral prophylaxis, cardinally stopping epizootic process is not registered, and etiopathogenetic therapy is not developed, therefore PED is a difficultly controlled emergent infection. With done up to three times a year over time appear stationary foci of infection, where the evolutionary changes in relationships occur quickly enough in the host-parasite system, since pigs are prolific and maturing animals and generational change. This leads to a significant variability in interpopulation relationships and the induction of biodiversity in the molecular mechanisms

[1] Candidate of Veterinary Sciences, Associate Professor,
Director Scientific Research Center of Biosafety
and Environmental Control Agro-industrial Complex,
Dnipro State Agrarian and Economics University, Ukraine
[2] Candidate of Veterinary Sciences, Senior Researcher, Scientific Research Center
of Biosafety and Environmental Control Agro-industrial Complex,
Dnipro State Agrarian and Economic University, Ukraine
[3] Candidate of Agricultural Sciences, Senior Researcher, Scientific Research Center
of Biosafety and Environmental Control Agro-industrial Complex,
Dnipro State Agrarian and Economic University, Ukraine

of adaptation and response of the viral genome. Clinically, genetic modifications of local variants of PEDV – populations are manifested in the form of changes in epizootic peculiar course of infectious pathology in different age groups of animals. Modifications of PEDV may be accompanied by a slight weakening of the intensity of the infectious process, a decrease in mortality and a decrease in the severity of the pathogenesis of diarrheal syndrome. At the same time, the age range of severe abdominal lesions expands from newborn piglets to fattening animals of older age groups of 28-32-70 days. Using a set of measures to combat the PED, including «reverse feeding» recycled infected biomaterial from convalescents pigs, eradication of the pathogen from the environment of host data with a stiff total disinfection and strict compliance with veterinary and sanitary rules of animal husbandry provides temporary positive results, but the theory is incorrect, since contamination animals leads to the dispersal of the virus and the formation of infection in endemic areas. The persistence of the virus in convalescent body is not observed, the external inanimate environment can only be mechanically pathogen transmission factor over time preserving the viability PEDV. Stabilization of the epizootic foci of infection is possible due to three factors: a) dissemination of the virus in «back feeding»; b) preservation of the virus in the external environment as a result of poor-quality disinfection; c) no occurrence of immune young gilts in which failure due to functional activity of a juvenile immune system formed accumulation of colostral antibodies in low titers, the viral antigen on the biomaterial at «back feeding». Due to the lack of «lactogenic immunity», neonatal pigs as biological indicator for the presence of PEDV in the environment, begin reproducing the virus in the enterocytes and develops a typical diarrheal syndrome PED.

1. Introduction

Porcine epidemic diarrhea (PED) is an emergent highly contagious viral zoonotic disease of pigs of all ages with the development of diarrhea syndrome. The causative agent is Porcine epidemic diarrhea virus (PEDV), gender is Alphacoronavirus, family is Coronaviridae. Virions are complex, pleomorphic, most often spherical in the range 95-130 nm, they consist of a nucleocapsid of helical symmetry and LOP (lipoprotein shell s. Supercapsid s. Peplos s. Envelope), on the surface of which there are radial club-shaped peplomers 18-23 nm long, forming " the solar corona" [7, p. 117].

The most commonly used synonyms for PEDV infection are: epidemic diarrhea of piglets – PED; pig epizootic diarrhea – PED; TGS-like disease; Womiting and wasting disease in piglets (English); Erbrechen und Kummern bein Saugferkel (German) [3, p. 1; 7, p. 117].

PEDV – infection in nonimmune newborn piglets proceeds according to the classical type of epizootic process with relay-free transmission of the pathogen and lethal abdominal phenomena, including debilitating watery diarrhea, dehydration and exhaustion, persistent vomiting, severe intoxication, infectious allergic processes, and mild cramp death [3, p. 1; 4, p. 2235].

Morbidity and mortality in newborn piglets up to 5 days of age can reach 100%, however, with an increase in age to 14-15 days, mortality decreases to 3-5%. The death of pigs as a result of PEDV infection is possible at any age, but fatalities in fattening animals are not widespread and pose no economic threat to the farm [2, p. 99; 11, p. 65].

