

Development and test of an associated vaccine Welshicolisan against gastrointestinal bacterioses of pigs

G. Ryzhenko,

Candidate of Biological Sciences

Gorbatiuk O.,

Andriyashchuk V.,

Zhovnir O.

Candidates of Veterinary Science

Ukhovska T.,

Tiutiun S.

Institute of Veterinary Medicine, NAAS

The purpose. To develop efficient associated vaccine against the most spread bacterioses of pigs. **Methods.** Microbiological, biological, serological, statistical. Microscopic studies are carried out, features of growth of cultures of isolates-originators of bacteriemic diseases are studied. **Results.** Probes have confirmed antigenic activity of the vaccine as at inoculated rabbits titres of specific antibodies were authentically enlarged. The adjuvanticity of the specimen ensured preservation of 83,3% of the vaccinated white mice after infestation with originators *C. Perfringens* and *E. coli*. Immunobiological reorganization of an organism of the vaccinated pigs is confirmed by titres of specific agglutinins, and economic efficiency — by preserved expenditures in pig-breeding equipments at rate of 240 – 320 thousand hrn for a year. **Conclusions.** Economically efficient antigen-active highly-immunifacient vaccine Welshicolisan against anaerobic dysentery, contagious enterotoxaemia, hydropic illness, and colibacteriosis of animals is developed.

Key words: *clostridiosis, associated vaccine, adjuvanticity.*

At present in Ukraine, the trade and economic relations and transport flows, there are risks of proliferation biological threats primarily diseases of bacterial etiology. Therefore, problems of the bakteriosis and their associated flow is relevant, because on animals constantly influencing factors associated with disorders of growing technology, way of maintenance, care and feeding of animals, which act as stress for the animals and lead to a reduction of non-specific resistance and bakteriosis in pigs [1–3]. In recent years bacteriological monitoring of confirmed special role in the pathogenesis associated microflora bakteriosis animals, because studies 2–3 and more isolated pathogens in microbiota and only partially - one pathogen isolates [4, 5].

To protect the animals from diseases of bacterial etiology and their complicated course there is an urgent need to develop new associated vaccines [6, 7]. The advantage associated vaccine preparations is that they can create intense immunity in the body of animals vaccinated simultaneously against several kinds of pathogens that contribute to the improvement and stabilization of the epizootic situation on a number of infectious diseases caused by bacterial pathogens [8–10].

Goal. Develop an effective associated vaccine against the most common bakteriosis of pigs, conduct laboratory quality control of drug research and production testing, to determine the cost-effectiveness of the vaccine after its use in farms.

Materials and methods. Research conducted at the laboratory of anaerobic infections of IVM NAAS, experimental-biological clinic of DU Institute of Cardiology M. D. Strazhesko in the farms: the corporation "Ukragroteh" Khrystynivka district, Cherkasy region.; PSP "Record" Koryukovka district, Chernigiv region.

Used microbiological, biological, serological methods of research, carried out statistical analysis of the results of studies [11–13].

Results. The pilot sample associated vaccine "Velshykolisan" against anaerobic dysentery young animals, infectious enterotoxemia oedematous disease, colibacillosis animals is a compound of antigens selected inactivated strains of epizootic *C. perfringens* and *E. coli* in optimal ratios and immunomodulatory drugs of

natural origin. Experimental vaccine has passed the quality control laboratory. Inbred white mice instilled test specimens vaccine preparation with subsequent infection of titrated minimal lethal doses of pathogens *C. perfringens* types B and C, and *E. coli*. The vaccine provided 83,3 % survival of animals vaccinated with unvaccinated death of 90,0 % white mice, indicating the formation of a high level of protection against specific pathogens above mentioned bacterial infections (Fig. 1).

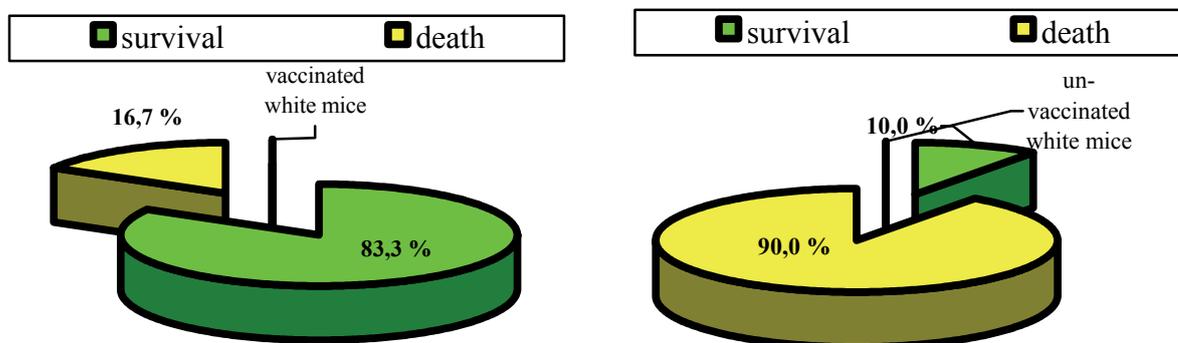


Fig. 1. – Survival of vaccinated and unvaccinated white mice after infection with diurnal *C. perfringens* cultures types B and C, and *E. coli*

