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EXPERIENCE IN CONTROLLING CLASSICAL SWINE FEVER USING BOOSTER VACCINATION

Sytiuk M. P.[1, *] (ORCID: 0000-0002-7492-9256), Obrazhei A. F.[2], Halka I. V.[3] (ORCID: 0000-0001-8701-3506), Gerilovych A.P.[4] (ORCID: 0000-0002-3280-4172)
1 – Institute of Veterinary Medicine of NAAS, Kyiv, Ukraine, *email: snp1978@ukr.net
2 – National academy for agrarian sciences of Ukraine, Kyiv, Ukraine
3 – State Research Institute for Laboratory Diagnostics and Veterinary and Sanitary Expertise, Kyiv, Ukraine
4 – One Health Scientific and Research Institute, PSI, Kharkiv, Ukraine

Abstract. *The article presents experimental scientific data of epidemiological investigation of classical swine fever (CSF) among domestic pigs in the private sector and the use of booster doses of CSF vaccines on piglets. The aim of the study was to conduct an epidemiological study of the incidence of CSF among domestic pigs in the private sector. To confirm the efficacy of booster (tenfold) doses of ASV and LK-M vaccines against classical swine fever using clinical, pathological, anatomical and laboratory methods for diagnosing CSF disease and evaluating the effectiveness of CSF disease in piglets.*

The experiments were conducted in the CSF chamber in the village of Khotiv, Kyiv-Svyatoshynskyi district of Kyiv, where 20 heads of unvaccinated piglets under two months of age weighing 10-12 kg, 1 piglet aged 7-8 months, 1 boar and 1 sow aged 1.5 years were kept. For the diagnostic purpose of the case of classical swine fever, in accordance with generally accepted methods, the hematological method with counting the number of blood leukocytes and the immunoperoxidase test for detection of antigens and antibodies to the swine fever virus were used. Piglets were vaccinated with dry lapinized ASV viral vaccine, and animals of the second group were vaccinated with LK-M viral vaccine.

Based on the results of the studies, it can be argued that in the outbreak of classical swine fever, the LK-M viral vaccine was more effective, which, unlike ASV, is able to stop the infectious process. In our opinion, this phenomenon is due to the fact that the live attenuated virus of the vaccine against classical swine fever in the amount of 100,000 ImD50 (tenfold dose of vaccination with the LK-M vaccine) probably exceeded the amount of field virus in the piglet's body and contributes to a more pronounced reaction of the immune system (formation of specific antibodies) and further neutralization of field virus and recovery of animals, whereas in the vaccine against classical swine fever the amount of live attenuated virus, even in a tenfold dose, was insufficient to stop the development of classical swine fever infection.

The analysis of the epizootic investigation of the case of classical swine fever in the private sector showed that the pathogen was introduced with food waste brought from canteens that had not been heat treated before feeding.

In the outbreak of classical swine fever, the LK-M virus vaccine proved to be more effective in stopping the infectious process due to the presence of a large amount of live attenuated vaccine virus in a tenfold dose, which exceeded the amount of field virus in the piglet's body and contributed to a more pronounced immunological response from the piglet's body and further neutralization of field virus and their recovery compared to the ASV vaccine.

In our opinion, in certain cases (e.g., preservation of breeds or breeding pigs), one of the promising measures in the event of the occurrence and elimination of a CSF outbreak is the use of tenfold doses of LK-M vaccine with the permission of the State Veterinary Service of Ukraine.

Keywords: classical swine fever, case investigation, diagnosis, vaccines.

Introduction. Classical swine fever (CSF) was first reported in England in 1826. Since then, CSF has spread quite rapidly, with outbreaks reported in 1890 in South Africa and since 1899 in South America (Terpstra, 1988). Currently, CSF is recorded in most regions of the world, both among domestic pigs (Postel, 2018; Luo, 2014) and wild boars (Rossi, 2015), but little is known about the situation in Africa (Blome, 2017).

The nature of clinical signs in CSF depends on the degree of susceptibility, general and immune status of animals, and the course of the disease can be ultra-acute, acute, chronic and asymptomatic (inapparent). There are reports that that in one herd of 0 pigs the disease occurs with different duration, manifestation of the disease and pathological changes (Jenckel, 2017; Shykov, 2002).