PED is recognized by the OIE and FAO as one of the five major viroses (ASF, PED, CoES, PRRS, circovirus), which pose the greatest economic danger to pig production. PEDV is an enveloped single-stranded RNA (ssRNA) virus that is prone to frequent mutations (like all ssRNA viruses), which leads to recurrence of diarrheal syndrome and stabilization of the epizootic focus of infection, with an endemic form of the infection process, and the pathogen is unusually highly contagious and its exceptional pathogenicity for newborn piglets, leads to the globalization of infectious diseases [5, p. 202].

PED was first registered in England in 1971, then in Belgium in 1978, where the reference strain CV 777 was isolated. From 1980 to 1990, PED was widely distributed in Europe. From 1995 to 2011, PED penetrated Japan, Korea, China, and Thailand. From China, North China PEDV strains entered the United States, which put the pig-breeding industry on the verge of an economic disaster. In 2005, PED was detected in the Russian Federation, in 2014 in Ukraine. The global spread of PEDV continues. The efforts of the OIE and national veterinary medicine services to localize and control PED are ineffective [4, p. 2235; 8, p. 18; 13, p. 1620; 14, p. 917].

The emergence of the pathogen and particularly large economic damage caused by hard-to-manage infectious pathology prompted researchers to intensively study the virus, therefore, the molecular biological characteristics of PEDV are well studied. It was found that the PEDV genome

consists of large (~ 28 kb) single-stranded sense RNA that contains 7 open reading frames (ORF1a, ORF1b, and ORF2-6). ORF 1a and 1b determine large polyproteins (pp1a and pp1b), which are cleaved by virus-encoded proteases into 16 non-structural proteins (nsp1-nsp16), which take part in the main mechanisms of transcription and replication of viral RNA. ORFs2-6 encode four structural proteins, including S-glycoprotein (Spike), integral envelope E-protein (envelope), membrane M-protein and nucleocapsid N-protein, as well as the auxiliary protein ORF3. This protein forms ion channels in the membranes of infected intestinal cells, which is one of the mechanisms of regulation of virus production. During the adaptation and propagation of the virus in CC, the ORF3 gene mutates and this phenomenon linearly correlates with the attenuation of the virus to the body of newborn piglets [10, p. 83].

Among the structural proteins, S-glycoprotein (150-220 kD) plays an important role in the adsorption of virions on intestinal enterocytes, the binding of the virus to cell receptors, in the fusion of cell membranes and peplos of the virus, and in addition it is the main target for virus-neutralizing antibodies (VNA), its composition identified 4 BHA epitope. For PEDV, a relationship was found between protein S, adaptation to reproduction in CC, and virulence. The S-protein gene is often subjected to an epidemiological study, including phylogenetic analysis and a number of molecular and serological diagnostic analyzes [1, p. 193; 10, p. 83].

Proteins M and N are widely used to develop molecular and serological diagnostic tests for PED. Protein M (20-30 kD) is involved in the assembly of virions, induces the synthesis of BHA, which can neutralize the virus in the presence of complement and ά-interferon. Protein N (57-58 kD), an alkaline phosphoprotein associated with the viral genome, is involved in the induction of cell-mediated immunity. The non-glycolized protein E of LPO together with S-glycoprotein plays an important role in the debut stages of virus reproduction [7, p. 117].

The development of technology for culturing the virus in the laboratory is a fundamentally important issue for the differentiation of individual resistant strains that spontaneously form during virus mutations. The first results of successful PEDV proliferation were obtained by oral inoculation of newborn piglets of the small intestine homogenate from sick animals. Subsequently, it was possible to adapt the virus to Vero CC in the pres-

ence of exogenous trypsin. Reproduction of the virus was accompanied by CPE, which was expressed in the vacuolization of cells and the formation of syncytium. Adapted to Vero CC, the virus can be maintained in CC lines of both porcine and non-porcine origin. There are reports of cultivation of duck fibroblasts treated with aminopeptidases in QC, as well as the ability of the MK strain to replicate in neurons of newborn mice after intracerebral infection with neurovirulence [9, p. 140].

After fecal-oral transmission, it is reproduced in the enterocytes of the small intestine and leads to the development of catarrhal-hemorrhagic inflammation with subsequent necrosis of the intestinal epithelium. After an incubation period of 12-24 hours to 3-6 days, clinical signs of the disease develop, characteristic of PED and include watery debilitating diarrhea, persistent vomiting, intoxication, dehydration, exhaustion, severe depression and high mortality in newborn piglets. In favorable cases, the disease lasts 5-10 days and ends with recovery with post-infectious asthenia and a decrease in productive potencies. The excretion of PEDV with feces is observed for 6 to 24-30 days. Then, the release stops and convalescents are freed from the virus [12, p. 265].

