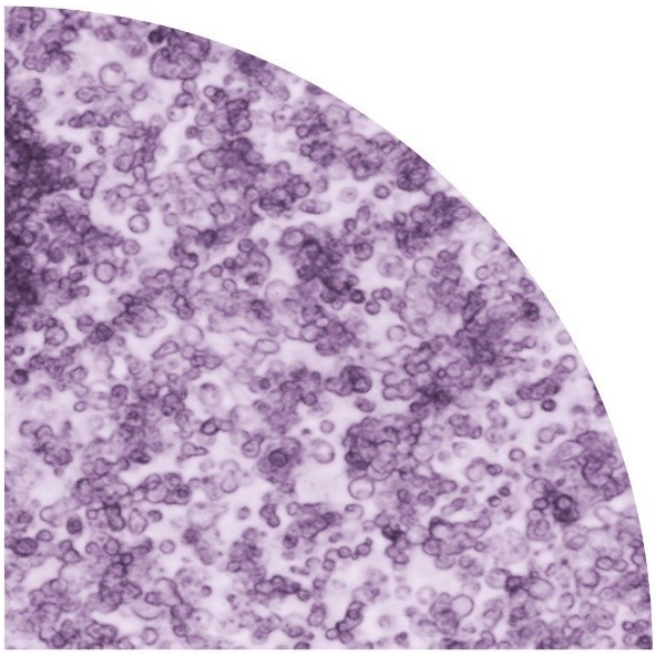
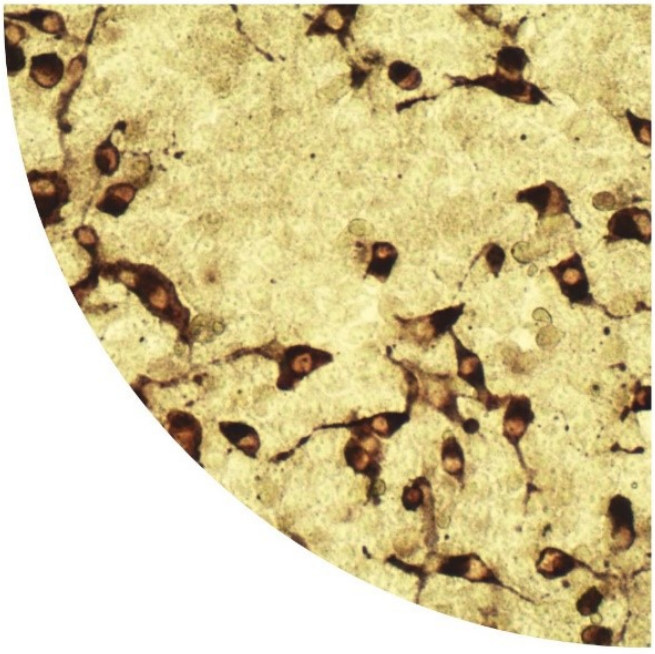
AFRICAN SWINE FEVER
RESEARCH RESULTS IN THE CZECH REPUBLIC

Team of Authors


VRI

VÚŽV Institute of Animal Science

Mendel University in Brno

Ministry of Agriculture of the Czech Republic

Státní veterinární ústav Jihlava

Universitas Veterinaria Brunensis

Forestry and Game Management Research Institute

VÚZT Research Institute of Agricultural Engineering, p. r. i.

CZU Czech University of Life Sciences Prague

AFRICAN SWINE FEVER
RESEARCH RESULTS IN THE CZECH REPUBLIC

Authors

MVDr. Martin **Faldyna**, Ph.D.; Mgr. Jakub **Hrdý**, Ph.D.; Mgr. Magdaléna **Krásna**;

Ing. Dr. rer. nat. Miroslava **Krzyžánková**; Mgr. Romana **Moutelíková**, Ph.D.; RNDr. Jana **Prodělalová**, Ph.D.; Mgr. Petra **Vašíčková**, Ph.D.

Veterinary Research Institute

MVDr. Kateřina **Mikulášková**; MVDr. Petr **Václavek**, Ph.D.

State Veterinary Institute Jihlava

prof. Ing. Luděk **Bartoš**, DrSc.; doc. Ing. Jitka **Bartošová**, Ph.D.; Ing. Gabriela **Malá**, Ph.D.; doc. MVDr. Pavel **Novák**, CSc.

Institute of Animal Science Prague

prof. MVDr. Vladimír **Celer**, Ph.D.; MVDr. Dagmar **Břínek Kolařová**

University of Veterinary Sciences Brno

Ing. Monika **Faltusová**; Ing. Miloš **Ježek**, Ph.D.; Ing. Václav **Silovský**

Czech University of Life Sciences Prague, Faculty of Forestry and Wood Sciences

Ing. Jakub **Drimaj**, Ph.D.; prof. Ing. Jiří **Kamler**, Ph.D. and Ing. Radim **Plhal**, Ph.D.

Mendel University in Brno, Faculty of Forestry and Wood Technology

Ing. Jan **Cukor**, Ph.D.; Ing. František **Havránek**, CSc.

Forestry and Game Management Research Institute

Ing. Antonín **Machálek**, CSc. and Ing. Josef **Šimon**, Ph.D.

Research Institute of Agricultural Engineering, p. r. i.

ISBN 978-80-7672-023-7

ISBN 978-80-7672-031-2 (anglické vydání)

The text was produced with the financial support from the National Agricultural Research Agency (Projects No. QK1920187, QK1920113, QK1920184 and QK1910462) and Institutional support projects (RO0118, RO0518, RO0618 and RO0718).

CONTENTS

1. Introduction

The first occurrence of African swine fever virus (ASFV) in the Czech Republic was confirmed on 26 June 2017. The virus was identified in two wild boars found dead in the cadastral territory of Příluky near Zlín. The State Veterinary Administration for the region imposed veterinary emergency measures associated with mass hunting and retrieval of animal carcasses, their laboratory examinations and safe disposal at rendering plants. The last positive case was detected on 15 April 2018, which was achieved through the collaboration of the local authorities, the armed forces, and farmers. Consequently, the EU Commission could issue an implementing decision on 12 March 2019 to officially confirm the successful completion of the eradication of ASF in the Czech Republic. Similarly, on 19 April 2019, the World Organisation for Animal Health (OIE) renewed the ASF-free status of the Czech Republic.

The emergence of this extremely harmful disease in the Czech Republic led the Ministry of Agriculture to announce topics for project proposals of the National Agency for Agricultural Research in order to increase the knowledge base of Czech professionals and preparedness of laboratories for dealing with African swine fever also from a non-diagnostic point of view.

The following projects were awarded grants in the competition:

African swine fever in the Czech Republic: A study of molecular epizootiology and biological properties of domestic isolates of the virus (QK1920187)

Veterinary Research Institute (RNDr. Jana Prodělalová, Ph.D.)

Co-investigators:

University of Veterinary Sciences Brno (prof. MVDr. Vladimír Celer, Ph.D.)

Institute of Animal Science Prague (doc. MVDr. Pavel Novák, CSc.)

in collaboration with the National Reference Laboratory for Classical Swine Fever and African Swine Fever,

State Veterinary Institute Jihlava (MVDr. Petr Václavek, Ph.D.)

African swine fever virus in meat and products thereof - detection methods and persistence studies (QK1920113)

Veterinary Research Institute (Mgr. Petra Vašíčková, Ph.D.)

Research and verification of the effectiveness of available technical and biological means and procedures for the prevention of African swine fever spread in the wild boar population in the Czech Republic (QK1920184)

Institute of Animal Science Prague (doc. Ing. Jitka Bartošová, Ph.D.)

Co-investigators:

Mendel University in Brno, Faculty of Forestry and Wood Technology (prof. Ing. Jiří Kamler, Ph.D.)

Forestry and Game Management Research Institute (Ing. František Havránek, CSc.)

Research Institute of Agricultural Engineering, p. r. i. (Ing. Antonín Machálek, CSc.)

Behavioral reaction of free-living wild boar on measures adopted against spreading of African swine fever virus (QK1910462)

Czech University of Life Sciences Prague, Faculty of Forestry and Wood Sciences (Ing. Miloš Ježek, Ph.D.)

Co-investigators:

Military Forests and Farms of the Czech Republic, state enterprise (Ing. Stanislav Dvořák, Ph.D.)

Recent developments in the global and European epidemiological situation further underline the importance of research and national preparedness. The presence of this disease in central Slovakia, but especially in Poland and Germany adjacent to the Czech border, raises significant concerns about the reintroduction of the virus into the Czech Republic. In addition to veterinary measures, including the possible threat of extinction of domestic pig herds, this would have a significant impact on the farm gate price of pork due to the disruption of supplier-customer relations and restriction on or redirection of exports and imports.

The purpose of this material is to provide basic information on the African swine fever virus and the disease it causes in an informative way, highlighting the results obtained thanks to financial support from the above mentioned projects. The information described is relevant as of the time of its writing. Additional information and extension publications are available from the authors of each chapter.

Gratitude is due to all contributors to this monograph, which will, hopefully, at least in part, help to address the situation caused by this infectious disease, a deadly infection with a major economic and social impact.

On behalf of all authors
Martin Faldyna
Veterinary Research Institute

Brno, 22 August 2022

2. Reviewer's comment

African swine fever (ASF) is a very topical issue, not only in the Czech Republic. It is a dangerous disease of pigs that has been spreading throughout the European Union since 2014 and has also affected the Czech Republic. In the event of an outbreak, ASF has very serious economic consequences due to the almost 100% mortality in infected pigs, or the ordered destruction of domestic pig herds, and also to the significant disruption of trade in pigs and pig products. Therefore, ASF is currently the most serious threat to the wild boar population as well as to domestic pig herds and the related processing industry.

After the successful eradication of ASF, the Czech Republic has been an ASF-free country since 2019, but the occurrence and spread of the disease in the neighbouring countries, namely Poland, Germany, Slovakia and Hungary, poses a persistent and even increasing risk of the possible reintroduction of ASF into this country.

The experience from recent years clearly shows that humans and their activities play a crucial role not only in the process of ASF spreading, especially over long distances, but perhaps even more so in the implementation of measures taken to control and eradicate ASF.

For this reason, it is very important to continuously raise awareness among the general public about the risks associated with this disease and, in particular, about the possibilities and effective measures to prevent the spread of ASF in the wild boar population and the transmission of ASF into domestic pig herds.

Scientific research plays an indispensable role in this endeavour, providing new knowledge on which a successful fight against ASF can be based, especially if research results are presented in a way accessible to the wider public.

The publication "African swine fever - Research results in the Czech Republic" provides a comprehensive set of information about ASF in its entirety, from the description of the causative agent of this disease and its characteristics and epidemiology, through clinical signs in diseased pigs, routes and possibilities of transmission and spread, laboratory diagnostics including sampling, to appropriate measures for the prevention of ASF transmission into domestic pig herds and spread in the wild boar population.

The material offers the basic characteristics of the causative agent of the disease, ASF virus, including the degree of its virulence and the related course of the disease, with specification of clinical signs and pathomorphological findings associated with different forms of infection, which is very important for expressing the suspicion of the infection and thus for early diagnostics. The detailed description of the collection of appropriate samples for laboratory examinations provides good instructions for their correct sampling, thus ensuring reliable results of the subsequent laboratory examinations.

The section of the publication focusing on ASF virus detection in meat and meat products is particularly useful for professionals, especially regarding the possibility of using the described method to distinguish between infectious and non-infectious viral particles detected in the sample examined.

The information on the occurrence and spread of ASF infection in Europe includes the first occurrence of this disease in the Czech Republic and its subsequent successful eradication but, above all, it highlights its dangerousness and the level of risk of possible spread of ASF to new territories. At the same time, the presentation of problems in developing an effective vaccine, which is not yet available, necessarily

leads the readers to the conclusion that the primary effort in the fight against ASF is to prevent its introduction.

The biosecurity as prevention of the spread of ASF and its transmission to domestic pig herds is appropriately presented in a very broad sense and includes the possible sources and routes of introduction of this disease into herds, as well as ways, methods and particular measures to reduce these risks. Moreover, the measures and recommendations are applicable as general rules for herd biosecurity, not only in the case of ASF. At the same time, biosecurity measures are also defined for the case of ASF occurrence in the wild boar population, and many of them are also valid for ASF-free areas.

The section dealing with disinfection is also very useful. It provides data on the extreme resistance of ASF virus in the environment and, above all, very practical information on appropriate and effective disinfection in pig herds, which will lead to the eradication of ASF virus and thus to the stopping its further possible spread.

