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For the Publisher / Za izdavača
dr Dragica Stojanović, Senior Research Associate, Director of the Institute

Reviewers / Recezenti
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Dr Tamaš Petrović
Scientific Veterinary Institute „Novi Sad“, Novi Sad, Serbia

Editor in Chief / Glavni i odgovorni urednik
Dr Tamaš Petrović, Senior Research Associate

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SPREČAVANJE ŠIRENJA KLASIČNE KUGE SVINJA U POGRANIČNOM REGIONU KROZ POBOLJŠANJE SANITARNIH STANDARDA I EDUKACIJU FARMERA (STOP – KKS)

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“PREVENTION OF CLASSICAL SWINE FEVER IN THE BORDER REGIONCROATIA – SERBIA (STOP – CSF)”
„SPREČAVANjE ŠIRENjA KLASIČNE KUGE SVINjA U POGRANIČNOM REGIONU HRVATSKA – SRBIJA (STOP – KKS)“

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QUALITY CONTROL OF TWO COMMERCIAL VACCINES AGAINST CLASSICAL SWINE FEVER

Dragica Stojanović[1], Radomir Ratajac[1], Tamaš Petrović[1], Jasna Prodanov-Radulović[1]

[1.] *Scientific Veterinary Institute „Novi Sad“, Novi Sad*

Abstract

In this paper we presented the results of quality control of attenuated live vaccines against classical swine fever (CSF) performed under controlled experimental conditions on experimental animals. The vaccines tested contain Chinese-strain of the CSF Virus and are used in systematic immunoprophylaxis in the Republic of Serbia. Identification of the vaccinia virus, examination of safety and potency was carried out according to guidelines of the European Pharmacopoeia (Ph. Eur. 6.0, 01/2008:0065) and guidelines of the World Organization for Animal Health (OIE) (Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, 5th Edition, 2008; Vol. 2, Chapter 2.8.3).

Two commercial vaccines against CSF were tested. Biological testing and identification of CSF virus in vaccine was performed on rabbits. The safety and efficacy/potency testing was performed on weaning piglets aged 7 weeks. For each vaccine, safety testing was performed on three piglets (which were administered 10-fold vaccine dose). Efficacy testing was performed on 12 piglets for each vaccine. The piglets were distributed into two groups of 5 animals, and a control group of two piglets. The piglets from Group 1 were vaccinated with the investigated attenuated live vaccines diluted 1:40, piglets from Group 2 with vaccine diluted 1:160, whilst piglets from the control group were not vaccinated. Fourteen days after vaccination all animals were artificially infected with highly virulent strain of CSF virus (Baker strain), and were continuously clinically observed (including body temperature monitoring) throughout subsequent 14 days.

Validity of the vaccine was confirmed in biological tests on rabbits, i.e. the classical swine fever virus was identified. Examination of vaccine safety on piglets revealed that examined vaccines are safe, even at multiple ten-fold dosage, and without appearance of clinical symptoms of the disease during entire monitoring period.

According to the obtained results on vaccine efficacy and using the Spearman-Kärber's formula, the protective doses (PD_{50}) per single dose were determined, being 182 for the vaccine Y and 105 for the vaccine X. Having in mind that one vaccine dose needs to have a protective value $\geq$100, it can be concluded that the examined vaccines are in accordance with the requirements of the European Pharmacopoeia and Manual of Diagnostic Tests and Vaccines for Terrestrial Animals of the OIE.

Key words: classical swine fever, attenuated live vaccine, identification, safety and potency testing

Introduction

Classical swine fever (CSF) is a contagious viral disease of domestic and feral pigs. The disease is eradicated from pig farms in Australia, Canada, North America and most countries in Western Europe; however, it still sporadically occurs in some Balkan countries. The causative agent is a virus particle with a single stranded RNA with positive polarity surrounded by a capsid, classified to the genus *Pestivirus* within the family *Flaviviridae* (Moening, 2000). The virus survives well in cold conditions and at low temperatures during the industrial food processing. Possible transmission routes include the trade in live pigs and pork meat, clothing, transport vehicles, insects, some worms, as well as swill feeding. CSF infection with weakly virulent strains can be difficult to recognize in saws, thus virus transmission via trading individual animals is likely. Purchasing pigs from different locations and uncontrolled farms is associated with particular risk of disease transmission because of their unknown CSF status (Dahle and Liess, 1992). Foetal infection may occur during all stages of pregnancy. Infection during late pregnancy can lead to the birth of clinically normal piglets; however, the piglets remain persistently viraemic and permanently spreading the virus within the herd, thus being a dangerous reservoir of the infection. Such piglets may show poor growth, sometimes with lethal outcome (Moenning et al., 2003).

Eradication strategies relying on vaccination programs have been applied worldwide since early 40-ties of the 20th century. Mass prophylactic vaccination resulted in eradication of the virus in the developed regions of the globe, and since the beginning of 1990-ies, the vaccination is discouraged in the European Union (Dong et al., 2007). The strategy of control and eradication in these countries involves the ,, stamping out" of affected animals and pigs located within an area of 1000 m, as well as strict restriction of animal movement and trade of pig products (Chenut et al, 1999, Moening et al., 2003). Keeping the disease under control is permanently the priority in the developing countries.

During the past 10-15 years, and particularly in the last few years, CSF has been a severe health and economic problem in our country. Economic losses caused by the disease are dramatic. There are data on the number of animals that were slaughtered with an aim to prevent the infection spreading, but the reliable data on the actual number of pigs died from CSF will most probably remain unknown.

Vaccination of pigs against CSF is mandatory in Serbia. The program of control and eradication of classical swine fever relies on vaccination of all pig categories (weaners, fattening pigs, breeding animals) with attenuated vaccine containing Chinese strain (C-strain) of the CSF virus. Upon identification of infection focus, the eradication is performed by safe depopulation of the infected herd along with application of measures prescribed by the Regulation. In spite of adherence to all aforementioned measures, classical swine fever still presents a serious problem.

Two vaccines developed by domestic manufacturers are registered and commercially available at our market. The efficacy (application of full dose after artificial infection) of domestic vaccines against CSF was examined in some previous research. The vaccines proved to be effective against CSF virus (Lazić, unpublished research).

In this paper, we presented the results of biological tests (identification, safety and potency) of two commercial vaccines available at our market. Both vaccines were produced using Chinese-strain of the CSF Virus by serial passage in rabbits. Examination of efficacy of the vaccines against classical swine fever was aimed at assessing the immunogenic potential of the vaccines by determining protective effects in conditions of artificial infection and by monitoring the effectors of the humoral immune response.

Material and methods

Vaccines

Two commercial vaccines against CSF were tested. Both vaccines are produced by serial passage in rabbits.

Animals

Biological testing of CSF vaccine virus identification was performed using *Panonski beli*-breed rabbits weighing about 2 kg, in good physical condition. For biological testing of safety and potency/efficacy of the vaccine the piglets, about 45 days of age were used. The piglets were purchased from the selected local pig-farm. The animals were housed in the experimental block of the Institute during one-week adaptation period. The water was available ad libitum, and feed was portion-controlled. Prior to inclusion into the experimental protocol of immunization and artificial infection piglets were examined for the presence of specific antibodies against CSF and against Bovine Viral Diarrhoea Virus (BVDV) applying immunoenzyme assay and serum-neutralization test, respectively. Neither assay revealed positive antibody finding.

Identification

For each vaccine examined, 10 rabbits were used, distributed into three groups. The Group 1 (4 rabbits) was inoculated intravenously (i.v.) with 1/10 dose, Group 2 (4 rabbits) was inoculated i.v. with 1/50 dose, and Group 3 (2 rabbits) was not vaccinated, serving as the Control Group. Rabbits were administered 0.1 ml of diluted vaccine by injection into the ear vain. In all rabbits, body temperature (rectal) was monitored daily throughout a 7-day period. On Day 8, full dose of the vaccine was administered to all rabbits (1 dose for pigs as recommended by the manufacturer) – 1 ml i.v. into the marginal ear vein. Body temperature was controlled twice daily throughout subsequent five days.

Safety

For each vaccine, three piglets, which satisfied the testing criteria (free from antibodies against CSF and BVD), were used. Ten-fold dose of reconstructed vaccine was administered i.m. to each piglet. The animals were monitored during 21 days.

Virus used for artificial infection

Artificial infection was induced using the standard highly pathogenic Baker-strain. It was previously established that application of 2-ml dose of the virus has resulted in death of all susceptible animals within 6 days.

Experimental design

The experiment was conducted pursuant to the European Pharmacopoeia (Ph. Eur. 6.0, 01/2008:0065). The animals were distributed into two groups of 5

animals. Five piglets were immunized with vaccine diluted 1:40 (1-ml dose, i.m.) and five piglets with vaccine diluted 1:160 (1-ml dose, i.m.). Two piglets were used as a control for artificial infection, i.e. they were not vaccinated, yet artificially challenged with pathogenic virus. Immediately before the artificial infection, blood samples were collected from all piglets to the purpose of detecting specific antibodies against classical swine fever virus. Fourteen days after vaccination, all animals were artificially challenged with the highly pathogenic CSF Virus (Baker-strain) by receiving 2-ml intramuscular dose. Throughout the entire investigation period continuous clinical observation of all experimental piglets has been performed. Body temperature was monitored and recorded daily until the end of experimental period, 14 days after artificial infection. Besides the rectal body temperature, the following clinical symptoms were monitored: behaviour of the animals (lethargy, apathy), changes of the skin and visible mucosa (erythema, cyanosis, bleeding), eye changes (conjunctivitis), respiratory changes (caught), changes of digestive tract (obstipation / diarrhoea) and locomotor apparatus, CNS disorders (paresis, paralysis, convulsions) as well as overall body condition (cahexia). After clinical observation, blood samples were collected from all experimental animals with an aim of detecting specific antibodies against CSF. On Day 14 after artificial infection, the survived animals were sacrificed with an aim of determining the pathomorphological changes. Samples of parenchymatous organs (portions of spleen, kidneys, mandibular lymph nodes and tonsils) were obtained for the purpose of detecting the CSF Virus.