All PED strains isolated in different countries of the world are almost identical in antigenic structure and represent one serotype of the virus. Comparison of the nucleotide sequences of the N and S genes, as well as the amino acid sequences of their products in the prototype strain CV 777 and Korean Chinju 99, and several field isolates of Korean and Japanese origin revealed a high homology up to 96.7% [15, p. 88].

Of particular danger is the mutational potential of the PEDV viral population, due to the formation of endemic foci of infectious pathology and the recurrence of diarrheal syndrome with an interval of 4-12 months in dysfunctional sites. The absence of effective specific cardinal interruptions of the epizootic chain of specific prophylaxis for any genetic variants of the field pathogen leads to an intensive spread of virosis in farms, making the epizootic situation difficult to manage with ineffective control and prophylaxis measures. Existing methods for creating lactogenic immunity using “reverse feeding” are archaic and theoretically undermine the foundations of biosafety and the fundamental principles of anti-epizootological control of infectious pathology. However, the real progressive spread of this infection shows the urgent need for the use of palliative methods to combat

PEDV, which may include components of the strategy of "reverse feeding" and veterinary-sanitary measures to eradicate the virus in the environment. In addition, existing vaccine biological products under conditions of widespread experimental use give mixed, sometimes negative or contradictory results, without providing reliable protective immunity in newborn piglets and industrial herds [5, p. 202; 6, p. 93; 11, p. 65; 12, p. 265].

Objective: to study the clinical and epizootological features of diarrhea syndrome induced by PEDV, monitor the endemic course of PED in a modern pig complex and characterize the etiopathogenesis of the disease in animals of different age groups while stabilizing the epizootic focus of infection.

2. Materials and research methods

Experimental studies of biomaterial from sick animals were carried out at the Research Center for Biosafety and Ecological Control of Agricultural Resources of the Dnipropetrovsk State Agrarian University. Detection, identification and differentiation of PEDV in biomaterial (intestines, internal organs, feces) and its quantitative characterization were carried out by PCR-RT method using the Bio-T kit®PEDV all – TGEV test system (Biosellal, France) on a thermocycler CFX 96 Real-Time System from BIO RAD (USA).

Serodiagnostics of PED was performed by ELISA using an ELx800 ELISA analyzer-photometer (BioTek, USA) and the ID Screen PEDV Indirect test system (ID.vet, France). According to the manufacturer's recommendations, sera are considered positive (containing specific immunoglobulins to virus antigens in a titer of 1: 200), according to the PED when the S / P value of the sample is above 0.4 Un.

The composition of the microbionts of the contents of the gastrointestinal tube of the piglets was studied using standard microbiological techniques by Gerhardt. The pathogenicity of isolated epizootic cultures of microorganisms was determined in a biological test on laboratory animals.

Statistical processing of the obtained research results was carried out using the Microsoft Office Excel application package.

3. The clinical manifestation of ped in neonatal piglets

Monitoring studies of the features of the enzootic course of PED during stabilization of the infectious focus were carried out in one of the pig farms of Zaporizhzhya region during 2015-17.

The farm maintained a high level of veterinary and sanitary culture of pig farming, standard biosafety measures were properly observed in accordance with the requirements of veterinary legislation and an epizootic situation. The animals were protected from external infection and were constantly monitored by the veterinary medicine service. The economy was safe for acute and chronic infections.

In the winter-spring period of 2015 (February-March) at the pig farm, where clinical and epizootological monitoring was carried out, the pigs developed an infectious pathology with diarrheal syndrome. The disease arose suddenly, with a massive coverage of the livestock, and previously a similar infectious pathology did not occur. The most vulnerable to infection were newborn piglets that died already on the 1-2 day of life, their incidence and mortality reached 100%. As the main symptomatic signs of PEDV infection, "watery diarrhea" and vomiting, as well as the rapid universal death of newborn piglets up to 5 days of age, are registered. With increasing age, mortality decreased significantly: 6-10 days – mortality was still very high and amounted to 50-60%; 10-15 days – decreased to 30%; ≥ 15 days ~ 3-4% for relatively mild diarrhea.

