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TESTING OF COMBINED VACCINES AGAINST AUJESZKY'S DISEASE, PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME AND PORCINE PARVOVIRUS INFECTION FOR THEIR ANTIGENICITY

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SUMMARY

Tests of inactivated emulsion anti-Aujeszky's disease, porcine reproductive and respiratory syndrome (PRRS) vaccine based on marker Aujeszky's disease virus strain, PRRS virus strain and inactivated emulsion anti-Aujeszky's disease and porcine parvovirus infection vaccine based on marker Aujeszky's disease virus strain and porcine parvovirus strain developed by the FGBI "ARRIAH" for their antigenicity are described in the paper. It is demonstrated that the said vaccines can be used successfully for PRRS and porcine parvovirus infection and Aujeszky's disease prevention. They are becoming of special significance when used in programs on Aujeszky's disease control and eradication. The use of the vaccine based on marker Aujeszky's disease virus strain allows differentiation between the animals that have antibodies to the marker vaccine strain (in the absence of antibodies to the glycoprotein gE in 100% of cases) from the animals with antibodies to field strains of the Aujeszky's disease virus in their sera. A comprehensive assessment of the use of associated vaccines developed in the FGBI "ARRIAH" (against Aujeszky's disease based on the marker strain, reproductive and respiratory syndrome, parvovirus infection), in the framework of anti-epidemic measures on a pig farm of the Russian Federation showed their safety, high antigenicity, immunogenicity.

Key words: Aujeszky's disease, marker virus strain, porcine reproductive and respiratory syndrome, porcine parvovirus infection, antigenicity.

INTRODUCTION

Porcine infectious respiratory diseases present a crucial challenge in veterinary field. They widely spread almost in all countries with well-developed pig industry and cause significant economic losses. In modern foreign and national literature, infectious diseases of pig respiratory organs are considered as a porcine respiratory disease complex (PRDC). Age of PRDC occurrence varies significantly. In the USA PRDC generally occurred in pigs at the age of 14–20 weeks. In the EU countries it affects growing pigs (8–10 weeks of age) or finishing pigs at the earlier fattening stages (10–15 weeks of age). In the Russian Federation infectious diseases affecting respiratory organs occur in pigs at the age of 35 days and older and in finishing pigs. In most cases, they occur in the form of concurrent (mixed) infection [4].

Viral respiratory pathogens are divided into several groups. The first group comprises major or primary pathogens causing lung lesions. As a rule, porcine reproductive and respiratory syndrome virus (PRRSV), porcine circovirus type 2 (PCV-2), swine influenza virus, Aujeszky's disease virus are classified to the first group. The second group comprises viruses playing minor role in the respiratory tract pathology but affecting reproducibility; parvovirus, paramyxovirus, adenovirus, reovirus, etc. are classified to the second group [2].

Porcine reproductive and respiratory syndrome virus (PRRSV) is a highly contagious disease characterized with late-term abortions, premature farrowing, birth of non-viable, mummified piglets and piglets with deformities, deaths of piglets within first days after their birth, sow infertility and lung lesions. Piglet stillbirth and infection rates in a litter depend on the virus isolate virulence and gestation term in which sow was infected. Stillbirth rate may vary from 0% to 100%; proportion of infected progeny varies from 4 to 75%, averaging 45%. The disease often occurs concurrently with other infections (Aujeszky's disease, parvovirus and enterovirus infections, encephalomyocarditis, influenza, leptospirosis, etc.) and it is a distinctive feature of PRRS [3, 4]. In the Russian Federation PRRS is reported annually. According to the data of the Information Analysis Centre of the FGBI "ARRIAH" PRRS outbreaks occurred in the Central (Lipetsk Oblast), Northwest (Murmansk Oblast), Siberia (Altai Krai and Omsk Oblast), Far East (Primorsky Krai) Federal Districts in 2015–2016 .

