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CURRENT APPROACHES TO THE VACCINE DEVELOPMENT FOR AFRICAN SWINE FEVER

(review)

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Abstract

African swine fever (ASF), first described in 1921 by R.E. Montgomery (R.E. Montgomery, 1921), has been a major problem in pig production for over 100 years. The search for effective and universal specific vaccine variants started back in 1933 (J. Walker, 1933). This article presents a literature review on the most important and successful events in the history of ASF vaccine development, presenting the approaches on developing attenuated (C. Mucoz-Pérez et al, 2021), inactivated (E. Cadenas-Fernández et al., 2021), subunit (J.G. Neilan et al., 2004) and live vectored (J.K. Jancovich et al., 2018) vaccines. The widespread use of naturally attenuated non-hemadsorbing isolates as vaccines in the second half of the 20th century in European countries led to a persistent chronic ASF infection in a big number of pigs (J. Manso Ribeiro et al., 1963). Successive passages of field isolates of the ASF virus in many cell cultures did not show the proper result in weakening the virulent properties of the pathogen, despite genetic changes in the virus genome (I. Titov et al., 2017). Only modern technologies (e.g., homologous recombination and CRISPR-Cas9 genome editing) for obtaining genetically modified virus ASFV-G-ΔI177L by deleting specific genes in the genome led to the creation of effective candidate vaccines (M.V. Borca et al., 2020). Inactivated, as well as subunit vaccines based on recombinant proteins, caused the formation of specific humoral immune responses in high titers, but did not confer protective properties (G. Burmakina et al., 2016). Live vectored vaccines have become a new milestone in the fight against infectious animal diseases, in particular ASF; human adenovirus 5 (rAd) and modified vaccinia Ankara (MVA) are among the vectors for the development of such vaccines (L.C. Goatley et al., 2020). Attenuated vaccines based on genetically modified viruses with a deletion of specific genes *I226R* and *18-7GD* require international expertise for further registration and use in veterinary practice.

Keywords: African swine fever, ASF, vaccines, inactivated vaccine, attenuated vaccine, DNA-vaccine, recombinant vaccine, CRISPR-Cas9

African swine fever (ASF) is a contagious, natural-focal, transboundary disease of domestic pigs and wild boars, which can be hyperacute, acute, subacute, chronic and asymptomatic. Its etiological agent is an enveloped virus containing double-stranded DNA (dsDNA) (African swine fever virus — ASFV, genus *Asfivirus*, family *Asfarviridae*) [1] (Fig. 1).

The virus genome consists of 170-193 kb and contains 151-167 open reading frames (ORFs) [2]. The pathogen has 9 seroimmunotypes identified in the

haemadsorption delay test (HAD) and in the immunoassay on susceptible animals, and 24 genotypes based on the variability of the *B646L* gene encoding the vp72 capsid protein [2-4]. Due to the conservatism of the latter, intragenotypic differentiation of ASF virus isolates is also carried out based on the analysis of three highly variable genes, the *B602L* (CVR) gene encoding a non-structural chaperone that is involved in the assembly of the capsid, as well as the *E183L* and *CP204L* genes encoding the structural proteins vp54 and vp30 [5, 6].

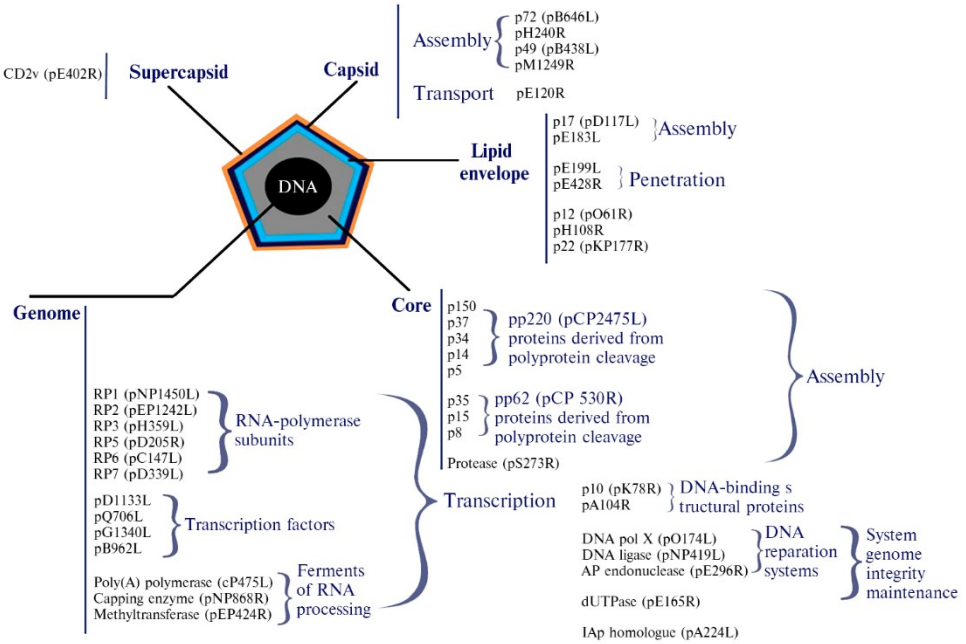
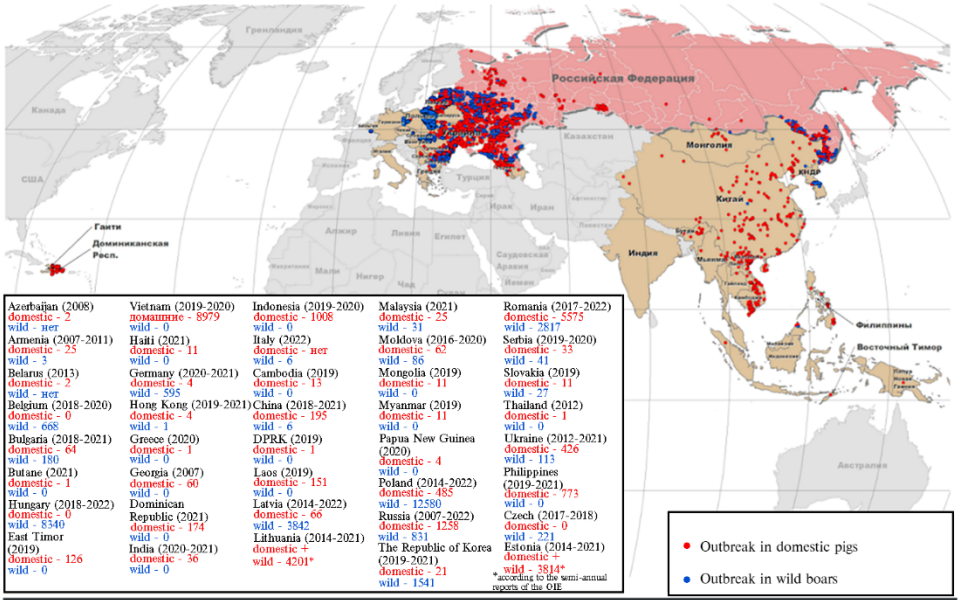


