African swine fever (ASF) is a contagious viral disease affecting swine and wild boars, characterized by hemorrhagic fever. ASF in animals may occur in a variety of forms ranging from peracute to inapparent. Mortality in case of infection with highly virulent isolates reaches 100%. To date, there is no ASF effective treatment and specific prevention [6, 10, 12, 14].

Russia has been permanently ASF-affected since the introduction of the pathogen into its territory in 2007. The main economic damage caused by the disease includes the costs of emergency slaughter of animals and the eradication of outbreaks, as well as the costs resulting from restrictions imposed on domestic and international trade. The absence of available preparations for ASF specific prophylaxis and treatment in pig farming indicates the need for the development of these preparations. This requires an extended study of factors affecting the immune response. Regulation of the immune response by cytokines plays an important role in the course of various pathological processes during viral infection [28]. An unbalanced immune response and excessive production of pro-inflammatory cytokines causes damage to the tissues of the host. This shall be considered when vaccinating animals with attenuated viruses.

One of the strategies of a macroorganism aimed at preventing such consequences of the immune response is the release of an immunosuppressive cytokine – interleukin-10 (IL-10) [22]. Interleukin-10 is a pleiotropic cytokine. It is one of the most important cytokines that regulates immunity and that was first characterized as a factor inhibiting the synthesis of cytokines. IL-10 shows a strong anti-inflammatory effect.
Addition of IL-10 to B-lymphocytes leads to their proliferation and a high level of immunoglobulin production as a type of dominant cytokines. After stimulation by interferon-gamma (INF-γ) and lipopolysaccharides, M1 macrophages are polarized, and they initiate Th1-cell response and protect against intracellular pathogens, destroying the affected cells.

In contrast, activation through IL-4 or IL-13 induces M2 macrophages, which produce a high level of anti-inflammatory cytokine IL-10 and are associated with mechanisms of immunosuppression and wound healing [25]. In addition to macrophages, IL-10 produces T- and B-cells, as well as mast cells, keratinocytes and some tumor cell lines.

The main effect of IL-10 is inhibition of activity of NK cells (natural killer cells) and T-cells, the cytokine production by TH1 cells (T-helper cells) and peripheral blood mononuclear cells, as well as the influence on the activity of macrophages. In macrophages, IL-10 inhibits the production of pro-inflammatory cytokines, such as tumor necrosis factor alpha (TNF-α) and IL-6 [17, 29]. On the other hand, IL-10 has an immunostimulatory effect on B-cells. Addition of IL-10 to B-lymphocytes leads to their proliferation and a high level of immunoglobulin production as a result of B-cells transformation into plasma cells [16]. The immunosuppressive functions of IL-10 suggest its possible clinical use for the suppression of transplant rejection. In addition, IL-10 can inhibit antigen-induced INF-γ production by NK cells.

Studies have shown that blocking the IL-10 signaling pathway during vaccination increases the induction of a cytotoxic T-cell immune response [23]. It has also been shown that transgenic mice with a deficiency of IL-10 demonstrate excessive production of inflammatory cytokines, which leads to chronic inflammation [5, 18, 21, 26, 32].

The regulation of cytokine production and the immune response in case of infection with ASFV isolates of different virulence has not been fully studied. When comparing in vitro and in vivo cytokine production by macrophages, it was established that infection with low virulent virus isolates leads to an immune response with a predominance of cytokines involved in cellular immunity, such as INF-α and IL-12p40, as compared with infection with highly virulent isolates [30]. Other studies demonstrate that there is no noticeable difference in the production of granulocyte-macrophage colony stimulating factor (GM-CSF), IL-2, IL-4, IL-6, IL-10, IL-12 and TNF-α by monocytes and macrophages infected with either avirulent or virulent strains [9].

S. Gil et al. have demonstrated that the infection with low-virulent ASFV isolates is accompanied by a significant increase in expression and earlier production of pro-inflammatory cytokines and cytokines inducing cell-mediated immune response. This does not happen in case of infection with highly virulent isolates. Thus, the virulence of ASFV isolates may depend on the capacity to regulate cytokine expression by macrophages [13].

The aim of this paper was to study the effect of African swine fever virus on the production of IL-10 in swine.

**MATERIALS AND METHODS**

Piglets of 15–20 kg body weight originating from farms free from infectious diseases of the Vladimir Oblast were used in experimental infection. The animals were kept in an isolated room of the FGBI "ARRIAH" animal facility which meets the requirements for work with biological agents of Pathogenicity group II (BSL-3). During a 9-day quarantine, the blood serum of animals was examined to confirm seronegative status for the agents of the main swine diseases. All animal experiments were carried out in accordance with the requirements of Directive 2010/63/EU of the European Parliament and of the Council of the European Union of 22 September 2012 on the protection of animals used for scientific purposes.

