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PORCINE REPRODUCTIVE AND NEONATAL INFECTIONS: IMPORTANCE AND THREATS OF BACTERIAL VIROPHORIA

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Summary. The provisions of the doctrine of transfer the epizootic process of dangerous viral infections to the enzootic process and their rooting in pig production through the integration of their pathogen into the pig microbiome in the form of comorbid viral and bacterial infections are substantiated. The aim of the study is to systematize the bacterial virophoria in the epizootology of porcine reproductive and neonatal infections (PRNI) as a component of the enzootic cycle of emergent infections in the pig industry of Ukraine. Classical swine fever (CSF) virus: attenuated strain 'IECVM-03'; Aujeszky's disease (AD) virus: epizootic strains of Ukrainian origin of AD virus '18v-UNDIEV'; Teschen disease (TD) virus: epizootic strain 'Bucha'. Epizootic strains: pasteurella bacteria, streptococcus, lacto- and bifidobacteria. According to the results of the study, it was found that the rotating magnetic field of the right direction promoted the adsorption of the CSF virus on pasteurella cells. The Aujeszky's disease virus was adsorbed on the bacteria *Salmonella choleraesuis* No. 34, *Bacterium bifidum* and *Lactobacillus casei* with an efficiency of 15–45% in the pH range of 8.5–9.5, at neutral pH (7.4) no more than 1.5% of the virus was adsorbed, and at acidic pH (3.0) the AD virus was not adsorbed et all. On bacteria *Pasteurella multocida* No. 7, AD virus was adsorbed in the pH range of 8.5–9.5 with an efficiency of no more than 1.5%; at neutral pH (7.4), up to 50% of the virus was adsorbed, and at acidic pH (3.0), no more than 1.5% of AD virus was adsorbed. The interaction of TD virus with bifidobacteria inhibited viral reproduction in the body of infected polecats, but preserved the reproductive activity of teschovirus in the presence of streptococci. The rooting of dangerous viral infections (AD and TD, circovirus and parvovirus infections, reproductive and respiratory syndrome, and endemic porcine diarrhea) in pig production has always been accompanied by the 'engraftment' of their pathogens in the microbiome of pig production facilities in the form of comorbid (i. e. clinically manifested) and/or associated infections (i. e. similar to the group of Minimal Residual Human Diseases — Maladie Résiduelle Minimale, MRD). A key role in the establishment of these diseases and the formation of their stationary centers in pig production is played by the virophoria of bacteria synergistic with their pathogens, in particular as part of the etiologic microflora of reproductive and neonatal infections in pigs

Keywords: virus adsorption, virulence, viral-bacterial infections, rooting of emergent pathogens

Introduction. Over the past decade, we have been studying the mechanisms of infectious diseases in industrial pig production associated with outbreaks of lethal fevers in rearing groups and reproductive disorders in the nucleus of the industrial pig herd. Already in the first 2–3 years of research, we became convinced that Aujeszky's disease (AD), Teschen's disease (TD), porcine circovirus (PCV) and porcine parvovirus (PPV) infections, reproductive and respiratory syndrome (PRRS), and porcine endemic diarrhea (PED), sometimes also certain viral vaccines against them, as well as classical swine fever (CSF) have been implicated in one way or another in the anamnesis of comorbid viral-bacterial pneumoenteritis in young animals and reproductive disorders in sows and boars.

Due to the similarity of epizootic manifestations and in order to standardize control protocols, we proposed to refer these diseases to porcine reproductive and neonatal infections (PRNI).

Unlike the bacterial component, the viral component of the etiology of these infections is clinically manifested almost exclusively in newborns and pigs after childbirth: although not always, and only for a short period of time — during exacerbation of PRNI. This exacerbation usually becomes emergent, as the use of antiepizootic drugs and measures periodically requires the search for unconventional approaches to antibiotic, chemotherapy, sero- or vaccine prophylaxis.

Another feature of PRNI is their 'tendency' to form stationary foci, in which periods of enzootic exacerbation are cyclically replaced by relative 'quiescence'. Today, few people pay attention to this, but in such foci PRNI manifest as comorbid infections of viral and bacterial etiology. Moreover, this manifestation depends not only on the composition of the etiologic viral-bacterial consortium of PRNI, but also on the action of a particular causative factor. In these cases, we often encountered the phenomenon of bacterial virophoria,

when certain bacterial species showed signs of adsorption of the corresponding viral agent from the consortium on their surface. Unfortunately, in our opinion, the above mentioned known data remain insufficiently systematized.

The aim of our work was to systematize bacterial virophoria in the epizootology of PRNI as a component of the enzootic cycle of rooting of pathogens of emergent infections in the pig industry of Ukraine.

Materials and methods. The mechanisms of virophoria formation were studied on the model of the oldest, according to scientific data, formerly exotic, but now familiar to the veterinary service of Ukraine viruses — the pathogens of AD and TD (Rudyk, 1995; Korolov, 2011; Derevianko, 2019).