Due to the fact that wild boars play a very important role in the spread of ASF in this country, a significant part of the publication is devoted to research results aimed at the wild boar population. The possibilities of locating wild boars and their carcasses in natural environment using thermal imaging and drones are described in detail, including the limiting factors of this method. Similarly, possible methods of controlling the movement of wild boars, in particular the use of electric fences, are based on the research results and offer very practical information on the methods and limitations of their use.

To conclude, the publication "African swine fever - Research results in the Czech Republic" provides a lot of useful information, including new findings following from scientific research. The monograph is primarily intended not only for professionals, but it is presented purposefully in a form accessible to and understandable by the general public. All the findings, recommendations and procedures are thus presented in a way that allows their easy application in practice. In particular, hunters and pig farmers will thus learn about the appropriate and effective methods for preventing the spread of ASF, but also about their possible applications in their everyday activities and the effects of different preventive measures they can expect, which I consider a significant benefit of this publication.

MVDr. Tomáš Jarosil
Animal Health and Welfare Department
State Veterinary Administration

Prague, 2 December 2022

3. Basic information about ASF virus

Jana Prodělalová

Veterinary Research Institute

What does the virus look like?

African swine fever virus (ASFV) is a large and complex enveloped virus with genetic information carried by double-stranded DNA (dsDNA). From a virological point of view, it is a completely unique virus and is therefore classified as the sole member of the Asfivirus genus within the *Asfarviridae* family. The virus contains more than 50 proteins with different functions, such as enzymes involved in the formation of a new viral particle (known as virion) in an infected cell. The proteins making up the virion structure are also important. A significant feature of the virion is the existence of an envelope acquired when leaving the host cell. The envelope contains lipids, making the ASF virus sensitive to most organic solvents, but resistant to some effector mechanisms of the immune system (Sánchez-Vizcaino et al., 2019) (Fig. 1).

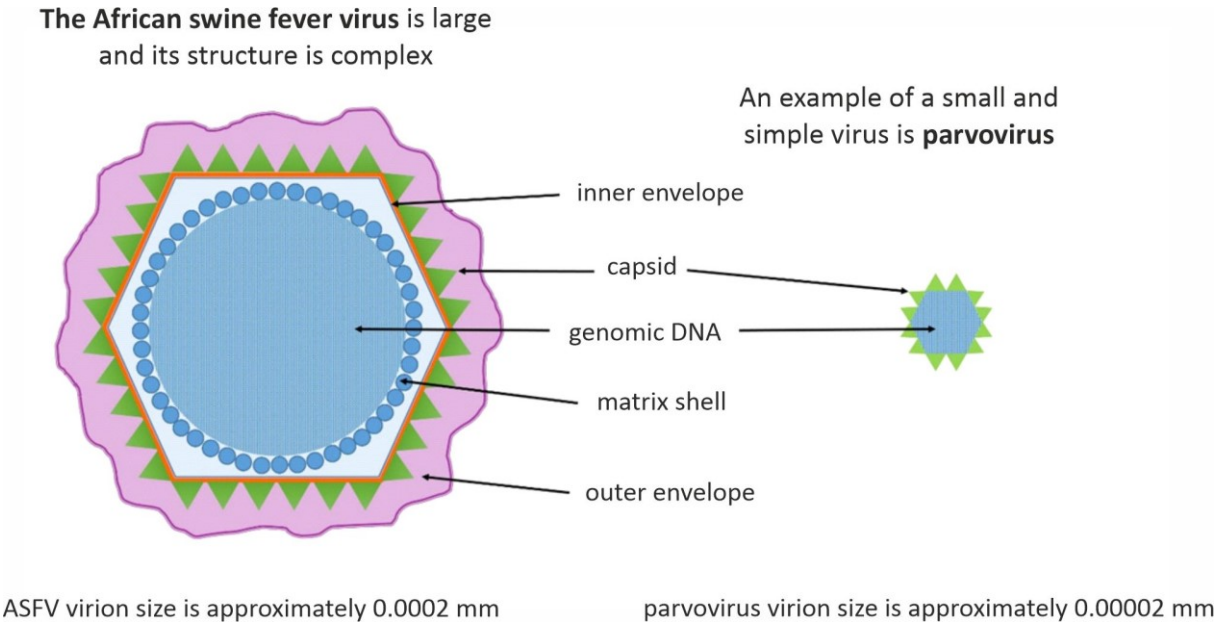


Figure 1: The complex structure of African swine fever virus in comparison with the ten times smaller and very simple parvovirus virion

As the name of the disease caused by ASF virus suggests, its course is very similar to that of classical swine fever, but virus is completely different. Based on genetic analyses of the gene encoding p72 protein, 24 genotypes of the virus have been identified so far. All these genotypes are found in Africa. Until 2006, genotype I was present in Europe and the Western Hemisphere in general. Genotype II strains are currently circulating in Eastern Europe and share a high degree of similarity. This genotype was transmitted from eastern Africa to Georgia in 2007 (probably by feeding contaminated food waste in the port of Poti) and then through Russia to Eastern Europe and EU countries.

How does African swine fever virus spread?

The natural hosts of ASF virus are European domestic pigs and wild boars (*Sus scrofa*) which become ill and usually die after infection with the virus. In contrast, asymptomatic infection is characteristic of

African wild pig species such as the warthog (Phacochoerus aethiopicus) and the bush pig (Potamochoerus porcus). These pigs act as a reservoir of ASF virus on the African continent, which is further transmitted by *Ornithodoros moubata* ticks to domestic pigs that become ill, and the disease is also spread by direct contact between sick and healthy animals. In the Eurasian continent, however, ticks are not carriers of the ASF virus and the infection spreads only between sick and healthy domestic pigs and wild boars. However, contaminated environment or food can also be a source of infection. The only exception to this was the ASF occurrence in the Iberian Peninsula in the past, when transmission occurred by the tick *Ornithodoros erraticus*, which is found in that area (Chenais et al., 2018; Plowright, 1981).

4. Disease pathogenesis depends on strain virulence

Kateřina Mikulášková and Petr Václavek

State Veterinary Institute Jihlava

Pathogenesis of ASF is a complex process

The pathogenesis of African swine fever virus is a very complex and not yet fully understood process in which the high affinity of the virus for lymphoid tissues plays a major role. In vertebrate bodies, the virus replicates in cells of the mononuclear phagocytic system, mainly in monocytes and macrophages.

The virus enters the host body through the tonsils and pharyngeal mucosa. Primary replication occurs in the mandibular and retropharyngeal lymph nodes. Primary viremia follows. The virus spreads systematically through the blood and lymph to other organs and tissues. In the blood, the virus is attached to the erythrocyte membrane. The highest titres of the virus are found in tissues containing cells of the mononuclear phagocyte system, especially in the spleen and lymph nodes. Secondary viral replication and subsequent secondary viremia occur in target organs. After 30 hours post-infection, the virus can be detected in almost all organs. It is excreted in all secretions and excretions at high concentrations (Blome et al., 2013).

Viremia usually occurs between day 2 and 3 (max. 8) after infection. Seroconversion, i.e. an increase in the levels of specific antibodies, occurs on days 7 to 9 after infection. The antibodies produced are not neutralizing (protective), which results in the long-term presence of the virus in the blood (even for several months). This phenomenon is particularly important in pigs that survive the acute phase of the disease and can then intermittently shed the virus into the environment for up to 90 days. Although the antibodies produced do not completely inactivate the virus, they still help alleviate clinical signs, protect pigs from mortality and reduce the intensity of viremia (Blome et al., 2013).

Most of the pathomorphological lesions are attributed to interactions driven by cytokines (small proteins used mainly by immune cells for intercellular communication) released by infected and activated monocytes and macrophages. ASF virus replicates in the cells but does not cause direct cell death. Infection with the virus leads to enhanced phagocytic and secretory activity of the cells. Thus the activated cells start to produce a wide range of mediators including pro-inflammatory cytokines such as interleukin 1 (IL-1), interleukin 6 (IL-6) and tumour necrosis factor α (TNF-α); complement components and arachidonic acid metabolites. The production of all these substances plays an essential role in the pathogenesis of African swine fever virus. These mediators are responsible for the massive destruction of leukocytes which occurs as a result of apoptosis known as programmed cell death, and directly influence the development of clinical signs. In particular, they cause activation of endothelial cells and coagulation systems, vascular alterations, impaired haemostasis, haemorrhagic diathesis, formation of immune complexes (in chronic infections), marked depletion of lymphoid tissues and also thrombocytopenia (low levels of platelets). Thrombocytopenia is likely the result of fragmentation of megakaryocytes - the cells from which platelets develop. As a consequence, haemorrhagic fevers,

oedema formation, marked immunosuppression and a number of other associated signs are observed. In the chronic phase of the disease where autoimmune processes also play a role, alterations may occur due to the deposition of immune complexes in the kidneys, lungs and skin and their subsequent binding to complement (Blome et al., 2013). Complement activation leads to local inflammatory changes, including organ enlargement.

The phenomenon of hemadsorption also occurs in infected cells. Hemadsorption is a phenomenon where characteristic "rosettes" of red blood cells, erythrocytes, form around the infected macrophages. This is a phenomenon specific to the ASF virus; none of the other swine viruses show the hemadsorption reaction (HAD). Most ASF strains have the hemadsorption ability. These properties of the virus are used in the disease diagnostics.

What is ASF virulence?

As mentioned above, various strains of ASF virus may differ in their virulence. In general, the strains can be classified as highly, intermediately, and low virulent. Each of the 24 described ASF genotypes is characterised by a particular virulence, but variation in virulence can exist between subtypes or strains of the virus within the same genotype. Virulence has a major impact on the clinical course of ASF disease and on the mortality rate (Fig. 2 adopted according to Beltrán-Alcrudo et al., 2017)

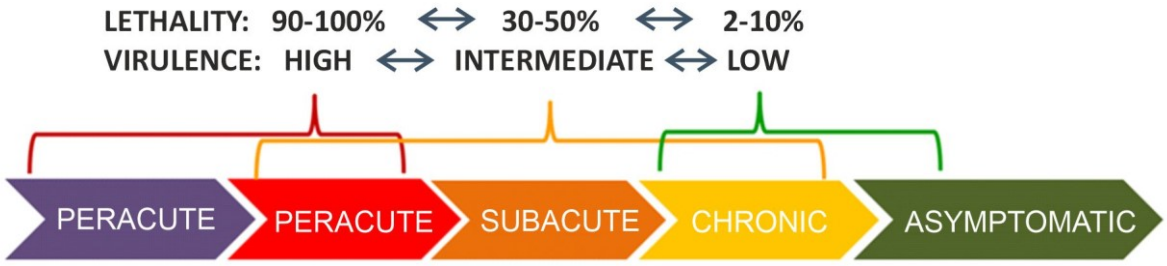


Figure 2: Relationship between ASF virus virulence and the clinical course of the disease

Highly virulent strains

Highly virulent strains of the virus cause the most aggressive form of infection accompanied by a peracute or acute course. Lethality is almost 100%. Death typically occurs 4-12 days after infection. From day 3 after infection, high concentrations of the virus are detected in the blood (Costard et al., 2009). The vast majority of infected pigs die before the onset of the antibody response (usually around day 7 to 9 after infection). Therefore, virological methods are the most suitable for the diagnosis of this form of the disease. If the virus circulates in the population for a long time, this form can change into a less virulent form of the virus.

Intermediately virulent strains

These strains cause acute and subacute forms of the disease with a lethality of 30-70% (Beltrán-Alcrudo et al, 2017). The course is very similar to infection with highly virulent strains. However, lower levels of the virus are detected in the blood of surviving pigs (10^4–10^6). According to the literature, infected pigs can spread the infection for up to 70 days (Guinat et al., 2016). The virus usually does not survive in the organs for more than 6 months. Viral DNA is still detectable 500 days after infection. In pigs infected with intermediately virulent strains of ASF virus, antibodies are also detectable for a long time.