An important indicator of the quality vaccine is the biosynthesis of specific antibodies, which characterizes the strength of immunity. In rabbits immunized with the vaccine test specimens defined titers of specific antibodies to antigens *C. perfringens* type B, *C. perfringens* type C and *E. coli* in the blood serum of animals. Analysis of the agglutination test accounting showed that during the period of the experiment the amount of specific antibodies increased significantly compared with previous indicators and rabbits in the control group. The maximum concentration of antibodies to the pathogen *C. perfringens* type B, observed 28 days after re-vaccination when titers of specific antibodies significantly by 51,8 % ($p < 0,001$) exceeded the indicators that have been received in 7 days after the first inoculation of rabbits .

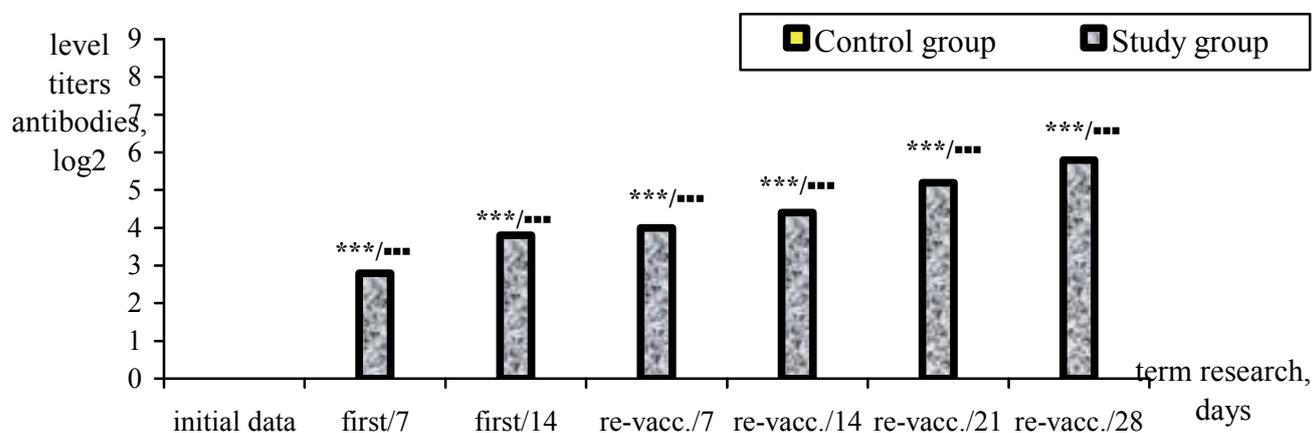


Fig. 2. - Titers of antibodies specific to *C. perfringens* type B in vaccinated rabbits

Note. ***/*** $p < 0,001$ compared to initial figures and animals in the control group.

About active immunogenesis testified agglutinins indicators, specific to the antigens of *E. coli*, because there was a probable increase their level at 51,9 % ($p < 0,001$), and the antigen *C. perfringens* type C, the level which significantly increased in 30,0 ($p < 0,001$) times, compared to the original figures and animals in the control group were found in them in 7 days after the first immunization.

For in-depth study of the impact of research on the organism vaccine preparation vaccinated pigs and feasibility of its use in households, we have conducted industrial tests of experimental sample associated vaccine "Velshykolisan" in farms Chernigiv region. - PSP "Record" v. V. Sloboda Koryukovka district and in Cherkasy region – Corporation "Ukragroteh" Khrystynivka district, where there was a high incidence of animals

that ran symptom of diarrhea by destruction of pigs of different ages and high mortality. After inoculation herds of pigs were conducted serological studies of blood serum of agglutination test by setting the appropriate antigens (Table 1).