This choice was made not only because of the indisputable long-standing rooting of these pathogens in Ukraine, but also because of the tradition of their use as surrogate models in the study of certain issues of epizootology of African swine fever (ASF) and foot-and-mouth disease (FMD), respectively (Frost et al., 2023; Harada et al., 2015).

Aujeszky's disease virus (ADV): epizootic strains of Ukrainian origin AD '18v-UNDIEV' isolated in 1968 (Poltava Region) and '1082' isolated in 2010 (Donetsk Region). These viruses, after passaging in laboratory mice, secrete hemagglutinins of mouse erythrocytes, which provides the possibility of in vivo studies in the format of a budget-saving epizootic model 'virus–mouse'. Before and during use, the viral suspensions were titrated in transplanted porcine cell cultures by the classical method (Reed and Muench, 1938) and in the haemagglutination assay (HA) and the hemagglutination inhibition assay (HIA) following Tetsu et al. (1989) * in accordance with the relevant standard operating procedure (SOP) of the NSC 'IECVM'. Viral suspensions with an activity of 6.5–7.5 lg $TCID_{50}$/ml (512–1,024 HAO_{50}/ml) were used to model virophoria and control its effect on pathogenicity of agents for mice.

Teschen's disease virus (TDV): epizootic strain 'Bucha' isolated in 2003 (Poltava Region). A trophovariant of the pathogen adapted to guinea pigs was used in a cost-effective epizootic model of peripheral neuropathogenicity of *Teschovirus* (Buzun, 2021); its infectious activity was controlled in continuous cultures of pig cells according to the relevant SOP of the NSC 'IECVM'. Viral suspensions with an activity of 7.0–7.5 lg $TCID_{50}$/ml were used in the experiments.

Classical swine fever virus (CSFV): attenuated strain 'IECVM-03' isolated in 2003 (Sumy Region) was grown and tested in the continuous culture of pig cells PK-15 according to the relevant SOP of the NSC 'IECVM'. Viral suspensions with an activity of 4.5–5.5 lg FFU_{50}/ml were used in the experiments. To control their infectious activity, as well as the activity of TD and AD pathogens (in accelerated variants), the method of fluorescent antibodies (FA) was applied, using appropriate diagnostics according to the SOP of the NSC 'IECVM'.

To model the virophoria of these pathogens, epizootic strains were used: of bacteria isolated in 2008 and 2012 from pigs — *Pasteurella multocida* No. 7 strain (2006, Poltava Region) and *Streptococcus suis* No. 53 strain (2009, Luhansk Region), *Salmonella choleraesuis* No. 34 strain (2011, Donetsk Region). Production strains of the probiotic bacteria *Bifidobacterium bifidum* and *Lactobacillus casei* ('resident microflora') were also used. The stock cultures of all bacteria were used at a concentration of 1.2×10^9 CFU/ml (based on the results of titration on dense medium).

Among the physical environmental factors that, according to the literature, can activate bacterial virorrhization (factors affecting particle agglutination) (Ortega-Vinuesa and Bastos-González, 2001), this series of studies tested (a) for the CSF virus and pasteurella, rotating magnetic fields of the right and left directions and (b) for AD and TD pathogens and pasteurella, streptococci, bifido- and lactobacteria, the acidity of the medium (pH). The generator of rotating magnetic fields was a device by Hrabina et al. (2009), and buffer solutions with appropriate pH and salt molarity served as acidity factors.

In the *in vivo* models, all manipulations with outbred laboratory mice and guinea pigs were performed in accordance with the 'European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes' (CE, 1986) and basic bioethical principles (Simmonds, 2017). The experiments were conducted in compliance with the principles of humanity stated in the European Council Directive 2010/63/EU (CEC, 2010).

Research results. *A. Study of the CSF virus virophoria on pasteurella.* The potential physical impact on the interaction between the CSF virus and pasteurella was studied using right (RR) and left (LR) rotating magnetic fields. To do this, we used the device of Hrabina et al. (2009) (Fig. 1a), which generates asymmetric magnetic fields. Virus-bacterial mixtures containing the CSF virus (strain 'IECVM-03'; final concentration 4.5–5.0 lg FFU_{50}/ml †) and pasteurella *P. multocida*

* With 0.5–1.0% mouse erythrocytes, total reaction volume — 0.075 cm^3, sample dilution step — 2, solvent — phosphate-gelatin buffer solution (pH 7.4), V-well plates, incubation at room temperature, recording of results after the control erythrocytes settle into the 'button'

† lg FFU_{50}/ml — the decimal logarithm of the extreme dilution of the virus-containing suspension under study, which still contains 50% of the test slides with focus-forming units of the CSF virus