The results of monitoring the affected cohort of animals showed that diarrhea was quite widespread in pigs of all ages, but vivid clinical manifestations in severe form were observed only in individual individuals, while death among pigs of older age groups was not recorded. The symptom complex of intestinal disorder included rapidly passing watery diarrhea and vomiting without severe pathophysiological organism deviations from the physiological norm, there was no temperature reaction, slightly decreased appetite, as evidenced by a decrease in the amount of food consumed, the amount of food consumed was slightly decreased, behavioral reactions did not change, signs of depression were not detected 5-7 days after the appearance of the first cases of a relatively mild clinical course of diarrhea syndrome, the health status of animals of older age groups returned to normal, gastrointestinal upset did not pose a danger to the life and productivity of animals, and restoration of disturbed physiological functions occurred spontaneously.

At the same time, it was revealed that diarrhea syndrome in newborn piglets proceeded superstroke, accompanied by frequent watery-mucous defecations of greenish-yellow color, progressive dehydration and exhaustion, excruciating vomiting and abdominal pains, rare tonic convulsions,

intoxication, general depression with complete retention of consciousness and tactile sensitivity. The temperature was within normal limits, before death it decreased.

4. Pathological changes in infected animals

Upon pathological examination, the corpses were in a state of cachexia and dehydration. The skin is thinned, wrinkled, inelastic, pale with a yellowish tint, with contouring bone formations from the muzzle to the ischial tubercles, the eyes are sunken. Signs of conjunctivitis and rhinitis have not been identified. On the skin of piglets, limited swelling of a purple-purple color with a jelly-like subcutaneous edema of an infectious-allergic nature was found.

Thus, one of the main causes of death of the infection is the metabolic depletion of the body of piglets. The infectious agent was highly pathogenic and obligatorily lethal for newborn animals, inducing critical disorders incompatible with the life of piglets. Pathological studies of fatal piglets showed signs of infectious pathogenesis (Figure 1).

Figure 1. Pathological changes in the internal organs of a piglet of 5 days of age, fallen from PED

The blood is not clotted, light, there are no hemorrhages on the skin and in the subcutaneous tissue. Pathognomonic changes were localized in the small intestine in the form of extremely intense catarrhal-hemorrhagic inflammation with desquamation of the epithelium and thinning of the intestinal wall. In piglets who died from diarrhea at 5 days of age, catarrhal hemorrhagic inflammation was also found in the colon. In the stomach,

pathological changes in the mucous membrane were not established, the filling of the stomach with mucous contents did not differ from the norm. Regional lymph nodes are swollen and hyperemic. In other internal organs, pathological disorders were not detected, in particular, the spleen was not enlarged, bright red; the liver is not enlarged, with slight grayish areas of protein-lipid dystrophy; kidneys and lungs were normal. However, in the tissue of the heart found areas of degenerative degeneration. In the internal cavities, a small amount of transudate.

The study of tissue samples of piglets that died from diarrhea syndrome on the 2nd day of life was carried out at the SRC DSAEU using PCR-RT in a joint homogenate from two small intestinal biomaterials. Samples of the small intestine of piglets revealed PEDV in the amount of 7.0 × 102 genome equivalents (g.) In one gram (g), which is a sufficiently substantial basis for establishing a positive result of *PEDV* infection.

In order to determine the spread of infection among susceptible animals, retrospective serological indication of IgG to PEDV capsid AH was performed in an ELISA. For serological studies, blood samples were taken from 10% of the pig population of the affected pigsty (n = 80) using random random sampling. The results of a serological study showed the presence of diagnostic titers in 42% of samples at a dilution of 1: 200, which confirmed a preliminary comprehensive diagnosis of PED. In 76% of feces samples from the same animals, PCR-RT was used to identify *PEDV* RNA at a concentration of 1.86 × 10 7 ± 0.14 × 10 6 (P≤0.01) g. in one g of biological material.

5. Intestinal microbiocenosis of newborn piglets

To study the composition of the microbial species composition of the intestinal tube, biomaterial (affected intestines and feces) was taken from 13 pigs 3-8 days old who died from PED and bacteriological studies were carried out using generally accepted methods for the species composition of microorganisms inhabiting the digestive tract. The obtained data of bacteriological studies of intestinal contents are presented in table 1.

