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Research Article

Influence of cell wall peptidoglycan of *Lactobacillus delbrueckii* on specific immunoprophylaxis of porcine epidemic diarrhea

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Abstract. Investigate the effect of peptidoglycan of *L. delbrueckii* cell wall on the specific immunoprophylaxis of PED. An experimental and control group of sows with 10 heads each. The sows of the experimental group at 60, 75 and 90 days of gestating were administered GMPP at a dose of 0.05 mg GMPP per 1 kg of weight. The sows of the control and experimental groups were immunized with autogenous inactivated vaccine against *PEDV* at 90 and 100 days of gestation. To determine specific IgG in sows and piglets born from them, blood samples were taken at 1, 5 and 10 days after farrowing and days of life respectively. The application of the GMPP preparation to sows under immunization against PED contribute to increases the seroconversion of IgG by 42% and their homogeneity by 17%, as well as contributes to the development of specific colostral immunity higher by 33% which is stored in more than 70% of the animals during the first 10 days of life in comparison to the control group. Application of the GMPP preparation contributes to increase the level of antigen specific IgG in the blood of sows and provides the development of higher levels of colostral immunity in piglets.

Key Words: *PEDV*, colostral immunity, IgG, peptidoglycan, piglets

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Introduction

In recent years, due to the active development of swine rearing, diseases that have not previously been registered in our country have become increasingly important. One of these infections is porcine epidemic diarrhea (PED), which is induced by an RNA genomic virus of the genus *Alphacoronavirus* of the *Coronaviridae* family. The disease was first reported on the territory of Ukraine in 2014 year (Dastjerdi et al 2015). Results of epizootic monitoring for 2014-2018 years indicate widespread distribution of PED among swine of agricultural enterprises in Ukraine (Masiuk et al 2020).

Swine of all ages are susceptible to the virus, but the most sensitive animals are the piglets first 10 days of life (Annamalai et al 2015; Jung et al 2015; Lin et al 2015). The infectious process is accompanied by the development of watery diarrhea and the dehydration of organism, which contributes to pigs' death, especially during the suckling period (Lin et al 2016). According to Annamalai et al (2015), infection with the pathogen of PED of piglets in the first 9 days of life can cause animal mortality within 2-4 days up to 100%, which contributes to significant economic losses. When infecting piglets older than 10 days of life the mortality rate decreases to 50%, and when inducing PED in groups of animals older than three weeks of age the mortality rate does not exceed 5-6% (Lin et al 2016). In view of this, it is urgent to conduct treatment-and-prophylactic measures aimed at preventing infections of young pigs in the first days of life in PED-positive farms.

One of the main measures to prevent the emergence and spread of infectious animal diseases is active immunoprophylaxis (Freitas Gerber et al 2016; Gimenez-Lirola et al 2017). Immunization of pregnant sows provides the appearance of colostral immunity specific to PED in newborn piglets, which prevents infecting of young pigs during the first weeks of life and accordingly contributes to the reduction of economic losses (Chen et al 2019). At present, the most common methods of active immunoprophylaxis of PED among the pig farms of Ukraine are oral and parenteral immunization of sows (Masiuk et al 2018). Each of these methods has certain disadvantages. In particular, oral immunization through chain intestinal-mammary gland-sIgA, provides more effective colostral immunity in newborn piglets, but promotes the spread of the virus in the environment (Bjustrom-Kraft et al 2016; Langel et al 2016). Parenteral immunization, especially with inactivated vaccines, does not lead to the virus replication in animals which breaks the epizootic chain, but the level of immune response to the action of PED virus antigens is much lower (Gerber & Opriessnig 2015; Clement et al 2016).

Recently, to enhance the immune response in animals for the action of antigens began to use immunomodulatory drugs (Yefimov et al 2016). Among the total number of immunotropic substances, the most pronounced immunostimulatory effect

have a components of the peptidoglycan of the bacteria cell wall – muramylpeptides (Kusumoto et al 2010; Kokarev & Masiuk 2017). Therefore, to increase the level of immune protection of newborn pigs for the purpose of immunoprophylaxis of PED we used the enzymatic hydrolyzate of the cell wall of *Lactobacillus delbrueckii* – glucosaminylmoramylpentapeptide (GMPP).