Aujeszky's disease (AD) is a viral infectious disease of pigs that are natural hosts of the agent but AD virus is able to affect cattle, sheep, cats, dogs and rats causing fatal disease. Definition "pig" means all *Sus scrofa* species both domestic and wild ones. AD is caused by herpesvirus and characterized by abortions in sows, central nervous system malfunctions in piglets, respiratory syndrome in weaned piglets. As a rule, AD occurs in animals in acute form. Incubation period lasts for 2 - 15 days sometimes longer. Susceptibility to the agent depends on pathogenic properties of the virus strain, animal age as well as stress factors. Convalescent pigs become life-long latent virus carriers. AD virus replicates in alveolar macrophages and monocytes and often is associated with necrotic rhinitis and thacheitis. However, AD can become severe particularly in growing and early-finishing piglets and

Table 1
Antigenicity of inactivated emulsion anti-AD and PRRS vaccine based on marker AD virus strain and PRRS virus strain
n = 15

Group of animals	Antibody level		
	PRRS	AD	
		gE	gB
Before vaccination	15/0* 0.15 ± 0.02**	15/0 0.95 ± 0.02	15/0 0.91 ± 0.02
On day 21 after the first vaccination	15/11 0.42 ± 0.02	15/0 0.91 ± 0.02	15/14 0.27 ± 0.02
On day14 after the second vaccination	15/15 0.64 ± 0.05	15/0 0.91 ± 0.02	15/15 0.11 ± 0.01
On day 40 after the second vaccination	15/15 0.83 ± 0.02	15/0 0.89 ± 0.02	15/15 0.09 ± 0.01

* number of tested/positive samples;
** mean value, $M \pm S$.
ELISA value for PRRS virus: $s/p < 0.4$ – specific antibodies are absent,
$s/p \geq 0.4$ – specific antibodies are present;
ELISA value for AD virus: $s/p \geq 0.7$ – specific antibodies are absent,
$s/p \leq 0.6$ – specific antibodies are present.

be complicated with bacterial microflora when PRRSV, PCV-2, parvovirus, swine influenza virus are present. Such epidemiological features make AD one the most economically significant diseases for commercial pig farming. The Russian Federation remains permanently AD- infected. AD ranks the fourth after classical swine fever, erysipelas and salmonellosis based on the vaccination coverage in pigs in the Russian Federation.

Porcine parvovirus infection (PPVI) is a contagious virus disease that is clinically apparent in pregnant sows only. Its clinical manifestations are as follows: reproductive failure, infertility, small litter, fetus death, mummified fetuses, stillbirths, birth of weak piglets and rarely abortions. In breeding boars the disease is subclinical. Currently, the disease is reported in 32 countries over the world [5, 6]. Serological analysis with hemagglutination inhibition test carried out by the FGBI "ARRIAH" confirmed that PPV circulates on the RF pig farms.

Out of the above-mentioned diseases, AD, is a notifiable to the World Organization for Animal Health (OIE). The status of AD freedom of country or zone from is taken into account during trade operations [1, 7]. According to Article 8.2.4 of the OIE Terrestrial Animal Health Code [1] country or zone may be considered free from Aujeszky's disease if the disease has not been reported for at least 25 years or if for at least 10 years measures for compliance with a set of requirements have been in place in the country or zone. Country or zone may be considered free from Aujeszky's disease providing that vaccination of domestic and captive wild pigs against Aujeszky's disease has been banned in the country or zone for at least 2 years unless there are means, validated by the OIE, of distinguishing between immune and infected pigs.

In our country application of vaccines inducing development of strong and long lasting immunity in pigs is one of the main components of veterinary and sanitary measures for control of the above-mentioned diseases. Conventional vaccine based on VGNKI virus strain is commonly used for vaccination against Aujeszky's disease. The vaccine is produced by various national manufacturers and has a relatively low price and sufficient efficacy. However, application of such vaccine does not allow the country or zone to gain AD freedom status since specific antibodies develop in pigs against the whole virus virion and their differentiation from post-infection antibodies is difficult.

Preventive anti-AD vaccination of pigs using vaccines prepared from gE-deleted AD virus strains (marker vaccine) is allowed for recovery of AD freedom status of a country or zone. This OIE provision offers commercial pig establishments an opportunity to increase export of their products by using anti-AD marker vaccine if their country is not free from AD.