Continuous cell culture-adapted ASFV isolate Vero25 was used for intramuscular infection at a dose of 10 HADU per animal. 12 piglets were infected with the virus-containing suspension and 2 more piglets were kept in the same room with the infected piglets as the controls. The piglets were challenged with the reference ASF virus isolate Arm 07 at a dose of 1,000 HADU per animal.

Thermometry and animal monitoring were performed daily. Blood samples were taken from the piglets to obtain serum at different time points after challenge. Serum was separated from the whole blood 18 h after collection and frozen until analyzed.

IL-10 amount in blood sera was determined using Invitrogen test systems (Thermo Fisher, USA) according to the manufacturer’s instructions. Analytical sensitivity of the test system is more than 3 pg/ml.

To determine the ability of the ASFV virus isolate to induce antibody production and the time of antibody detection, an enzyme-linked immunosorbent assay (ELISA) was performed using the Ingezim PPA Compac K3 kit (Ingenasa, Spain) in accordance with the manufacturer’s instructions.

The polymerase chain reaction (PCR) was used to determine the challenge effectiveness based on the presence of the ASFV genetic material in blood. “Test system for the diagnosis of African swine fever by polymerase chain reaction with real-time hybridization-fluorescence detection” (FGBi "ARRIAH", Russia) was used.

**RESULTS AND DISCUSSION**

On days 8–15 post infection, five of the twelve infected animals died demonstrating ASF clinical signs and pathology. Seven infected animals survived, and some of them showed only a slight increase in temperature, which indicated that the isolate was moderately virulent. Both control piglets survived without showing any ASF clinical signs, which demonstrates the absence of contact transmission of the virulent virus, which is confirmed by the PCR results (see Table).

Seven surviving and two control piglets were challenged 30 days after the start of the experiment. On days 6–7 post challenge, the control pigs died showing ASF clinical signs, which indicates the absence of protective immunity.

Prior to infection, animals had different IL-10 serum levels. In three of the five dead animals, the level of the tested system used (4–5 pg/ml). In two dead animals, its level before infection was 5–8 pg/ml, which does not confirm the conclusion of J. Post et al. [20] on the effect of high IL-10 levels prior to infection on the survival rate of animals. However, when dividing animals into groups, including dead and survivors, the correlation between the IL-10 serum level after infection and the death of animals has been established. In surviving animals, the IL-10 amount was at the lower sensitivity limit of the test system used (4–5 pg/ml). A higher IL-10 level (15.8–173 pg/ml) was observed in blood sera of all five dead animals.
The IL-10 level in one control animal increased slightly on day 14 after the start of the experiment (29.8 pg/ml), reaching the value of 163.4 pg/ml by day 28. At the same time, ASF virus genome and anti-ASFV antibodies were not detected in blood samples of piglets throughout the experiment until the challenge, which indicates the influence of other factors, for example, other infectious pathology.

The second control animal had a low IL-10 level before the challenge (7.1 pg/ml), which increased after challenge, reaching 28.3 pg/ml before the death. Animals died showing clinical signs and postmortem changes characteristic of ASF.

Among a variety of IL-10 impacts on the organism, it can be assumed that the ASF pathogenesis is mainly influenced by:

1. Inhibition of TNF-α production by monocytes and macrophages. The onset of ASF clinical picture is accompanied by pro-inflammatory activation of monocytes and macrophages. Activated monocytes and macrophages secrete a wide range of mediators, including pro-inflammatory cytokines, among which TNF-α is one of the most important [2, 19].

TNF-α can induce vascular changes, including vaso-dilation and increased vascular permeability, as well as change the coagulation status of the vascular endothelium. An increase in TNF-α production together with IL-1α, IL-1β, and IL-6 is one of the mechanisms involved in the development of fever, vascular damage, and changes in lymphoid structures [8].

Various researchers have found that ASF infection is associated with TNF-α production, and this cytokine probably regulates the main clinical manifestations of the disease. Infection of porcine macrophages with ASF virus leads to an increase in TNF-α expression in vitro. Besides, a high level of TNF-α is found in the serum of animals infected with ASF virus. When infected with a highly virulent virus, significant production of TNF-α is observed already in the early stages [27].

Besides, pulmonary intravascular macrophages are affected by ASF. Their infection leads to the activation and expression of IL-1α and TNF-α. These cytokines are involved in the mechanism of development of interstitial edema and the formation of microthrombi in the capillaries of the alveolar septa; this plays a leading role in the appearance of lung lesions [2, 7, 31].

### Table

**Results of detection of ASF virus genome and neutralizing antibodies**

<table>
<thead>
<tr>
<th>Animal number</th>
<th>Days post infection</th>
<th>PCR</th>
<th>ELISA</th>
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<tr>
<td></td>
<td>3</td>
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<td>28</td>
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<td>42 (12 dpc)</td>
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<tr>
<td></td>
<td>50 (20 dpc)</td>
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</table>

**Legend:**

- PCR: «+» – cycle threshold (Ct) corresponds to a positive result; «-» – Ct corresponds to a negative result; «±» – Ct corresponds to an equivocal result;
- ELISA: «+» – positivity percentage corresponds to positive result; «-» – positivity percentage corresponds to negative result.