The same experimental procedure was applied for both vaccines. The experiment was approved by the *Medicines and Medical Devices Agency of Serbia*. The average protective dose (PD/50) for the vaccines tested was determined using the Spearman-Kärber's formula and according to the guidelines of European Pharmacopoeia

Detection of antigens and antibodies

In both experiments, the CSF antigen and specific antibodies was detected applying ELISA test. The antigen was detected in pooled organ samples (spleen, kidneys, mandibular lymph nodes, tonsils) obtained after section. The test was performed according to manufacturer's instructions.

Results

Identification

The CSF virus was identified in the vaccines because of its specific pyrogenic character, as it resulted in elevation of body temperature on Days 3 and 4 post vaccination with diluted doses, as well as in non-immunized rabbits on Days 2 and 3 after administration of full-dose of the vaccine rectal body temperature was over 40.5^0C. In rabbits previously inoculated with diluted vaccine, the temperature remained within the physiological range after applying the full dose.

Safety

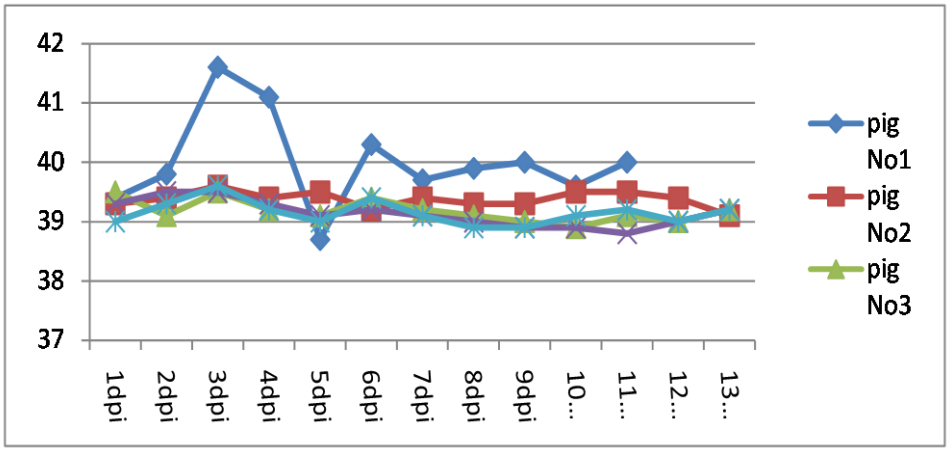
Animals were observed during a 21-day period. In both investigated vaccines, animals were in good overall condition, revealing normal growth, and the

temperature curve remained within physiological range throughout the monitoring period

Potency - efficacy

To the purpose of objectivity and impartiality, the vaccines were labelled X and Y. In piglets vaccinated with vaccine X diluted 1:40, increase of body temperature (41.6^{0}C) was observed in one animal (Graph. 1.), and in the group administered vaccine diluted 1:160 body temperature was increased in 3 animals (41.2^{0}C, 41.6^{0}C and 41.6^{0}C) (Graph. 2.). In other animals from this group, the body temperature remained within physiological range. Animals with elevated body temperature manifested decrease or loss of appetite, apathy, lethargy, conjunctivitis, obstipation and decumbency on Days 3 and 4 after AI. Diarrhoea was noticed on Days 4 and 5 post infection. On Day 6 (in one piglet) and Day 7 (in other diseased piglets), locomotor disorders with signs of ataxia and posterior paresis with intermittent convulsions were apparent. On Day 12, the disease resulted in lethal outcome in one piglet manifesting most pronounced clinical symptoms. The piglet was from the group vaccinated with vaccine dilution 1:40. Moreover, diseased piglets manifested skin changes (cyanosis, erythema and bleeding) that persisted since Day 5 until death, i.e. sacrifice. In 4 piglets from the group vaccinated with dilution 1:40 and in 2 piglets vaccinated with dilution 1:160 there were neither clinical signs of the disease nor changes with respect to water and food intake, as compared to the acclimatization period.

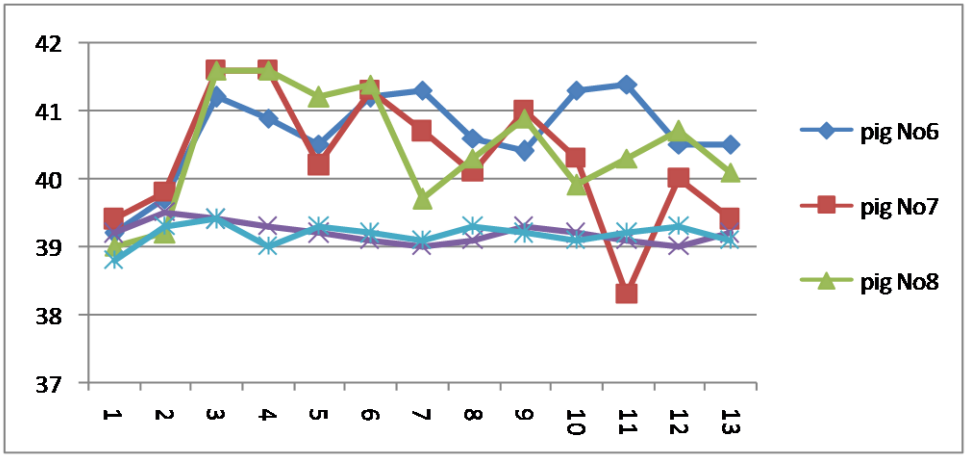
Graph 1. Rectal temperature (^{0}C) of artificially infected piglets 14 days after immunization with 1:40 dilution of vaccine X



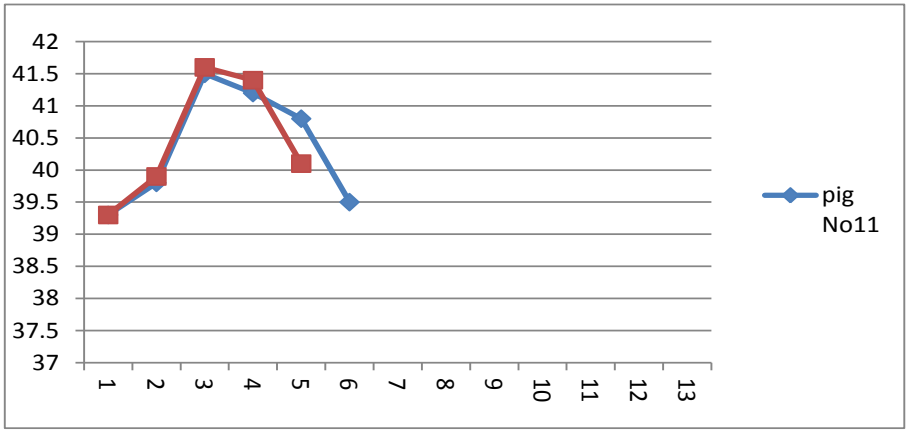
In the control group, the increase of body temperature (41.5^{0}C and 41.6^{0}C) was first observed on Day 3 after challenge with CSF virus (Graph. 3.). Body temperature of piglets has persistently been increased during the entire experimental protocol, reaching physiological range immediately before death. Clinical symptoms such as appetite decrease / loss, apathy, lethargy, conjunctivitis, obstipation and decumbency were observed on Days 2 and 3. Diarrhoea was noticed on Day 3 after infection, and locomotor disorders associated by ataxia and posterior paresis with

intermittent convulsions dominated on Day 4. Furthermore, skin changes (cyanosis, erythema and pronounced bleeding) were noticed in both piglets, starting from Day 4 until death. In both piglets, the course of the disease was severe, and death occurred on Days 6 and 7 after AI.

Graph 2. Rectal temperature (^{0} C) of artificially infected piglets 14 days after immunization with 1:160 dilution of vaccine X



Graph 3. Rectal temperature (^{0} C) of artificially infected piglets in the Control group (vaccine X)



In blood sera collected before artificial infection, presence of specific antibodies against CSF was detected in one animal from group vaccinated with dilution 1:40 (Table 1.), as well as two suspect reactions (in one piglet from the same group and in one piglet from the group vaccinated with dilution 1:160). Examination of blood sera from other piglets revealed negative finding of specific antibodies against CSF virus. Specific antibodies against CSF were detected in blood sera obtained before sacrificing (Day 14 after vaccination) in 3 animals vaccinated with 1:40 dilution and in 3 animals vaccinated with vaccine dilution 1:160. Suspect reaction was observed in two animals from each experimental group,

whilst all other animals were negative. Reaction to presence of antigens against CSF in organ samples was positive in dead piglet from group vaccinated with 1:40 dilution, as well as in all three diseased piglets from the second vaccinated group. Suspect reaction was noticed in one sample originating from the group vaccinated with vaccine diluted 1:160. In other piglets, antigens against CSF could not be detected in organ samples. ELISA test demonstrated presence of antigens against CSF in all examined organ samples originating from piglets from the control group (portions of spleen, kidneys, mandibular lymph nodes and tonsils).