The analysis of the data presented are given in table. 1 showed that an bacteriological study of the intestinal contents of piglets that died from PED did not isolate physiologically beneficial microbionts, such as aerococci (Aerococcus viridans), lactobacilli and bifidobacteria. Molds and yeast were not detected in the intestinal contents. Intestinal microflora was

Table 1

The species composition of the microbiocenosis of the intestinal tube of piglets that died from PED (n=13)

Microbiont name	Biomaterial number												
	1	2	3	4	5	6	7	8	9	10	11	12	13
A. viridans	-	-	-	-	-	-	-	-	-	-	-	-	-
Lactobacillus	-	-	-	-	-	-	-	-	-	-	-	-	-
Bifidobacterium	-	-	-	-	-	-	-	-	-	-	-	-	-
E. coli	+	+	+	+	+	+	+	+	+	+	+	+	+
P. vulgaris	+	+	+	+	+	+	+	+	+	+	+	+	+
C. perfringens	+	+	+	+	+	+	-	+	-	+	+	+	-
Bac. cereus	-	-	-	-	+	+	+	+	-	+	+	+	+
Bac. subtilis	-	-	-	+	-	+	-	-	+	+	+	+	+
Bac. mycoides	+	-	+	+	+	-	-	+	+	-	+	+	-
Bac. megaterium	-	-	+	+	+	+	+	-	+	-	+	-	+
Enterococcus	+	+	+	+	-	+	-	+	-	+	+	+	-
Staphylococcus	+	+	-	-	+	-	+	+	+	-	-	+	-

note: + presence of microbiont; - lack of microbiont

represented by standard transient microflora, which got into the digestive tract of piglets from the environment and mainly consisted of putrefactive microorganisms with high enzymatic activity, of different taxonomic subordination. Bacteria of the Enterobacteriaceae, Bacillaceae, Coccaceae family were isolated from the intestinal contents. Isolated cultures of prokaryotes possessed morpho-tinctorial, cultural, and biochemical properties typical of their species. C. perfringens cultures were characterized by high glycolytic activity; cultures of anthracoid bacilli, E. coli, and P. vulgaris possessed pronounced proteolytic potentials. In a biological study of the pathogenic potencies of identified cultures of prokaryotes on white mice, the death of infected animals was not observed within 5 days of observation, and when crops were sown with 5% blood MPA, hemolysis was not detected, which is an indicator of the pathogenicity of intestinal microflora.

The results of a comprehensive diagnosis of PED-positive samples among a previously successful pig population were taken as the main argument in choosing a solution to reorganize the economy from registered zoonosis by the method of “reverse feeding” and eradication of the pathogen from the pig population livestock ecosystem without using vaccines.

At the first stage, the concentration of the virus in the feeding biomaterial was determined using PCR-RT. The tissues of the small intestine of twelve 2-3-day-old piglets that died from PED were subject to investigation. The results of molecular genetic analysis showed the following experimental values of the quantitative content of PEDV in the small intestine of newborn piglets that died from PED, which are presented in the form of ranked series in CE / g:

$1{,}69\times10^5$; $4{,}55\times10^5$; $5{,}29\times10^5$; $8{,}30\times10^5$;
$1{,}01\times10^6$; $1{,}42\times10^6$; $2{,}60\times10^6$; $4{,}79\times10^6$;
$4{,}70\times10^7$; $9{,}67\times10^7$; $9{,}79\times10^7$;
$1{,}01\times10^8$.

The results of quantitative PEDV content in the tissues of the small intestine of newborn piglets that died from PED are presented in Figure 2.

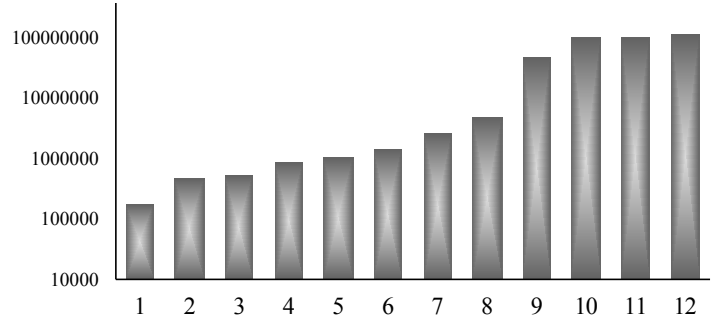

Figure 2. Concentration of PEDV genome equivalents in small intestine samples of newborn piglets that died from PED

The obtained quantitative data on the content of PEDV virions in tissue samples from dead piglets allow us to note that the viral load in the affected tissues varies widely, from 5 to 8 lg of virions in a gram of biomaterial, with the range $M \pm m = 4.11 \times 106 \pm 3.42 \times 105$ CE / g ($P \leq 0.001$).