Fig. 1. Morphology of the African swine fever virus virion [1].

Epizootic situation for African swine fever virus in the Russian Federation, Europe, Asia and America, 2007-2022
(OIE emergency report data of 01/17/2022)



Azerbaijan (2008)
domestic - 2
wild - нет

Armenia (2007-2011)
domestic - 25
wild - 3

Belarus (2013)
domestic - 2
wild - нет

Belgium (2018-2020)
domestic - 0
wild - 668

Bulgaria (2018-2021)
domestic - 64
wild - 180

Butane (2021)
domestic - 1
wild - 0

Hungary (2018-2022)
domestic - 0
wild - 8340

East Timor (2019)
domestic - 126
wild - 0

Vietnam (2019-2020)
домашние - 8979
wild - 0

Haiti (2021)
domestic - 11
wild - 0

Germany (2020-2021)
domestic - 4
wild - 595

Hong Kong (2019-2021)
domestic - 4
wild - 1

Greece (2020)
domestic - 1
wild - 0

Georgia (2007)
domestic - 60
wild - 0

Dominican Republic (2021)
domestic - 174
wild - 0

India (2020-2021)
domestic - 36
wild - 0

Indonesia (2019-2020)
domestic - 1008
wild - 0

Italy (2022)
domestic - нет
wild - 6

Cambodia (2019)
domestic - 13
wild - 0

China (2018-2021)
domestic - 195
wild - 6

DPRK (2019)
domestic - 1
wild - 0

Laos (2019)
domestic - 151
wild - 0

Latvia (2014-2022)
domestic - 66
wild - 3842

Lithuania (2014-2021)
domestic +
wild - 4201*

Malaysia (2021)
domestic - 25
wild - 31

Moldova (2016-2020)
domestic - 62
wild - 86

Mongolia (2019)
domestic - 11
wild - 0

Myanmar (2019)
domestic - 11
wild - 0

Papua New Guinea (2020)
domestic - 4
wild - 0

Poland (2014-2022)
domestic - 485
wild - 12580

Russia (2007-2022)
domestic - 1258
wild - 831

The Republic of Korea (2019-2021)
domestic - 21
wild - 1541

Romania (2017-2022)
domestic - 5575
wild - 2817

Serbia (2019-2020)
domestic - 33
wild - 41

Slovakia (2019)
domestic - 11
wild - 27

Thailand (2012)
domestic - 1
wild - 0

Ukraine (2012-2021)
domestic - 426
wild - 113

Philippines (2019-2021)
domestic - 773
wild - 0

Czech (2017-2018)
domestic - 0
wild - 221

Estonia (2014-2021)
domestic +
wild - 3814*

*according to the semi-annual reports of the OIE

Fig. 2. African swine fever (ASF) epizootic situation in the world, 2007-2022 [11].

In the 21st century, in a relatively short period of time, ASF has become a global problem. Over the past 8 years, the rapid spread of infection in Europe (95% of outbreaks in the wild boar population) and Southeast Asia (97% of outbreaks in the domestic pig population), previously free from this disease, has become catastrophic. In the modern ASF panzootic (2007-2022), the first foci of infection officially appeared on the territory of Georgia at the beginning of 2007, when meat products contaminated with the ASF virus, delivered to the port of Poti on ships from the countries of South-East Africa, were sold among the local population . After that, during the spring-summer season around the city of Poti, mass deaths of pigs were recorded, including those that were free-range [7]. In November 2007, the first outbreak of ASF was registered in the Russian Federation in the Chechen Republic among wild boars [8]. In 2012, ASF outbreaks occurred in Ukraine, in 2013 in Belarus, in 2014 in Poland and Estonia, in 2017 in the Czech Republic, in 2018 in Hungary, Belgium and China, in 2020 in Germany , in 2021 in Malaysia, the Republic of Haiti and the Dominican Republic [9, 10]. According to OIE, as of 2021, ASF has been reported in 13 countries in Europe and 11 countries in Asia (Fig. 2) [11]. Between January 2020 and November 2021, the total losses from ASF in Europe and Asia amounted to 1,168,354 and 373,693 domestic pigs, respectively [12].