In view of this, the purpose of our study was to investigate the effect of peptidoglycan of *L. delbrueckii* cell wall on the specific immunoprophylaxis of porcine epidemic diarrhea.

Material and methods

The study was performed in the Scientific research center of biosafety and environmental control agro-industrial complex of the Dnipro State Agrarian and Economic University in accordance with the Scientific theme "Determination of theoretical aspects of epizootic process taking into account the genetic variants of porcine epidemic diarrhea virus strains" (No. of state registration is 0117U004293).

Description of the experiment. The experimental part of the study was carried out in one of the pig-breeding enterprises of Ukraine of the farrowing-fattening cycle, which is permanently PED-positive. The total number in the farm is over 20 000 pigs. More than 2 800 piglets are born every week at the swine complex.

For research an experimental and control group of sows of 2nd-3th farrowing was formed, with 10 heads each. From sows of the control group, 146 pigs were born. From the sows of the experimental group 151 pigs were born.

The sows of the control and experimental groups were parenterally immunized with against porcine epidemic diarrhea at 90 and 100 days of gestation autogenous inactivated vaccine at a dose of 2 ml. The injection was performed intramuscularly in retroauricular the area.

The sows of the experimental group at 60, 75 and 90 days of gestation additionally were administered enzymatic hydrolysate of the cell wall of *L. delbrueckii* intramuscularly at a dose of 10.5 mg glucosaminylmoramylpentapeptide per of the sows.

All manipulations with animals were conducted in accordance with the rules of handling of experimental animals, described in "European Convention for the Protection of Vertebrate Animals used for experimental and other scientific purposes" (Strasburg, 1986).

Samples collection. To assess the formation of post-vaccination immunity to IgG, blood was taken from sows 1, 5, and 10 days after farrowing. To investigate the formation of colostral immunity to PED and its duration, blood was collected after 1, 5, and 10 days of piglet life. Blood collection pigs were randomly selected from 3 animals from each nest.

To determine the specific IgG, blood was taken in a volume of 1 ml from each animal. Blood samples were taken without anticoagulant. Blood samples were placed in a thermostat at a temperature of 37 C for 30-40 minutes to form a clot. After separation of the blood serum, it was carefully put into a in a separate tube. To remove the erythrocyte suspension the remaining, the serum tubes were centrifuged for 10 min at 1500 rpm. The supernatant was transferred to a new tube. Serum samples were stored frozen at -18 ...- 22 ° C until testing.

PEDV antibody detection assays. The determination of serum specific IgG levels in sows and piglets was performed by ELISA using an ELx800 analyzer ("BioTek", USA) and G2b-PEDV NP-based ID Screen® PEDV Indirect Screening test (IDvet, France) was used according to the manufacturer's label instructions. According to the guideline to the test system sera were tested in a diagnostic titer of 1:200. An S/P % ratio ≥40% was considered antibody positive and <40% was considered negative.

Statistical analysis. Variation and statistical processing of the obtained results were performed by using the Statistica 6.0 specialized software (StatSoft Inc., USA). The significance of differences was assessed after verifying the experimental data which was obtained by using Student t-test or its non-parametric counterpart – the Wilcoxon test. Selective parameters presented in the work have the following designations: x is the sample average; SE is the standard error of the average value; CV is the coefficient of parameter variation in the group.

Results

As a result of the study the peculiarities of the development of specific immunity in sows and piglets as part of active immunoprophylaxis of porcine epidemic diarrhea by inactivated autogenous vaccine were found out. The effect of GMPP on seroconversion of post-vaccine serum IgG on the background of the use of an inactivated autogenous PED vaccine has been established.

As a result of the studies, it was found that under sows' immunization by inactivated autogenous vaccine the specific IgG in titer 1:200 found in 80% of animals on the first day after farrowing. Amongst the animals of the experimental group found 90% of sows with PEDV-specific antibodies in a titer of 1:200 (Table 1). The S/P rate indicates that the serum specific IgG level in the animals of the experimental group is greater by 42% and the coefficient of variation is lower by 26% than in the animals of the control group.

On the 5th day after farrowing the number of seropositive sows in the control and experimental groups reached to 70%. The level of S/P at day 5 after farrowing in control animals decreased by 25.2% and in sows of the experimental group by 28.6%, compared to the values of the first day.