Table 1. Results on presence of clinical symptoms of CSF, Ab presence before and after AI and Ag presence in pooled samples from piglets vaccinated with vaccine X

Piglet No.	Piglets vaccinated with vaccine X				
	Presence of antibodies against CSF before artificial infection	Clinical symptoms	Day of death after artificial infection	Day 14 post artificial infection	
				Presence of antibodies against CSF in serum**	Antigen against CSF in pooled organ samples**
	Vaccine dilution 1 : 40				
1.	- At	+	day 12		+ Ag
2.	+ At	-	*	+ At	- Ag
3.	- At	-	*	± At	- Ag
4.	± At	-	*	+ At	- Ag
5.	- At	-	*	+ At	- Ag
	Vaccine dilution 1 : 160				
1.	- At	+	*	± At	+ Ag
2.	- At	+	*	+ At	+ Ag
3.	- At	+	*	- At	+ Ag
4.	± At	-	*	+ At	- Ag
5.	- At	-	*	+ At	± Ag
	Control (non-vaccinated piglets)				
1.	- At	+	day 7		+ Ag
2.	- At	+	day 6		+ Ag

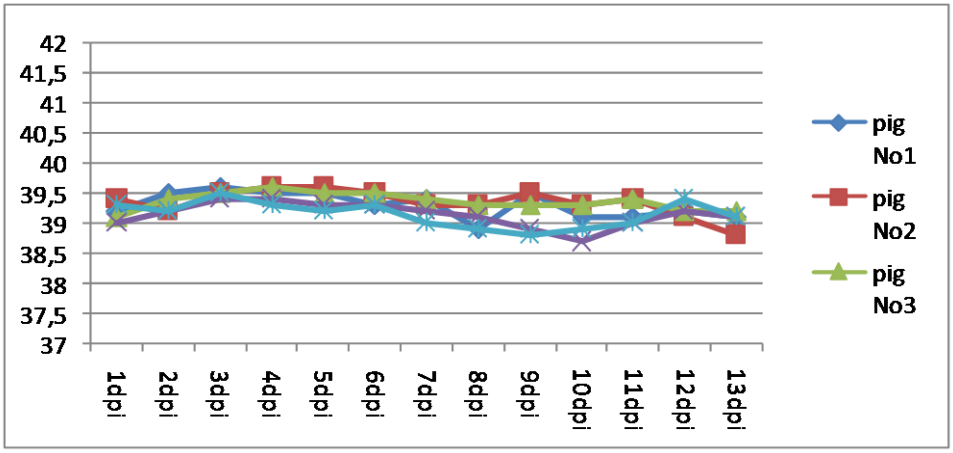
* sacrificed 14 days after artificial infection; ** Ab (positive +, negative - , suspect ±) in the serum, Ag (positive +, negative - , suspect ±) in pooled organ samples

Pathomorphological examination of dead and diseased piglets revealed changes indicating CSF virus infection. The changes included hemorrhagic infarctions in the spleen, lymph node bleeding (mandibular, mesentherial, inguinal), subcapsular bleedings and bleeding at kidney cross-section and in renal pelvis as well as hemorrhagic-necrotic tonsillitis.

Using the Spearman-Kärber's formula, the protective dose (PD_{50}) per single dose was determined for the vaccine X, being 105. In the experimental group vaccinated with the vaccine Y, clinical symptoms of CSF were not observed in animals vaccinated with dilution 1:40 (Graph. 4.), whereas two piglets vaccinated with 1:160 dilution demonstrated clearly pronounced symptoms of CSF. One piglet died on Day 7 after artificial infection. In two non-vaccinated piglets from the control group, typical acute form of CSF was apparent, resulting in death of the animals 7 days after infection. In the group vaccinated with vaccine diluted 1:160,

increase of body temperature (40.3^{0}C and 41.3^{0}C) was observed in two animals on Day 3 (Graph. 5.), whereas appetite loss, apathy, lethargy, conjunctivitis, obstipation and decumbency were noticed on Days 3 and 4. Diarrhoea was apparent on Day 5. In one piglet, locomotor disorders associated by ataxia and posterior paresis with intermittent convulsions occurred on Day 6 resulting in death of the animal on Day 7 after infection. Skin changes manifested as cyanosis, erythema and pronounced bleeding were apparent in both piglets starting from Day 5, and persisted until death, i.e. sacrifice. In the control group, the increase of body temperature (41.3^{o}C and 41.4^{o}C) was first observed on Day 3 after artificial infection (Graph. 6.). Body temperature was elevated throughout entire experimental period, reaching physiological range immediately before death. First clinical symptoms, such as decrease in appetite / appetite loss, apathy, lethargy, conjunctivitis, obstipation and decumbency were observed in the control group on Days 2 and 3, post infection. Diarrhoea was noticed on Day 4 post infection, whilst pronounced signs of aphonia were apparent on Day 6. Later on, clinical picture was characterized by dominant locomotor disorders associated by ataxia and posterior paresis with intermittent convulsions. Skin changes including cyanosis, erythema and pronounced bleeding were apparent starting from Day 5 until death of the animal.

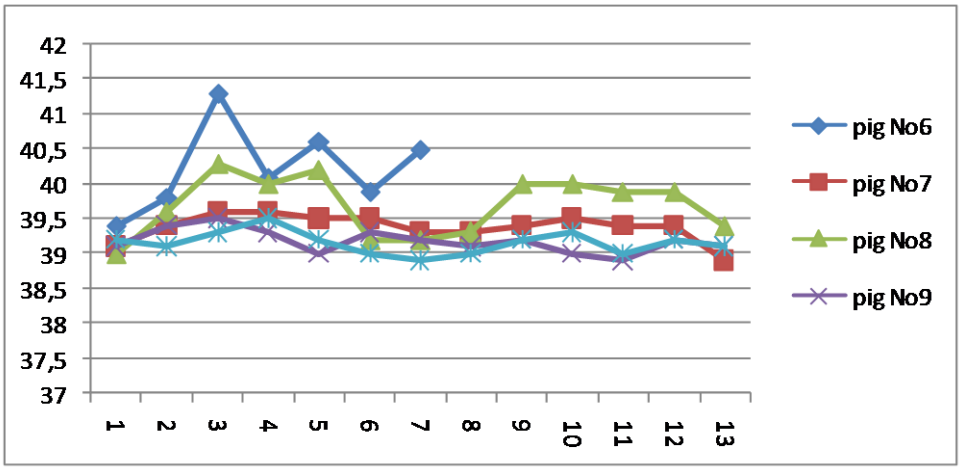
Graph 4. Rectal temperature (^{0} C) of artificially infected piglets 14 days after immunization with 1:40 dilution of vaccine Y



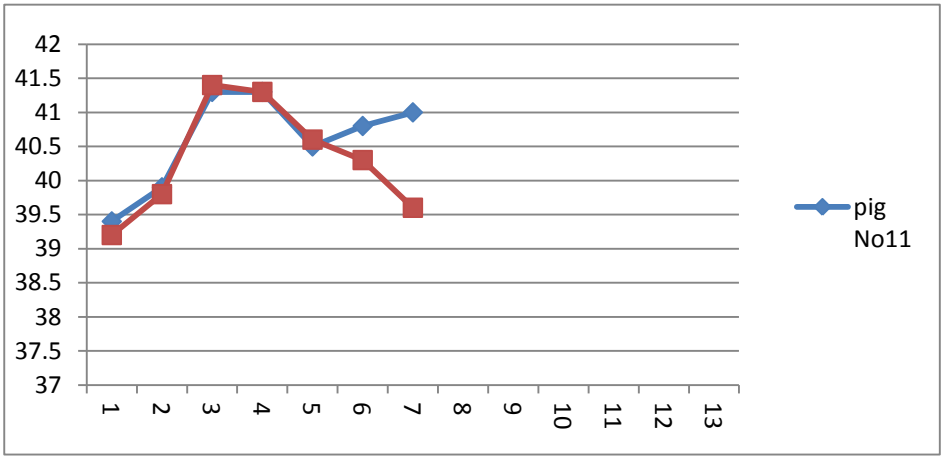
In the blood sera obtained before artificial infection, presence of specific antibodies against CSF virus was confirmed in one animal vaccinated with dilution 1:40 (Table 2.), as well as one suspect reaction in one animal from the same group. Positive finding of specific antibodies against CSF virus was obtained also in two piglets vaccinated with vaccine dilution 1:160. Negative findings were obtained in all other animals. Blood samples obtained 14 days after artificial infection revealed presence of specific antibodies against CSF virus in all vaccinated piglets that have survived the challenge. Reaction to presence of antigens in organ samples was positive in a dead piglet from group vaccinated with 1:160 dilution. Suspect finding was obtained in two animals from the same group. Other examined organ samples

originating from vaccinated piglets did not reveal presence of the antigen against classical swine fever.

Graph 5. Rectal temperature (^{0} C) of artificially infected piglets 14 days after immunization with 1:160 dilution of vaccine Y



Graph 6. Rectal temperature (^{0} C) of artificially infected piglets in the Control group (vaccine Y)



After sacrificing, pathomorphological examination was carried out revealing changes indicating infection with CSF virus in only two piglets vaccinated with vaccine diluted 1:160. ELISA test revealed presence of antigen against CSF virus in all examined organ samples originating from diseased piglets from the control group (portions of spleen and kidneys, mandibular lymph node and tonsils).

Using the Spearman-Kärber's formula, the protective dose (PD_{50}) per single dose was determined for the vaccine Y, being 182.