Various methods are used for the laboratory diagnosis of CSF: fluorescent antibodies (Saunders, 1977), enzyme-linked immunosorbent assay (Xia, 2015; Meyer, 2022), immunoperoxidase test (Liu, 1991), polymerase chain reaction (Kit, 2021), and in the early stages of the disease it is customary to use a hematological method of research (Sytiuk, 2002). Thus, the number of leukocytes in the blood of pigs normally ranges from 9 to 21 thousand per 1 μL (Dales, 1973), and in the acute course of swine fever their number decreases to 2 thousand or less per 1 μL of blood (Sobko, 1999).

Today, live virus vaccines from attenuated strains characterized by sufficient immunogenicity are used worldwide for the prevention of CSF: lapinized (ASV), cultured (VGNKI, LK-VNIIVVIM, LK-K, LK-M) and recombinant (KS) (Pryskoka, 1995).

In practice, in addition to single doses of vaccination in case of classical swine fever, especially in pig farms, some experts recommend using an increased number of recommended vaccine doses (five or more) for a single vaccination. There are reports that with a 10-fold increase in doses, the protection of pigs increased by 44 and 72%, and 100 times to 85 and 100%, respectively (Kolomyitsev, 1995). According to (Makarov, 2001), when immunizing pigs in 5 and 10-fold doses with LC-K viral vaccine, a decrease in piglet mortality was observed in a week. Pryskoka (2000) proposed to overcome the barrier of passive antibodies with the "KS" vaccine or 10-fold doses of the "LK-VNIIVViM" vaccine.

In general, the European Union countries do not vaccinate against CSF, but in 2016, in accordance with the joint program (OIE WAHIS), 22 countries officially announced that they were conducting a temporary campaign of mandatory vaccination of pigs against CSF to prevent the spread of this disease.

The main purpose of the work was to conduct an epizoothological investigation of the case of CSF among pigs in the private sector, confirm the disease of pigs with CSF by clinical, pathological, anatomical and laboratory diagnostic methods, as well as to compare the effectiveness of booster (tenfold) doses of ASF and LK-M vaccines against classical swine fever in the focus of the disease in piglets, which, in our opinion, will help to stop the death and manifestation of the disease in pigs.

Materials and methods. The study was conducted in the center of CSF in Kyiv-Sviatoshynskyi district, Kyiv region, where the following were kept: 1 boar and 2 sows older than 1 year, 7 gilts, and 20 piglets of two months of age.

For the diagnostic purposes of the CSF case, in accordance with generally accepted methods, the hematological method with counting the number of blood leukocytes (Liess, 1987) and the immunoperoxidase test for detection of antigen and antibodies to the CSF virus (Sobko, 1998) were used.

Piglets of one group were vaccinated with dry laponized vaccine ASV series No. 1, produced by Sumy Biological Factory, and animals of the second group were vaccinated with vaccine LC-M series No. 14, produced by LLC "SPE Bio-Test Laboratory".

Statistical processing of the results was performed according to the method (Chistiakov,1966).

Results and discussion. The work was carried out in a private farm (Kyiv-Svyatoshynsky district, Kyiv region), where the disease of classical swine fever began in the last decade of July 2002 and was diagnosed in August. The animals were not vaccinated against classical swine fever and were housed separately in three rooms located 5-10 meters apart. The pigs were fed kitchen waste from various Kyiv canteens and concentrate feed. One sow, one boar and 5 gilts died during the disease period.

The preliminary diagnosis of a seven-month-old gilts that was forcibly slaughtered was made on the basis of clinical signs (lack of appetite, depression, impaired coordination of

movements, bleeding and body temperature of 40.1 - 41.1 °C) and the results of pathological autopsy: multiple hemorrhages of the skin of the underarm, abdominal wall, outer and inner thighs, base of the ears, tips of the hooves (Fig. 1); hemorrhagic inflammation of the submandibular lymph nodes, hemorrhages in the epiglottis (Fig. 2); splenic infarctions (Fig. 3); spilled hemorrhages in the bladder (Fig. 4).