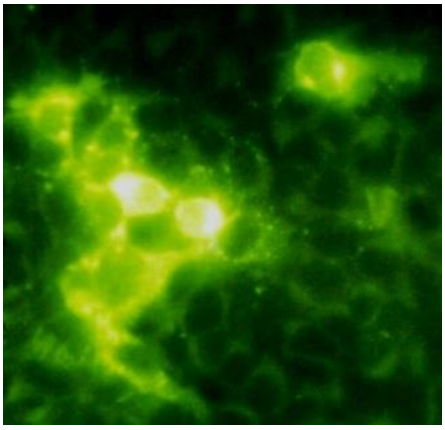
(strain '7', activity 5×10^5 BC_{50}/ml[‡]) on a buffered saline solution, each in volumes of V = 5.0 and 7.0 cm^3, were exposed to a rotating magnetic field of θ =0.2 mTl. The samples were exposed to the right (n = 17) and left (n = 11) rotating magnetic fields for 25 min. The control samples (CS) (n = 17) were identical to the experimental ones in all respects: they were exposed for 25 min with the device turned off. After exposure, all precipitates of the mixtures (pasteurella) were washed in buffered saline solution by a single centrifugation (1,000 gX, 30 min). The washed bacterial suspensions of the RR samples were divided into three parts: the first of them was controlled for the presence of the CSF virus in the bacterial precipitate in parallel with the precipitates of the LR and CS. The second and third parts of the RR samples were immediately exposed in a thermostat at 40.0 ± 0.5 °C for 3 and 5 days, respectively, after washing, and only then were they monitored for the presence of the virus. In all cases, the titration of CSF virus was performed according to the standard procedure by FA after virus isolation in the continuous PK-15 cells on slides (Fig. 1b). Two of the 17 RR-precipitates were tested by polymerase chain reaction (PCR) for CSF, using commercial primers to identify CSF virus adsorbed on pasteurella (Fig. 1c).

By both tests, CSF virus was detected on pasteurella only in the bacterial RR sediments: in 11 samples out of 15 examined by FA and in one out of two by PCR. The geometric mean titer of the virus in 9 positive RR-precipitates was 2.5 ± 0.3 $lgFFU_{50}$/ml (p < 0.01). On the 3rd and 5th day after exposure at 37 °C, RR-precipitates of virophoric bacteria contained remnants of infectiously active CSF pathogen: the geometric mean virus titers were 1.1 ± 0.8 $lgFFU_{50}$/ml (n = 4) and 1.0 ± 0.9 $lgFFU_{50}$/ml (n = 2), respectively.

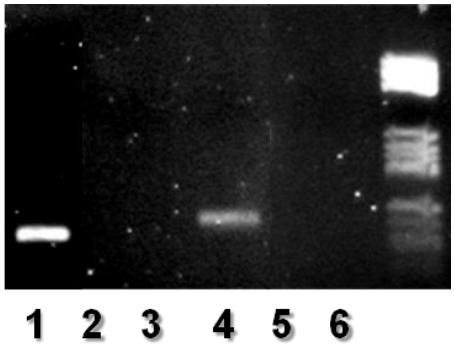
At the same time, the initial viral suspension of the pathogen at 40 °C was completely inactivated after 35 hours of exposure (n = 3, p < 0.01). All LR- (n = 11) and CS-precipitates (n = 17) were negative for CSF in FA and PCR tests. Moreover, 7 out of 11 LR sediments did not even contain live bacteria. These results to some extent coincide with the data on the phenomenon of 'Gränder's water'[§], which is manifested in bacteria-contaminated water under the influence of weak magnetic irradiation from a device patented in Austria (Liu et al., 2022).

Thus, a rotating magnetic field of the right direction with a power of θ = 0.2 mTl promotes the adsorption of the CSF virus on pasteurella cells — stimulates the virophoria of these bacteria. At the same time, the virus adsorbed on bacteria acquires significant resistance at temperatures up to 40 °C inclusively.

a



b



c

Figure 1. Summary data on the use of a right-rotating magnetic field for modeling the virulence of the CSF pathogen on pasteurized bacteria: a — device of Hrabina et al. (2009); b — focus-forming unit of the CSF pathogen adsorbed on pasteurized bacteria; c — genetic material (track 4) of the CSF virus in the bacterial mass of the RR-sediment.

These results indicate a certain probability of an important causal role of the magnetic irradiation factor in the epizootology of the CSF.

B. Study of the virophoria of AD and TD pathogens on pasteurella, streptococci, bifido- and lactobacteria, with epizootological modeling of its consequences on laboratory rodents. The general scheme of the study is shown in Fig. 2.

[‡] BC_{50}/ml — concentration of bacterial cells according to the results of densitometry (by McFarland)

[§] https://www.grander.com/intl-en/international/grander-water/what-is-grander-water