Table 2. Results on presence of clinical symptoms, Ab presence before and after AI and Ag presence in pooled samples from piglets vaccinated with vaccine Y

Piglet No.	Piglets vaccinated with vaccine Y				
	Presence of antibodies against CSF before artificial infection	Clinical symptoms	Day of death after artificial infection	Day 14 post artificial infection	
				Presence of antibodies against CSF in the serum**	Antigen against CSF in pooled organ samples **
	Vaccine dilution 1 : 40				
1.	- At	-	*	+ At	- Ag
2.	- At	-	*	+ At	- Ag
3.	- At	-	*	+ At	- Ag
4.	+ At	-	*	+ At	- Ag
5.	± At	-	*	+ At	- Ag
	Vaccine dilution 1 : 160				
1.	± At	+	*	+ At	± Ag
2.	- At	-	*	+ At	- Ag
3.	± At	-	*	+ At	- Ag
4.	- At	-	*	+ At	± Ag
5.	- At	+	Day 7		+ Ag
	Control (non-vaccinated piglets)				
1.	- At	+	Day 7		+ Ag
2.	- At	+	Day 7		+ Ag

* sacrificed 14 days after artificial infection; ** Ab (positive +, negative - , suspect ±) in the serum, Ag (positive +, negative - , suspect ±) in pooled organ samples

Discussion

Our experiment demonstrated that two tested commercial vaccines against CSF have satisfied the criteria of the European Pharmacopoeia 01/2008:0065, as the PD_{50} value was higher than 100. Chinese-strain has been widely used for protection of pigs against CSF. The strain passages were performed on cell cultures or in rabbits. The virus attenuated by passage through rabbits is termed lapinized live apathogenic strain of classical swine fever virus, and has since years been used in production of commercial products. Vaccines based on Chinese-strain provide long-term protection and good tolerance in young animals and pregnant saws (Dong et al., 2007). Meindi-Boehmer and Markus-Chisel (2006) investigated the immunogenic properties of the *Cholerevac – Plivak* vaccine against CSF. In their experiment, a protective dose 50 (PD_{50}) reaching $\geq$ 320 was achieved after artificial challenge with highly pathogenic Koslov-strain. Clinical symptoms in piglets resulting from Koslov-strain infection were similar to those presented in our experimental results. Clinical symptoms of CSF resulting from Baker-strain infection in our research are similar to previously published results of Milanov et al. (Milanov et al., 2002). In this research, animals were 3-4 months old at the time of artificial infection. The piglets used in the experiment originated from non-vaccinated saws and were not vaccinated themselves. Three of twelve piglets have survived the infection, thus it is likely that they have been exposed to a moderately virulent CSF virus before artificial challenge or to a BDVD infection. This is supported by serologically positive result of ELISA test in these three animals, performed before artificial infection.

In this experiment, three piglets vaccinated with the vaccine Y diluted 1:40 proved negative for the presence of antibodies; however, they have been protected against infection with highly pathogenic virus. In a group vaccinated with vaccine Y diluted 1:160 two animals that were negative to presence of antibodies at the moment of artificial infection did not manifest any clinical symptoms of the disease. In two piglets from the group administered vaccine X diluted 1:40 and in one piglet from the group vaccinated with 1:160 dilution, neither presence of antibodies before artificial challenge nor clinical signs of CSF were observed. In these animals, antigen against CSF could not be detected in organ samples using ELISA test. Suspect finding was obtained only in one animal vaccinated with vaccine X diluted 1:160 and one animal vaccinated with vaccine Y diluted 1:160. Our results on the presence of antibodies obtained in ELISA test did not correlate with protection against CSF virus. More sensitive tests, such as NPLA or immunofluorescent assay, might have been more effective in antibody detection. In the experiment of Meindl-Boehmer and Markus-Chisel (2006), the NPLA revealed low antibody titre values after 13 days post infection. During the course of CSF virus infection, cell-mediated immunity is activated in the first stage, followed by maturation of B-cells and production of antibodies. An increase in IgM cells 9 days after AI, as well as increase in immunoglobulins of B-cells(C-□$^{+}$) after infection with highly pathogenic Alforth 187-strain (Sanchez-Cordon et al., 2006) was apparent; however, it was not associated with detectable levels of virus neutralizing antibodies 14 days after AI. The authors also reported that IL4 production increased on 11-14 days post AI resulting in transformation of T1 cell-mediated immune response to the T2. These cytokines induce maturation of B-cells into plasma cells producing immunoglobulins (Sanchez Cordon et al., 2005a). Such a scenario could be an explanation for delayed production of antibodies in the presented experiment. Satisfactory protection level is most likely due to a cell-mediated immune response to the vaccinia strain. Suradhat et al. (2001) offered evidence for strong interferon gamma (IFN γ) as early as 6 days post vaccination. Their experiment revealed a good correlation between production of IFN γ and protection of pigs against highly virulent CSF virus in artificial infection.

Interpretation of the results obtained by ELISA test can be difficult in field conditions in a view of assessment of the protection of vaccinated pigs. If vaccinated pigs are exposed to the wild-non-vaccinia virus strain, serologic monitoring of the infection could be difficult, even impossible. ELISA test, though easy to apply and suitable for large number of samples, is not always accurate and false positive result may occur. During a CSF outbreak in Holland in 1997/98, about 1.5 million samples were examined using ELISA test. Among ELISA-positive samples, positive CSF finding was confirmed in VN test in 15% of sera, whereas 35% reacted to Bovine diarrhoea and BVD virus. VN test revealed negative finding in 50% samples (de Smit et al., 2000).

In these experiments, all animals that were Ag-ELISA positive demonstrated clinical symptoms of the disease. This test, when animals are antigen-positive, undoubtedly indicates recent infection. In one animal with manifest clinical symptoms, which was vaccinated with vaccine Y 1:160, the results obtained in ELISA test were suspect. Detection of antigens was performed in pooled organ samples. An uneven virus distribution (probably it was present only in the thymus)

in those animals is likely, thus detection limit was considered low. Vaccinia virus is detectable by the use of Real time PCR only in thymus, up to 42 days post inoculation, which was demonstrated in the experiments of Koenig et al., 2007. Examination of individual organs using highly sensitive tests would enable better understanding of the distribution pattern of a wild non-vaccinia virus type in vaccinated animals.

Clinical symptoms induced by artificial infection with Baker-strain were similar to those reported by other researchers. ELISA test reveals high degree of probability in detecting recent infections, whilst antibody detection in this test was not reliable enough.

Results of the examination of two commercial vaccines against CSF indicated that they are safe and harmless. The protective effect – efficacy was confirmed by vaccine application using various dilutions, according to the requirements of European Pharmacopoeia.

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KONTROLA KVALITETA DVE KOMERCIJALNO DOSTUPNE VAKCINE PROTIV KLASIČNE KUGE SVINJA

Dragica Stojanović[1], Radomir Ratajac[1], Tamaš Petrović[1], Jasna Prodanov-Radulović[1]

1. Naučni institut za veterinarstvo Novi Sad, Novi Sad

Kratak sadržaj

U radu su prezentovani rezultati kontrole kvaliteta atenuiranih živih vakcina protiv klasične kuge svinja (KKS) u kontrolisanim eksperimentalnim uslovima na životinjama. Ispitivane vakcine u svom sastavu sadrže Kina soj virusa KKS i u Republici Srbiji se koriste za sistemsku imunoprofilaksu. Identifikacija vakcinalnog virusa, ispitivanje neškodljivoti i potentnosti vakcina izvršeno je u skladu sa smernicama Evropske farmakopeje, 6. izdanje, 2008. (01/2008:0065) i smernicama koje je objavila Međunarodna kancelarija za epizootije (OIE) (Priručnik o dijagnostičkim testovima i vakcinama za kopnene životinje, 5. izdanje, 2008.; Vol. 2, Poglavlje 2.8.3.).

Ispitivane su dve komercijalno dostupne vakcine protiv KKS. Biološki test identifikacije virusa KKS u vakcinama je urađen na kunićima. Testovi neškodljivosti i efikasnosti-potentnosti vakcina su izvedeni na zalučenoj prasadi, uzrasta 7 nedelja. Za svaku ispitivanu vakcinu za test neškodljivosti korišćena su tri praseta (kojima je aplikovana desetostruka doza vakcine), a za ispitivanje efikasnosti 12 prasadi po vakcini koja su bila podeljena u dve grupe od 5, odnosno 2 praseta u trećoj kontrolnoj grupi. Prasad prve grupe su vakcinisana ispitivanim atenuiranim živim vakcinama u razređenju 1:40, a prasad druge grupe su vakcinisana istim vakcinama u razređenju 1:160, dok prasad treće kontrolne grupe nisu vakcinisana. Četrnaest dana nakon vakcinacije sve životinje su veštački inficirane sa visoko virulentnim sojem virusa KKS – soj Baker, a tokom dve nedelje nakon veštačke infekcije, životinje su svakodnevno klinički opservirane uključujući i termometriranje.

Biološki testovi na kunićima pokazali su da su vakcine valjane, odnosno identifikovan je virus KKS. Ispitivanja neškodljivosti na prasadima ukazuju na sigurnost primene ispitivanih vakcina i u višestrukim-desetostrukim dozama bez pojave kliničkih simptoma bolesti tokom perioda posmatranja.

Primenom jednačine po Spearman-Karberu**,** na osnovu dobijenih rezultata ispitivanja efikasnosti vakcina, utvrđeno je da zaštitna vrednost (PD_{50}) jedne doze vakcine Y iznosi 182, a vakcine X 105. Ako se ima u vidu da jedna vakcinalna doza treba da sadrži protektivnu vrednost ≥100, može se zaključiti da efikasnost ispitivanih vakcine ispunjava zahteve Evropske farmakopeje i zahteve OIE Priručnika o dijagnostičkim testovima i vakcinama za kopnene životinje.