Fig. 1 Multiple subcutaneous hemorrhages

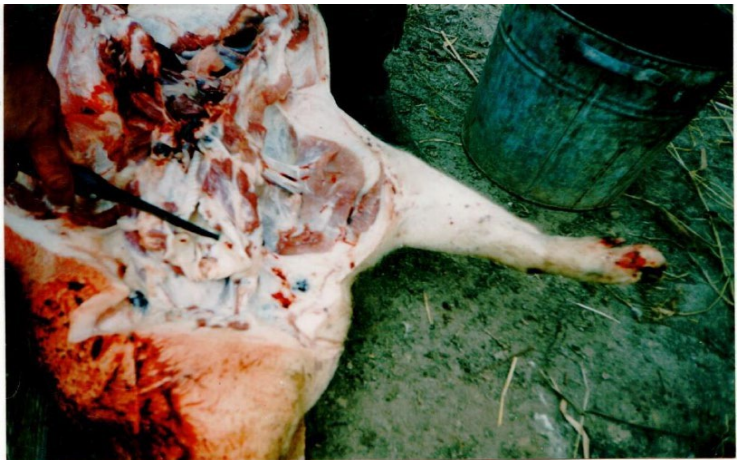

Fig. 2 Hemorrhagic inflammation of the submandibular lymph nodes and hemorrhages in the epiglottis

Lymph node samples, pieces of spleen, kidney, breastbone and lungs were taken from the euthanized piglet, and blood was collected from the live piglets for hematological studies.

Six of the 20 piglets showed an increase in body temperature from 40.1 to 41.1°C, depression, and decreased appetite.

To compare immunogenicity and study the protective properties of viral vaccines, two groups of 10 piglets were formed. The piglets of the first group were vaccinated with dry laponified ASV viral vaccine, and the animals of the second group were vaccinated with LK-M viral vaccine. Both vaccines were administered to piglets in a 10-fold dose intramuscularly in the upper third of the neck in a volume of 2 cm^3.

Blood samples were taken before and 7, 14, and 30 days after vaccination.

A decrease in the number of leukocytes (6.24, 7.19, 6.45 thousand/μL) was detected in the blood of three piglets of the first group (No. 2, No. 6 and No. 9) before the administration of the ASV vaccine, and further testing revealed the presence of CSF virus in titers: 2.2, 2.2, and 1.7 (2.03 ± 0.14) lg (Table 1).

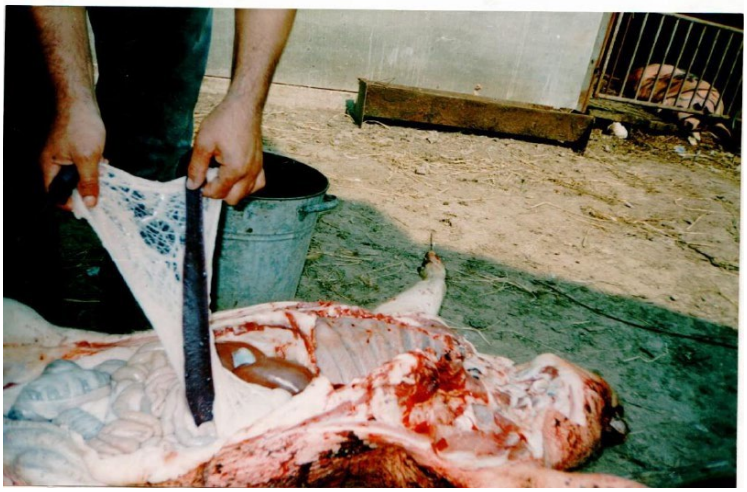

Fig. 3 Hemorrhagic infarctions of the spleen

Fig. 4 Spilled hemorrhages in the bladder

Further progression of the disease, development of leukopenia (2.27-4.77 thousand leukocytes per 1 μL) and accumulation of virus in the blood up to 3.53 ± 0.14 lg were observed in these virus-carrying animals. Within 16 days after vaccination, these piglets died. At autopsy, they showed pathological changes characteristic of CSF. In the rest of the piglets, on the seventh day after vaccination with the ASV vaccine, a slight decrease in the number of blood leukocytes by 5-9 thousand/μL was observed within the physiological norm. The leukocyte content of the piglets' blood increased from day 14 and normalized by day 30 of observation. 14 days after vaccination, the presence of specific antibodies in the sera of five vaccinated piglets № 3, 4, 5, 7 and 10 was recorded in titers of 2.2-2.2

Titres in two pigs - No. 1 and No. 8 were on the level of 3.28-3.53 (2.81 ± 0.15 log 2). On the 30th day after vaccination, antibody titers increased to 3.75 ± 0.13 log 2.

Samples of organs and tissues (spleen, lymph nodes, breastbone, kidney) were collected from the dead animals and tested for the presence of antigen to the CSF virus (Table 2).