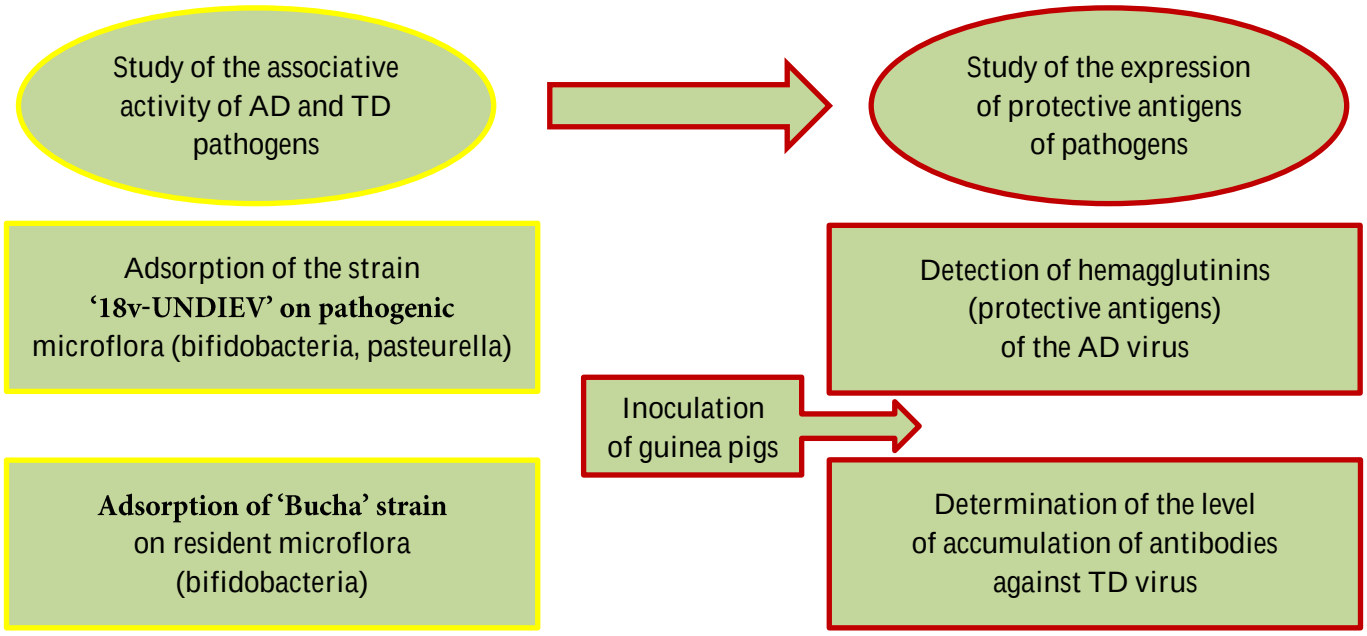


Figure 2. Scheme of the study of bacterial virophoria of the pig microbiome as a factor in the pathogenesis of AD and TD.

The adsorption of AD and TD pathogens on bacteria of the resident and pathogenic microflora of the pig was studied in the context of the study of the associative activity of these infectious agents and its effect on the expression of protective antigens of AD and TD viruses. Table 1 summarizes the results of studies of the intensity of adsorption of the mouse trophovariant strain '18v-UNDIEV' on the reference strains of bacteria of the species *Pasteurella multocida* No. 7, *Salmonella choleraesuis* No. 3–4 (pathogenic microflora), *Bifidobacterium bifidum* and *Lactobacillus casei* (resident microflora) under alkaline (pH 8.5), neutral (pH 7.4) and acidic (pH 3.0) conditions.

Table 1 — Results of the study of adsorption of the strain '18v-UNDIEV' of ADV on bacteria of pathogenic and resident microflora at different acidity of the medium

No.	Samples under test		ADV titer (lg CFU_{50}/ml) in sample fractions at the pH of the corresponding buffer solutions:		
	The kind of sample	Sample fraction	8.5	7.4	3.0
1	Virus suspension	All fractions [1)]	4.5	4.5	4.5
A mixture of ADV strain '8v-UNDIEV' and bacteria of strain No. 7 of *Pasteurella multocida*					
2	Virus-bacterial mixture	Supernatant [2)]	3.5	3.8	2.3
		Sediment [2)]	2.7	4.2	1.0
		adsorption, %	≤ 1.5	≤ 50	0
A mixture of ADV strain '18v-UNDIEV' and bacteria of strain No. 34 *Salmonella choleraesuis*					
3	Virus-bacterial mixture	Supernatant [2)]	3.2	3.8	2.3
		Sediment [2)]	3.8	2.7	0
		adsorption, %	≤ 20	≤ 1.5	0
A mixture of ADV strain '18v-UNDIEV' and bacteria *Bifidobacterium bifidum*					
4	Virus-bacterial mixture	Supernatant [2)]	3.1	3.8	1.0
		Sediment [2)]	4.1	2.7	0
		adsorption, %	≤ 40	≤ 1.5	0
A mixture of ADV strain '18v-UNDIEV' and bacteria *Lactobacillus casei*					
5	Virus-bacterial mixture	Supernatant [2)]	2.8	3.8	2.3
		Sediment [2)]	3.7	2.5	0
		adsorption, %	≤ 15	≤ 1.0	0

Notes: [1)] titer values before the preparation of the mixtures, which are assumed to be 100%; [2)] titer values after preparation and storage of samples, based on the results of 2–3 series of experiments for each parameter ($p < 0.010–0.017$).