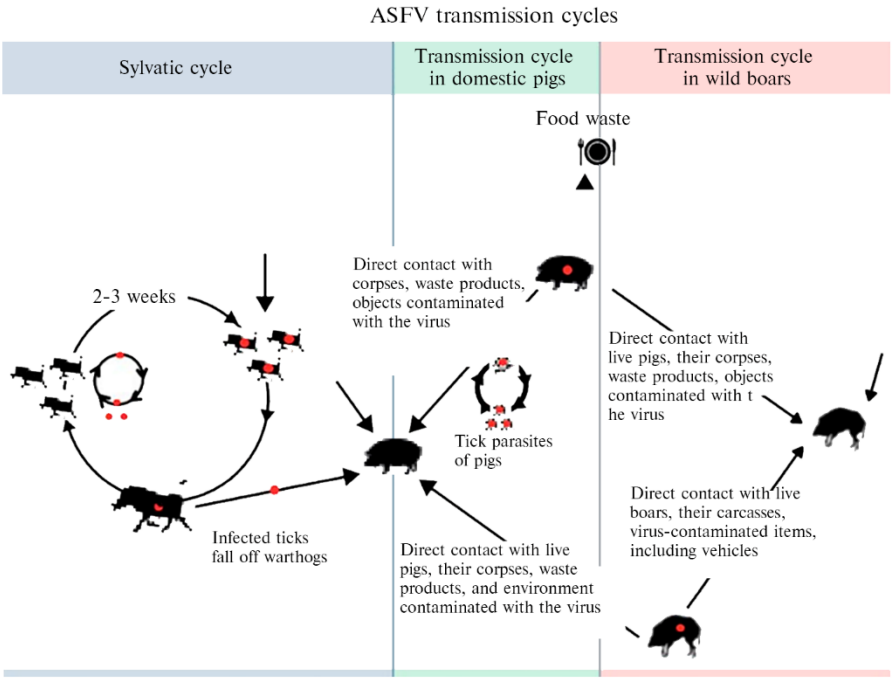


Рис. 3. African swine fever virus (ASFV) transmission [13].

The mechanism of transmission of the ASF virus occurs in three main ways: through soft ticks (sylvatic cycle: countries of South and Southeast Africa, Sardinia), contact transmission and alimentary infection, the last two involving both domestic pigs and wild boars (Fig. 3) [13]. In most regions of Africa, the mutualistic relationship between Ornithodoros moubata (a tick of the family Argasidae) and the warthog Phacochoerus africanus forms a stable sylvatic cycle, thus maintaining the circulation of the virus in nature [14]. In northern hemisphere conditions, wild boars can transmit the virus to domestic pigs by contact [15]. In addition, another mechanism is anthropogenic and associated with active

human activity, including the transportation of pig products and hunting products contaminated with the ASF virus, its entry into the feed of healthy susceptible animals [16].

A distinctive feature of the infection is the formation of a large number of antibodies, but the complete elimination of the virus from the body of a sick animal does not occur [17]. Moreover, due to the presence of the FCγ receptor in a macrophage, an antibody-dependent increase in infection can be observed, leading to excessive synthesis of IL-10, and, as a result, activation of Th2 cells [16, 18-20]. It has been experimentally established that the role of antibodies is to reduce the titers of primary viremia and, as a result, to delay the manifestation of clinical signs [21].

Due to the complexity of studying the mechanism of immune responses in the body of pigs in response to infection with the ASF virus, the search for approaches to the development of effective and safe means of specific prevention continues to this day [22]. In recent years, in the context of the unprecedented spread of ASF in the world, unfortunately, little progress has been made in creating an effective vaccine.

In this regard, in our review, we will consider issues related to the current state of affairs in the development of attenuated, inactivated and recombinant vaccines against ASF.

The role of cellular and humoral immunity in protection against ASF. Most of the available evidence points to the critical role of NK cells in the development of the immune response against ASF. An in vitro study found that virulent ASFV isolates inhibited NK cell activity [23]. A high level of cytotoxic CD8+ T lymphocytes, which destroy infected macrophages in the body, plays an equally significant role in the immune response in pigs [24]. However, hypergammaglobulinemia, as well as increased levels of plasma cells and cytotoxic T-lymphocytes, can cause both specific immunosuppressive and mediated response of the body through IL-4 and IL-10 [25, 26]. To date, attenuated vaccines obtained by deletion of certain genes and recombinant vaccines that induce strong cellular immunity, which is due to the early activity of NK cells and cytotoxic CD8+ T lymphocytes, have shown themselves to be the most effective. The main difficulty associated with the use of DNA vaccines is precisely to ensure the formation of cellular immunity associated with the early activity of NK cells and cytotoxic CD8+ T lymphocytes, which, of course, plays a greater role than short seroconversion to ASF virus proteins. , which are expressed in host cells [27].

Currently, there are works proving the existence of virus-neutralizing antibodies [17]. Convalescent sera obtained after immunization of pigs with the E75CV1-4 variant attenuated in the CV-1 cell culture protected animals from infection with the original E75 isolate, as well as E70, Lisbon 60, Malawi Lil 20/1 isolates of the ASF virus in 86-97% of cases [28]. In pigs passively immunized with purified immunoglobulins against the ASF virus, a delay in primary viremia was observed for 3 days compared with the control group of animals [29]. A study of the virus-neutralizing activity of antibodies in cultures of Vero cells and porcine alveolar macrophages showed 80% neutralization of the radiolabeled ASF virus, while its internalization into cells continued [30]. Antibodies to the cytoplasmic dynein domain DLC8 of the vp54 protein played a leading role in the neutralization of the ASF virus in the Vero cell culture [31, 32]. Despite the inconsistency of the data obtained regarding both the level and the very fact of the formation of humoral immunity against the ASF virus, most researchers agree that specific antibodies are important in the hemadsorption delay reaction upon re-infection with

the ASF virus and in the delay of primary viremia, but they do not play a protective role [17].

Attenuated vaccines. Attenuated vaccine preparations are based on naturally attenuated (or attenuated) viruses obtained in the laboratory through successive passages on sensitive cell cultures, as well as by genetic modification of a virulent virus as a result of the deletion of certain genes.