Table 1

Results of hematological and serological studies of piglets vaccinated with 10-fold doses of ASV vaccine against CSF

# of experimental animals	Time of blood sampling and testing											
	before vaccination			after vaccination								
				7 days			14 days			30 days		
	lg antigen	lg antibodies 2	leukocytes (thousand/μL)	lg antigen	lg antibodies 2	leukocytes (thousand/μL)	lg antigen	lg antibodies 2	leukocytes (thousand/μL)	lg antigen	lg antibodies 2	leukocytes (thousand/μL)
1	-	-	20.70	-	-	11.76	-	3.53	15.92	-	4.28	19.95
2	2.2	-	6.24	3.7	-	2.27	died					
3	-	-	18.47	-	-	10.66	-	2.62	14.29	-	3.62	18.36
4	-	-	19.78	-	-	10.30	-	2.53	15.22	-	3.53	18.53
5	-	-	20.36	-	-	11.19	-	2.78	14.24	-	3.87	14.41
6	2.2	-	7.19	3.7	-	4.77	died					
7	-	-	16.71	-	-	11.74	-	2.33	17.61	-	3.28	16.01
8	-	-	18.75	-	-	13.91	-	3.28	17.12	-	4.12	14.66
9	1.7	-	6.45	3.2	-	2.53	3.2	-	1.28	died		
10	-	-	17.74	-	-	12.61	-	2.62	18.56	-	3.53	17.27
M ± м	2.03 ± 0.14		15.24 ± 1.82	3.53 ± 0.14		9.17 ± 1.29	3.2	2.81 ± 0.15	14.28 ± 1.81		3.75 ± 0.13	17.03 ± 0.73
P <	0.01						0.005					

When examining samples of the selected material (spleen, lymph nodes, kidney and breastbone) in cell culture, the highest titers of CSF virus were found in the lymph nodes and spleen of animals, and the lowest in the kidney and breastbone (Table 2).

The results of hematological and serological studies of blood from piglets vaccinated with 10-fold doses of cultured virus vaccine LK-M against CSF are shown in Table 3.

Table 2

Results of testing of organ samples from dead piglets for the presence of antigen to the CSF virus

# of experimental animals	Pathological material	Laboratory tests for the detection of CSF virus antigen using the immunoperoxidase test, lg TCKID50/cm3	M±m
2	spleen	3.2	3.33 ± 0.11
	lymph nodes	3.7	
	kidney	3.2	
	breastbone	3.2	
6	spleen	2.7	2.58 ± 0.21
	lymph nodes	3.2	
	kidney	2.2	
	breastbone	2.2	
9	spleen	3.7	3.33 ± 0.21
	lymph nodes	3.7	
	kidney	3.2	
	breastbone	2.7	

Table 3

Results of hematological and serological studies of blood from piglets vaccinated with 10-fold doses of L K - M vaccine against CSF

# of experimental animals	Time of blood sampling and testing											
	after vaccination			after vaccination								
				7 days after vaccination			7 days after vaccination			7 days		
	lg antigen	lg antibodies 2	leukocytes thousand/μL	lg antigen	lg antibodies 2	leukocytes thousand/μL	lg antigen	lg antibodies 2	leukocytes thousand/μL	lg antigen	lg antibodies 2	leukocytes thousand/μL
1	2.7	-	5.50	1.2	1.03	9.27	-	4.62	11.43	-	6.33	14.45
2	-	-	19.25	-	2.12	12.26	-	6.53	13.81	-	9.03	20.80
3	-	-	18.38	-	2.12	10.18	-	5.53	13.20	-	7.28	19.68
4	-	-	14.21	-	1.03	11.79	-	5.33	14.47	-	8.12	16.38
5	1.7	-	7.95	-	1.12	10.39	-	4.78	12.80	-	6.28	17.17
6	-	-	15.60	-	1.28	12.68	-	5.33	16.40	-	7.03	16.26
7	2.2	-	6.79	1.2	1.03	9.72	-	4.53	11.67	-	6.62	21.50
8	-	-	22.53	-	2.33	15.19	-	6.03	18.62	-	8.78	20.70
9	-	-	16.51	-	2.53	12.53	-	6.28	16.53	-	8.62	17.25
10	-	-	17.60	-	2.33	11.27	-	5.62	17.82	-	7.32	19.0
M ± м	2.20 ± 0.24		14.43 ± 1.73	0.80 ± 0.33	1.69 ± 0.19	11.53 ± 0.53		5.46 ± 0.21	14.68 ± 0.76		7.54 ± 0.31	18.32 ± 0.7
P <	0.05				0.001							

In three piglets of this group (No. 1, No. 5 and No. 7), before the vaccine administration, an increase in body temperature, a decrease in the number of leukocytes in the blood (5.50, 7.95, 6.79 thousand/μL) and the presence of CSF virus in the blood at a titer of 2.7, 1.7 and 2.2 (2.2 ± 0.24) lg were also recorded.