It was found that on the bacteria *Salmonella choleraesuis* No. 34, *Bacterium bifidum* and *Lactobacillus casei*, AD virus was adsorbed with an efficiency of 15–45% in the pH range of 8.5–9.5. At a neutral pH (7.4), no more than 1.5% of the virus was adsorbed, and at an acidic pH (3.0), AD virus was not adsorbed at all and was inactivated within 3–4 hours. On bacteria *Pasteurella multocida* No. 7, AD virus was adsorbed in the pH range of 8.5–9.5 with an efficiency of no more than 1.5%; at neutral pH (7.4), up to 50% of the virus was adsorbed, and at acidic pH (3.0), no more than 1.5% of the AD virus was adsorbed.

AD virus adsorbed on pasteurella was inactivated at 37 °C for 4 hours by 0.25 lg TDC_{50}/ml (0.004%), while unadsorbed virus lost 2.25 lg TDC_{50}/ml of infectious activity (0.4%) under the same conditions.

Table 2 summarizes the data on the effect of AD virus adsorption on bifidobacteria on its pathogenicity for mice. It was found that the adsorbed virus does not enter the mouse brain under the experimental conditions ($p < 0.01$), but retains pathogenicity due to damage to parenchymal organs. This may be associated with the selective adsorption of pathogen variants with the indicated tropism to mouse tissues under experimental conditions.

On the other hand, such a model of AD-pasteurellosis comorbidity pathogenesis may result from increased sensitivity of mouse tissues to pasteurella toxins under the influence of the AD pathogen.

Table 3 summarizes the results of the study of the effect of pH on the adsorption of teschovirus on pasteurella, streptococcus and bifidobacterium. These data show that TD virus is actively adsorbed on bacteria only at alkaline pH (8.5). It is most active on streptococci (item 6 of Table 3, $p < 0.01$). The teschovirus adapted to the organism of guinea pigs, adsorbed on bifidobacteria cells, was inactivated by 1.5 lgTCD_{50}/ml (i. e., by 0.001 %) during 5–9 hours of incubation at 50 °C, and its free form — by 4.0–5.0 lgTCD_{50}/ml, i. e., by 42% ($n = 8$, $p < 0.01$). Thus, in the pathogenesis and epizootology of TD, the virophoria of the resident rodent intestinal microflora can be considered a natural mechanism of protection of the pathogen from adverse factors.

The following consequences of teschovirus adsorption on bacteria can be seen in Table 4 — the effect of the latter on the infectious activity of the pathogen in the body of the guinea pig. At a probability level of $p < 0.01$ ($n = 9$), it was found that mice infected with TD virus adsorbed on bifidobacteria at a dose of 6.0 lgTCD_{50}/ml showed no signs of disease, gave normal offspring (not shown in the table) and showed no signs of viral infection (no virus accumulation in target organs and no seroconversion to TD pathogen).

Thus, the interaction of the TD virus with bifidobacteria inhibits viral reproduction in the body of infected guinea pigs and, accordingly, the formation of a persistent infection, which is typical for rodents infected with the free form of the TD pathogen. Virophorous pasteurella and streptococci do not lose their pathogenic properties for rodents. In guinea pigs that survived streptococcal infection from virophorous bacteria ($n = 2$ out of $n = 5$), antiviral antibodies in significant titers were detected 3 weeks after intraperitoneal infection. This indicates that the reproductive activity of teschovirus is preserved in the presence of streptococci, unlike bifidobacteria and pasteurella.

In the context of the above results, it is advisable to present a clinical case of AD in a boar with reproductive disorders, in the semen of which, during the examination, the causative agent of this disease was detected. Initially, a positive result was obtained when staining semen smears according to the protocol for the direct method of fluorescent antibodies against the AD virus (Fig. 3). Then the presence of the virus genetic material was confirmed by PCR.

Based on many years of NSC 'IECVM' experience in studying bacterial virophoria, we 'blindly' sent a sample of primary bacilli from this ejaculate for PCR analysis to another department of our research center. As expected, we received a positive result: the bacterial culture (mainly *Neisseria* and *Pasteurella*) contained the AD pathogen genome. A similar result was obtained during the examination of the ejaculate of a boar carrying the virus from another pig farm (Fig. 3b).

Table 2 — Results of the study of the effect of resident and pathogenic bacteria on the pathogenicity of the AD pathogen for mice

Samples (30% suspensions)	AD virus hemagglutinin titer / its infectious titer (lg TCD_{50}/g)		
	Mice after injection of bifidobacteria (n = 6)	Mice infected with virophorous bifidobacteria (n = 16)	Mice infected with AD virus (n = 12)
24 hours after infection			
of the brain	0 / 0	0 / 0	0 / ≤ 2.0
spleen	0 / 0	0 / ≤ 2.0	1:40–1:80 / ≤ 3.0
96 hours after infection			
of the brain	0 / 0	0 / 0	1:20–1:40 / ≤ 7.0
spleen	0 / 0	1:40–1:320 / ≤ 5.0	1:80–1:320 / ≤ 7.0
Time / percentage of mouse deaths			
	0 / 0	5–9 days / 43.8%	2–3 days / 91.7%

Note: [1)] intranasal infection.