Ključne reči: klasična kuga svinja, atenuirana živa vakcina, identifikacija, ispitivanje neškodljivosti i potentnosti

Uvod

Klasična kuga svinja (KKS) je kontagiozna bolest domaćih i divljih svinja. Bolest je iskorenjena sa farmi svinja u Australiji, Kanadi, Severnoj Americi i u većini zemalja zapadne Evrope, ali na Balkanu se još uvek javlja sporadično. Uzročnik bolesti je virus, sa jednolančanom RNK pozitivnog polariteta, koja se nalazi unutar kapsida, koji pripada rodu *Pestivirus*, familiji *Flaviviridae* (Moening, 2000). Virus preživljava hladnoću i tretman niskom temperaturom tokom obrade mesa. Trgovina, odeća, oprema, transportna vozila, insekti, neki crvi i ishrana pomijama su mogući putevi širenja virusa. Ako je krmača zaražena sa sojevima niske virulencije, infekcija KKS može biti neprimećena i da se raširi putem trgovine jedinkama. Kupovinom svinja sa različitih lokacija, sa farmi koje nisu pod odgovarajućim nadzorom i veterinarskom kontrolom, se ispostavilo da je veoma riskantno zato što je teško kontrolisati njihov KKS status (Dahle i Liess, 1992). Fetus može biti inficirani u bilo kom periodu tokom graviditeta, ali ukoliko se infekcija desi u kasnom stadijumu, prasad će biti klinički zdravi ali će širiti virus i predstavljaju permanentni rezervoar infekcije u zapatu. Takva prasad mogu biti zakržljala i mogu uginuti (Moenning et al., 2003).

Strategija iskorenjivanja bolesti putem vakcinacije je bila zastupljena širom sveta još od ranih četrdesetih prošlog veka. Na ovaj način virus je bio iskorenjen u razvijenim delovima sveta i od početka 1990 vakcinacija je povučena u Evropskoj Uniji (Dong et al., 2007). Strategija kontrole i eradikacije u ovim zemljama obuhvata „ stamping out" obolelih životinja i svinja koje se nalaze u okviru 1000 m oko zaražene farme, kao i stroga zabrana kretanje svinja i prometa svinjskih proizvoda (Chenut et al, 1999, Moening et al., 2003). Međutim, usled intezivne trgovine u Evropi epidemije KKS se pojavljuju svremena na vreme (Moening, 2000). Nerazvijene zemlje stalno pokušavaju da drže bolest pod kontrolom.

KKS je tokom proteklih 10-15 godina, a posebno poslednjih nekoliko godina predstavljala značajan zdravstveni i ekonomski problem u našoj zemlji. Štete koje su nastale pojavljivanjem ovog oboljenja su ogromne, a verovatno još i nesagledive. Postoje podaci o broju ubijenih svinja koje je izvršeno radi sprečavanja širenja infekcije, ali verodostojni podaci o broju uginulih svinja od klasične kuge verovatno da nisu poznati i nikada se neće ni saznati.

Vakcinacija svinja protiv KKS u Srbiji je obavezna. Program suzbijanja i iskorenjivanja klasične kuge svinja se zasniva na vakcinaciji atenuiranom vakcinom koja sadrži Kina (K) soj virusa klasične kuge svinja i to svih kategorija (zalučena prasad, tovljenici i priplodna grla), a nakon ustanovljavanja žarišta infecije suzbijanje se vrši neškodljivim uklanjanjem inficiranog zapata, kao i na primeni svih ostalih mera propisanih pravilnikom. Uprkos sprovođenju svih navedenih mera, klasična kuga svinja i dalje predstavlja problem.

Dve vakcine domaćih proizvođača protiv KKS su registrovane-dostupne na tržištu. U nekim ranijim istraživanjima je ispitivana efikasnost (aplikacijom pune doze) vakcina protiv KKS, domaćih proizvođača, posle veštačke infekcije. Ispitivane vakcine su se pokazale efikasnim u zaštiti od oboljenja virusom KKS (Lazić, neobjavljena istraživanja).

U ovom radu prezentovani su rezultati bioloških testova (identifikacije, neškodljivosti i potentnosti) dve komercijalne vakcine dostupnih na našem tržištu, obe proizvedene od Kina soja virusa KKS, koji je pasiran kroz kuniće. Ispitivanja

efikasnosti vakcina protiv klasične kuge svinja imala su za cilj da se stekne kompletan uvid u imunogeni potencijal vakcina utvrđivanjem efekata zaštite u uslovima veštačke infekcije i praćenjem efektora humoralnog imunog odgovora.

Materijal i metode

Vakcine

Ispitivane su dve komercijalne vakcine protiv KKS, dostupne na tržištu. Obe vakcine su proizvedene serijskim pasažama na kunićima.

Životinje

U biološkom testu identifikacije vakcinalnog virusa KKS korišćeni su kunići rase Panonski beli telesne mase oko 2kg, u dobroj kondiciji. U biološkom testu ispitivanja neškodljivosti i potentnosti-efikasnosti vakcine korišćena su prasad starosti oko 45 dana. Kupljena su sa odabrane lokalne odgajivačke farme svinja. Životinje su držane u eksperimantalnom bloku Instituta tokom nedelju dana da se adaptiraju. Voda im je davana bez ograničenja, a hrana im je uobročena. Kod prasadi, pre uključivanja u eksperimentalni protokol vakcinacije i veštačke infekcije, imunoenzimskim testom nije utvrđeno prisustvo specifičnih antitela protiv virusa KKS, takođe serum-neutralizacionim testom nije utvrđeno prisustvo antitela protiv virusa bovine virusne dijareje (BVDV).

Identifikacija

Za svaku ispitivanu vakcinu korišćeno je 10 kunića, koji su bili podeljeni u tri grupe. Prva grupa od 4 kunića je vakcinisana sa 1/10 doze intra venski (i.v.), druga grupa od 4 kunića je vakcinisana sa 1/50 doze i.v. i treća grupa od dva kunića nije bila vakcinisana i služila je kao kontrola. Kunići su bili vakcinisani sa razređenom vakcinom u količini od 1 ml u ušnu venu. Telesna temperature (rektalna) je praćena svakodnevno tokom 7 dana kod svih kunića. Osmi dan je aplikovana svim kunićima puna doza vaccine (1 preporučena doza proizvođača za svinje) - 1 ml i.v. u marginalnu ušnu venu. Narednih pet dana je telesna temperature kunićima merena dva puta dnevno.

Neškodljivost

Korišćena su tri praseta za svaku vakcinu, koja su bila u saglasnosti sa zahtevima za test (slobodna od antitela za KKS i BVD). Desetostruka doza rekonstruisane vakcine je aplikovana intra muskularno svakom prasetu. Životinje su posmatrane tokom 21 dan.

Virus korišćen za veštačku infekciju

Za veštačku infekciju korišćen je standardni visoko patogeni soj Baker. Prethodno je utvrđeno da 2 ml virusa uzrokuje smrt kod svih osetljivih životinja tokom 6 dana.

Eksperimentalni dizajn

Eksperiment je urađen u skladu sa Evropskom Farmakopejom (Ph. Eur. 6.0, 01/2008:0065). Formirane su dve grupe životinja (5 životinja po grupi) i to 5 prasadi je vakcinisano sa vakcinom koja je razređena u odnosu 1:40, a 5 prasadi je vakcinisano sa vakcinom koja je razređena u odnosu 1:160, intramuskularno u količini od 1 ml. Dok su dva praseta bila kontrola veštačke infekcije (nisu bila vakcinisana ali su veštački inficirana sa patogenim virusom). Neposredno pred izvođenje veštačke infekcije izvršeno je uzorkovanje krvi od svih prasadi radi

utvrđivanja specifičnih antitela protiv virusa klasične kuge svinja. 14 dana nakon vakcinacije sve životinje su veštački inficirane sa visoko patogenim sojem virusa klasične kuge svinja (Baker soj) u količini od 2ml (intramuskularnom aplikacijom). Tokom celog perioda ispitivanja vršena je kontinuirana klinička opservacija svih eksperimentalnih prasadi. Telesna temperature je beležena svakodnevno do završetka eksperimenta, 14 dana posle veštačke infekcije. Pored praćenja rektalne telesne temperature, praćeni su i sledeći klinički simptomi: ponašanje životinja (apatija, letargija), promene na koži (eritem, cijanoza, krvarenja) i vidljivim sluzokožama, promene na očima (konjunktivitis), promene respiratornog (kašalj) i digestivnog (opstipacija/dijareja) trakta i lokomotornog aparata, poremećaje funkcije od strane CNS (pareza, paraliza, konvulzije), kao i telesnu kondiciju (kaheksija). Kada se završila klinička opservacija, uzorkovana je krv kod svih eksperimentalnih životinja u cilju utvrđivanja specifičnih antitela protiv virusa KKS. 14. dana nakon veštačke infekcije izvršeno je žrtvovanje preživelih svinja u cilju utvrđivanja patomorfoloških promena, i uzorkovanje tkiva parenhimatoznih organa (deo slezine, bubrega, mandibularni limfni čvor i tonzile) radi utvrđivanja prisustva virusa KKS.

Ista eksperimentalna procedura je primenjena za ispitivanje obe vakcine. Eksperiment je odobren od Agencije za lekove i medicinska sredstva. Utvrđena je srednja protektivna doza (PD/50) za vakcine koje su ispitivane po zahtevima Evropske farmakopeje, a primenom jednačine po Spearman-Kaerberu.

Detekcija antigena i antitela

U oba eksperimenta antigen KKS i specifična antitela su detektovna korišćenjem ELISA testa. Antigen je detektovan iz zbirnih uzoraka organa (slezina, bubrezi, mandibularni limfni čvorovi, krajnici) uzorkovanih posle sekcije. Test je urađen u skladu sa uputstvima proizvođača.

Rezultati

Identifikacija

Virus KKS u vakcinama je identifikovan zbog svog specifičnog pirogenog karaktera, jer je doveo do porasta telesne temperature 3. i 4. dan nakon vakcinacije sa razređenim dozama, kao i kod ne-imunizovanih kunića, 2. i 3. dan posle davanja pune doze vaccine, rektalna temperature je bila preko $40,5^0$C. Kod prethodno vakcinisanih kunića sa razređenom vakcinom temperature je ostala u fiziološkim granicama nakon aplikacije pune doze.