Naturally attenuated vaccines. The first data on the use of attenuated vaccines were published in 1933 by J. Walker [33]. The percentage of surviving pigs when immunized with such vaccines was low, and the administration of convalescent sera to intact pigs did not protect against infection with a virulent virus. The author then suggested that the low immune protection of pigs against ASF could be due to the high antigenic variability of the isolates due to the large number of strains of the pathogen compared to the classical swine fever virus [33]. Subsequently, it was found that immunization with attenuated vaccines could protect against infection only with a homologous genotype isolate, while infection with a heterologous virus isolate develops the clinical picture of ASF [34, 35].

In the work of F. Boinas et al. [36], pigs were injected with a naturally attenuated ASF virus isolate OURT88/3 (genotype I), isolated from the tick Ornithodoros erraticus of the family Argasidae and showing no haemadsorbing properties, followed by infection with a virulent haemadsorbing virus OURT88/1 (genotype I) as a punch strain. At the same time, neither viremia nor clinical signs of ASF were noted in animals. However, after infection of such pigs with Lisbon 57 isolate (genotype I), the death of the entire population occurred in 10-14 days [36].

When pigs were infected with a non-hemadsorbing NH/P68 isolate (genotype I), isolated in 1968 in Portugal from pigs with a chronic form of ASF, an asymptomatic course of the disease was observed, while early activity of NK cells (from day 7), late viremia (14 days after) and a high level of specific IgM, IgG1, IgG2 and IgA and cytotoxic T lymphocytes detected on days 7-18 after infection. Pigs that showed inapparent infection, early activity, and high NK cell counts were resistant to challenge with the virulent ASF L60 isolate (genotype I). However, in animals with chronic ASF, the level of NK cells was low and approached that of pigs from the control group [37, 38].

K. King et al. [39] immunized pigs with the avirulent OURT88/3 isolate and then with the original naturally attenuated low-virulence strain OURT88/1 of the ASF virus. When such pigs were infected with virulent isolates Benin 97/1 (genotype I) and heterologous Uganda 1965 (genotype X), the level of immune protection was 85.7 and 100%, respectively. Moreover, 78% of immunized pigs challenged with Benin 97/1 isolate and 50% of pigs challenged with Uganda 1965 isolate showed no viremia and no clinical signs of disease [39].

During immunization of pigs with a non-hemadsorbing naturally attenuated isolate of the ASF virus Lv/17/WB/Rie1 (genotype II), isolated from a wild boar in Latvia in 2017, followed by contact with pigs infected with the virulent Arm07 virus (genotype II), in 50% animals of the experimental group noted clinical signs and viremia, and 50% remained clinically healthy, but the viremia persisted. When pigs of the control group were infected with the related hemadsorbing virulent isolate Lv17/WB/Zieme3, death occurred on day 12 [40]. Oral vaccination of wild boars with the Lv/17/WB/Rie1 strain showed 92% immune protection against challenge with Arm07 [41]. A vaccine candidate based on Lv/17/WB/Rie1 is currently being evaluated for safety in the wild boar population. Different survival rates of animals were noted after vaccination and revaccination at doses of

10^3 TCD_{50} and 10^4 TCD_{50}, which shows the importance of further studies of this vaccine prototype [42].

Therefore, naturally attenuated vaccine variants of the ASF virus show wide variability in terms of protective properties even against challenge by homologous genotype isolates [17]. This feature hinders the development of a unified drug with a wide range of protective activity.

Laboratory attenuated vaccines. Cultural vaccines. When attenuating the virulent isolate BA71 of the ASF virus (genotype I), 36 passages were performed on porcine macrophages, followed by 23 passages on a transplanted Vero cell culture. This resulted in the BA71V variant, which proved to be the standard for ASF virus titration by plaque forming units (PFU), but this variant did not reduce the lethality of pigs upon infection [43].

After 50 successive passages of the virulent K49 ASF virus isolate (genotype I) on a transplantable porcine embryonic kidney (SPEV) cell culture and 262 passages on a primary culture of porcine bone marrow cells, the avirulent KK262 strain was obtained. After two injections of KK262 to the pigs of the experimental group (on the 1st and 21st days), viremia was observed in 33% of the animals on day 28, but when infected with the initial virulent K49 isolate, on the 42nd day from the start of the test, all pigs remained alive [44].

When studying the biological properties of the Odintsovo 02/14 isolate (genotype II) isolated from wild boar, three successive passages of the virus were performed on a culture of porcine bone marrow cells (PBC). It is important to note that the initial field isolate isolated from the spleen of a fallen wild boar caused 87.5% lethality in domestic pigs after the isolate was administered to five animals intramuscularly at a dose of 10 HAD_{50} and to five animals intranasally at a dose of 50 HAD_{50} [27, 45]. Adaptation of the Odintsovo 02/14 isolate on a transplanted CV-1 cell culture for 30 consecutive passages (the virus was named ASF/ARRIAH/CV-1/30) resulted in a decrease in hemadsorption from 40-50 to 20-30 erythrocytes attached to an infected cell. Infection of pigs with this variant showed a decrease in mortality to 16.7% and the resistance of surviving animals to control infection with the virulent Arm 07 isolate [46-48].

When adapting the Georgia 2007/1 isolate, 110 passages were carried out in Vero cell culture. At the 80th passage, the ASFV-G/VP80 variant showed a 10-fold reduced ability to replicate in a primary trypsinated porcine macrophage cell culture, and at the 110th passage, the ASFV-G/VP110 variant showed a 10^5-10^6-fold decrease in replication. When pigs were immunized with the ASFV-G/VP110 variant, no clinical signs of the disease were noted during 21 days of observation. However, control infection with the Georgia 2007/1 isolate did not show the effectiveness of such immunization, since all pigs showed clinical signs of ASF and death was recorded on day 9 [49].

The results of the conducted studies on laboratory attenuation of field isolates allow us to conclude that, as a rule, there is no such weakening of the virulent properties of the ASF virus in order to further use it for safe immunization. However, in the development of vaccines, targeted modification of the genes of attenuated variants of the ASF virus using molecular biology methods may be successful.