Table 3 — Results of the study of adsorption of TD virus strain 'Bucha' on bifidobacteria, pasteurella and streptococci at different pH

No.	Samples under test		ADV titer (lg TCD_{50}/ml) in sample fractions at the pH of the corresponding buffer solutions:		
	The kind of sample	Sample fraction	8.5	7.4	3.0
Adsorption on *Bifidobacterium bifidum*					
1	Virus suspension	All fractions [1)]	6.5	6.5	6.5
2	Virus-bacterial mixture	Supernatant [2)]	4.8	4.8	6.0
		Sediment [2)]	5.8	2.7	0
		adsorption, %	≤ 20	≤ 10	0
Adsorption on *Pasteurella multocida*					
3	Virus suspension	All fractions [1)]	6.5	6.5	6.5
4	Virus-bacterial mixture	Supernatant [2)]	5.0	6.0	6.0
		Sediment [2)]	3.5	0	0
		adsorption, %	≤ 10	0	0
Adsorption on *Streptococcus suis*					
5	Virus suspension	All fractions [1)]	6.5	6.5	6.5
6	Virus-bacterial mixture	Supernatant [2)]	4.5	5.0	6.0
		Sediment [2)]	6.5	3.0	0
		adsorption, %	≤ 80	≤ 10	0

Notes: [1)] titer values before the preparation of the mixtures, which are assumed to be 100%; [2)] titer values after preparation and storage of samples, based on the results of 3 series of experiments for each parameter ($p < 0.010–0.017$).

Table 4 — Results of the study of the effect of bacteria of the pig microbiome and guinea pigs on the reproductive activity of the TD pathogen in guinea pigs

No	10–30% of the suspension of target organs in guinea pigs	Characteristics of infected guinea pigs [1)]		
		Virus titer TD (lg TCD_{50}/ml)	Death of infected guinea pigs	Virus-neutralizing antibody titer
Intraperitoneal infection with a viral suspension of the 'Bucha' strain				
1	duodenum	0 (n = 4)	0 (n = 4)	1:4–1:8 (n = 4)
2	ileum	1.0–3.0 (n = 4)		
Intraperitoneal infection with virophorous bifidobacteria				
3	duodenum	0 (n = 5)	0 (n = 5)	0 (n = 5)
4	ileum	0 (n = 5)		
Intraperitoneal infection with virophorous pasteurella				
5	duodenum	0 (n = 4)	75% (n = 4)	0 (n = 1)
6	ileum	1.0–1.5 (n = 4)		
Intraperitoneal infection with virophorous streptococci				
7	duodenum	1.0–1.5 (n = 5)	60% (n = 3)	1:8–1:32 (n = 2)
8	ileum	3.0–3.5 (n = 5)		

Notes: [1)] recording within 21 days after infection.

This gives reason to believe that the semen of boars carrying the virus is contaminated not so much with the AD virus as with virophorous bacteria, which, in turn, adsorbed on spermatozoa. After all, the AD pathogen is neurotropic: although it affects almost all organs, in all cases it affects only their nervous tissue (Fan et al., 2019), which is not present in sperm. Nevertheless, microbes, including virophorous ones, are actively adsorbed on spermatozoa, as shown in Fig. 3.

Discussion *(Substantiation of the doctrine of transfer the epizootic process of dangerous viral infections to the enzootic one and their rooting in pig production through the integration of their pathogen with the pig microbiome in the form of comorbid viral and bacterial infections).* Back in the 1960s, Prof. Kulesko and his students developed a method of immunization of wild and domestic pigs against CSF based on the virophoria of its pathogen on bacteria of the vaccine strain of erysipelas.

Due to the tropism of erysipelas bacteria to the tonsils, oral vaccination with this drug practically saved the wild boar population of the Belovezhskaya Pushcha Reserve from imminent death (Kulesko and Lichtman, 1961).

Since then, the NSC 'IECVM' has established a tradition of researching and analyzing animal infectious pathology and epizootology in the format of biocenology — from the angle of interaction of viruses and bacteria with each other and their biological hosts, taking into account the influence of environmental factors. In the late 1980s, discussions between scientists from Ukrainian Research Institute of Experimental Veterinary Medicine (former name of the NSC 'IECVM') and Research Agricultural Institute of the Ministry of Agriculture of USSR (Gvardeyskiy, Kazakhstan) began on the virophoria of bacteria with certain types of herpes-, morbilli-, pox-, picorna- and rhabdoviruses, the staff of the institute formed and developed a powerful school of studying the epizootology of associated viral and bacterial infections in cattle (Fuks, 1993, 1994, 1999; Fuks et al., 1993, 1994).

One of the achievements of this school was undoubtedly the establishment of the phenomenon of reproduction of herpesvirus of infectious rhinotracheitis in *Bacillus alvei* — bee bacteria (Fuks, 1994). Already in the period 2015–2022, during epizootic surveys and research on relevant academic topics, we often observed the phenomenon of bacterial virophoria in the analysis of samples of pathological material, semen and feed from pig facilities contaminated with microbial associations containing PRNI pathogens: circoviruses, parvo-, teschoviruses, Aujeszky's disease and PRRS viruses, smallpox and PED. These unpublished data are fully consistent with the hypothesis of Katharina Ribbeck from Harvard University that viruses use bacteria and sperm as a factor in the transmission of viral infections in humans and animals (Ribbeck, 2009). Only after systematizing our similar epidemiological data on comorbid infections with African swine fever virus in the StopAfSFVmix doctrine, we came to the conclusion that the 'biocenological melting pot' of the above-mentioned associated reproductive and neonatal infections of pigs is a natural tool for transfer the acute epizootic process of monoinfections of pathogens exotic to pig production to the enzootic process of PRNI. Such infections were certainly CSF, AD, PCV-2, porcine parvovirus, PRRS and PED, which now fall under the definition of PRNI. In our opinion, unfortunately, the situation with ASF is developing in the same way.