C – control animal.

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An increase in IL-10 level at the final stage of acute ASF infection is the result of the anti-inflammatory response [27]. The inhibiting effect of IL-10 on TNF-α and IL-1 is critical for its anti-inflammatory activity, since these cytokines have a synergistic effect on inflammation, enhancing it through the induction of prostaglandins, platelet activating factor and cytokine-activating factor [4]. IL-10 not only induces inhibition of these effectors, but also enhances the expression of their antagonists [22].

Hemorrhagic syndrome in case of ASF causes serious damage to the organism [1]. TNF-α inhibition in the late stages of acute ASF is a factor that shall lead to a more favorable prognosis, as shown by J. Post et al. [20]. However, in this experiment, all dead animals had a high level of IL-10, probably due to the ability of IL-10 to have other effects on the immune system.

2. The participation of IL-10 in the regulation of proliferation and differentiation of B-cells, which leads to a higher level of production of immunoglobulins.

A high level of immunoglobulins in serum leads to a high frequency of binding of cytotoxic cell receptors to Fc fragments of antibodies adsorbed on the surface of infected cells.

It has previously been shown that infection with ASF virus can lead to excessive stimulation of B-lymphocytes both in vitro and in vivo [3, 33], which, if the virus is not neutralized by antibodies, can enhance the dissemination of the virus in the body and lead to the development of antibody-dependent cellular cytoxotoxicity. Thus, in some cases, animals that received specific antibodies to ASF virus before infection, demonstrate more rapid development of clinical signs of the disease, as well as more pronounced post-mortem changes, since antibodies can have a potentiating effect on the entry of the virus into the target cells of the macrophage series [1]. Thus, apparently, antibodies can accelerate the death or recovery of the animal, depending on the ability of other components of the immune system to effectively fight ASF, without leading to the neutralization of the virus. In this experiment, no correlation between the speed of appearance of specific antibodies and IL-10 serum levels has been established.

3. Inhibition of activation and effector function of T-cells [22]. Cellular immunity plays a major role in protection against ASF in convalescent animals. The results of experiments with the CD8+ lymphocyte depletion of immune pigs by monoclonal antibodies confirm the important role of cytotoxic lymphocytes in protection against ASF virus [17]. IL-10 inhibits the activation of Th1 and NK cells [13]. Thus, the IL-10 induction can play a significant role in the pathogenesis of African swine fever and animal death.

In the experiments of J. Post et al. [20] animals surviving after ASFV infection had significantly higher IL-10 serum levels. It was demonstrated that at the beginning of the experiment the presence of TNF-α, IL-12 and IL-10 differed depending on the age of an animal. In adult pigs (18-week-old as compared with 12-week-old), the level of these cytokines was higher before the experiment. After distribution of animal groups by mortality, differences were observed only in IL-10. Surviving animals had high IL-10 levels. The authors suggest that this is due to IL-10 ability to prevent uncontrolled production of pro-inflammatory cytokines and tissue damage, which allows T-cells to effectively fight the virus. Moreover, a high number of γδ-T-cells and activated NK cells were detected in surviving animals, which may indicate the IL-10 effect [15].

A high number of cytotoxic cells in surviving animals may be associated not with IL-10 effect, but with some independent reason, especially taking into account the inhibiting effect of IL-10 on T-cells. The high IL-10 level in dead animals during the experiment described in this work may be associated both with a high level of virus replication, which leads to infection and activation of a large number of macrophages producing IL-10, and with the IL-10-associated change of infection from acute to subacute [27]. However, the absence of effective anti-ASF components of immune components did not allow animals to survive.

A similar picture is observed in case of Ebola virus infection in humans. There is a clear difference in the expression of cytokines in surviving and dead patients. The presence of IL-10 and high levels of neopterin and receptor for IL1A after the onset of clinical signs indicates a fatal infection, while the presence of IL-1β and increasing plasma concentrations of IL-6 during the phase of the clinical manifestation of the disease are the markers of non-fatal infection [11, 24].

In addition, the IL-10-associated differences in the development of the disease may be caused by polymorphism of coding genes [25] or differences in the virulence of the studied isolates.

All this indicates the need for further study of the regulation of inflammation (expression of pro-inflammatory and anti-inflammatory cytokines) in various forms of ASF.

**CONCLUSION**

A higher IL-10 level (15.8–173 pg/ml) was observed in blood sera of the dead animals infected with a moderately virulent African swine fever virus, as compared with the surviving piglets (4–5 pg/ml).

Further studies are needed to establish a causal relationship, including study of the expression of various cytokines during infection with both low- and highly virulent virus isolates.

The challenge does not affect the IL-10 serum level, probably because the disease proceeds without any severe inflammation, which has to be regulated by the organism, as in the case of a primary immune response.

No noticeable effect of the IL-10 serum level prior to infection on the survival rate of animals has been observed.

**Conflict of interest.** The authors declare no conflict of interest.

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