The procedure of "reverse feeding" was carried out using biomaterial from the fallen newborn piglets. A homogenate of the biomaterial of the intestinal tube in the state of catarrhal-hemorrhagic inflammation was made using a tissue grinder PT-2. From the obtained ex tempere homogenate, an

aqueous suspension was prepared with the pathogen content in the range of 6 lg virions per gram of the final product.

In order to form effective colostral immunity in newborn piglets, all pregnant sows, no later than two weeks prior to farrowing, were infected per os with a prepared homogenate in the form of an aqueous suspension containing a known concentration of the epizootic PEDV variant.

Along with measures aimed at creating collective immunity in animals, in order to reduce the level of pressure and prevent the spread of PEDV in the pig farm, according to the recommendations of the veterinary legislation of Ukraine, extremely thorough mechanical cleaning of livestock buildings and the strictest total disinfection, with terminal quality control of disinfection and confirmation of the eradication of PEDV from the environment using PCR-RT. The causative agent was identified only on one of the elements of the internal equipment, namely, in flushings from a plastic floor without drain for urine.

Most of the animals that died from PED were disposed of as a source of infection. To confirm or deny the fact of the persistence of PEDV in the body of animals-convalescents, PCR-RT was used to study 68 samples of feces from sick pigs of various age groups, ranging from pigs on suction to pregnant sows and repair young animals. The results of the study showed the absence of PEDV in the samples. In addition, the 14 least productive animals of convalescence, classified as sanitary marriage, selected from all technological groups of contents were tested using PCR-RT for the presence of PEDV in the intestinal mucosa, regional lymph nodes, liver, spleen and bone marrow. No virus was detected in any of the studied objects.

Vigorous measures taken to vaccinate the epizootic effect on the functioning of the epizootic chain have led to the fact that diarrheal syndrome of PEDV etiology has been clinically stopped.

However, unstable epizootic well-being remained relatively short, for 6 months, until the time that inseminated repair pigs were introduced into the main herd. In 4 out of 64 nests, a typical clinical picture of PED appeared, with subsequent spread of infection to other susceptible animals. The PED outbreak among newborn piglets continued for two weeks. All diseased animals were killed and disposed of, biomaterial (intestines) was used for “reverse feeding”. A thorough total disinfection was carried out with terminal confirmation of the quality of rehabilitation in PCR-RT. As a

result of the taken anti-epizootic measures, the infectious PED process was eliminated and the pig complex was improved.

6. Discussion

The results showed that genetic modifications of the field virus, when the epizootic focus of infection is stabilized, induce wide variability of the clinical and epizootic course of the infectious process in a population of different ages, and also provide the possibility of suppressing the immune and biological resistance of the macroorganism. The rapid change of farrowing rounds and the multiplicity of sows create a dense and susceptible biological environment for the passage and selection of the most transmissible and actively reproducing genetic variants of the pathogen that can colonize the ecological niche of habitat – pig intestinal enterocytes, with the development of lethal, super-acute diarrheal syndrome in newborn piglets. Age-related processes in a macroorganism increase immunobiological resistance and provide resistance to the development of an acute infectious process, which leads to a significant decrease in mortality and a decrease in the severity of diarrheal syndrome, which coincides with experimental data published by [9, p. 140; 11, p. 65].

Based on the results of research and analysis of literature, it was found that the virus does not persist, the macroorganism is completely freed from the presence of foreign genetic information in the space controlled by its own immune system [7, p. 117; 12, p. 265]. The survival of the virus is possible only on objects of external inanimate nature with the subsequent infection of susceptible organisms and the resumption of the reproduction cycle in the form of a recurrence of the endemic process in a stabilized focus of a viral infection. In this paradigm of the PEDV life cycle, the weakest link in the vaccine-free method of breaking the epizootic chain is repair pigs, since a juvenile, physiologically immature animal organism under conditions of malnutrition cannot guarantee the synthesis of the required amount of colostral Ig, and thereby create a full and intense lactogenic immunity in newborn piglets. Offspring from such animals represent a high-risk group and may become the debut link in the epizootic chain of PED [5, p. 202; 6, p. 93].