Low-virulent strains

Regarding the infection of pigs with low-virulent strains, it is rarely fatal (2-10%) (Beltrán-Alcrudo et al, 2017). The infection is subclinical or chronic. If viremia occurs, virus concentrations are low (10^2–10^3). The virus is demonstrated in tissues. Intermittent shedding into the environment occurs, which, according to recent evidence, lasts up to 90 days (Blome et al., 2013). The study of possible persistent infection and long-term virus shedding is a current research topic. For example, in one experimental study it was confirmed that there is a certain percentage of pigs (30% in the study) that are able to recover completely from infection. According to the authors, such animals can then become a source of infection for other animals and thus contribute to the persistence of the virus in the pig population, especially in endemic areas. This claim was established on the basis of an experiment in which healthy pigs were exposed to direct contact with recovered, experimentally infected pigs that showed no signs of disease. Two of the twelve healthy pigs developed acute infection after exposure, confirming the hypothesis of the potential hazard posed by the survivors (Eblé et al., 2019). In contrast, another study analysing data from 39 publications reported that there was no evidence of epidemiological significance of surviving pigs or virus carriers. The study describes the existence of two types of survivors –the chronically infected animals and the virus carriers. Chronically infected animals are pigs that develop a persistent infection accompanied by periodic viremia with frequent signs of the subacute or chronic disease. Viral shedding occurs in these animals only in the context of infection relapse. The second category of 'virus carriers' are pigs that recovered from infection irrespective of the degree of virulence of the virus. According to the authors, these animals are not persistently infected and do not shed the virus for a long time (max. 30-40 days). This study shows that none of the categories of surviving pigs can be considered as "healthy" carriers of the infection, i.e. animals without any clinical signs (Stähl et al., 2019).

5. Clinical signs and pathomorphological findings depend on the "speed" of the disease

Kateřina Mikulášková and Petr Václavek

State Veterinary Institute Jihlava

What does a pig infected with African swine fever look like?

Clinical signs together with pathomorphological changes show a considerable variability in infected pigs depending on the speed of the disease course. The main factors influencing the course of infection are: the level of the virus virulence, the infectious dose and resistance of the target organism, and probably also the breed of the animal. Based on these three factors, the course of infection can be categorised as follows:

Peracute form of infection

The peracute course is characterised by non-specific clinical signs including high fever (41-42°C), inactivity and inappetence, and sudden deaths usually in a good physical condition with almost no pathological lesions. Only hyperaemia of internal organs and lymph nodes only is often observed. Splenic enlargement is regularly detected. The incubation period lasts for 1-3 days (Beltrán-Alcrudo et al., 2017).

Acute form of infection

In pigs with the acute course of infection we can observe fever (40-42°C), inappetence, drowsiness, weakness, recumbency, increase in the respiratory rate, discharge from the nostrils and eyes, diarrhoea, vomiting, constipation, in pregnant sows also abortions. Infected pigs usually die between 6-15 days after infection, depending on the virulence of the virus. The lethality in this form reaches 90-100% (Beltrán-Alcrudo et al., 2017). The acute course of infection with ASF virus is very similar to that of other infections such as classical swine fever, swine erysipelas, intoxications, salmonellosis and others. Typical pathomorphological findings are haemorrhages on the skin (especially on the ears, abdomen and hind limbs) and on internal organs (mainly lymph nodes, spleen, kidneys, heart muscle, urinary bladder and intestinal mucous membranes, Fig. 3). The spleen is enlarged, tender, dark red to black in colour with rounded edges. The lymph nodes are enlarged, oedematous and haemorrhagic, resembling blood clots. Multiple petechial haemorrhages are seen in the renal cortex. The lungs and liver are congested and oedematous, and there may be a straw-coloured or bloody exudate in the thoracic and abdominal cavities (Beltrán-Alcrudo et al., 2017).

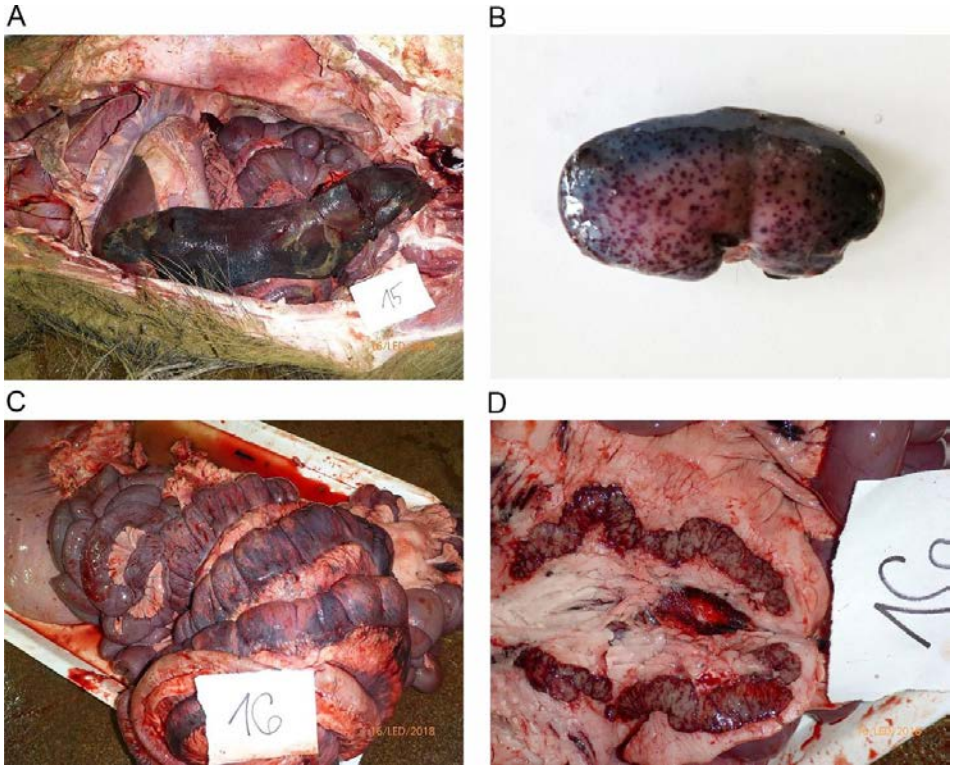


Figure 3: Pathomorphological findings in a pig that died due to ASF: A - hyperaemic splenomegaly; B – petechiae on the kidney; C - enlarged oedematous haemorrhagic mesenteric lymph nodes; D - petechial haemorrhages on the intestine (photo MVDr. Kateřina Brázdová - A, C, D and MVDr. František Kostka, Ph.D. - B)

Subacute form of infection

This form of infection is most common in endemic areas or after infection with less virulent strains. Lethality rates range from 30 to 70% and death occurs 15-45 days after infection. Surviving pigs may recover. Clinical signs are similar to the acute form of the disease, but are usually milder. Intermittent fever, depression, inappetence, haemorrhages, oedema, swelling of the joints, pneumonia and pericarditis appear. Even the pathomorphological findings correspond to a milder form of the acute course. Pneumonia, serofibrinous pleurisy or pericarditis are other common findings (Beltrán-Alcrudo et al., 2017).

Chronic form of infection

This form results from infection with low-virulent strains of the virus, or may be an expression of an endemic form of the disease in a population partially adapted to the infectious agent. Therefore, it is described mainly in countries with long-term persistence of the virus in the environment (Spain, Portugal, Angola). The chronic form of the disease is characterized by only a few typical clinical signs and the pathological and anatomical findings are also the least developed and often atypical. Intermittent low-grade fever, inappetence and depression, cutaneous necrosis and joint inflammation associated with lameness and caseous necrotizing pneumonia and fibrinous pericarditis may be observed (Beltrán-Alcrudo et al., 2017). The infected animals die within 2-5 months or survive (mortality <20%). The nutritional condition of pigs which die is rather poor. Some animals overcome the infection and may become carriers of the virus. The chronic or asymptomatic course of infection, the role of ASF survivors transmitting the virus, their immune response to infection and the mechanisms of virus persistence in the infected pig body are the timely topics in the study of ASF.

6. The immune system responds to the infection and the findings are used to develop a vaccine

Martin Faldyna

Veterinary Research Institute

Vladimír Celer

University of Veterinary Sciences Brno

Can the immune system recognise that the pig is infected?

Firstly, it should be emphasized that the immune mechanisms responsible for protection against infection are not clearly recognized. As with any other infection, the immune response includes non-specific response and antigen-specific response and involves both cellular and soluble factors. These play different roles depending on the pathogen type.

It is important to note here that African swine fever is a viral infection. Viruses must enter the host cell in order to use its protein-making mechanisms (proteosynthesis) to replicate. The virus particle, or its uniquely composed nucleic acid, is recognized by intracellular receptors (termed as TLR receptors) in the infected cells. This leads to the production of interferons and the activation of mechanisms by which the infected cell attempts to destroy the virion and at the same time limit the production of new virions (Golding et al., 2019). Such an infected cell is recognised by the "natural killer" or NK cell. It is able to destroy the cell. This leads to an inflammatory response and the attraction of other immune cells that are responsible for the development of antigen-specific immunity. It has the advantage of being targeted at a specific part of the viral particle, making its response more precise. However, during this response, immune memory is formed, which is responsible for the fact that the individual who has undergone this immune response is better prepared to cope with another infection in the future. And this is the principle of vaccination.

The specific immune response has two mechanisms – cell-mediated and antibody-based. The cell-mediated immune response is represented by the activity of T-lymphocytes. These can be functionally divided into cytotoxic lymphocytes (Tc), which are able to find a cell infected with a virus and, like the NK cell, destroy the cell. This mechanism is generally considered to be important in the immune response against viruses. In addition to Tc, there are helper T-lymphocytes (Th) that produce cytokines, small signalling proteins that influence the activity of other cells (Figure 4). There are Th1 lymphocytes,

which help the aforementioned Tc lymphocytes or NK cells in their activity. However, there are also Th2 lymphocytes that rather help another population of lymphocytes, termed as B-lymphocytes, which are responsible for the production of antibodies. From the point of view of protection against viral infection, antibodies play mainly a neutralizing role. If antibodies bind to the surface structure of the viral particle, which is necessary for virion entry into the cell, the ability of the virion to infect the cell decreases.

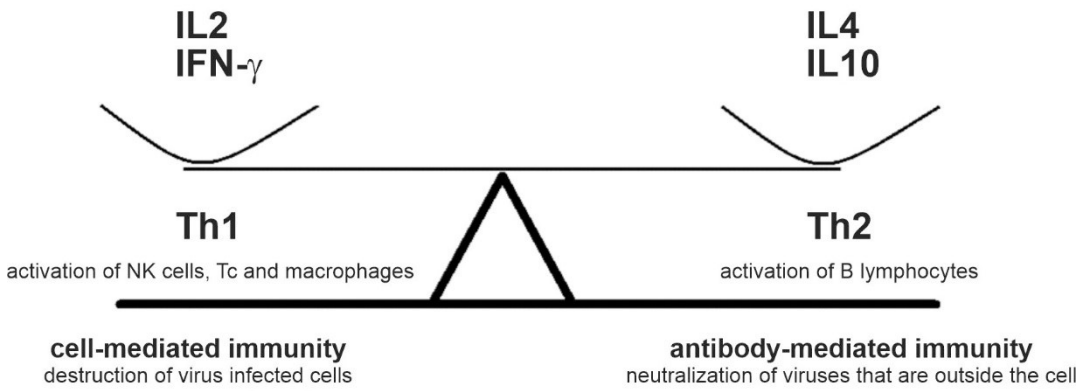


Figure 4: Role of cell-mediated and antibody-mediated immune responses in protection against viral infection

Now that we know how it should work, where are we with the development of ASF vaccines?

To understand the directions of vaccine development, it is important to realize that there are diverse types of vaccines that differ, among other things, in their ability to rather stimulate cell-mediated immune response or immune response associated with antibody production (Sanchés-Cordón et al., 2017, 2020; Urbano and Ferreira, 2022; Xie et al., 2022).

To date, two groups of ASF vaccines have been developed and tested:

The first group are the whole-cell inactivated vaccines, which contain whole virus particles that have been killed that is inactivated. Their advantage is that the inactivated virus cannot return to a fully virulent form that would be capable of causing disease in vaccinated animals or be shed by vaccinated animals into the environment and become a source of infection. However, this safety is associated with lower immunogenicity, i.e. the ability to induce an immune response. In addition, efficacy testing has shown that these vaccines mainly induce antibody production but are not associated with animal protection.

The second group are recombinant vaccines, which contain selected proteins/antigens that have been produced in laboratories as recombinant proteins. Vaccines constructed in this way are also safe, but have not yet been able to induce protective immunity. This can be related to the fact that, like inactivated vaccines, they mainly elicit an antibody response. Another cause of failure may be the choice of the protein against which the immune response is directed.

The next group of experimental vaccines are the live attenuated vaccines. These are based on a vaccine strain that is attenuated naturally or by repeated multiplication under laboratory conditions. The advantage of these vaccines is their ability to induce a strong immune response - both antibody and cellular, they have a significant protective function, but are shed by vaccinated animals into the environment and retain the ability to return to a fully virulent form.