Vaccines based on genetically modified viruses. One promising new approach to obtaining safe attenuated vaccines involves the deletion of specific ASF virus genes encoding proteins that serve as virulence factors [22, 50]. However, in this case, significant difficulties are associated with the fact that the functions of many proteins have not been studied. For example, deletion of the

KI69R gene encoding thymidine kinase resulted in a loss of virulence in the Malawi Lil-20/1 isolate (genotype VIII), but retained virulence in the Georgia 2007/1 isolate (genotype II), which, apparently, is explained by compensatory mutations in other parts of the genome [51, 52].

Removal of six genes of the *MGF360* and *MGF505* multigene families from Georgia 2007/1 (*MGF505-1R*, *MGF360-12L*, *MGF360-13L*, *MGF360-14L*, *MGF505-2R*, and *MGF505-3R*) completely weakens the virulence properties of the virus. Immunization of animals with this deleted variant at doses of 10^2 and 10^4 HAD_{50} did not cause the development of clinical signs of ASF, and after control infection with the virulent Georgia 2007/1 strain, no signs of the chronic course of the disease were noted, however, moderate viremia was detected for approx. 7.5 days [53]. When immunized with the Georgia 2007/1 variant with a deletion of the *9GL* gene encoding phosphorylase, a higher level of protection was observed when combined with a deletion of the *UK* (*DP96R*) gene [50, 54]. However, simultaneous deletion of the *MGF360* and *MGF505* genes encoding type I interferon inhibitors and *9GL* did not result in a protective effect during immunization [55].

Pigs immunized with the BA71ΔCD2 variant with a deletion of the *EP402R* gene obtained by homologous recombination were resistant to infection with the original virulent BA71 isolate (genotype I), as well as virulent E75 isolates (genotype I), Georgia 2007/1, RSA/11/2017 (genotype XIX), Ken06.Bus (genotype IX) [56, 57].

Deletion of the DP148R gene in the Benin 97/1 ASF virus isolate (genotype I) resulted in a complete loss of virulence. In pigs immunized with BeninΔDP148R, intramuscular infection with the original isolate showed 100% protection, while intranasal infection was 83.3% [58].

When immunizing pigs with NH/P68DA238L-COS7 variants with a deletion of the *A238L* gene, NH/P68DA224L-COS7 with a deletion of the *A224L* gene, NH/P68DEP153R-COS7 with a deletion of the *EP153R* gene at a dose of 10^6 TCD_{50} and NH/P68DA276R-PAM at a dose of 10^2 TCD_{50} followed by a control infection of Arm07 strain at 10 HAD_{50}, the weakening of the virulent properties was achieved only for a deletion of the *A224L* gene. Immune protection in this case reached 100% [59].

In studies on the deletion of the *MGF505-1R*, *MGF505-2R*, *MGF505-3R*, *MGF360-12L*, *MGF360-13L*, *MGF360-14L*, *EP402R*, *9GL*, *DP148R* genes encoding seven different proteins, Chinese scientists [60] obtained a modified version of the ASF virus HLJ/18-7GD, after intramuscular inoculation of which the pigs remained clinically healthy for 3 weeks of observation. When pigs were infected with the virulent HLJ/18 isolate (genotype II) at 200 LD_{50}, animals immunized with 10^3 TCD50 HLJ/18-7GD developed fever for 3-9 days with a maximum rise in temperature up to 42 °C, however, in pigs immunized with 10^5 $TCD5^0$ HLJ/18-7GD, a slight rise in temperature to 40.7 °C occurred only during the first day [60].

In 2020, a group of scientists from the USA published data on 100% protection of the pig population immunized with an attenuated ASFV-G-ΔI177L variant of the ASF virus, obtained from the original virulent isolate of ASFV-G (Georgia 2007/1) as a result of the deletion of the I177L gene, which had previously The study was not typical of such experiments. In the experimental group, pigs were injected with ASFV-G-ΔI177L at 10^2 HAD_{50} after which insignificant titers were recorded during viremia ($10^{1.8}$-10^5 HAD_{50}/cm^3 on day 4, peak at 10^4-$10^{7.5}$ HAD_{50}/cm^3 on day 11 followed with a decrease to $10^{2.3}$-10^4 HAD_{50}/cm^3 up

to day 28) and the absence of any clinical signs of ASF during 28 days of observation. These pigs were subsequently challenged with 10^2 HAD_{50} of the original ASFV-G isolate. Within 21 days, the animals had no clinical signs of ASF, viremia developed, titers did not exceed those in the first observation period, and the virulent virus was not detected in the blood by RT-PCR (qPCR) [61]. The work of M.V. Borca et al. [61] was the first report on the formation of sterile immunity against ASF in the history of the study of this infection. Adaptation of ASFV-G-ΔI177L on PIPEC (Plum Island porcine epithelial cells) cell culture resulted in a stable isolate of ASFV-G-ΔI177L/ΔLVR with a deletion of the *MGF* and *X69R* genes, protecting 100% of pigs from infection with virulent ASFV-G, which can be used in the production of the universal ASF vaccine [62]. Later, the authors showed the effectiveness of an experimental vaccine based on ASFV-G-ΔI177L when administered orally, which is of great importance from the point of view of the prospects for immunization of wild pigs (especially the European boar *Sus scrofa*). At the same dose with intramuscular inoculation, viremia titers for oronasal administration were significantly lower, while IgG1, IgG2 and IgM titers remained at the same level [63]. A recent study showed that an experimental vaccine based on ASFV-G-ΔI177L successfully induced immune protection in Vietnamese pigs against ASF virus (genotype II) field isolates in Vietnam [64].