Neškodljivost

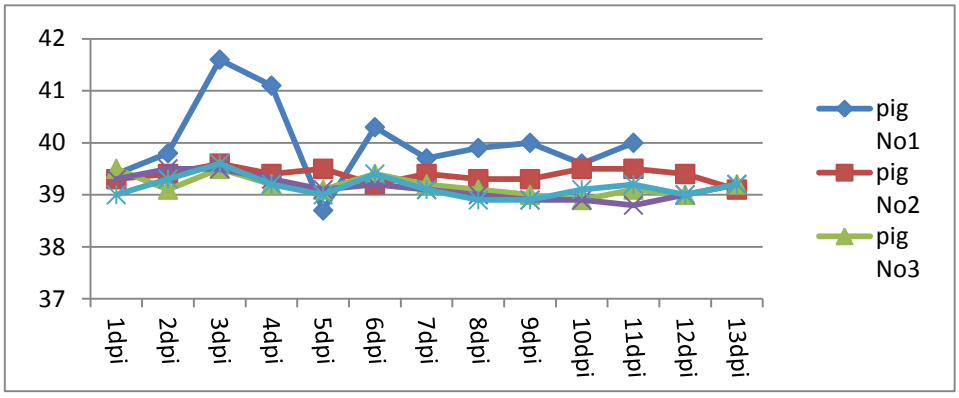
Životinje su posmatrane tokom 21 dan. Za vreme tog perioda temperaturna kriva je ostala u fiziološkim granicama i životinje su ostale dobrog zdravlja, sa normalnim prirastom, kod obe ispitivane vakcine.

Potentnost – efikasnost

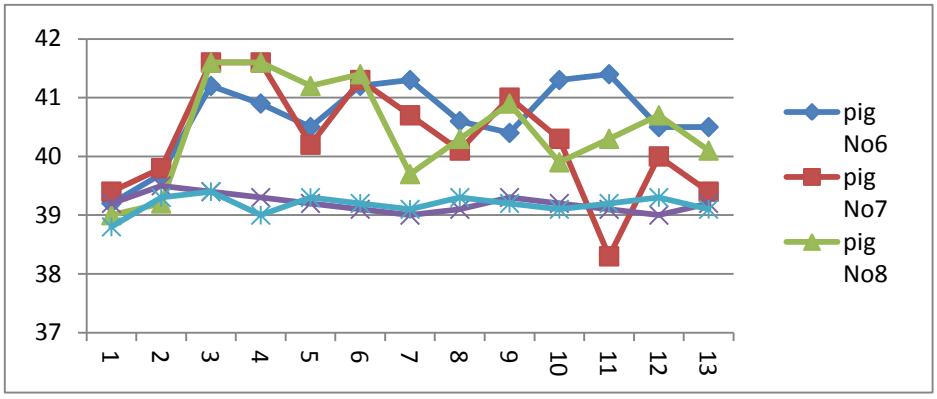
Ispitivane vakcine su obeležene sa X i Y, radi nepristrasnosti prema proizvođačima vakcina. Kod prasadi kod kojih je obavljena vakcinacija sa vakcinom X, 3. dana posle veštačke infekcije zabeležen je kod jedne jedinke porast telesne temperature ($41,6^0$C) u grupi koja je vakcinisana sa razređenom vakcinom u odnosu 1 : 40 (Graf.1.), a u grupi koja je vakcinisana sa vakcinom koja je razređena u odnosu 1 : 160 kod tri jedinke je zabeležen porast temperature (41,2; 41,6 i $41,6^0$C) (Graf.2.), dok se kod ostalih eksperimentalnih životinja kretala u granicama

fizioloških vrednosti. Kod životinja koje su imale povišenu telesnu temperaturu, 3. i 4. dan posle veštačke infekcije zapažen je smanjen apetit i gubitak apetita, apatija, letargija, konjunktivitis, opstipacija i ležanje. Pojava proliva je zabeležena 4. i 5. dan posle infekcije. Šestog dana kod jednog praseta, a 7. kod ostale obolele prasadi pojavili su se lokomotorni poremećaji sa znacima ataksije i posteriorne pareze, povremeno sa konvulzijama, da bi 12. dana uginulo jedno prase kod koga su bili najizraženiji klinički znaci bolesti i to u grupi koja je vakcinisana sa vakcinom koja je razređena u odnosu 1 : 40. Takođe, kod obolele prasadi su zabeležene promene na koži u vidu cijanoze, eritema i krvarenja od 5. dana pa sve do uginuća, odnosno žrtvovanja. Kod 4 praseta iz grupe koja je vakcinisana sa vakcinom koja je razređena u odnosu 1 : 40 i kod 2 praseta iz grupe koja je vakcinisana sa vakcinom koja je razređena 1 : 160 nisu primećeni klinički znaci bolesti, niti promene u konzumaciji vode i hrane u odnosu na zapažanja tokom perioda aklimatizacije.

Graf 1. Rektalna temperatura (^{0} C) prasadi veštački inficiranih 14 dana posle vakcinacije sa vakcinom X razređenom 1:40



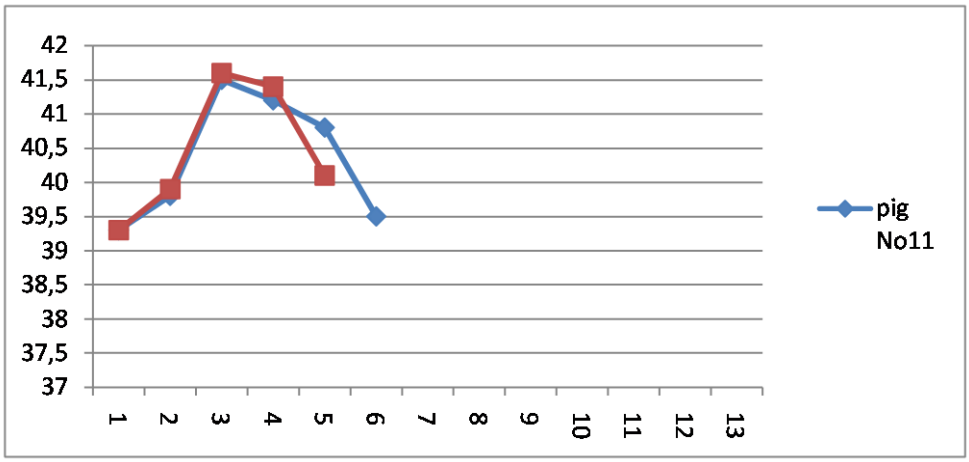
Graf 2. Rektalna temperatura (^{0} C) prasadi veštački inficiranih 14 dana posle vakcinacije sa vakcinom X razređenom 1:160



U kontrolnoj grupi, nakon veštačke infekcije virusom KKS, prvi porast telesne temperature je zabeležen 3. dana (41,5 ^{o}C i 41,6 ^{o}C) (Graf.3.). Telesne temperature prasadi su bile kontinuirano povišene tokom eksperimentalnog

protokola, a dostigle su fiziološke vrednosti neposredno pred uginuće. Klinički simptomi kao što su smanjen apetit i gubitak apetita, apatija, letargija, konjunktivitis, opstipacija i ležanje, zabeleženi su 2., odnosno 3. dana bolesti. Proliv je zabeležen 3. dana posle infekcije, a 4. dana u kliničkoj slici su dominirali lokomotorni poremećaji sa znacima ataksije i posteriorne pareze, povremeno sa konvulzijama. Takođe, kod oba praseta su zabeležene promene po koži u vidu cijanoze, eritema i izraženih krvarenja od 4. dana pa sve do uginuća. Tok bolesti je kod oba praseta bio jakog intenzitata sa uginućem 6. odnosno 7. dana posle veštačke infekcije.

Graf 3. Rektalna temperature (^{0} C) veštački inficiranih prasadi u Kontrolnoj grupi (vakcine X)



U ispitanim uzorcima krvnih seruma uzetih pre veštačke infekcije utvrđeno je prisustvo specifičnih antitela protiv virusa KKS kod jedne jedinke u grupi koja je vakcinisana sa vakcinom koja je razređena u odnosu 1 : 40 (Tabela 1.), kao i dve sumnjive reakcije, kod jednog praseta iz iste grupe i kod drugog praseta koje je poreklom iz grupe koja je vakcinisana sa vakcinom koja je razređena u odnosu 1 : 160. U uzorcima krvi ostalih prasadi rezultat na specifična antitela za virus KKS je bio negativan. U ispitanim uzorcima krvnih seruma pre žrtvovanja (14. dana posle vakcinacije) utvrđeno je prisustvo specifičnih antitela protiv virusa KKS kod tri jedinke iz grupe koja je vakcinisana sa vakcinom koja je razređena u odnosu 1 : 40 i kod tri jedinke iz grupe koja je vaksinisana sa vakcinom koja je razređena u odnosu 1 : 160. Sumnjiva reakcija je bila kod dve jedinke iz obe ogledne grupe, a kod ostalih jedinki reakcija je bila negativna. Reakcija na prisustvo antigena KKS u uzorcima ispitivanih organa bila je pozitivna kod uginulog praseta iz grupe koja je vakcinisana sa vakcinom koja je razređena u odnosu 1 : 40, kao i kod tri obolela praseta iz druge vakcinisane grupe. Suspektna reakcija je utvrđena u jednom uzorku koji je bio poreklom iz grupe koja je vakcinisana sa većim razređenjem vakcine (1:160). Kod ostalih jedinki nije utvrđeno prisustvo antigena KKS u ispitivanim organima. U svim ispitivanim uzorcima organa poreklom od prasadi iz kontrolne grupe (deo slezine, bubrega, mandibularni limfni čvor i tonzile), ELISA testom je utvrđeno prisustvo antigena virusa KKS.