The fourth group are the vector vaccines, in which a gene for the ASF virus protein against which we want to induce an immune response is inserted into a carrier, i.e. vector. The existing results show a good immune response but poor protection. The cause may be, as with recombinant vaccines, an incorrect choice of the antigen.

The latest group of vaccines being developed and tested are live vaccines with a deleted gene. A gene for a protein that is important for the development of clinical symptoms is deleted from the genome of the vaccine strain using molecular biology techniques. This creates a live but safe vaccine that elicits both antibody and cellular immune responses. However, up to now, even these vaccines do not lead to an immune response associated with protection of vaccinated animals. In addition, it is a GMO vaccine.

The development of an ASF vaccine is problematic for several reasons. It is an extremely large virus with a large number of genes and proteins produced, each playing a role in the pathogenesis of disease. The second reason is financial - development and testing have to be carried out in high biosafety level laboratories, which are limited in number and very expensive to operate. Despite some progress in the development of ASF vaccines, most of the potentially promising results have been achieved when testing the homologous immunity, i.e. situation where the same strain of virus from which the experimental vaccine is prepared is used for testing. When the heterologous immunity was tested, low efficacy was observed in all experiments.

The last important fact about vaccine development is that all vaccines under development are administered into the body of the vaccinated animal by injection (or a needle-free injection system). However, this is very problematic in the case of wild boar populations. Therefore, a separate chapter will be devoted to a different route of vaccine administration. Perhaps by feeding GMO plants producing viral proteins? Leaving aside the fact that these are GMOs again, it is very difficult to induce an immune response after oral administration, and it is actually unsuccessful even under laboratory conditions (Barasona et al., 2019; Criscuolo et al., 2019).

7. Epidemiology of African Swine Fever

Petr Václavek

State Veterinary Institute Jihlava

Where was the virus first discovered and where has it been found since?

African swine fever virus is endemic in the majority of sub-Saharan Africa, including Madagascar. The disease was first described in Kenya in 1909, following the introduction of European, locally exotic, domestic pigs. Subsequently, it was found that ASF virus was present in most countries in southern and eastern Africa for a long time in wild boars in which it does not cause clinical disease. The first spread of ASF outside the African continent occurred in 1957, when the virus was transmitted from West Africa (Angola) to Portugal (Lisbon). This outbreak was soon eradicated, but two years later (1957) the virus was reintroduced to Lisbon, followed by the spread of the disease to Spain and other countries in Europe: Andorra (1975), Belgium (1985), France (1964, 1967 and 1974), Malta (1978), the Netherlands (1986) and Italy (1967, 1969 and 1983). Several countries in South America and the Caribbean were also affected: Cuba (1971, 1980), Brazil (1978), the Dominican Republic (1978) and Haiti (1979). Eradication of the virus in Cuba entailed the slaughter of 400,000 pigs. In some cases, the complete depopulation of all domestic pig farms in a given country (Malta, Dominican Republic) had to be carried out. In Spain and Portugal, ASF virus became endemic and complete eradication took more than 30 years (1960-1995). All countries in Europe and the Americas, with the exception of the island of Sardinia, succeeded in eradicating the virus. In many European countries, these were only sporadic small outbreaks in domestic pig farms (Belgium, the Netherlands, France), which were relatively easy to tackle by depopulating the infected farm. The rapid eradication of ASF virus in these countries, without

detailed information on the extent and course of the outbreak, leads to the misconception that the disease can be easily eradicated. Currently, the virus is still endemic in Sardinia, where it was introduced in 1978. The eradication efforts failed, with traditional backyard pig farming playing an important role.

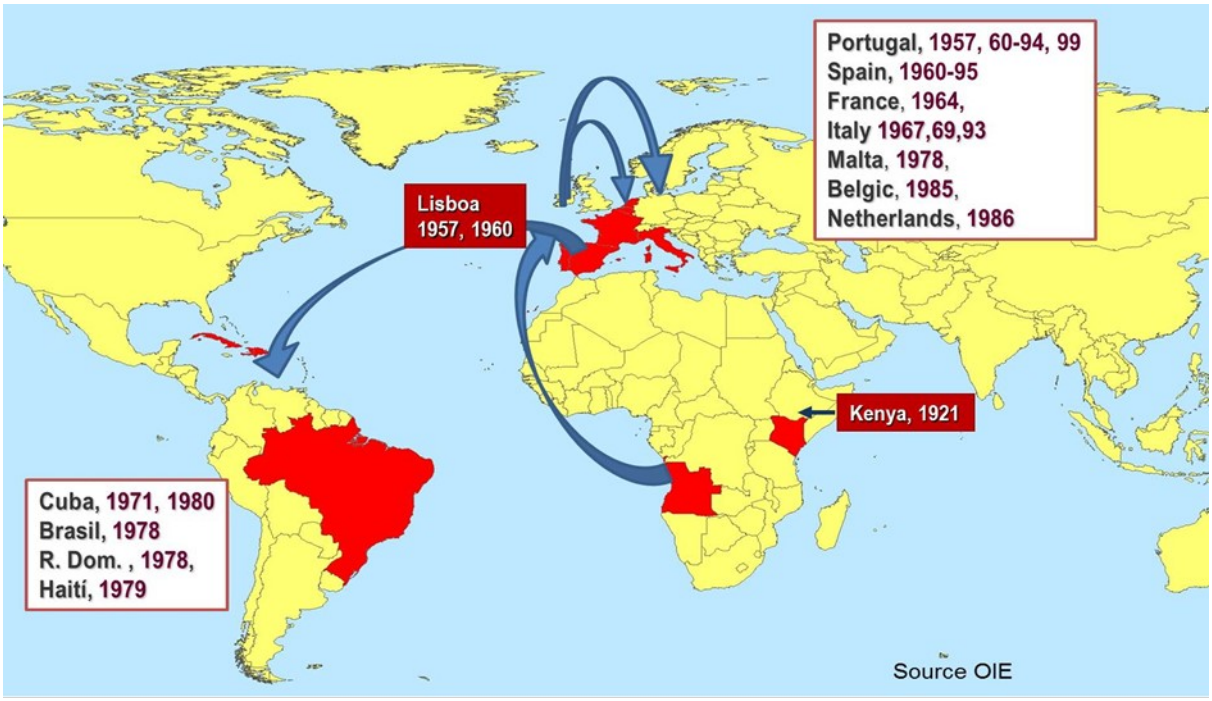


Figure 5: Spread of ASF virus outside the African continent in the 20th century (Source: OIE)

How and where did the current ASF epidemiological situation begin to deteriorate once again?

Based on nucleotide sequence analysis of the B646L gene region (p72), there are currently 24 basic genotypes of ASF virus. Isolates transmitted from West Africa to Europe, the Caribbean and South America in the late 1970s which are still present in Sardinia belong to genotype I. The isolates of ASF virus known as "Caucasian" now found in Europe and Asia belong to a highly virulent strain of genotype II, which was originally isolated in East Africa (Mozambique and Zambia 1993-2002, Madagascar 1998). The current ASF outbreak started in Georgia in 2007. The source of the infection was most likely non-heat treated pork from a ship in the Black Sea port of Poti. By June 2007, the infection was transmitted to most districts of Georgia. In the following months, ASF further spread to the neighbouring states - Armenia (8/2007), Russian Federation (11/2007) and Azerbaijan (1/2008). The disease became endemic in the Russian Federation and gradually spread further northwestwards. From the Russian Federation, the virus spread to Ukraine (7/2012) and Belarus (6/2013). In the course of 2014, the disease spread to the European Union (EU) countries of Lithuania (1/2014), Poland (2/2014), Latvia (5/2014) and Estonia (8/2014). In 2016, the virus was introduced to Moldova and in 2017 to the Czech Republic and Romania. In April 2018, the first outbreak of ASF occurred in wild boars in Hungary and the virus started to spread uncontrolled among wild boars and domestic pigs in Romania. The virus also spread to Bulgaria (8/2018) and an outbreak of ASF in wild boars in Belgium near the border with France was surprising (9/2018). The disease became endemic in the Baltic States and Poland. In the Russian Federation, the ASF virus has also gradually spread eastwards and since August 2018, China, the world's largest pork producer (approximately half of world production), has also been affected. In 2019, the first outbreaks of ASF were reported in Mongolia (1/2019) and Vietnam (2/2019).

Figure 6: AFS outbreaks in China (purple) including Taiwan (red), Mongolia (light blue) and Vietnam (dark blue) (Source: OIE, 2019 Google maps data, INEGI).

What is the current situation in the Czech Republic and the rest of the world?

In 2021, no specific antibodies to ASF virus were detected in domestic pigs throughout the Czech Republic. Virological testing of 3626 samples from domestic pigs was conducted in 2020 with negative results. In wild boars, no specific antibodies to ASF virus were detected in any of 2655 samples tested. Virological testing for ASF virus was negative in all 9673 in wild boar samples tested across the Czech Republic.

ASF virus was last detected in a hunter harvested wild boar in the Czech Republic on 15 April 2018. The last detection of specific antibodies to ASF virus was recorded on 17 October 2018, also in a sample from a hunter harvested wild boar. There were no positive cases of ASF virus infection in 2019. Based on these results, the European Commission officially confirmed the successful completion of ASF eradication in the Czech Republic on 12 March 2019 by the Commission Implementing Decision (EU) 2020/404. Similarly, on 19 April 2019, the World Organisation for Animal Health (OIE) officially declared that the Czech Republic had been free from ASF.

The current global situation of ASF development remains very worrying. In 2018, the infection was transmitted to the Asian continent, first to China. During 2019, ASF spread to Mongolia, Vietnam (The situation in 2019 is demonstrated in Figure 6), Cambodia, Hong Kong, the Democratic People's Republic of Korea, the Republic of Korea, Myanmar, the Philippines, East Timor, Indonesia and Laos. In 2020, ASF spread to Papua New Guinea, India, Malaysia and Bhutan. In 2021, the ASF virus was transmitted to Central America to the Dominican Republic and Haiti.

In Europe, 11 EU Member States (Lithuania, Latvia, Estonia, Poland, Romania, Bulgaria, Italy, Hungary, Slovakia, Greece and Germany) and 4 other European countries (Ukraine, Moldova, Belarus, Serbia) are affected. Belgium has again become an AFS-free country after successfully eradicating an outbreak in the southern part of the country. In January 2022, an outbreak of AFS in wild boars was detected in the Piedmont region of northern Italy. The situation on 1 July 2022 is illustrated in Figure 7.

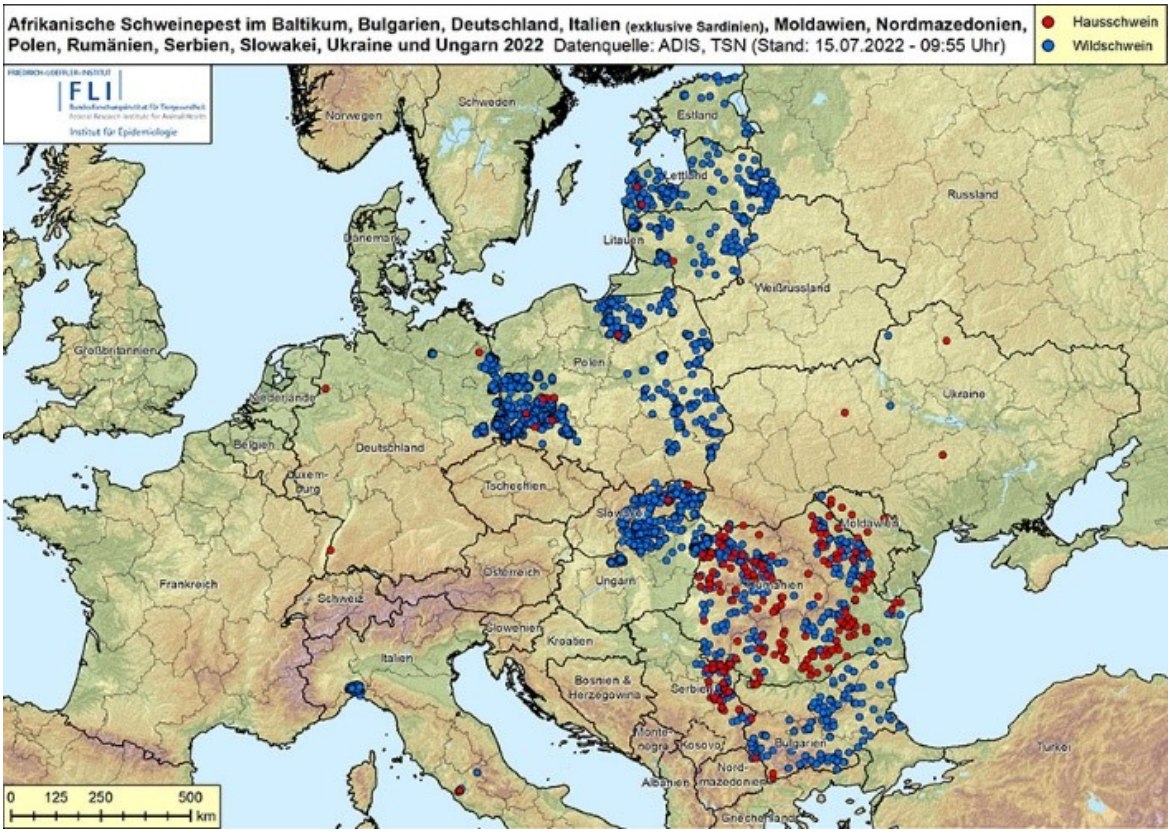


Figure 7: Epidemiological situation in Central Europe – 1 July 2022 (FLI - https://www.fli.de/de/aktuelles/tierseuchengeschehen/afrikanische-schweinepest/karten-zur-afrikanischen-schweinepest/)

In Slovakia, ASF was spreading westwards in 2021 and, currently, Zvolen, Banská Bystrica, Liptovský Mikuláš and some other districts are infected (see Figure 8).