In Fig. 4a, we summarized the PRNI enzootic chain, which is fully consistent with and, in our opinion, clarifies the French-American doctrine of the establishment of emergent pathogens (Morse, 1995; Desenclos and De Valk, 2005) in new nosoarea territories (Fig. 4b). Our refinement concerns the final node of the chain of establishment of an exotic infectious agent (Fig. 4b, c).

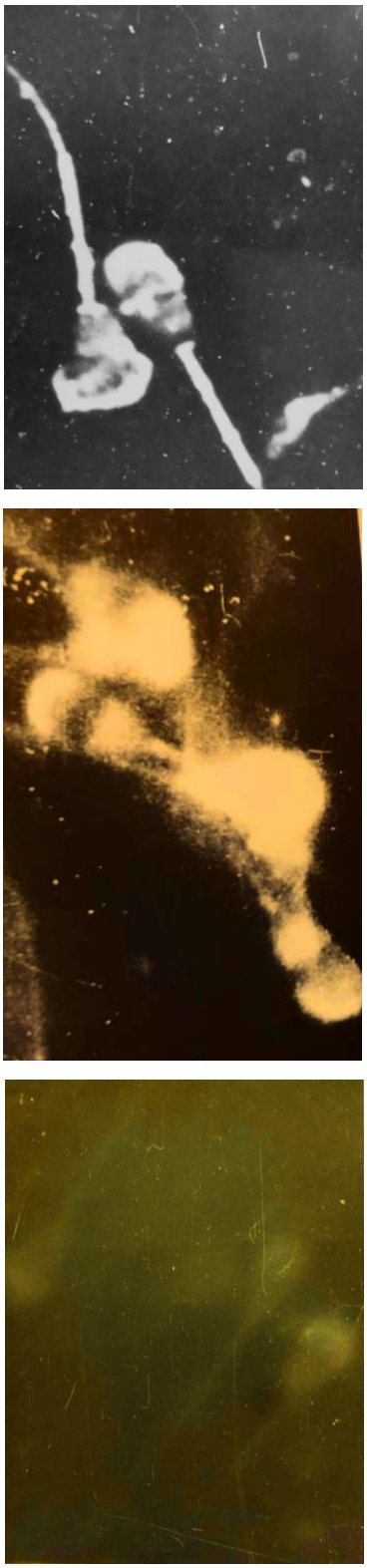


Figure 3. Results of examination of ejaculate swabs from boars No. 1 (a and c) and No. 2 (b) by direct fluorescent antibody method for AD virus carriage. In photos a and b — native smears (ethanol fixation), in photo c — a smear of ejaculate from boar No. 1, which before ethanol fixation was exposed for 15 min in a buffer solution with a pH 3.0 to elute virophorous bacteria from sperm.