The CV parameters in the animals of the control group increased by 17.9% at 5th day of lactation and in sows of the experimental group increased by 10.3% relative to the values obtained in the first day after farrowing

It should be noted that the level of S/P in the animals of the experimental group exceeds the same value in the animals of the control group by 35.9% and the indicator CV is lower by 8.7%.

On the 10th day after farrowing only 30% of seropositive sows of the control group and 40% of the experimental group were detected. The value of S/P, both in the experimental and control animals, was the lowest during the study period at this time, and the CV parameters is the highest for all 10 days after farrowing. It should be noted that the tendency to increase the S/P parameters in sows of the experimental group remains and reach to 25% relative to the values of animals in the control group.

Therefore, immunization of sows by an autogenous inactivated PED vaccine promotes seroconversion of serum G immunoglobulins in a titer of 1:200 in 80% of animals with an average S/P value of 73.3% and a coefficient of variability of 39%. The application of the GMPP preparation to sows under immunization against PED contribute to increases the seroconversion

Table 1. The level of specific IgG in blood serum of sows under the active immunoprophylaxis of PED (x ± SE, n = 10)

Time after farrowing, days	Control group			Experimental group		
	Number of seropositive animals	S/P, %	CV, %	Number of seropositive animals	S/P, %	CV, %
1	8	73.3 ± 8.9	39	9	104.4 ± 9.5*	29
5	7	54.8±7.9	46	7	74.5±9.8	42
10	3	37.4±5.2	44	4	46.7±5.3	36

Note: changes are considered to be statistically significant at * – P<0,05 relative to the parameters of the animals of the control group

Table 2. The level of specific IgG in the blood serum of suckling piglets under the active immunoprophylaxis of PED in sows (x ± SE, n = 30)

Age of piglets, days	Control group			Experimental group		
	Number of seropositive animals	S/P, %	CV, %	Number of seropositive animals	S/P, %	CV, %
1	27	119.0±8.7	40	28	147.1±9.3**	35
5	21	104.6±9.7	51	25	139.7±10.5*	41
10	14	49.1±5.1	56	22	69.8±6.4*	50

Note: changes are considered to be statistically significant at * – P<0,05 relative to the parameters of the animals of the control group

of specific IgG in the titer of 1:200 by 42% and their homogeneity by 17% compared to the animals of the control group.
The results of the detection of IgG specific for the PED virus in the blood serum of piglets on the 1st day of life indicates the development of colostral immunity in 90.0% of animals in the control group and in 93.3% of animals in the experimental group (Table 2).
The level of specific immunoglobulins is significantly higher by 23.6% (P<0.01) in the piglets of the experimental group in the first day of life and the variability parameters is lower by 12.5% relative to the animals of the control group.
On the 5th day of life, the number of seropositive piglets in the control group reached to 70.0%, which is lower by 22.2% than the values in the first day. Among the piglets of the experimental group, the number of seropositive animals on the 5th day of life is decreased by 10.7% compared to the values on the 1st day of life and amounted to 83.3%. S/P indicators declining in piglets of the control and experimental groups on the 5th day of life by 12.1% and 5.0% respectively, compared with the values obtained on the 1st day of life.
At 10th day of life, specific IgG was detected in 46.7% of the control piglets and in 73.3% of the animals of the experimental group, which is higher in 1.6 times than the value of the control group. At the same time, the S/P parameters in the animals of the experimental group is greater by 42.2% (P<0.01) relative to the animals of the control group. Also, there is a tendency to decrease the variability of antibody level, which is lower in the animals of the experimental group by 20.7% than the value in piglets of the control group.