Table 1. Titers of specific antibodies in pigs for the experimental sample application associated vaccine "Velshykolisan"; ($M \pm m, \log_2, n = 100$)

Term research	Multiplicity vaccinations	Days after vaccination	Types of antigens	A household name, district, region				
				corporation "Ukragroteh" Hristinovka Khrystynivka district Cherkasy region.		PSP "Record" V. Sloboda Koryukovka district Chernihiv region.		
				Groups of pigs				
				sows	piglets (2-4 months)	sows	piglets (2-4 months)	
Number of animals in the group				35	25	20	20	
Initial data		<i>Cl. perfringens</i> type B		0,5±0,03	0,4±0,04	0,6±0,05	0,6±0,02	
		<i>Cl. perfringens</i> type C		0,5±0,03	0,4±0,04	0,5±0,05	0,6±0,08	
		<i>E. coli</i>		1,1±0,09	0,8±0,11	1,2±0,10	1,0±0,08	
After vaccination, over days	first	14	<i>Cl. perfringens</i> type B		4,1±0,16 ***	3,9±0,20 ***	4,4±0,10 ***	4,1±0,13 ***
			<i>Cl. perfringens</i> type C		4,4±0,14 ***	4,1±0,17 ***	4,7±0,10 ***	4,1±0,30 ***
			<i>E. coli</i>		5,0±0,16 ***	4,9±0,17 ***	5,4±0,15 ***	4,7±0,30 ***
	Re-vaccination	14 (28)•	<i>Cl. perfringens</i> type B		5,4±0,16 ***	4,9±0,17 ***	6,0±0,15 ***	4,6±0,20 ***
			<i>Cl. perfringens</i> type C		5,6±0,11 ***	5,4±0,15 ***	5,9±0,15 ***	5,4±0,20 ***
			<i>E. coli</i>		6,6±0,13 ***	6,3±0,17 ***	7,2±0,20 ***	6,0±0,06 ***
		28 (42)	<i>Cl. perfringens</i> type B		6,6±0,13 ***	6,3±0,17 ***	7,3±0,15 ***	5,7±0,20 ***
			<i>Cl. perfringens</i> type C		6,8±0,11 ***	6,6±0,11 ***	7,2±0,15 ***	6,3±0,20 ***
			<i>E. coli</i>		8,2±0,15 ***	8,1±0,15 ***	8,6±0,25 ***	7,7±0,20 ***

Note. () • – the number of days after the first vaccination; *** – $p < 0,001$ compared with initial values.

According to records of the results established that titers of specific agglutinins in sows and piglets have started to grow significantly in 14 days after the first vaccination.

With the end of the experiment in the sow, which belonged to the corporation "Ukragroteh" and PSP "Record", titers of antibodies specific to *C. perfringens* type B were significantly higher at 12,3 ($p < 0,001$) and 13,2 ($p < 0,001$); in pigs 2–4 months age – in 9,5 ($p < 0,001$) times and 15,8 ($p < 0,001$), respectively. Arguably growing quantitative indicators antibodies specific to *C. perfringens* type C because sows on both farms increased their titles in more than 13,6 ($p < 0,001$) and the pigs – 10,5 ($p < 0,001$) and 16,5 ($p < 0,001$) times, respectively. Level agglutinins specific to *E. coli*, grew in sow all farms significantly more than 7,2 ($p < 0,001$); in piglets – more than 7,7 ($p < 0,001$) times, compared to the initial data.

For analysis of epizootic surveys and economic indices after vaccination of livestock Corporation "Ukragroteh", Cherkasy region. the incidence of pigs decreased by an average of 43,0 % and deaths decreased

in 2,0 times. The use of vaccine research boosted the average live weight of pigs by 21,0 % and the overall economic effect of the drug was about 240 thousand UAH in a year.

In PSP "Record", Chernihiv region., after application of associated vaccine "Velshykolisan" incidence in pigs decreased twice, mortality – by 38,0 %, rising average live weight gain of 15,0 %, the economic effect was about 320 thousand UAH per year.

Conclusions.

Developed associated inactivated vaccine "Velshykolisan" against anaerobic dysentery, infectious enterotoxemia, oedematous disease, colibacillosis animals, laboratory quality control and mounted high antigenic activity and immunogenicity of the drug because it provided 83,3 % survival of mice grafted after their infection titered DLM, against by 90,0 % death unvaccinated mice.

Confirmed immunobiological alteration in the body of vaccinated sows and young pigs, because the use of associated vaccine "Velshykolisan" in sows boosted their level of antibodies specific to *C. perfringens* type B in 12,3–13,2 ($p < 0,001$) times; *C. perfringens* type C – to 13,6 ($p < 0,001$) and *E. coli* – in 7,2 ($p < 0,001$) times. After the vaccine piglets 2–4 months age, increased titers of antibodies to *C. perfringens* type B – at 9,5 ($p < 0,001$) and 15,8 ($p < 0,001$); *C. perfringens* type C – 10,5 ($p < 0,001$); to *E. coli* – 7,7 ($p < 0,001$) times, respectively, indicating the formation of specific protection in vaccinated pigs to pathogens *Perfringens* and *Escherichia*.

Prospects for further research focused on the use of metal nanoparticles in biotechnology manufacturing associated vaccines to enhance their effectiveness.

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