In 7 days after vaccination, a decrease in the level of CSF virus was observed in the blood of 2 piglets (No. 1 and No. 7), and on day 14, no CSF virus was detected in the blood of all virus-carrying animals.

The content of blood leukocytes after vaccination in piglets in which the presence of CSF virus was not detected also decreased to 2-9 thousand per 1 μL of blood. However, on the 14th day after vaccination, the content of blood leukocytes in these animals increased and on the 30th day after vaccination amounted to 18.32 ± 0.7 thousand/μL of blood.

In 7 days after the administration of the LK-M vaccine in a 10-fold dose, the level of antibodies against CSF was 1.69 ± 0.19 log2 with the parallel presence of antibodies in the blood serum of virus-carrying animals in a titer of 1.03 log2. Subsequently, on the 14th day after vaccination with LC-M vaccine in a 10-fold dose, the average titer of specific antibodies was 5.46± 0.21 log2 and reached a level of 7.54 ± 0.31 log2 30 days after vaccination.

It should be noted that in virus-carrying piglets, after administration of a 10-fold dose of LK-M vaccine, along with the elimination of field virus from the blood and the formation of virus-neutralizing antibodies, the leukocyte counts in the blood normalized by day 30.

It should be noted that one recommended vaccination dose of LK-M vaccine contains 10,000 IMD50, and 10 times that of 100,000 IMD50. One recommended dose of the ASV vaccine contains 20 IMD50, and 10 times that amount - 200 IMD50. Apparently, for the same reason, the ASV vaccine (even in a 10-fold dose), due to the lower titer of the virus in it, does not provide sufficient protection of pigs after vaccination.

A statistical comparison of both vaccines showed that the increase in antibody titers after

vaccination with ASV against CSF is reliable, since the P value< is 0.005, and the effectiveness of antibody levels' increase after vaccination with LK-M vaccine is more than reliable since P < 0.001.

Discussion. Thus, two virus vaccines at ten times the recommended doses were used in the focus of CSF in virus-carrying animals in a comparative aspect. The results of the study revealed insufficient effectiveness of vaccination of piglets carrying viruses with 10 times the recommended doses of ASV vaccine. All virus-carrying piglets (3 heads) died between 14 and 30 days after vaccination with this vaccine with a temperature reaction, leukopenia and clinical and pathological changes characteristic of CSF.

In contrast, after vaccination of three piglets with 10-fold dose of LK-M vaccine, starting from the 7th day after vaccination, a decrease in body temperature and restoration of leukocyte content in the blood of piglets to physiological norms were observed, and 14 days after vaccination, no CSF virus was detected in the blood of experimental animals.

Thus, based on the results of the studies, it can be concluded that in the center of classical swine fever, the LK-M vaccine proved to be more effective, which, unlike the ASV vaccine, is able to stop the infectious process when used at 10 times the recommended dose. This, in our opinion, is due to the fact that live attenuated vaccine CSF virus in the amount of 100,000 ImD50 (at ten times the recommended dose for vaccination with LK-M vaccine) probably exceeded the amount of field virus in the piglet's body and contributed to a more pronounced reaction of the immune system (formation of specific antibodies) and further neutralization of field virus and recovery of animals, whereas the amount of live attenuated virus in the ASV vaccine, even at ten times the recommended dose, was insufficient to stop the development of CSF infection.

Our results are in line with the data of a number of researchers (Kolomyitsev, 1995; Makarov, 2001), which indicate a decrease in the incidence of CSF in pigs when using increased inoculated doses of live cultured viral vaccines whose activity is 10-100 times higher than lapinized ones, in particular, the ASV vaccine.

Conclusions and perspectives for further research.

1. The analysis of the epizootic investigation of the case of classical swine fever in the private sector showed that the introduction of the pathogen occurred with food waste from canteens that were not subjected to heat treatment before feeding.

2. In the center of classical swine fever, the LK-M vaccine proved to be more effective than the ASV vaccine, which is able to stop the development of the infectious process due to the presence of a sufficient amount of live attenuated vaccine CSF virus in a tenfold dose - 100,000 ImD50, which exceeded the amount of field virus in the piglet's body and contributed to a more pronounced immunological reaction from the piglet's body and further neutralization of field virus and their recovery.