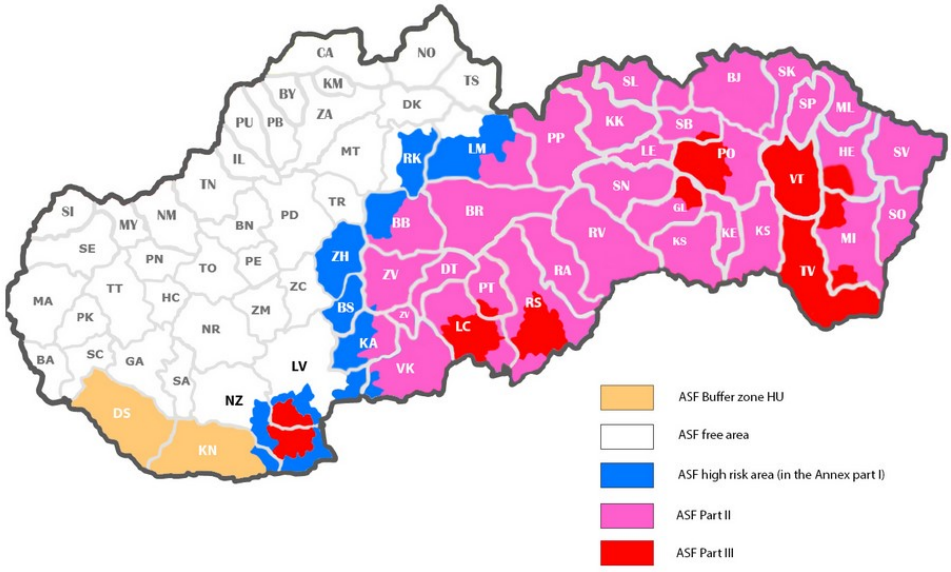


Figure 8: ASF Infected zone and buffer zone in the Slovak Republic (1/2022)

The major transmission of ASF in 2021 occurred in western Poland and eastern Germany along the Polish border (Figures 9 and 10). The nearest outbreaks of ASF in wild boars at the end of June 2022 were 5-10 km away from the Czech border - specifically from the Frýdlant Hook on the Polish side and the Šluknov Hook on the German side. ASF transmission to the Czech Republic in the aforementioned areas can be expected in 2022 through the wild boar population.

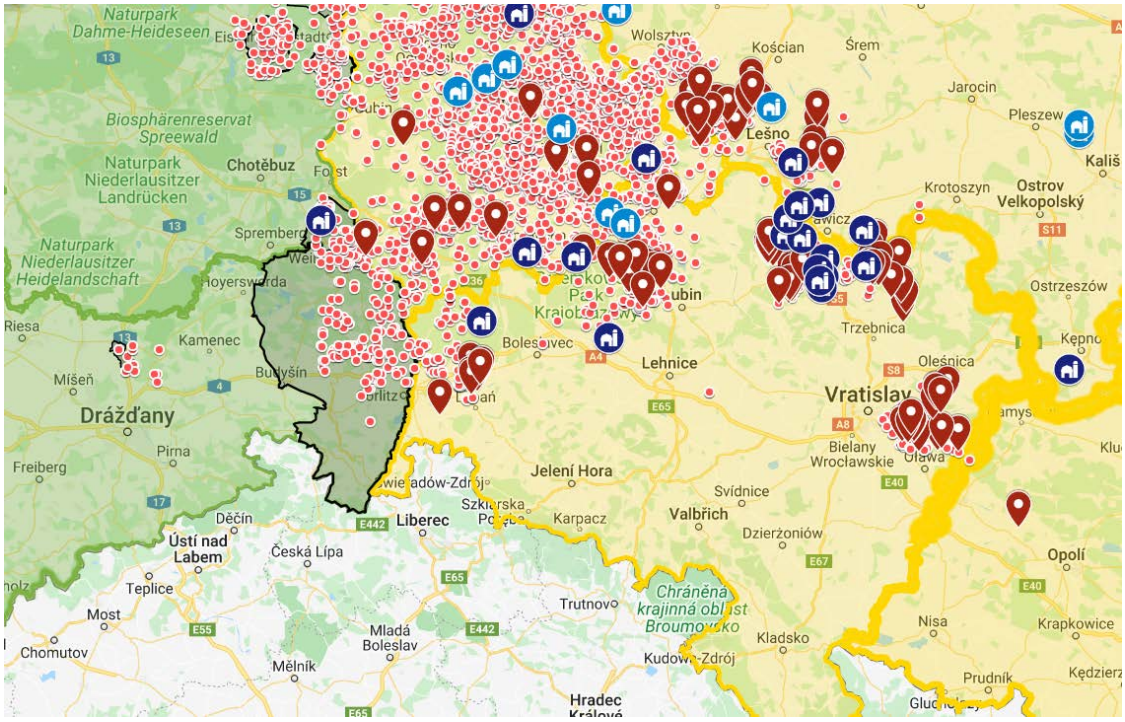


Figure 9: ASF outbreaks in Germany and Poland adjacent to the border with the Czech Republic at the end of 2021

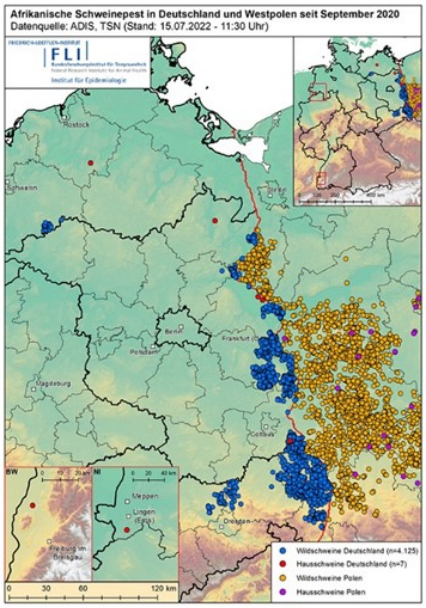


*Figure 10: ASF outbreaks in Germany and Poland adjacent to the border with the Czech Republic – 15 July 2022 (FLI-*https://www.fli.de/de/aktuelles/tierseuchengeschehen/ afrikanische-schweinepest/karten-zur-afrikanischen-schweinepest/)

As a precautionary measure and for early detection of the disease, an active surveillance zone with intensive wild boar hunting has been adopted on 16 November 2020 along the border with Germany and Poland. This zone covered an area of 1,440 km2 in the Liberec Region and the Ústí nad Labem Region (Figure 11). In the whole area, all hunted wild boars (hunting reward 3 000 CZK/each animal) and all wild boars found dead (increased reward 3 000 CZK/each animal) were examined. During 2021, this area was extended along the entire Polish border with the Czech Republic and now covers a total area of 7200 km2 (Figure 12).

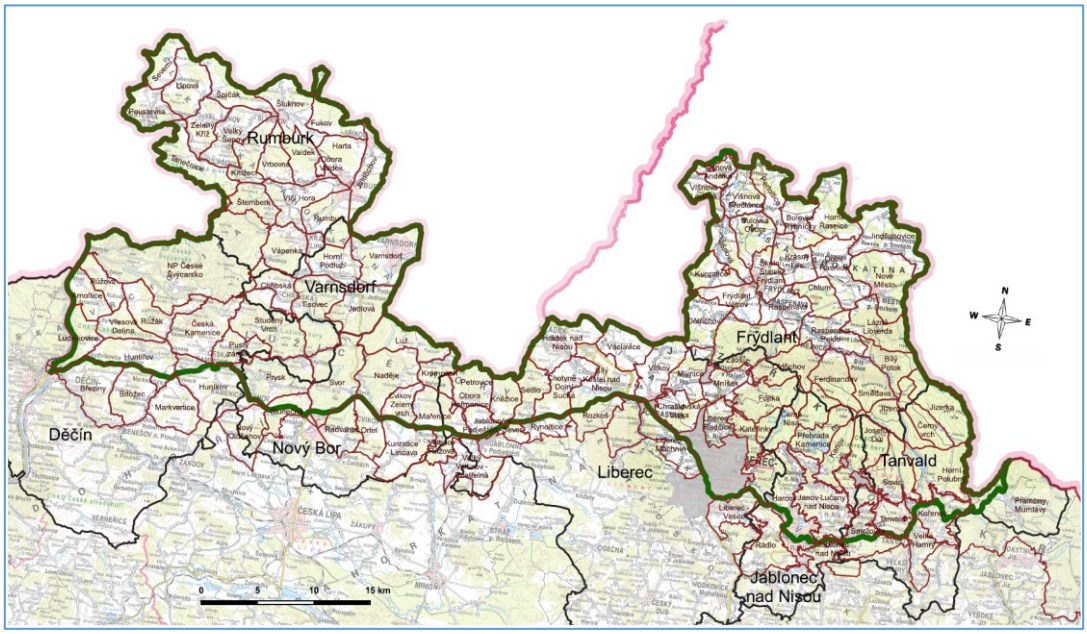


Figure 11: Emergency veterinary measures against ASF - intensive hunting area - active surveillance zone (since 16 November 2020)

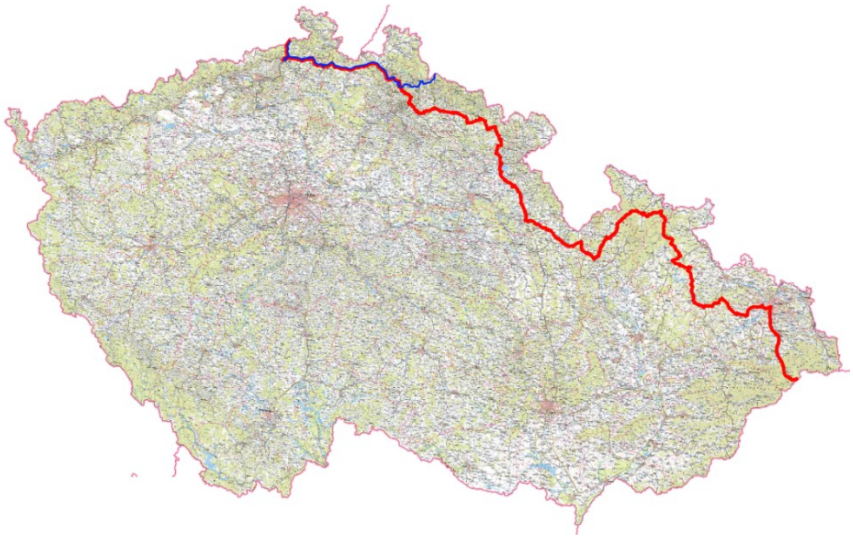


Figure 12: Emergency veterinary measures against ASF - intensive hunting area - active surveillance zone (since December 2021)

8. Sampling and Laboratory Diagnostics

Kateřina Mikulášková and Petr Václavek

State Veterinary Institute Jihlava

Vladimír Celer a Dagmar Břínek Kolařová

University of Veterinary Sciences Brno

What samples can be used and how can they be taken?

Careful sampling is an important factor affecting the accuracy and validity of the result. Several sample types can be used for diagnosing African swine fever. These include whole blood, blood serum, organs and tissues, or ticks as disease vectors.