Tabela 1. Prikaz prisustva kliničkih siptoma KKS, serološki nalaz At pre i posle VI i nalaz Ag u pulovanim uzorcima organa prasadi vakcinisanih sa vakcinom X

Broj praseta	Prasad vakcinisana sa vakcinom X				
	Prisustvo antitela protiv KKS u serumu pre veštačke infekcije	Klinički simptomi	Dan uginuća posle veštačke infekcije	14. dan posle veštačke infekcije	
				Prisust. antitela protiv KKS u serumu**	Antigen KKS u pulovanim uzorcima organa**
	Razređenje vakcine 1 : 40				
1.	- At	+	12 dan		+ Ag
2.	+ At	-	*	+ At	- Ag
3.	- At	-	*	± At	- Ag
4.	± At	-	*	+ At	- Ag
5.	- At	-	*	+ At	- Ag
	Razređenje vakcine 1 : 160				
1.	- At	+	*	± At	+ Ag
2.	- At	+	*	+ At	+ Ag
3.	- At	+	*	- At	+ Ag
4.	± At	-	*	+ At	- Ag
5.	- At	-	*	+ At	± Ag
	Kontrola (ne-vakcinisana prasad)				
1.	- At	+	7 dan		+ Ag
2.	- At	+	6 dan		+ Ag

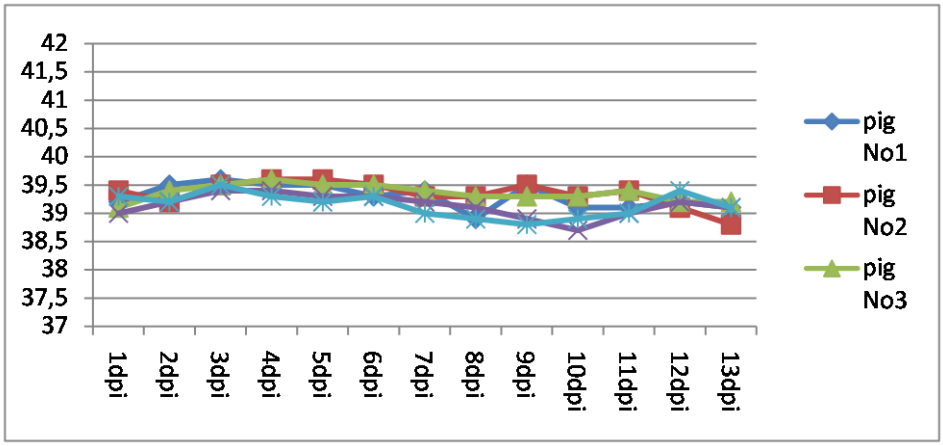
* žrtvovani 14 dana posle veštačke infekcije; ** At (pozitivan +, negativan - , sumnjiv ±) u serumu, Ag (pozitivan +, negativan - , sumnjiv ±) u pulovanim (zbirnim) uzorcima organa

Patomorfološkim pregledom uginulih i obolelih prasadi utvrđene su promene koje ukazuju na infekciju virusom KKS, a obuhvatale su hemoragične infarkte na slezini, krvarenja u limfnim čvorovima (mandibularni, mezenterijalni, ingvinalni), subkapsularna krvarenja i krvarenja na poprečnom preseku bubrega, krvarenja u bubrežnoj karlici i hemoragično-nekrotični tonzilitis.

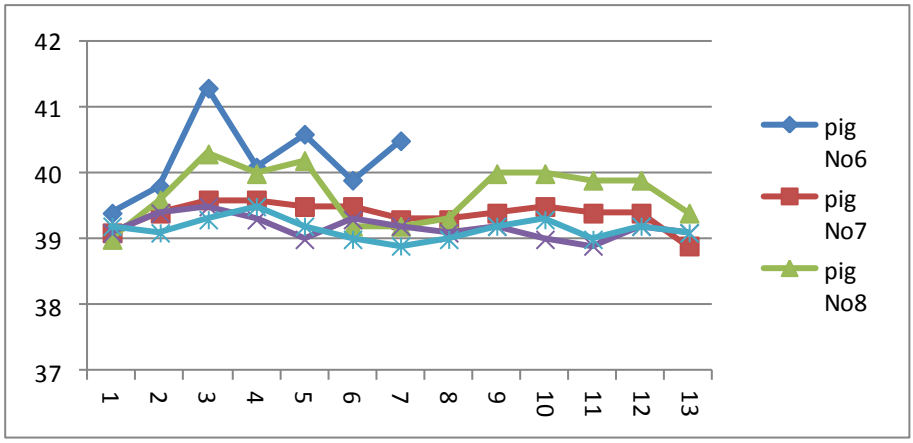
Primenom jednačine po Spearman-Kaerberu**,** utvrđeno je da zaštitna vrednost (PD_{50}) jedne doze vakcine X iznosi 105. U eksperimentalnim grupama životinja koje su vakcinisane sa vakcinom Y pri razređenju 1:40 nisu ustanovljeni klinički znaci oboljenja karakterističnih za KKS (Graf. 4.), dok su u grupi koja je vakcinisana pri razređenju 1:160 dva praseta obolela sa jasno izraženim kliničkim znacima KKS, od kojih je jedno uginulo 7. dana nakon sprovedene veštačke infekcije. U kontrolnoj grupi životinja, odnosno kod dva nevakcinisana praseta došlo je do pojave karakteristične akutne forme KKS i uginuća u roku od sedam dana nakon infekcije. Kod vakcinisane prasadi u grupi koja je vakcinisana sa vakcinom koja je razređena u odnosu 1 : 160, 3. dana posle veštačke infekcije kod dve jedinke zabeležen je porast telesne temperature (40,3 i 41,3^{0}C) (Graf.5.), a 3. i 4. dan posle veštačke infekcije zabeležen je smanjen apetit i gubitak apetita, apatija, letargija, konjunktivitis, opstipacija, ležanje, dok je pojava proliva zabeležena 5. dana. Šestog dana kod jednog praseta su se javili lokomotorni poremećaji sa znacima ataksije i posteriorne pareze, povremeno sa konvulzijama, do uginuća je došlo sedmi dan od infekcije. Kod oba praseta su zabeležene promene na koži u vidu cijanoze, eritema i izraženih krvarenja od 5. dana pa sve do uginuća, odnosno žrtvovanja. U kontrolnoj grupi, prvi porast telesne temperature je zabeležen 3. dana (41,3 □C i 41,4 □C) (Graf.6.) nakon veštačke infekcije, a kontininuirano su bile povišene tokom celog

eksperimenta, da bi pred uginuće dostigle fiziološke vrednosti. Prvi klinički simptomi kao što su smanjen apetit i gubitak apetita, apatija, letargija, konjunktivitis, opstipacija i ležanje kod kontrolne grupe su zabeleženi 2. i 3. dana posle infekcije. Pojava proliva je zabeležena 4. dana posle infekcije, dok su izraženi znaci afonije zabeleženi 6. dana, a posle ovog perioda u kliničkoj slici su dominirali lokomotorni poremećaji sa znacima ataksije i posteriorne pareze, povremeno sa konvulzijama. Promene na koži u vidu cijanoze, eritema i izraženih krvarenja zabeleženi su od 5. dana pa sve do uginuća.

Graf 4. Rektalna temperatura (^{0} C) prasadi veštački inficiranih 14 dana posle vakcinacije sa vakcinom Y razređenom 1:40



Graf 5. Rektalna temperatura (^{0} C) prasadi veštački inficiranih 14 dana posle vakcinacije sa vakcinom Y razređenom 1:160



U ispitanim uzorcima krvi uzetih pre veštačke infekcije utvrđeno je prisustvo specifičnih antitela protiv virusa KKS kod jedne životinje iz grupe koja je vakcinisana sa razređenom vakcinom 1 : 40 (Tabela 2.), kao i jedna sumnjiva reakcija kod jedike iz iste grupe, ali i kod dve jedinke iz grupe koja je vakcinisana sa razređenom vakcinom 1 : 160. Kod ostalih jedinki nalaz je bio negativan. U

ispitanim uzorcima krvi koja je uzorkovana 14 dana posle veštačke infekcije, utvrđeno je prisustvo specifičnih antitela protiv virusa KKS kod svih preživelih vakcinisanih jedinki. Reakcija na prisustvo antigena u uzorcima ispitivanih organima bila je pozitivna kod uginulog praseta iz grupe vakcinisane sa razređenom vakcinom 1 : 160, kao i dva suspektna nalaza kod dve jedinke iz iste grupe. U ostalim ispitivanim uzorcima organa vakcinisane prasadi nije utvrđeno prisustvo antigena virusa klasične kuge svinja.