Similar studies were carried out in the PRC in 2021, where the SY18ΔI226R isolate with a deletion of the previously undescribed functional I226R gene encoding the conserved pI226R protein, localized in the virosome of the cell cytoplasm ("viral factories"), was used as a candidate vaccine. After application of 10^4 TCD_{50} and 10^7 $TCD5_0$ SY18ΔI226R to pigs of two test groups the animals showed no increase in body temperature above 40.1 °C and no clinical signs of the disease. When the first group was infected with the initial isolate SY18 (genotype II) at $10^{2.5}$ TCD_{50}, a 2-day fever was recorded with a maximum temperature increase of up to 41.4 °C. In the second group, when infected with 10^4 TCD_{50} SY18, no fever or other clinical signs of ASF occurred during the observation period with 0% mortality in both groups [65].

1. Genetically modified variants of African swine fever virus (ASFV) used as promising vaccine strains

Experimental ASFV	Delited genes	Control isolate (genotype)	Infected/survived (protection effect, %)	References
BA71ΔCD2, 10^6 HAD_{50}	*EP402R* (CD2v)	BA71 (I)	6/6 (100 %)	[56]
BA71ΔCD2, 10^3 HAD_{50}	*EP402R* (CD2v)	BA71 (I)	6/2 (33 %)	[56]
BA71ΔCD2, $3{,}3\times10^4$ or 10^6 HAD_{50}	*EP402R* (CD2v)	E75 (I)	12/12 (100 %)	[56]
BA71ΔCD2, 10^3 HAD_{50}	*EP402R* (CD2v)	E75 (I)	6/1 (17 %)	[56]
BA71ΔCD2, $3{,}3\times10^4$ or 10^6 HAU_{50}	*EP402R* (CD2v)	Georgia 2007/1 (II)	18/18 (100 %)	[56]
BA71ΔCD2, $3{,}3\times10^4$ or 10^6 HAD_{50}	*EP402R* (CD2v)	RSA/11/2017 (XIX)	6/5 (83.3 %)	[57]
BA71ΔCD2, $3{,}3\times10^4$ and 10^6 HAD_{50}	*EP402R* (CD2v)	Ken06.Bus (IX)	8/4 (50 %)	[57]
HLJ/18-7GD	*MGF505-1R*, *MGF505-2R*, *MGF505-3R*, *MGF360-12L*, *MGF360-13L*, *MGF360-14L*, *EP402R*, *9GL*, *DP148R*	HLJ/18 (II)	4/4 (100 %)	[60]
BeninΔDP148R	*DP148R*	Benin (I), intramuscularly	11/11 (100 %)	[58]

Continued Table 1

BeninΔDP148R	*DP148R*	Benin (I), intranasally	6/5 (83.3 %)	[58]
ASFV-G-ΔI177L, 10^2 HAD_{50}	*I177L*	Georgia 2007/1 (II)	10/10 (100 %)	[61]
ASFV-G-ΔI177L, 10^4 HAD_{50}	*I177L*	Georgia 2007/1 (II)	5/5 (100 %)	[61]
ASFV-G-ΔI177L, 10^6 HAD_{50}	*I177L*	Georgia 2007/1 (II)	5/5 (100 %)	[61]
ASFV-G-ΔI177L (oronasal immunization)	*I177L*	Georgia 2007/1 (II)	10/10 (100 %)	[63]
SY18ΔI226R	*I226R*	SY18 (II)	10/10 (100 %)	[65]
Georgia 2007/1	*9GL* (*B119L*) and *UK* (*DP96R*)	Georgia 2007/1 (II)	5/5 (100 %)	[50]
Pr4Δ9GL	*9GL* (*B119L*)	Pr4 (XX)	4/4 (100 %)	[76]
NH/P68DA238L-COS7, 10^6 TCD_{50}	*A238L*	Arm07 (II)	4/0 (0 %)	[59]
NH/P68DA224L-COS7, 10^6 TCD_{50}	*A224L*	Arm07 (II)	4/4 (100 %)	[59]
NH/P68DEP153R-COS7, 10^6 TCD_{50}	*EP153R*	Arm07 (II)	4/0 (0 %)	[59]
NH/P68DA276R-PAM, 10^2 TCD_{50}	*A276R*	Arm07 (II)	5/0 (0 %)	[59]
ASFV-G-ΔMGF, 10^2 HAD_{50}	*MGF505-1R*, *MGF360-12L*, *MGF360-13L*, *MGF360-14L*, *MGF505-2R*, *MGF505-3R*	Georgia 2007/1 (II)	10/10 (100 %)	[55]
ASFV-G-ΔMGF, 10^4 HAD_{50}	*MGF505-1R*, *MGF360-12L*, *MGF360-13L*, *MGF360-14L*, *MGF505-2R*, *MGF505-3R*	Georgia 2007/1 (II)	10/10 (100 %)	[55]

Published data on ASFV vaccine preparations, including genes to be edited and survival rates after challenge, are presented in Table 1. This summarizes the past decade of ASFV genetic modification studies using the best characterized and studied virulent isolates (in particular, Georgia 2007, Benin, E75).

It can be seen (Table 1) that due to the bioinformatic analysis of the ASF virus genome, the choice of target genes for deletion in order to obtain a candidate vaccine has expanded. When pigs were immunized with experimental viruses, the doses ranged from 10 to 10^6 HAD_{50}. The results obtained so far indicate that deletions in various genes (e.g., *I177L*, *9GL* and I226R) reduce the virulence of the virus and provide sufficient protection against re-infection. The effectiveness of a drug may vary depending on the genotype of the parent isolate, the dose of immunization, and even the route of administration of the virus. Moreover, the deletion of a number of genes can affect the virulence of the virus and the degree of protection against re-infection in different ways, depending solely on the method of immunization. These results demonstrate the difficulty of determining the best vaccination route for ASF control.

Inactivated vaccines. Inactivated vaccines are biological preparations in which the replication activity of the virus is suppressed under the action of a chemical. Attempts to develop an effective inactivated vaccine against ASF, similar to other infectious animal diseases, were made simultaneously with the creation of attenuated variants of the virus. However, already in 1967, when studying the immune response to an inactivated vaccine, it was found that immunized pigs did not develop resistance to infection with a virulent isolate (66). When immunized with a culture vaccine inactivated by glyceralaldehyde, resistance to spleen injury was noted in some animals, but protective immunity was not formed when infected with a virulent ASFV isolate (67). Despite initial setbacks, research continued.