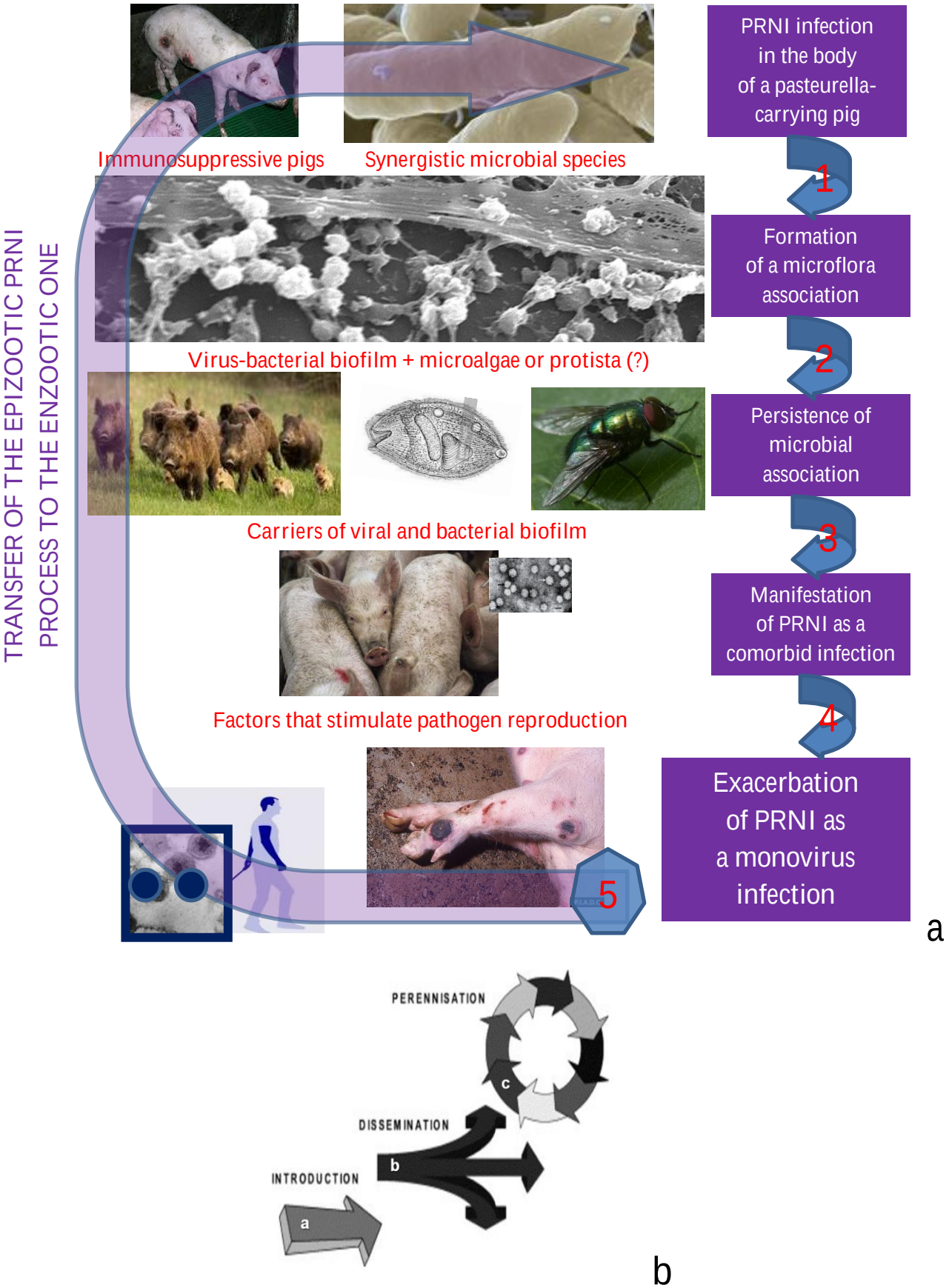


Figure 4. Determination of the role of bacterial virophoria as a key element in the formation of stationary PRNI foci (a) and in transfer acute phases of the enzootic process to chronic/comorbid ones (b, node 'c').

The 'Perennization' node (Fig. 4b, c) of the epizootic chain of emergent infections schematically summarizes the enzootic cycle of gradual exacerbation (dark arrows in shades from black to light gray) and 'normalization' of the epizootic situation (white arrow). This pattern is also characteristic of PRNI. Therefore, our concept (Fig. 4a) explains the interaction of an infectious agent new to a given pig herd with the pig's microbiome and the environment of a given territory/stationary focus. This interaction can lead to the elimination of the exotic agent and then the epizootic process will not become enzootic (Fig. 4b, b). If the agent 'finds a partner' in the above microbiome, it can be temporarily stored in microbial biofilms in the pig's body or in the environment, which at stage 2 (Fig. 4a, arrow 2) during the formation of a stationary enzootic focus can enter ecological niches in various ways to interact with microbially fed protists cells and other reservoir hosts of the pathogen. Under the influence of environmental factors (solar activity, weather factors, etc.) and the pig's body (e. g., immunosuppression factors), the target agent is activated (Fig. 4a, arrow 3), which leads to an exacerbation of the epizootic situation in the form of comorbid PRNI — different levels of intensity (Fig. 4b, c). In the next phase of the enzootic cycle (Fig. 4a, arrow 4), the target agent acquires, according to its inherent nature, a sufficient level of pathogenicity for the pig and is clinically manifested as a corresponding monoinfection, which corresponds to the black arrows in Fig. 4b, c.

We realize that there are many 'white spots' in our model of the rooting of emergent infectious agents in the PRNI microbiome, but the key importance of bacterial virophoria for triggering the scenario of transfer the epizootic process of emergent porcine viruses to an enzootic one is obvious. We see further development of the doctrine of transfer the epizootic process of dangerous viral infections to the enzootic one in the study of the possible persistence of PRNI pathogens in the protozoa (especially in their cysts), as well as in the study of the nature of the increase in the virulence of infectious agents of the pig in stationary foci of PRNI.

Conclusions. 1. The rooting of dangerous viral infections (Aujeszky's and Teschen's diseases, circovirus and parvovirus infections, reproductive and respiratory syndrome, and endemic porcine diarrhea) in pig production has always been accompanied by the 'engraftment' of their pathogens in the microbiome of pig production facilities in the form of comorbid (i. e. clinically manifested) and/or associated infections (i. e. similar to the group of Minimal Residual Human Diseases — Maladie Résiduelle Minimale, MRD).

2. The key role in the rooting of these diseases and the formation of their stationary foci in pig production is played by the virophoria of bacteria synergistic with their pathogens, in particular, as part of the etiological microflora of reproductive and neonatal infections in pigs.

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