Graf 6.Rektalna temper. (^{0}C) veštački inficiranih prasadi Kontrolna grupa (vakcinaY)

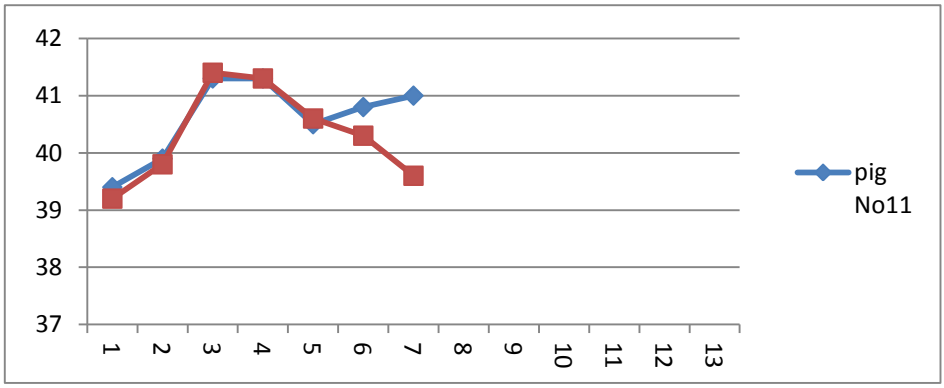


Tabela 2. Prikaz prisustva kliničkih siptoma KKS, serološki nalaz At pre i posle VI i nalaz Ag u pulovanim uzorcima organa prasadi vakcinisanih sa vakcinom Y

Broj praseta	Prasad vakcinisana sa vakcinom Y				
	Prisustvo antitela protiv KKS u serumu pre veštačke infekcije	Klinički simptomi	Dan uginuća posle veštačke infekcije	14. dan posle veštačke infekcije	
				Prisust. antitela protiv KKS u serumu**	Antigen KKS u pulovanim uzorcima organa**
	Razređenje vakcine 1 : 40				
1.	- At	-	*	+ At	- Ag
2.	- At	-	*	+ At	- Ag
3.	- At	-	*	+ At	- Ag
4.	+ At	-	*	+ At	- Ag
5.	± At	-	*	+ At	- Ag
	Razređenje vakcine 1 : 160				
1.	± At	+	*	+ At	± Ag
2.	- At	-	*	+ At	- Ag
3.	± At	-	*	+ At	- Ag
4.	- At	-	*	+ At	± Ag
5.	- At	+	7 dan		+ Ag
	Kontrola (ne-vakcinisana prasad)				
1.	- At	+	7 dan		+ Ag
2.	- At	+	7 dan		+ Ag

* žrtvovani 14 dana posle veštačke infekcije;** At (pozitivan +, negativan - , sumnjiv ±) u serumu, Ag (pozitivan +, negativan - , sumnjiv ±) u pulovanim (zbirnim) uzorcima organa

Nakon žrtvovanja prasadi obavljen je patomorfološki pregled, kada su utvrđene promene koje ukazuju na infekciju virusom KKS samo kod dva praseta koja su vakcinisana sa vakcinom koja je razređena u odnosu 1:160. U svim ispitivanim uzorcima organa poreklom od obolele prasadi iz kontrolne grupe (deo

slezine, bubrega, mandibularni limfni čvor i tonzile), ELISA testom je utvrđeno prisustvo antigena virusa KKS. Primenom jednačine po Spearman-Kaerberu**,** utvrđeno je da zaštitna vrednost (PD_{50}) jedne doze vakcine Y iznosi 182.

Diskusija

Ovaj eksperiment pokazuje da su ispitivane vakcine zadovoljile kriterijume Evropske farmakopeje 01/2008:0065, pošto je PD_{50} veća od 100. Kina soj se, dugi niz godina, široko koristi za zaštiti svinja od KKS. Soj je pasiran na kulturi ćelija ili kroz kuniće. Virus koji je pasiran kroz kuniće se naziva lapinizovani živi apatogeni soj virusa svinjske kuge i dugi niz godina se koristi za proizvodnju komercijalnih proizvoda. Vakcine proizvedene od Kina soja obezbeđuju dugu zaštitu i dobro su tolerisane od strane mladih jedinki i suprasnih krmača (Dong et al., 2007). Meindi-Boehmer i Markus-Cizelj (2006) su izvršili ispitivanje imugenosti vakcine protiv KKS Cholerevac – Plivak. U njihovim eksperimentima dobijena protektivna doza 50 (PD_{50}) posle veštačke infekcije sa visoko patogenim sojem virusa – Koslov je bila $\geq$ 320. Klinički simptomi bolesti kod prasadi izazvani infekcijom sa Koslov sojem su bili slični sa našim prezentovanim eksperimentalnim rezultatima. Klinički simptomi KKS sa Baker sojem u ovom eksperimentu su takođe slični sa ranije objavljenim rezultatima istraživanja (Milanov et al., 2002). U njihovim istraživanjima, u vreme veštačke infekcije životinje su bile starosti 3 do 4 meseca. Prasad korišćena u tom eksperimentu nisu vakcinisana i poticala su od nevakcinisanih krmača. Tri od dvanaest jedinki su preživele infekciju i predpostavilo se da su te životinje morale doći u kontakt sa virusom KKS umerene virulencije pre veštečke infekcije ili su bile izložene BVDV infekciji. To je potkrepljeno serološki pozitivnim ELISA nalazom kod te tri životinje pre veštačke infekcije.

U ovom eksperimentu tri praseta vakcinisana sa vakcinom Y razređenom 1:40 su bila negativna na prisustvo antitela, ali su bila zaštićena protiv infekcije sa visoko patogenim virusom. U grupi vakcinisanoj sa vakcinom Y u razređenju 1:160 kod dve jedinke koje su bile negativne na prisustvo antitela u vreme veštačke infekcije nisu se pojavili klinički znaci bolesti. Kod dva praseta iz grupe vakcinisane sa X vakcinom u razređenju 1:40 i jednog praseta iz grupe 1:160 takođe nisu dokazana antitela pre veštačke infekcije, i nisu se pojavili klinički znaci KKS. Kod ovih životinja antigen KKS nije nađen u organima ELISA testom, po završetku ogleda, izuzev kod jedne jedinke iz grupe vakcinisane X vakcinom u razređenju 1:160 i jedne jedinke iz grupe vakcinisane sa Y vakcinom 1:160, koje su bile sumnjive. Prisustvo antitela koje je ispitivano ELISA testom, u našim ispitivanjima, nije bilo u korelaciji sa zaštitom od virusa KKS. Testovi koji su više osteljivi kao što su NPLA ili imunofluorescentni test možda bi omogućili detekciju antitela. U eksperimentu Meindl-Boehmera i Markus-Cizelja (2006) NPLA titar antitela je bio nizak 13 dana posle infekcije. Tokom infekcije sa virusom KKS, prvo se razvija ćelijski imunitet, a zatim dolazi do maturacije B ćelija i razvoja antitela. Posle infekcije sa visoko patogenim sojem virusom Alforth 187 (Sanchez-Cordon et al., 2006) se pokazalo da dolazi do povećanja IgM ćelija 9 dana posle VI, kao i do povećanja imunoglobulina B ćelija (C-□$^{+}$), ali to nije praćeno sa detektabilnim virus neutralizacionim antitelima kao odgovorom 14 dana posle VI. Autori su takođe našli da prilikom imunološkog odgovora T ćelije tipa 1 prelaze u tip 2 posle povećanja produkcije IL4, 11 do 14 dana posle VI. Ovi citokini indukuju maturaciju B ćelija u

plazma ćelije koje proizvode imunoglobuline (Sanchez Cordon et al., 2005a). Takav scenario možda može objasniti odlaganje pojave antitela u prezentovanom eksperimentu. Zadovoljavajuća zaštita je verovatno zbog ćelijski posredovanog imunog odgovora na vakcinalni soj. Suradhat et al., 2001 pružili su dokaze za snažnu interferon gama (IFN□) produkciju već 6 dana posle vakcinacije. U njihovom eksperimentu bila je dobra korelacija između IFN□ produkcije i zaštite svinja protiv visoko virulentnog virusa KKS kod veštačke infekcije.

Interpretacija rezultata dobijenih ELISA testom u smislu procene zaštite svinja posle vakcinacije može biti problematična u terenskim uslovima. Ukoliko vakcinisane svinje dođu u kontakt sa divljim-nevakcinalnim sojem virusa, biće teško ili nemoguće vršiti monitoring infekcije serološkim testovima. ELISA test mada je lak za izvođenje i pogodan za veliki broj uzoraka nije uvek ubedljiv, pošto može takođe da da lažne pozitivne rezultate. U Holandiji, 1997/98. godine tokom izbijanja zaraze KKS, je bilo ispitano 1,5 milion uzoraka seruma koristeći ELISA test, među ELISA pozitivnim uzorcima VN test je dokazao pozitivne rezultate na KKS u 15 % seruma, 35% je odreagovalona na Bovinu dijareju i BVD virus i 50% uzoraka su bili negativni u VN testu (de Smit et al., 2000).

U ovim eksperimentima sve životinje koje su bile antigen Ag-ELISA positivne su pokazale kliničke simptome bolesti. Ovaj test, ukoliko su jedinke antigen pozitivne, nedvosmisleno otkriva skoru infekciju. Kod jedne životinje u grupi vakcinisane sa vakcinom Y 1:160, kod koje su bili klinički simptomi dobijeni rezultati ELISA testa su bili sumnjivi. Detekcija antigena je urađena u pulovanim-zbirnim uzorcima organa. Postoji mogućnost da je kod ovih jedinki distribucija virusa bila neravnomerna, možda je bio prisutan samo u timusu, iz tih razloga limit detekcije je bio nizak. Vakcinalni virus može biti detektovan, primenom Real time PCR, samo u timusu do 42 dana posle inokulacije što je pokazano u eksperimentima istraživača Koenig et al., 2007. Ispitivanja pojedinačnih organa sa veoma osetljivim testovima bi pružila bolji uvid o distribuciji divljeg-nevakcinalnog tipa virusa kod vakcinisanih životinja. Klinički simptomi uzrokovani veštačkom infekcijom sa Baker sojem virusa su bili slični sa drugim objavljenim radovima o veštačkoj infekciji. ELISA test otkriva skorašnje infekcije sa visokom verovatnoćom, dok detekcija antitela u ovom testu nije bila pouzdana. Rezultati ispitivanja vakcina, ukazuju da je njihova primena sigurna – neškodljiva, a njihov zaštitni efekat - efikasnost je potvrđena, prilikom aplikacije vakcina u različitim razređenjima prema zahtevima Evropske farmakopeje.

Zahvalnica

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