When collecting whole blood, it is appropriate to use tubes containing the anticoagulant EDTA. It is important not to fill the tube above illustrated maximum blood volume line. Failure to comply with the maximum volume may cause a significant dilution of the anticoagulant and blood clotting. The sampling site in pigs are the *vena jugularis, vena cava cranialis* or *vena auricularis*. Post-mortem blood can be taken directly from the heart or thoracic cavity as soon as possible after the death of the animal. The use of heparin should be avoided. This anticoagulant may cause inhibition of PCR analysis or induce false positive reactions in the haemadsorption test (HAD) for virus identification. The whole uncoagulated blood is used as a sample for virological diagnostics (PCR, virus isolation). Plasma obtained by centrifugation can also be used, especially for the detection of specific antibodies (indirect immunoperoxidase test - IPT; indirect fluorescence test - IFAT). The minimum required volume of blood collected is 1 ml.

Blood serum is obtained from blood drawn as described above into a tube (usually HEMOS) without anticoagulants. Serum is preferably tested fresh by both serological and virological methods. A complication may be the occurrence of haemolysed serum, which is particularly common in samples taken from carcasses. Haemolysis can cause false positive reactions in ELISA test. The minimum serum volume required for virus detection is 500 µl, equivalent to approximately 1 ml of blood, and for serological diagnosis, 1 ml of serum, equivalent to approximately 2 ml of blood.

Although all organs and tissues can be used to diagnose ASF virus, the spleen and lymph nodes are among the most commonly used in practice, followed by the liver, tonsils, heart, lungs and kidneys. In animals found dead, bone marrow is also very suitable for laboratory diagnosis as it is often the only available material and contains very high concentrations of the virus. In the case of low-virulent ASF isolates, intra-articular tissues are also used for diagnosis. The harvested organs are stored at 4°C and should be sent to the laboratory within 24 hours of collection. If samples cannot be delivered within this time, it is advisable to freeze them at -80°C or store them in liquid nitrogen. Samples for histological diagnosis are stored in 10% formalin. Such samples are not suitable for virus isolation, but can be used for PCR diagnostics and immunohistochemistry. The minimum recommended amount of tissue is 5 g/1 organ. Tissue samples can also be used for serological diagnostics (IPT, IFAT), for example, exudates obtained from spleen, liver and lung.

The disease vectors, ticks of the genus Ornithodoros, can also be used to test for the presence of ASF virus. These arthropods are mainly found in subtropical and tropical regions, so this is a rather unlikely sample for the Czech Republic. As alternative sample types, e.g. saliva could be used for serological diagnosis. Compared to the detection of specific antibodies in serum, this sampling method was less sensitive, which can be related to the different dynamics of production of antibodies that are produced into plasma/serum and that are produced to mucosal surfaces. However, saliva testing could serve as a non-invasive method of obtaining samples when conducting surveillance. According to some studies, blood smears or faecal samples can also be used.

What diagnostic options exist to confirm ASF virus infection?

The diagnostics of African swine fever virus involves direct detection of the virus and, equally important, serological detection of specific antibodies. The circulation of the virus and the presence of specific antibodies in the blood show dynamics over time after infection. The incubation period of the disease usually lasts for 4 to 19 days. Animals shed the virus during the incubation period, about two days before the first clinical signs appear. The intensity of shedding depends on the degree of virulence of the virus. Seroconversion occurs approximately 7 to 9 days after infection (dpi) and the specific antibodies produced persist for a long time, from several months to several years.

The following methods can be used to detect the virus, viral antigen or ASF virus genome:

- detection of the genome by polymerase chain reaction (PCR)
- virus isolation and hemadsorption reaction (HAD)
- antigen detection by Direct Fluorescent Antibody Test (FAT)
- antigen detection by ELISA test
- loop-mediated isothermal amplification (LAMP) with recombinant polymerase

The following methods can be used to detect antibodies to ASF virus:

- ELISA
- immunoblot (IB) assay
- indirect fluorescent antibody test (IFAT)
- indirect immunoperoxidase test (IPT)
- pen-side testing

The description of principles of these methods is beyond the scope of this handbook. All these methods have their advantages, limitations and explanatory value in different situations. Therefore, the World Organisation for Animal Health (OIE) in its diagnostic manual lists the methods and the purpose of their use (Table 1).

Table 1: Diagnostic methods recommended by the OIE for the diagnostics of ASF and their suitability for use

	PURPOSE OF USE				
	Healthy population	Preventive testing of individual animals	Eradication programme	Confirmation of clinical cases	Surveillance
DETECTION of VIRUS					
Virus isolation / HAD	N	N	++	+++	++
Direct immunofluorescence	N	N	++	++	+
ELISA	+	++	+	+	+
Conventional PCR	++	++	++	++	++
Real-time PCR	+++	+++	+++	+++	+++
DETECTION OF SPECIFIC ANTIBODIES					
ELISA	+++	+++	+++	+	+++
Indirect immunoperoxidase test	+++	+++	+++	+	+++
Indirect fluorescence test	+++	+++	+++	+	+++
Immunoblot	++	++	++	+	++

Legend: +++ recommended method; ++ suitable method but may require further verification; + method can be used in some situations, but is limited by the cost, reliability or other factors; N not recommended.

Are the diagnostic methods under development also intended for research purposes?

The development of diagnostic methods - especially those suitable for detecting specific antibodies against a particular antigen – naturally goes hand in hand with the development of vaccines, especially those based on recombinant proteins. Within the QK1920187 project, an ELISA assay was developed and subsequently validated to detect antibodies against the ASF virus C-type lectin protein.

9. African Swine Fever in meat and meat products – Detection methods

Petra Vašíčková, Magdaléna Krásna, Jakub Hrdý, Miroslava Krzyžánková

Veterinary Research Institute

Is it possible to detect ASF virus in meat?

ASF virus is classified among viruses that exhibit high stability in the environment outside the host body. The virus is able to retain infectivity for a relatively long time in a protein-rich environment, in chilled meat for several weeks to months, and in frozen meat for several years. This stability, together with other factors including irresponsible human behaviour, has a significant impact on the massive spread of ASF. The possibilities for controlling this disease are limited, which encourages the development of reliable methods for the causative agent detection, and the adoption of measures to prevent its further spread. For this purpose, a number of methods have been developed. The EU Reference Laboratory for African swine fever (EURL ASFV) in Madrid has several defined standard operating procedures (SOPs) for the detection of ASF virus. The EURL ASFV procedures for the rapid detection of the presence of DNA of ASF virus are defined for different types of biological samples; e.g. blood, serum, tissues and cell cultures. However, the matrix in the form of meat and meat products is not mentioned there. The detection of viral agents in food, and meat products in particular, is generally problematic, but it shows to be absolutely necessary in view of the above data.

The main objective of the project QK1920113 was to develop, optimize and validate technical solutions for the rapid detection of ASF virus, especially in meat and meat products. The selection of methods was based on their sufficient sensitivity and specificity as well as their practical applicability and affordability. Validated methods were prepared in the form of functional samples, thus providing modern tools for rapid and routine analyses of meat and meat products for the presence of ASF virus. Within the project, information on virus survival in meat, selected meat products and during their processing was also obtained and verified. For this purpose, conventional methods of virus cultivation on cell lines were used. At the same time, the suitability of using the less time-consuming molecular biological methods to distinguish infectious and non-infectious viral agents was verified.

The detection of ASFV using molecular biological methods (especially qPCR) is highly sensitive, specific and rapid. Using them, not only detection but also quantification of the monitored agents (genome) in different types of samples is possible. The actual detection step is preceded by a nucleic acid isolation step, in which DNA must be obtained in sufficient quantity and purity. Non-heat-treated meat and meat products are complex matrices consisting of increased protein and fat content. Both of these substances have an undesirable effect on the detection process, and if an inappropriate nucleic acid isolation method is used, residues of these components may contaminate the sample and cause skewed results.

The method for the isolation and detection of ASF virus DNA in meat matrices and non-heat-treated meat products was optimized in the form of a functional sample and introduced. A significant advantage of the method is the analysis of a large sample size (5 g) compared to other possible procedures where only mg of the matrix is analysed, thus significantly increasing the probability of virus detection. The method has the advantage of inactivating the virus in the first step of nucleic acid isolation. This step

allows that only the initial work, i.e. the rinsing of the sample, during which the viral particles are released into the elution buffer and inactivated, can be carried out in a biosafety level 3 (BSL3) laboratory. The actual isolation of nucleic acids can then follow in a laboratory with a lower biosafety level, using commercially available kits with the possibility of full automation (using an automated analyser), which can significantly speed up and simplify the entire analytical procedure and reduce its laboriousness. For the optimized method, a detection limit was set as 400 target agents (or genomic equivalents of DNA) in 5 g of a sample, which is specified as the lowest number of detected agents with 100% probability. In order to check the accuracy of the procedure, minimise false positive or false negative results and thus ensure valid results, the control system is clearly defined in the method described.

The nucleic acid isolation is followed by the detection of the virus of interest. Primarily, for the detection and possible quantification of the virus genome, qPCR was introduced and optimized based on the EURL SOP for ASF with the introduced internal amplification control, which monitors the proper course of qPCR detection and thus contributes to the discrimination of false negative results. An innovative possibility of further, independent determination of the presence of ASF virus (or this virus DNA) is the use of the xMAP technology (Luminex corporation, USA, Texas) in combination with multiplex oligonucleotide ligation reaction and PCR. This technology allows a relatively broad multiplex analysis of samples, where up to 50 possible targets/agents can be identified in a single reaction. The whole procedure is an "open" system, to which additional detection targets can be added almost randomly according to current needs (e.g. classical swine fever agents). Therefore, it can be used for comprehensive and rapid one-step screening and an effective risk analysis tool.

The analysis of the presence of viral DNA using electrochemical methods can also be added to the innovative procedures introduced in the framework of the above-mentioned project implementation. Although the method does not include nucleic acid amplification, the analysis shows potentially sufficient sensitivity to detect the virus. The elimination of nucleic acid amplification significantly reduces the resulting time required for detection of the agent of interest (in the order of minutes) as well as the cost of the analysis, and does not require an equipped molecular biology laboratory.

How long does the virus persist in meat?

Viral agents cannot replicate in the environment outside the specific cells of the host organism, they do not metabolise and, therefore, do not increase in number in the contaminated raw material/food/feed as is in the case of bacteria. Due to this fact, it does not alter sensory properties of the raw material/food/feed. The potential for the spread of viruses depends largely on their ability to persist in the environment outside the host organism. This ability is influenced by a number of biological, physical and chemical factors and their combinations.

According to previously published data, ASF virus has the ability to survive in a protein-rich environment and remains stable at pH 4-10 for a long time. This virus shows high resistance to acidic pH; at pH 3.1 the virus is inactivated after 22 hours. Strong alkaline pH (>11.5), using NaOH or Ca(OH)2, also causes rapid inactivation of the virus. Thermal inactivation of ASF virus requires exposure to temperatures of at least 56°C for 70 minutes or 60°C for at least 20 minutes. The virus is relatively stable at 4, 22, and 40°C for 24 hours, and is resistant to refreezing. If the virus is present in the blood, it loses infectivity after 30 minutes when heated to 60°C. The virus can survive in carcasses for up to seven months at 4-8°C and is present in dried blood and frozen meat for years. ASFV has been shown to persist in carcass tissues for several months; probably for the longest time in lymphoid tissues (lymph nodes and spleen, tonsils) where the highest concentration of the target cells for the virus (macrophages and monocytes) are found. A common characteristic is the prolonged persistence time in the environment/food at lower temperatures (e.g. refrigerator temperatures), in frozen meat or food where the ASF virus is able to remain in an infectious state for years. The persistence of ASF virus is not affected by meat maturation

processes, and thus meat from pigs slaughtered during the infectious phase of the disease or those which spontaneously died is a potential source of infection.

And is the virus actually living?

On the basis of the above data, the objectives of the studies carried out within the project were to define the viability of ASF virus in meat under the conditions of common storage of meat and meat products, i.e. storage in freezers and refrigerators. For this purpose, conventional methods of virus cultivation on cell lines were used. The results obtained showed a minimal decrease in the amount of infectious virus during storage in a freezer (-25°C). In meat stored at common refrigerator temperature, a decrease in infectivity was observed after 30 days, but even after 14 months of storage the amount of infectious virus still reached levels at which pigs can become infected after eating such meat.