For this purpose, the FGBI "ARRIAH" has developed and produces combined emulsion vaccines for veterinary use based on the strains of PRRS virus (European genotype), PPV isolated in the RF territory as well as based on marker AD virus strain. The main advantage of inactivated vaccines is their epidemiological safety, namely they are incapable of transmitting vaccine virus strain horizontally and vertically. Therefore, the said vaccines can be used for immunization of sows, boars and piglets both in the disease-free holdings and in areas at the disease risk. They have broad biological, antigenic and immunogenic activity and are capable of inducing strong and long lasting immunity in vaccinated animals.

At the initial stage of AD eradication in the EU marker monovaccines against AD alone or in combination with vaccinations against PRRS and PPV were used. In order to induce stronger immunity anti-AD vaccination schedule recommended in some countries includes immunization of sows with inactivated vaccines and immunization of their offspring with live vaccines.

The study was aimed at testing the above-mentioned vaccines comprising marker AD virus strain for their antigenic properties when they were applied on RF pig farms.

MATERIALS AND METHODS

Vaccines:

– inactivated emulsion vaccine against Aujeszky's disease and porcine parvovirus infection based on the marker AD virus strain. The vaccine is prepared from marker gE-deleted VK strain of Aujeszky's disease virus and BL-94 strain of porcine parvovirus grown in domestic goat gonad and porcine kidney cell cultures inactivated with aminoethylethylenimine and supplemented with oil adjuvant up to 70% of the vaccine volume;

– inactivated emulsion vaccine against Aujeszky's disease and porcine reproductive respiratory syndrome. The vaccine is prepared based on marker gE-deleted VK strain of Aujeszky's disease virus and KPR-96 strain of porcine reproductive respiratory syndrome virus (PRRSV) grown in continuous domestic goat gonad cell culture and rhesus monkey kidney cell culture, a trophovariant of MA-104 cell culture, inactivated with aminoethylethylenimine and supplemented with oil adjuvant up to 70% of the vaccine volume.

Animals. Clinically healthy pigs at the age of 2 months weighing 20–25 кг obtained from AD, PRRS and PPVI-free farms, rabbits weighing 2.5–3.0 kg and white mice were used for tests.

The following test-kits were used for differentiation test of porcine sera for specific antibodies against AD: HerdChek PPV gB Antibody Test Kit and HerdChek PPV

gI Antibody Test Kit (IDEXX, USA), against PRRS virus – Porcine Reproductive and Respiratory Syndrome Virus Antibody Test Kit (IDEXX, USA), against PPV – Test-Kit for Serological Diagnosis of Porcine Parvovirus Infection with Hemagglutination Test (FGBI "ARRIAH"). The results were recorded according to the test-kit manufacturer instructions.

The proposed vaccines were tested for their efficacy on pig farms. The vaccines were applied in accordance with the directions for their use.

RESULTS AND DISCUSSION

To test the developed vaccines for their reactogenicity, each tested vaccine was injected intramuscularly to 10 white mice, 6 rabbits and 2 gilts at the dose of 0.5; 2.0 and 5.0 cm^3, respectively. The animals were clinically observed for 14 days, observation results were scored based on the swelling at the site of the vaccine injection. No significant systemic and local reactions were detected. Slight local rise in body temperature and swelling of 1 cm in diameter at the site of injection occurred in rabbits. These signs completely disappeared by the end of observation period. Short-term body temperature rise by 0.7 °C as well as slight swelling at the site of injection disappearing in 3-4 days were observed in some piglets only when they had been injected intramuscularly at the dose that was twice higher than recommended one (according to the international requirements for safety test of inactivated vaccines). At the end of the observation period animals were euthanized and tissue lesions were scored based on criteria proposed by H. Stone.

At the initial stage, we tested inactivated emulsion anti-AD and PRRS vaccine based on marker AD virus strain and PRRS virus strain for its antigenic activity. Tests were performed in ADV- and PRRSV-seronegative piglets. The vaccine was administered intramuscularly to the upper third of piglet's neck at the dose of 2 cm^3; piglets were revaccinated 21 days after. The animals were clinically observed for 60 days and bled before vaccination, 21 days after the first vaccination as well as on day 14 and 60 after the second vaccination (Table 1).