In our opinion, in some cases (preservation of valuable breeds or breeding pigs), one of the promising measures in the event of an outbreak of CSF may be the use of tenfold doses of LK-M vaccine with the permission and approval of the State Service of Ukraine for Food Safety and Consumer Protection.

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ДОСВІД БОРОТЬБИ З КЛАСИЧНОЮ ЧУМОЮ СВИНЕЙ З ВИКОРИСТАННЯМ БУСТЕРНОЇ ВАКЦИНАЦІЇ

Ситюк М. П.[1, *] (ORCID: 0000-0002-7492-9256), Ображей А. Ф.[2], Галка І. В.[3] (ORCID: 0000-0001-8701-3506), Герілович А. П.[4] (ORCID: 0000-0002-3280-4172)
1 – Інститут ветеринарної медицини НААН, Київ, Україна, *email: snp1978@ukr.net
2 – Національна академія аграрних наук України, Київ, Україна
3 – Державний науково-дослідний інститут лабораторної діагностики та ветеринарної і санітарної експертизи, Київ, Україна
4 – Науково-дослідний інститут єдиного здоров'я, ПНУ, Харків, Україна

Резюме. *У статті представлено експериментальні наукові дані епізоотологічного дослідження випадку класичної чуми свиней (КЧС) серед домашніх свиней у приватному секторі та застосування бустерних доз вакцин проти КЧС на поросят-вірусоносіях. Метою роботи було проведення епідеміологічного дослідження захворюваності на КЧС серед домашніх свиней у приватному секторі. Підтвердити ефективність бустерних (десятикратних) доз вакцин ASV та LK-M проти класичної чуми свиней за допомогою клінічних, патологоанатомічних, анатомічних та лабораторних методів діагностики захворювання на КЧС та оцінки ефективності захворювання на КЧС у поросят.*
Експерименти проводилися в камері КЧС у селі Хотів Києво-Святошинського району міста Києва, де утримувалося 20 голів невакцинованих поросят до двомісячного віку вагою 10-12 кг, 1 порося віком 7-8 місяців, 1 кнур та 1 свиноматка віком 1,5 року. Для діагностичної мети випадку класичної чуми свиней, відповідно до загальноприйнятих методів, було використано гематологічний метод з підрахунком кількості лейкоцитів крові та імунопероксидазний тест для виявлення антигенів та антитіл до вірусу чуми свиней. Поросят вакцинували сухою лапінізованою вірусною вакциною ASV, а тварин другої групи – вірусною вакциною LK-M.
На основі проведених досліджень можна стверджувати, що у спалаху класичної чуми свиней більш ефективною була вірусна вакцина LK-M, яка, на відміну від ASV, здатна зупинити інфекційний процес. На нашу думку, це явище пов'язане з тим, що живий атенуйований вірус вакцини проти класичної чуми свиней у кількості 100 000 ImD50 (десятикратна доза вакцинації вакциною LK-M) ймовірно перевищував кількість польового вірусу в організмі поросят і сприяє більш вираженій реакції імунної системи організму (утворенню специфічних антитіл) та подальшій нейтралізації польового вірусу та одужанню тварин, тоді як у вакцині проти класичної чуми свиней кількість живого атенуйованого вірусу навіть у десятикратній дозі була недостатньою для зупинки розвитку інфекції класичної чуми свиней.

Аналіз епізоотичного розслідування випадку класичної чуми свиней у приватному секторі показав, що збудник був занесений з харчовими відходами, принесеними з закладів харчування, які не пройшли термічну обробку перед годуванням.

В умовах спалаху класичної чуми свиней вакцина проти вірусу ЛК-М виявилася більш ефективною, здатною зупинити інфекційний процес. через наявність великої кількості живого атенуйованого вакцинного вірусу в десятикратній дозі, що перевищувало кількість польового вірусу в організмі поросят та сприяло більш вираженій імунологічній реакції з боку організму поросят та подальшій нейтралізації польового вірусу та їх одужанню порівняно з вакциною АСВ.

На нашу думку, у певних випадках (наприклад, збереження порід або племінне розведення свиней) одним із перспективних заходів у разі виникнення та ліквідації спалаху КЧС є застосування десятикратних доз вакцини ЛК-М з дозволу Державної ветеринарної служби України.

Ключові слова: класична чума свиней, розслідування випадків, діагностика, вакцини.

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