The experiments also targeted the currently popular culinary treatment of meat, sous-vide. It is a heat treatment of meat in which the food is prepared in an airtight plastic container in a temperature-controlled water bath. It usually takes several hours (sometimes up to 72 hours) at a temperature below the boiling point, most often between 55 and 60 °C. The intention is to preserve the natural flavour, juice and texture of quality food by heating it evenly in a precisely temperature-controlled water bath. Although the experiments were originally designed to be performed in several temperature (55, 56, 57, 58 and 60°C) and time ranges (1, 6, 12 and 24 hours), infectious virus was not detected in the sample when the first heat/time combination (55°C/1 hour) was used. These results showed a lower stability of the ASF virus than previously assumed (recommended heat treatment of the matrix at a minimum of 56°C for 70 min).

Can the infectious virion be detected by a method other than culturing on cell lines?

Even though qPCR is a rapid, highly sensitive and specific method for the detection of ASF virus DNA, it is unable to distinguish between infectious and non-infectious viral particles. On the other hand, culturing of the virus on cell lines, which can solve this qPCR deficiency and is thus suitable for determining the existent risk of infection, can be time consuming. Therefore, current research into viral agents aims at the development of new, alternative methods. Due to their speed, these methods are usually based on a qPCR system preceded by sample preparation; elimination of non-infectious viral particles. For this purpose, it is possible to use specific enzymes (benzonase, proteinases or nucleases) or other chemical compounds (e.g. propidium monoazide, ethidium monoazide, platinum or palladium salts) that have the ability to penetrate the capsid (or envelope) of a non-infectious viral particle, bind to its nucleic acid and subsequently block the amplification/detection of the monitored agent in the qPCR reaction.

On the basis of published data and previous experience, a procedure based on the pretreatment of the sample with palladium compounds was optimized and verified. To validate the procedure, conventional virus cultivation on cell lines was used as a reference, i.e. control method. Artificially contaminated samples of meat and meat products that were subjected to freezing, refrigerator temperature and heat treatment were analysed using this method. The results obtained by the two methods applied to the samples were comparable. The best efficiency of sample pretreatment was achieved with the PdCl2COD compound. The obtained results thus indicate the suitability of this method, i.e. the use of qPCR together with PdCl2COD sample pretreatment, to discriminate between infectious and non-infectious ASF virus particles and thus its use as a possible alternative tool to analyse the actual risks of infection with this virus.

10. Preventive measures to increase the biosafety level against the spread of African swine fever

Pavel Novák and Gabriela Malá

Institute of Animal Science Prague

Wild boars, including their carcasses, are important potential sources of African swine fever infection for domestic pig herds (de la Torre et al., 2015). The virus is transmitted not only by direct contact with infected animals, or through their faeces and secretions, but also indirectly through carriers capable of transmitting the virus, e.g. humans and their clothing and footwear, wildlife, insects, contaminated objects and materials, feed, vehicles, etc. (Olesen et al., 2017).

ASF virus spreads naturally in the environment at a rate of approximately 30-50 km per year. ASF spread via aerosol is of minimal importance and only occurs over short distances, especially in a very close contact. ASF virus in aerosol does not remain infectious for a long time and a certain infective dose of virus is required for successful infection, which is usually insufficient in aerosol. Thus, a sounder of wild boars does not become infected immediately (in one or two days), but it is a rather slow process lasting for weeks or months. The infection would most likely be just as slow in domestic pig herds, where pigs would become infected one by one.

The ASF virus can easily be transmitted by infected objects, but also by objects stained with body fluids (secretions and excretions) of infected pigs, where the ASF virus can persist for a relatively long time, as well as in non-heat-treated meat products.

Risk factors influencing the spread of ASF in the wild boar population include the density and size of the population, age and gender of the infected population, season of year when the population becomes infected, as well as inappropriate hunting methods, including non-compliance with biosafety principles when hunting.

In the epizootiology of ASF disease, there is no minimum boar density threshold at which ASF infection can no longer spread. ASF is not a completely density-dependent infection; density is only one of many factors. Despite a lower density of wild boars means fewer contacts with each other, the analysis of outbreaks in Russia, Ukraine and Belarus does not suggest that ASF infection would disappear if the density of wild boars drops to 0.5 pig/ km^2.

The virus never infects 100% of the wild boar population in a given area, usually only 30% of the wild boar population is positive.

An average of 3.9% (PCR test) and 6.6% (ELISA test) of wild boars hunted in the infected area are found to be infected. The probability of detecting ASF virus in an outbreak area is about 55 times higher in animals found dead than in hunted animals. Pigs that died due to ASF and their carcasses have not been found are another risk factor in the wild.

However, human factor plays a major role in the transmission of the ASF virus, specifically by the dense population in a defined area and the free movement of people in nature (hikers, mushroom and berry pickers, people walking dogs). Attention should also be paid to hunters with dogs in hunting areas. The free movement of dogs may also contribute to the spread of ASF virus in the area.

The main routes of African swine fever virus transmission include direct contact between wild and domestic pigs, vectors (ticks, blood-sucking insects), farm staff including visitors, genetic material (semen), ingestion of contaminated feed (especially when kitchen waste is fed), slurry, manure, faeces, vehicles, clothing, boots and other personal protective equipment, contaminated breeding equipment, tools, equipment, instruments and, in exceptional cases, air. African swine fever virus is usually transmitted by direct contact between infected and susceptible animals, mainly pigs, or by eating meat or non-heat-treated meat products made from infected meat.

For the aforementioned reasons, strict compliance with the principles of biosafety aimed primarily at preventing wild boars from entering the farm premises and coming into contact with feed and bedding is essential (Guinat et al., 2016; Lewerin et al., 2015).

How can ASF virus get into herds of domestic pigs?

Potential sources and routes of transmission of African swine fever virus to herds of domestic pigs are shown in Figure 13.

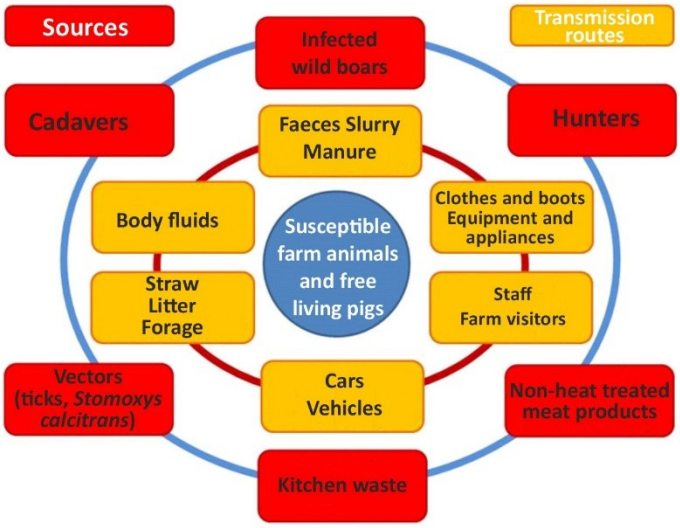


Figure 13: Sources and routes of ASF virus transmission

Animals, humans, vehicles, contaminated objects, technological systems, feed, water, bedding, aerosols, as well as wildlife including insects and rodents pose a potential risk of transmission of infectious disease agents into pig herds.

Introducing the most important risk factors

Animals

The biggest threat to biosafety is the purchase of new pigs and their inclusion in the stud herd, or own animals returning after travel to offsite facilities such as exhibitions, shows, auction markets, etc. As even clinically healthy pigs can be carriers of various infectious and parasitic diseases, new animals should only be purchased from farms with a better or similar epidemiological status.

Regular monitoring of the health status of the herd allows early detection of health disorders in animals, rapid diagnosis and timely treatment by a veterinarian.

Separate housing must be established in each herd for the housing of newly purchased pigs prior to their introduction to the stud herd (quarantine facility) and animals that are ill or suspected of being ill or infected (isolation facility). During the quarantine, prevention measures are adopted and diagnosis is made, treatment is provided and health status is monitored; in addition, during isolation, diagnosis is established and, if necessary, treatment is provided to prevent the spread of diseases.

The optimum preventive measure against the transmission of infection into pig farms by animals is a closed herd turnover with the all-in all-out system.

The detailed veterinary inspection of market animals, their carcasses and organs after slaughter and basic processing is a unique and unrepeatable opportunity to obtain information on the health situation of the pig population in different farms.

Humans

All visitors who move around the farm premises or enter the animal facility and may come into direct or indirect contact with living animals (consultants from different breeding, feeding and other distribution companies, advisors, etc.) represent a high potential risk of transmission of infection into pig herds; as well as all persons involved in ensuring the healthcare and reproduction of pigs (veterinarians, technicians for artificial insemination, inspectors of the State Veterinary Administration, etc.)

People visiting several pig farms on the same day (animal transporters, veterinarians, insemination technicians) pose the greatest risk from a biosecurity point of view. Appropriate biosecurity measures can reduce the risk of entry and spread of disease, e.g. the use of protective disposable overalls and gloves; or, in the case of pig farms with a high level of biosecurity, the use of hygiene loop, including the provision of clean 'farm clothing and boots' before entering the farm area, and in some cases changing them between the housing facilities or sections with different age groups of pigs, at least during the transition from the reproduction section (farrowing house, facility for farrowing and gestating sows, including the final stage of piglet rearing) to the production section (pre-fattening and fattening).

For farm workers, replacement of personal protective equipment, washing hands or showering are among the effective preventive measures to reduce the risk of pathogen transmission between different categories of pigs.

An important tool for preventing the transmission of microorganisms, including pathogens on the boots of workers or visitors, are disinfectant mats, which can be placed both at the entrance to the animal facilities and also at the entrance to different sections. Their effectiveness depends not only on the disinfectant used and the frequency of their refilling, but also on the time-length of contact between the disinfectant and the boots.

Animal caretakers and other staff, including visitors to the farm, who raise pigs in their backyard, pose a high risk in terms of biosecurity.

At the same time, all workers and visitors to pig farms who are active hunters must not come into contact with domestic pigs for at least 48 hours after chasing and hunting during periods of deteriorated epizootiological situation in the region (e.g. African swine fever).

Vehicles

Another risk factor in terms of biosecurity is the vehicles (cars, trucks, machinery and other equipment which are in contact with pigs or their faeces). As a general rule, only dedicated means of transport shall be used for the transport of animals. The simplest way to minimise the potential risk requires the inclusion of cars, trucks and other agricultural technology in the biosecurity plan of the farm. This primarily involves:

- no entry of unauthorized vehicles into the farm;
- restrictions on the movement of vehicles carrying feed or bedding;
- allowing the entry of vehicles for the removal of manure/dung/semi-liquid manure;
- establishing the border between the black and white zone for cars;
- ensuring that vehicles can be cleaned, disinfected and dried.

No unauthorised vehicles should enter the farm premises. Personal vehicles of the employees and visitors should be parked outside the farm. Vehicles used for animal transportation, feed, bedding and faeces can contribute significantly to the spread of pathogens (e.g. swine fever, *Actinobacillus* spp., *Streptococcus* spp., viral gastroenteritis, *Salmonella* spp., etc.). These vehicles must be washed and disinfected between two transportations. In the event of a worsened epizootiological situation or in herds with a high level of biosecurity, all vehicles should enter the farm via a disinfection bath, frame or

mat. In addition, all vehicles intended for the transportation of pigs must be thoroughly cleaned, washed and disinfected at the end of one transportation before the next one. Compliance with the above transportation guidelines is a particular problem in small-scale farms. Compound feed silos should be located close to the outer fence of the farm so that vehicles loading the compound feed do not have to enter the farm premises. Rendering trucks providing the removal of carcasses of pigs that died represent a high potential risk of pathogenic microorganisms entering pig farms, especially in small farms where the rendering box is located on the farm premises. Unlike on large-scale farms, where the rendering box on most farms is located on the boundary between the production (white) zone and the waste (black) zone, the carcasses of pigs that died are loaded into it through a gate in the white zone side and taken for loading onto vehicles for transport to the rendering plant from the black waste zone.

Optimisation of technological systems

Compliance with the principles of good husbandry practices, including technological procedures in all sections of the farm operation, is one of the essential measures of internal biosecurity on pig farms. In order to minimise the risk of pathogen transmission, full attention should be paid to the most susceptible age categories of pigs, namely piglets with sows in farrowing houses and piglet rearing, followed by pregnant sows, up to the least susceptible age category, namely fattening pigs.

Housing facilities must provide pigs with protection from adverse climatic conditions (extreme weather conditions) and at the same time create a suitable environment to ensure the physiological functions of the body, including rest. Housing conditions have a major impact on the health and welfare of housed pigs. In general, the principles of biosecurity are easier to implement in new modern housing facilities.

Technological farming systems directly determine the possibility of using and comply with the individual principles of biosecurity.