All animals were ADV- and PRRSV-seronegative before vaccination. Specific anti-ADV and PRRSV antibodies were detected in 73% and 93% gilts, respectively, starting from day 21 after immunization. The mean s/p value of specific anti-PRSSV antibodies was 0.42 ± 0.02 and became 0.83 ± 0.02 on day 60 (observation period); the mean s/p value of specific anti-ADV antibodies to gB glycoprotein was 0.27 ± 0/02 and became 0.09 ± 0.01 on day 60 (observation period) that was indicative of sufficient vaccine antigenicity. No antibodies to gE glycoprotein were detected in serum samples collected on all specified dates that evidenced development of specific antibodies after the marker vaccine application.

Tests for postvaccinal immunity level based on specific antibody level were performed in non- AD and PRRS vaccinated animals on a pig farm. Piglets were immunized intramuscularly twice with the tested vaccine at the dose of 2 ml at the age of 100 days and 3 weeks after the first immunization. Blood samples were collected from pigs before vaccination, before revaccination (3 weeks after the first vaccination) and 2 weeks after revaccination (Table 2).

Table 2

Results of testing sera collected from pigs after their immunization with inactivated emulsion anti-AD and PRRS vaccine based on marker AD virus strain and PRRS virus strain.

n = 80

Group of animals	Antibody level		
	PRRS	AD	
		gE	gB
before vaccination	80/0* 0.15 ± 0.02**	80/0 0.95 ± 0.02	80/0 0.91 ± 0.02
day 21after vaccination	80/64 0.49 ± 0.02	80/0 0.86 ± 0.02	80/73 0.15 ± 0.02
day 14 after revaccination	80/73 0.76 ± 0.05	80/0 0.98 ± 0.03	80/80 0.09 ± 0.01

* number of tested/positive samples;
** mean value, $M \pm S$.
ELISA value for PRRS virus: $s/p < 0.4$ – specific antibodies are absent, $s/p \geq 0.4$ – specific antibodies are present;
ELISA value for AD virus: $s/p \geq 0.7$ – specific antibodies are absent, $s/p \leq 0.6$ – specific antibodies are present.

Table 3

Results of testing sera from pigs of different age groups for specific antibodies after their immunization with inactivated emulsion anti-AD and PPV vaccine based on marker ADV strain and PPV strain

Age group	Number of vaccinated animals	Specific antibody level				
		before vaccination		40 days after vaccination		
		PPV	ADV (gB)	PPV	ADV	
					gB	gE
Piglets at the age of 40–60 days	80	5.8 ± 0.3	0.96 ± 0.08	10.7 ± 0.3	0.23 ± 0.05	0.92 ± 0.02
Replacement gilts	80	7.6 ± 0.5	0.82 ± 0.08	11.8 ± 0.4	0.33 ± 0.08	0.97 ± 0.02
Sows	20	10.5 ± 0.3	0.83 ± 0.08	12.2 ± 0.5	0.42 ± 0.0	0.87 ± 0.02
Boars	10	9.2 ± 0.4	0.88 ± 0.05	11.8 ± 0.6	0.36 ± 0.06	0.81 ± 0.02

* number of tested/positive samples;
** mean value, $M \pm S$.
ELISA value for AD virus: $s/p < 0.7$ – specific antibodies are absent, $s/p \geq 0.6$ – specific antibodies are present;
HI test value for PPV: >7.0 log_2 – specific antibodies are present.

Table 4
Results of testing sera collected from pigs after administration of inactivated emulsion anti-AD and PPV vaccine based on marker ADV strain and PPV strain
n = 50

Group of animals	Antibody level		
	PPV	ADV	
		gE	gB
before vaccination	50/0* 7.8 ± 0.2**	50/0 1.02 ± 0.02	50/0 0.98 ± 0.01
day 21after vaccination	50/45 9.8 ± 0.15	50/0 1.0 ± 0.04	50/42 0.37 ± 0.08
day 14 after revaccination	50/50 10.6 ± 0.3	50/0 0.92 ± 0.03	50/50 0.12 ± 0.005

* number of tested/positive samples;
** mean value, *M* ± *S*.
ELISA value for AD virus: $s/p < 0.7$ – specific antibodies are absent,
$s/p \geq 0.6$ – specific antibodies are present;
HI test value for PPV: >7.0 $\log_2$ – specific antibodies are present.