Discussion

The work was carried out with the aim of determining the formation of the immune response of sows and colostral immunity in newborn piglets with active immunoprophylaxis of epidemic diarrhea by the method of parenteral immunization of pregnant sows with an inactivated autogenous vaccine. There are reports in the literature that the swine epidemic diarrhea virus induces intestinal infection (Lin et al 2016; Hanke et al 2017; Masiuk et al 2019). The most difficult infection occurs in newborn piglets (Annamalai et al 2015). Despite this, the specific immunoprophylaxis strategy should focus on inducing mucosal immunity in newborn piglets to protect targeted intestinal enterocytes. The immunity induced in pregnant sows and passively transferred to sucking piglets through colostrum and milk (colostral immunity) is crucial for protecting newborn piglets from PED (Bjustrom-Kraft et al 2016).
Our studies show the immunostimulating effect of peptidoglycan of *Lactobacillus delbrueckii* against the background of specific immunoprophylaxis of PED with an inactivated autogenous vaccine. This is consistent with the results of a study of specific IgG to antigen *PEDV*. Its level in the blood of sows of the experimental group is higher at 42 % compared to the values of control animals.
An increase in S/P variability was found in the sows of the control group during the first 10 days of lactation indicates a low level of immune response of their organism (Ouyang et al 2015). The application of the GMPP preparation to sows under immunization against PED contribute to increases the seroconversion of specific IgG in the titer of 1:200 by 42% and their homogeneity by 17% compared to the animals of the control group. That may be due to immunomodulatory effect of lactobacillus GMPP (Kusumoto et al 2010; Kokarev & Masiuk 2017).
According to Ogawa et al (2016) immune protection in piglets against intestinal infections is formed locally by secretory SIgA and antigen specific IgG, which upon contact with the target antigen opsonize it and contribute to neutralization by phagocytosis by immune cells (Hou et al 2018; Wang et al 2019). Therefore, the level of specific antibodies to *PEDV* antigens in newborn piglets is an integral indicator of immune defense formation.
The difference in S/P parameters between the groups indicates that the level of specific immunoglobulins is significantly higher in the piglets of the experimental group in the first day of life and the variability parameters is lower relative to the animals of the control group. This indicates a higher and more homogeneous level of antigen specific IgG in the blood of newborn

piglets of the experimental group compared to control animals (Gerber et al 2016).

On the 5th day of life the number of seropositive piglets in the control group lower by 22.2% than the values in the first day of life. Among the piglets of the experimental group, the number of seropositive animals on the 5th day of life is decreased by 10.7% compared to the values on the 1st day of life, which is associated with a decrease in the level of colostral antibodies in neonatal piglets (Clement et al 2016; Chen et al 2019). It is consistent with the results of the determination of S/P parameters, which indicate its decrease in piglets of the control and experimental groups on the 5th day of life by 12.1% and 5.0% respectively, compared with the values obtained on the 1st day of life.

The decrease in the number of seropositive animals as well as reduction in the level of colostral IgG to *PEDV* antigens may be related to the natural catabolism of colostral antibodies in neonatal piglets (Clement et al 2016).

Therefore, immunization of sows by an autogenous inactivated *PEDV* vaccine provides specific immune protection in 90% of the piglets born from them, which is stored in 46.7% of the animals within up to 10 days of life. The application of GMPP on the background of specific immunoprophylaxis contributes to the development of specific colostral immunity in 93.3% of piglets, which is stored in more than 70% of the animals during the first 10 days of life and is significantly higher by 33.1% in comparison to the same values in the animals of the control group. It should be noted that according to Scherba et al (2016) piglets born from sows that were parenterally immunized against PED and containing specific IgG in the blood do not show clinical signs of infection during the first week of life under oral infection. On the other hand, animals that have been inoculated with the virus at an older age (5 days of life) have a milder course compared to the outbreak of the disease recorded in the first days of life. Poonsuk et al (2016), who found that circulating antibodies did not protect piglets from PED infection by 100%, but provided a milder disease course and significantly reduced the mortality rate of piglets, obtained similar results.

In view of this, the detected increase in the level of antigen-specific IgG in the blood of sows promotes the development of a higher level of immune protection in the newborn, which will interfere with the replication of the virus in the intestinal epithelium cells of the piglets (Lin et al 2015; Freitas Gerber et al 2016).

Conclusion

Parenteral immunization of gestating sows by an autogenous inactivated vaccine against PED provides the induction of antigen specific IgG in a titer of 1:200 in 80% of animals and promotes the development of colostral immunity in 90% of newborn piglets.

Specific immunoprophylaxis of sows by an autogenous inactivated vaccine against PED on the background of the application of the GMPP preparation contributes to increase the level of antigen specific IgG in the blood of sows and provides the development of higher levels of colostral immune protection in newborn piglets, which will interfere with the replication of the virus in the intestinal epithelium cells of the piglets.

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