Thus, when using an inactivated ASF virus isolated from an extract of the spleen of diseased pigs and N-octylglucoside as an adjuvant, immunized pigs showed resistance to infection with a homologous isolate, but were sensitive to a heterologous isolate [68]. In 2021, new and extremely active adjuvants (Silicaoil, mGNE, etc,) were used for virus inactivation with binary ethyleneimine at low temperatures. However, infection of immunized pigs with a virulent ASFV isolate did not develop protective immunity [69].

Because of the strong evidence that this type of ASF vaccine is not appropriate, control experiments are being conducted to prove that inactivated ASF vaccines are not viable [70].

As a result, it should be noted that all approaches used to develop an inactivated vaccine were not successful. This, in turn, raises a number of important questions regarding the genetic and antigenic variability of the virus, which require detailed study.

Subunit vaccines. In the late 1990s, the attention of scientists was focused on recombinant proteins obtained in the baculovirus expression system for the immunization of pigs against ASF.

In the experiment, the CD2v protein synthesized in this way was administered to pigs in different doses. After infection of pigs with intact virus, a temporary delay in haemadsorption and a temporary dose-dependent delay in the development of infection were observed, but protective immunity was not formed [71]. Administration of the recombinant vp12, vp30 and vp54 proteins produced specific immunoglobulins in pigs that delayed the entry of the virus into target host cells, but high antibody titers did not result in resistance in pigs when infected with virulent ASFV isolate [72, 73].

The use of the vp54/30 chimeric protein, which was obtained by expression of the *CP204L* gene integrated into the restriction site of the *E183L* gene in the baculovirus system, in porcine macrophage cell culture resulted in 50% neutralization of the ASF virus by specific antibodies. A bioassay with immunization with the vp54/30 chimeric protein and subsequent infection with the E75 isolate showed 100% survival of pigs in chronic ASF [74]. When using chimeric CD2v proteins and type C lectin encoded by the *EP153R* gene, the role of specific antibodies protecting against infection with a homologous ASFV isolate was proven [75].

In 2004, an experiment was conducted on susceptible animals to compare subunit vaccines and vaccines based on genetically modified ASFV, using two groups of pigs: the first was immunized with the Pr4Δ9GL isolate with a deletion of the *B119GL* gene, the second was injected with recombinant proteins vp30, vp54, vp72 and vp22, expressed in the baculovirus system. After infection with the original Pr4 isolate (XX genotype), the animals of the first group showed clinical signs of ASF and viremia with an insignificant titer (2.9±0.6 TCD_{50}/ml), however, death did not occur, while in the second group, viremia developed with a delay on day 2 after which the virus titer was 9.1±0.3 TCD*50*/ml, and on day 8.5±0.5 after infection the animals died [76]. Such data cast doubt on the existence of virus-neutralizing antibodies involved in the formation of immune protection against ASF.

As Table 2 shows, various recombinant proteins can be used as candidate subunit vaccines for immunization of pigs, but almost all protein combinations fail to protect immunized animals from re-infection, and all the results obtained so far are unpromising. The only exception was the vp54/30 chimeric protein,

immunization with which ensured the survival of pigs during the control infection with a virulent isolate, however, ASF took a chronic course and the virus was still isolated from the body of pigs. Similar works related to the use of subunit vaccines as candidates cannot yet be considered promising either. This is largely due to the lack of knowledge of many antigens that can protect pigs from infection with the ASF virus.

2. Used African swine fever (ASFV) **subunit vaccine prototypes**

ASFV proteins	Expression system	Protective effect	References
vp12	Baculovirus	No protection	[73]
CD2v	Baculovirus	No protection	[71]
vp54 and vp30	Baculovirus	No protection	[73]
Chimeric proein vp54/30	Baculovirus	0 % mortality (2/2), chronic ASF	[74]
vp30, vp54, vp72 and vp22	Baculovirus	No protection	[76]
CD2v and lectin type C	Baculovirus	Partial protection	[75]

Recombinant (vector) vaccines. In connection with the development of genetic engineering and molecular biology, the development of recombinant vaccines, including against ASFV, in which plasmids and heterologous viruses can be used as a vector, has become methodically accessible [77-79).

For example, when using the plasmid construct pCMV-UbsHAPQ, encoding the chimeric proteins vp54, vp30 and CD2v coupled with ubiquitin to increase the possibility of expression together with MHC class I molecules on the cytoplasmic membrane of target cells of pigs, as expected, there was a high level of cytotoxic CD8+ T lymphocytes with a peak on the 3rd day after immunization. After infection with the virulent E75 isolate, partial protection against ASF was observed in pigs with a 66.0% mortality [78]. Plasmid BacMam-sHAPQ can be used as a promising vector for the expression of vp54, vp30 and sHA proteins, the combination of which, when immunized pigs, showed high levels of cytokines in four out of six animals [78].

When constructing modified adenoviruses to express ASFV proteins, the *A151R*, *B119L*, *B602L*, *EP402RΔPRR*, *B438L*, *K205R*, and *A104R* genes of the Georgia 2007/1 isolate were incorporated into the adenoviral genome. In cultured porcine bone marrow cells (PMC), this recombinant construct induced a high level of γ-interferon on day 7 after inoculation, but due to the lack of tests on susceptible animals, it cannot be concluded that there is immune protection against virulent ASF isolates [80]. Immunization of wild boars with 35 ASFV antigens expressed using human adenovirus 5 as a vector showed no immune protection when challenged with the virulent Arm 07 isolate [78].