Considering that PEDV is an exclusively contagious, highly pathogenic, and obligately lethal infectious agent for non-immune newborn piglets, in

which, during an initial outbreak of PED among a successful pig population, it induces a super-sharp emergent course of diarrhea syndrome according to the classic type of epizootic process with non-relay transmission of the pathogen and 100% infected piglets –consider the most important direction of further biological research – monitoring of the epizootological process in PED. In this case, it is necessary to focus efforts on an in-depth study of the molecular biology of the pathogen, the correlation between the genetic characteristics of PEDV field isolates and the clinical and epizootological features of the virus infection process in different age groups of piglets with spatio-temporal stabilization of the focus of infection with a deepening in the molecular epizootology of the inter-population interaction of PEDV and the body newborn piglets [1, p. 193; 15, p. 88].

Stevenson et al. believe that the practice of pig farming requires effective and specific prophylaxis based on the biology of the infectious agent. During the development of such agents, they recommend the use of various lightweight stamping-aut variations aimed at eradicating the pathogen and creating an immune background available in ways that impede the reproduction of the virus and thereby break the epizootic chain. This statement is consistent with our findings and recommendations on the fight against PED.

The study of the molecular epizootology of PEDV, which induces the classical type of infection without relaying the pathogen to susceptible livestock – newborn pigs, which are PEDV bioindicators, presents a unique opportunity to trace the process of the interaction of two antagonistic populations of micro– and macroorganisms and to elucidate the general biological patterns of coexistence and co-existence of organisms and coexistence of populations [7, p. 117].

A paradigmatic epizootological feature of the etiopathogenesis of PED is a narrow time range of lethal damage to sensitive intestinal target cells of nonimmune animals with elimination of the virus after reproduction, that is, a short life cycle of the virus in the macroorganism with subsequent complete elimination of the pathogen, which reduces the strategy of combating PED to create an immune macroorganism (population immunity) for the virus and its eradication in the external technological environment.

In conclusion, it must be emphasized that at the present stage of the evolutionary development of PEDV, the biological interspecific lethal antagonism

between the virus and non-immune newborn piglets is not overcome, the PEDV population and the organism of newborn piglets are biologically, like unsung genetic systems, are incompatible, and cannot coexist in a single biotope.

7. Conclusion

1. The persistence of PEDV in the body of convalescence animals is not fixed, the macroorganism is completely freed from the pathogen after the disease. The formation of a stable endemic focus of infection is possible due to the preservation of the virus at technological facilities of the environment and infection of non-immune animals, especially newborns.

2. Newborn piglets obtained from repair pigs present an increased risk group, since juvenile animals with a low level of immuno-biological resistance with poor feeding after "reverse feeding" can form lactogenic immunity of insufficient tension, and non-immune newborn piglets as a bioindicator in the external environment PEDV, become the debut link in the epizootic chain of PED.

3. When the epizootic focus of PED is stabilized in time and space, the area of infection increases as a result of the extension of the age range of the lesion to older age groups – 28-30-70 days, while the severity of pathogenesis slightly decreases and mortality decreases. However, pathogenesis does not emerge from emergent quality, which indicates the initial stage of adaptation and weak attenuation of the PEDV population to the ecosystem of the organism of piglets.

References:

1. Annamalai, T., Saif, L. J., Lu, Z., Jung, K. (2015). Age-dependent variation in innate immune responses to porcine epidemic diarrhea virus infection in suckling versus weaned pigs. Veterinary Immunology and Immunopathology, 168(3): 193–202. DOI: https://doi.org/10.1016/j.vetimm.2015.09.006

2. Bjustrom-Kraft, J., Woodard, K., Giménez-Lirola, L., et al. (2016). Porcine epidemic diarrhea virus (PEDV) detection and antibody response in commercial growing pigs. Bjustrom-Kraft et al. BMC Veterinary Research, 12: 99–107. DOI: https://doi.org/10.1186/s12917-016-0725-5