The animals were continuously observed for clinical signs. Slight inflammation symptoms accompanied with slight painless swelling at the site of injection were observed in 19% of the pigs on the next day after vaccination. Therewith, no pyrexia, refusal to feed and dullness were observed in vaccinated animals.

All animals were seronegative to PRRS and AD viruses before vaccination. Three weeks after vaccination specific antibodies to PRSS virus and AD virus were detected in 80% and 91% of animals, respectively. Mean *s/p* ratio for gB glycoprotein was 0.15 ± 0.02 on day 21 after vaccination and 0.09 ± 0.01 on day 14 after revaccination that was indicative of sufficient antigenic activity of the vaccine. Two weeks after revaccination, 91.3% of animals had specific antibodies against PRRS virus and 100% of animals had specific antibodies against AD virus. No anti-gE glycoprotein antibodies were detected in sera collected on all specified days that evidenced that specific antibodies were developed after the marker vaccine administration. Thus, single and double vaccination induced apparent immune response in vaccinated pigs.

At the next stage, inactivated and emulsion anti-AD and PPVI vaccine based on marker ADV strain and PPV strain was tested for its antigenic activity and reactogenicity. Tests were carried out in animals of different ages kept on pig farms. Blood samples were collected from pigs before vaccination and 40 days after vaccination (Table 3). Vaccination was performed according to the directions for its use.

Clinical observations showed that body temperature and main physiological characteristics of vaccinated pigs remained normal, slight swelling up to 1-2 cm of diameter was palpated at the site of the vaccine injection in some animals.

Analysis of the data given in Table 3 showed that before vaccination specific antibodies against parvovirus were detected only in groups of sows and boars. All animals were seronegative to AD virus. Specific antibodies against parvovirus (in dynamics) and ADV gB (at sufficient levels) were detected in animals of all groups whereas no antibodies against gE glycoprotein were detected in the animals 40 days after vaccination that confirmed that only specific antibodies against vaccine ADV strain were present. The administered vaccine induced strong immunity in animals of all vaccinated groups.

Tests for postvaccinal immunity level based on specific antibody level were performed on a pig farm where a group of animals was formed. The group comprised 50 piglets at the age of 2-4 months not vaccinated against AD and PPVI. The animals were immunized twice intramuscularly at the dose of 2 ml. Revaccination was performed 3 weeks after. Blood samples were collected form piglets before vaccination and 2 weeks after revaccination (Table 4).

The animals were continuously observed for clinical signs. Body temperature and main physiological characteristics of vaccinated pigs remained normal within the whole observation period.

All animals were seronegative to porcine parvovirus and AD virus before vaccination. Specific antibodies against parvovirus were detected in 90% of pigs 21 days after vaccination; group mean titre was 9.8 ± 0.15 $\log_2$; specific antibodies against AD virus were detected in 84% of pigs; mean *s/p* ratio for gB was 0.37 ± 0.08. Two weeks after revaccination, 100% of pigs immunized with the proposed vaccine had specific antibodies against both parvovirus and vaccine ADV strain that was evidenced by absence of antibodies against gE glycoprotein.

CONCLUSION

Combined inactivated emulsion anti-AD and PPV vaccine based on marker ADV strain and PPV strain as well as combined inactivated emulsion anti-AD and PRRS vaccine based on marker ADV strain and PRRS strain developed by the FGBI "ARRIAH" are safe and highly effective. The proposed biological can be used for preventive immunization of pigs on PRRS-, AD- and PPV-free pig farms, pig farms at risk of and infected with PRRSV, ADV and PPV. Administration of the said vaccines allows differentiation between animals with antibodies to vaccine (marker) strain and field strains of Aujeszky's disease virus. Therefore, it is also recommended to use the said vaccines within programmes on AD control and eradication on farms of various ownership for AD freedom status recovery.

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