Cloning of 47 antigens of the ASF virus to plasmids and vaccinia virus as vectors did not give notable results in the search for recombinant vaccines, since when pigs immunized with the recombinant variant were infected with the virulent Georgia 2007/1 isolate at 10^4 HAD_{50}, in all animals of the experimental group, tere was an acute form of the disease, despite the high level of production of γ-interferon by cytotoxic CD8+ T lymphocytes in vitro (6×10^5 cells) [81].

In a similar study, the *Escherichia coli* pGEX 4T-1 plasmid served as a vector, in which the genes of the ASF virus were cloned in two variants: in one, encoding proteins CD2v, vp32, vp72, and vp17; in the other, proteins vp15, vp35, vp54, and vp17. The level of γ-interferon synthesized in blood mononuclear cells sensitized with the recombinant variant barely exceeded that in the control sample, and 100% mortality was observed when immunized pigs were infected with the virulent ASF virus isolate Arm 07 [82].

Very encouraging results were obtained from a study in which eight genes

of the ASFV isolate OURT88/3 (*B602L*, *B646L*, *E183L*, *E199L*, *CP204L*, *F317L*, *EP153R*, and *MGF505*-5R) were incorporated into human adenovirus 5 (rAd) and modified vaccinia virus Ankara (MVA) vectors. When pigs were injected with the recombinant rAd virus at a dose of 1.5×10^{10} IU and the recombinant MVA virus at a dose of 2×10^{8} PFU and re-infected with the virulent ASF isolate OURT88/1, viremia was observed for 6 days while neither animal died [83].

An important step in the development of molecular biology was the CRISPR/Cas9 technology, which has already experimentally shown results in the expression of the vp30 protein encoded by the ASFV *CP204L* gene [84]. A recombinant variant of the highly virulent isolate ASFV-Kenya-IX-1033 (genotype IX) with the deletion of the *A238L* gene was synthesized using the CRISPR/Cas9 system [85]. This method is considered as the most promising for editing the genome of viruses, including the causative agent of ASF.

The available generalized information on genetically engineered ASF vaccines is presented in Table 3. From the data in this table, it can be seen that the use of recombination of various genes expressed using plasmid and viral vectors is one of the most interesting and promising approaches in the development of an ASF vaccine. Different genes can be pooled to achieve the best result, which ranges from complete lack of protection to 100% protection (see Table 3). The success of such results depends both on the combination of genes used and on their influence on each other.

3. The use of recombinant constructs as a potential vaccine against African swine fever

ASFV proteins	Expression system, vector	Protective effect	References
Ubiquitin-CD2v, vp54 and vp30	Plasmid pCMV-UbsHAPQ	Partial protection (33 %)	[78]
vp54, vp30, sHA	Plasmid BacMam-sHAPQ	Partial protection	[77]
7 antigenes: vpA151R, vpB119L, vpB602L, vpEP402RΔPRR, vpB438L, vpK205R and vpA104R	Human adenovirus 5	Биопроба не проводилась	[80]
47 antigenes	Plasmid pCMVi-LS and modified vaccinia virus	No protection	[81]
CD2v, vp32, vp72, vp17	Plasmid *Escherichia coli* pGEX 4T-1	No protection	[82]
vp15, vp35, vp54 and vp17	Plasmid *Escherichia coli* pGEX 4T-1	No protection	[82]
35 antigenes	Human adenovirus 5	No protection	[79]
8 antigenes: vpB602L, vpB646L, vpE183L, vpE199L, vpCP204L, vpF317L, vpEP153R, vpMGF505-5R	Human adenovirus 5	Complee protection (100 %)	[83]
8 antigenes: vpB602L, vpB646L, vpE183L, vpE199L, vpCP204L, vpF317L, vpEP153R, vpMGF505-5R	Modified vaccinia virus Ankara (MVA)	Complee protection (100 %)	[83]

Thus, it can be concluded that, despite the considerable accumulated experience in studying the ASF virus genome, as well as the functional and immunological characteristics of viral proteins, today there is not a single certified ASF vaccine in the world. After a relatively long lull, a new ASF epizootic in 2007-2021 resulted in multi-billion dollar losses across all swine and hunting sectors. In China, losses from ASF in 2018-2019 amounted to 0.78% of the country's gross product [86]. In this regard, the issue of the need for effective protection of farms from ASF has again become acute, not only by methods of general prevention, but also by vaccination. An independent evaluation of ASF-specific prophylactic drugs requires further research into potential vaccine candidates in commercial swine production in different countries in order to avoid the chronic course of ASF that persists in a particular country [87]. To implement the strategy for differentiating infected vs. vaccinated animals (DIVA), it is proposed to use a vaccine variant of

the ASF virus with an induced deletion of the conserved E184L gene of the immunogenic protein, which makes it possible to clearly determine the absence of antibodies to this protein in vaccinated individuals [88].

So, the development of effective and safe vaccines against African swine fever (ASF) has been going on for almost 90 years. The widespread use in the middle of the 20th century of attenuated vaccines against homologous isolates of the ASF virus in many European countries (Spain, Portugal) led to large-scale circulation of the pathogenic virus in the population of domestic and wild pigs and to an increase in the number of livestock with a chronic course of the disease. An attempt to weaken the virulent properties of the virus by successive passages on many primary and continuous cell culture lines in order to create a vaccine was not successful. Inactivated and also subunit vaccines based on recombinant proteins had high immunogenicity, but they did not have protective properties, regardless of which adjuvants and inactivants were used. The use of homologous recombination technology to obtain clones of the ASF virus with the deletion of certain genes has led to the creation of successful candidate vaccines. Human adenovirus 5 (rAd) and modified vaccinia Ankara (MVA) virus have proven themselves as vector constructs for transferring ASFV DNA in the development of effective recombinant (vector) vaccines. The most promising, from our point of view, are experimental vaccines based on genetically modified viruses ASFV-G-ΔI177L and SY18ΔI226R, demonstrating 100% protection of pigs, at least against homologous ASFV genotype II isolates. Similar expectations are raised by the HLJ/18-7GD candidate vaccine.

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