3. Carvajal, A., Argüello, H., Martínez-Lobo, F.J.,Costillas, S., Miranda, R., de Nova, P.J.G., Rubio, P. (2015). Porcine epidemic diarrhoea: new insights into an old disease. Porcine Health Management, 1:12, 1–8. DOI: 10.1186/s40813-015-0007-9

4. Dastjerdi, A., Carr, J., Ellis, R. J., Steinbach, F. & Williamson, S. (2015). Porcine Epidemic Diarrhea Virus among Farmed Pigs, Ukraine. Emerging Infectious Diseases, 21(12): 2235–2237. DOI: 10.3201/eid2112.150272

5. Goede, D. & Morrison, R.B. (2016). Production impact & time to stability in sow herds infected with porcine epidemic diarrhea virus (PEDV). Preventive Veterinary Medicin, 123(1): 202–207. DOI: https://doi.org/10.1016/j.prevetmed.2015.11.010

6. Langel, S.N., Paim, F.C., Lager, K.M., Vlasova, A.N. & Saif, L.J. (2016). Lactogenic immunity and vaccines for porcine epidemic diarrhea virus (PEDV): Historical and current concepts. Virus Research, 226 (0): 93–107. DOI: https://doi.org/10.1016/j.virusres.2016.05.016

7. Li, W., Kuppeveld, F.J.M., He, Q., Rottier, P.J.M., Bosch, B.J. (2016). Cellular entry of the porcine epidemic diarrhea virus. J. of Virus Research, 226: 117–127. DOI: https://doi.org/10.1016/j.virusres.2016.05.031

8. Masiuk, D.M., Kokariev, A.V., Sosnytsky, O.I., Vasilenko, T.O., & Hustova, Y.V. (2018). Features of humoral immunity formation under oral and parenteral immunoprophylaxis of porcine epidemic diarrhea. Theoretical and Applied Veterinary Medicine, 6(3), 18–22. DOI: 10.32819/2018.63004

9. Ouyang, K., Shyu, D.L., Dhakal, S. et al. (2015). Evaluation of humoral immune status in porcine epidemic diarrhea virus (PEDV) infected sows under field conditions. Veterinary Research, 46: 140–151. DOI: https://doi.org/10.1186/s13567-015-0285-x

10. Poonsuk, K., Zhang, J., Chen, Q., Gonzalez, W., Correa da Silva Carrion, L., Sun, Y., Ji, J., Wang, C., Main, R., Zimmerman, J. (2016). Quantifying the effect of lactogenic antibody on porcine epidemic diarrhea virus infection in neonatal piglets. Veterinary Microbiology, 197: 83–92. DOI: https://doi.org/10.1016/j.vetmic.2016.11.002

11. Strizhakova, O.M. (2013). Isolation and identification of epizootic diarrhea virus in pigs under outbreak at a large farm. Sel'skokhozyaistvennaya Biologiya [Agricultural Biology], 4, 65–69. DOI: 10.15389/agrobiology.2013.4.65eng

12. Tun, H.M., Cai, Z. & Khafipour, E. (2016). Monitoring Survivability and Infectivity of Porcine Epidemic Diarrhea Virus (PEDv) in the Infected On-Farm Earthen Manure Storages (EMS). Frontiers in Microbiology, 7: 265–276. DOI: 10.3389/fmicb.2016.00265

13. Vlasova, A. N., Marthaler, D., Wang, Q., Culhane, M.R., Rossow, K.D., Rovira, A., Collins, J. & Saif, L.J. (2014). Distinct Characteristics and Complex Evolution of PEDV Strains, North America, May 2013 – February 2014. Emerging Infectious Diseases, 20 (10): 1620–1628. DOI: 10.3201/eid2010.140491

14. Wang, L., Byrum, B. & Zhang, Y. (2014). New Variant of Porcine Epidemic Diarrhea Virus, United States. Emerging Infectious Diseases, 20(5): 917–919. DOI: 10.3201/eid2005.140195

15. Zhang, X., Pan, Y., Wang, D., Tian, X., Song, Y. & Cao1, Y. (2015). Identification and pathogenicity of a variant porcine epidemic diarrhea virus field strain with reduced virulence. Virology Journal, 12: 88–93. DOI: 10.1186/s12985-015-0314-4