The optimisation of production technological systems from the point of view of biosecurity must create the preconditions for consistent compliance with the turnover system, which is a prerequisite for maintaining the good health condition of pigs and at the same time minimising the risk of spreading the causative agents of disease and the possibility of maintaining an adequate level of hygiene. At present, the turnover system is used in pig herds mainly in farrowing, rearing and fattening facilities. This method of pig farming is based on the all-in all-out system. The precondition is the possibility of creating homogeneous groups of animals of the same origin, closely matched in age and with comparable weight, which are housed in the same space. The time taken to form a group should not exceed 21 days. The housing space should remain empty for at least 7 days between two rounds so that it can be cleaned, washed and disinfected before the next group of pigs is introduced. Maintaining a 7-day interval between two rounds is particularly problematic on large-scale farms, where often only 3 days are left to sanitize the facility.

On the other hand, the continuous farming system is mainly used for non-pregnant and pregnant sows. The animals are continuously introduced to and removed from the facility. As the housing is never empty of animals, the effectiveness of preventive disinfection is limited. However, even with this farming system, it is necessary to ensure that the pig housing area is thoroughly cleaned, washed and disinfected at least once a year.

Infectious pressure in animal housing facilities increases with the increasing density of animals and the length of time they stay in the animal housing. As a consequence of the above, growth depression and health problems occur in housed animals. Compliance with the hygiene standards of the breeding environment is one of the basic preventive measures on livestock farms; it is an integral part of the principles of good breeding practices and the biosecurity plan. The cleaning process reduces the total number of mesophilic bacteria from the surface by 2 to 3 log orders; disinfection by 1.5 to 5 log orders. The effectiveness of sanitation (cleaning, washing and disinfection) directly determines the level of infectious pressure exerted to the newly introduced animals. The time between turnovers (i.e. between

removal and subsequent stocking of animals) is an essential part of the prevention of transmission of disease agents, particularly diarrhoea in weaned piglets.

Implements and tools

Each animal facility, each category of pigs and, where possible, sections should be equipped with their own tools (shovels, brooms, rakes, portable fencing, etc.) which are regularly cleaned and disinfected.

When performing veterinary treatment, it is necessary to prevent the transmission of infection between different animals by no repeated sharing of needles, and between different groups of animals by no sharing of both needles and syringes. Similarly, instruments used in the treatment of animals should be disinfected or sterilised.

Cleaning and disinfection of the tools and facilities significantly reduces the risk of pathogens spreading between animal facilities/sections. In order to maintain an appropriate level of biosecurity, it is important not to use the same equipment and tools for feeding and manure removal.

Feed and water

The farmer should ensure appropriate management of nutrition and feeding to meet the physiological requirements of all categories of animals kept on the farm with regard to the amount and composition of different nutrients in the ration, including supplements, minerals and vitamins, in order to maintain the best condition of the animals throughout their production and reproduction cycle. A potential risk of indirect transmission of pathogens to a pig herd is posed by contaminated feed, water and bedding, which are entered by microorganisms after their excretion from the host and are able to survive for a very long time. Indirect contamination of feed and water can also occur through biological vectors such as rodents and birds. Similarly, feed mixtures can be contaminated during their production in mixing plants. Regular monitoring of the quality of water used for animal drinking and water used in the primary production process is another important preventive measure in all livestock farms. A higher risk of feed water contamination is found in pig farms that use their own water sources for drinking.

Technological systems of feeding and watering need to be regularly checked and cleaned because microorganisms grow and multiply not only in feed troughs, feeders and waterers, but of course also in the feed and water distribution systems in sections/pigsties and feed storage (silos) and water tanks, thus gradually increasing the level of microbial contamination of feed and water to a level that can cause disease in pigs.

The risk of gastrointestinal disorders around weaning caused by deficiencies in management and feeding methods or technology can be reduced by gradually making the animals used to a change in feed mixture, adjustment of ration composition, feed structure and feeding frequency.

Free living animals

Free living and domestic animals can be sources of serious viral, bacterial, mycotic and parasitic infections. The basic principle of biosecurity is to limit the possibility of contact between domestic animals and wild animals. Currently, the possibility of African swine fever virus transmission to domestic pig herds via infected wild boars, which have been present in high numbers throughout Europe in recent years, poses a high potential risk. ASF virus is transmitted not only by direct contact with infected animals, or their faeces and secretions, but also indirectly by carriers capable of transmitting the virus (e.g. free living animals, rodents, insects, etc.). For the above reasons, strict compliance with the principles of biosecurity aimed primarily at preventing wild boars from entering farm premises or coming into contact with feed and bedding is essential. The basic measures to prevent the transmission of pathogens into pig herds by deer and game to which the hunting act applies, including wild boars, involve compact continuous fencing of the entire farm, including closing all entrance gates and gates

for the entry of people into the farm area, which is more feasible especially in large pig farms. In some farms, odour fences are also installed on the outside of the fence. However, the effectiveness of these measures requires both strict closing of gates and regular inspection of the outer fence of the farm.

The measures aimed at preventing the entry of birds and insects (disinsection) into pig housing facilities consist in ensuring the repair of windows, the installation of window nets, or nets for air inlets and outlets, and their maintenance.

Effective rodent control (extermination) consists, on the one hand, in securing objects against rodent intrusion, preventing their settlement and nesting, removing food sources, repelling rodents (electromagnetic waves, coating with odour-active compounds), and on the other hand in the actual extermination of rodents in places of their occurrence. The use of disinsection and extermination in pig farms leads to a significant improvement in the level of biosecurity of the farm.

Stray dogs and cats, guard dogs in kennels and cats in animal facilities are potential sources of disease for farmed pigs. Their regular vaccination and deworming is a prerequisite for their presence on the farm.

Air

The number of farms and the number of pigs in the region in the immediate vicinity of the farms determines the risk of pathogen transmission between farms primarily through microbial aerosol and vector animals, including insects.

Protection zones and veterinary protection zones are a set of passive measures to prevent the spread of animal diseases. Protection zones address the location of new farms at a prescribed distance from public facilities (roads, railways, high voltage power lines, transformers, etc.). While the veterinary protection zones indicate the recommended distances of a herd from other herds of the same species or other species of animals, distances between the housing facilities on a farm with the same species of animals are not stipulated in a directive in all cases. However, it is necessary to keep such a distance that the ventilation of the housing facilities is not disturbed, i.e. the exhaust air from one facility must not be drawn into another, i.e. emissions from neighbouring facilities must not endanger others. The establishment of buffer zones and veterinary buffer zones in the construction project design for new farms or prior to the renovation of the existing farms is essential.

Biofiltration is an effective method, especially for removing low concentrations of harmful and undesirable substances from waste gases. Biofilters with a solid or fluidized bed are most commonly used to remove odour-causing compounds and some inorganic pollutants (i.e. ammonia, odorous compounds, dust) from the air discharged from animal facilities. Other systems used are biological scrubbers.

From a practical point of view, it is essential to design an individual biosecurity plan based on the existing conditions of each farm and the requirements of the farmer; to focus on the critical biosecurity control points including the interactions between them with regard to the possibilities of direct and indirect spread of infectious agents (Figure 14).

Are there any biosecurity principles in the wild boar population?

The basic measures against the spread of the ASF virus, i.e. biosecurity principles, also apply to hunters and members of hunting associations.

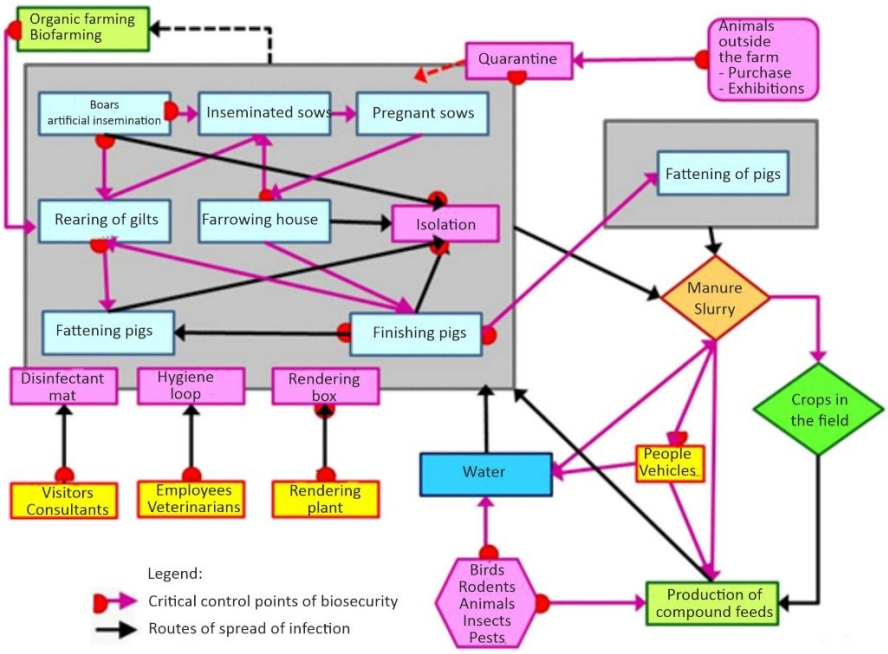


Figure 14: Potential routes of entry and spread of pathogens in pig farms

The key in the fight against ASF virus is the consistent adherence to principles of controlling wild boar numbers, based on actual numbers in different hunting grounds, including targeted searches for wild boar carcasses and their safe removal.

Wild boar population sizes in natural habitats should be maintained in accordance with food availability in the environment where they occur, taking into account the disruption of natural regulatory mechanisms - the presence of large carnivores and unfavourable climatic conditions - by a lack of food at certain times of the year. However, nowadays, after the decline of large carnivores, wild boars have lost their main predators. Of the large beasts of prey, the most important role was played by the wolf, whose occurrence is very limited in our cultural landscape. The same is true of the bear. At present, the sporadic occurrence of the lynx also has a negligible impact on the control of wild boar populations. The current climate and the structure of the landscape, especially the way of farming, also have an impact on the increase of wild boar numbers. The large-scale cultivation of cereals, rape, maize and root crops creates ideal feeding conditions for wild boars. As a result, their numbers are gradually increasing.

From an epizootiological point of view, the increasing numbers of wild boars and the related higher population density lead, despite their relative resistance to diseases, not only to an increase in the frequency of parasitic diseases (trichinosis), but also to an increase in the potential risk of outbreaks of contagious diseases (Aujeszky's disease, African swine fever) and their spread. Furthermore, we must not forget to mention the increase in damage to agricultural land and forest plantations, direct contacts with humans and traffic accidents caused by the increasingly frequent migration of wild boars across transport routes, including motorways, are not negligible.

Wild boar reproduction is influenced by a number of factors, which often interact with each other. Intrinsic factors include the genetically determined reproductive capacity of the species or the capability of reproductive adaptation to different environmental conditions. The external factors are mainly the food supply throughout the year and optimal living conditions based on the favourable climate in winter and spring in Central Europe (very short periods of frosty winters with high snow cover), as well as insufficient, inconsistent, often unsystematic regulation of numbers. In addition to the above factors, inappropriate supplementary feeding of wild boars and inappropriate social structure of wild boar sounders due to improper hunting, which causes population rejuvenation associated with uncontrolled reproduction, also affect wild boar density. For the aforementioned reasons, the consistent observance

of the social structure of the hunted wild boars, aimed at a significant reduction in the number of young animals, selective reduction of adult animal numbers as the basis of the standardized numbers and consistent culling of all old animals, plays an indispensable role in individual hunting grounds.

If a wild boar carcass is found in an area infected with ASF, it must be removed as soon as possible and, above all, safely, by competent personnel from the area, including disinfection of the site. The found carcasses should only be handled by staff of the State Veterinary Administration in cooperation with other components of the integrated safety system and locally competent state authorities in accordance with the legislation in force and the applicable emergency veterinary measures issued for the area.

Wild boar carcasses act as a significant source of infection and after sampling for laboratory testing for the presence of ASF virus should be handed over to a rendering plant collection employee for safe disposal. Disinfection of all material and utensils that have come into contact with the carcass, including sanitisation of the site of finding (with effective disinfectants), must be an integral part of compliance with biosecurity principles.

What are the preventive measures against the spread of African swine fever by wild boars?

In the event of a deteriorated epizootiological situation or when the State Veterinary Administration (SVS) issue emergency veterinary measures for a given area (in the outbreak area and its immediate surroundings), hunters and hunting associations must comply with the following measures:

1. Reduce the population size of wild boars in and around the outbreak area to a minimum.
2. Remove dead animals. Active search for wild boar carcasses throughout the year, with all carcasses found to be safely removed as soon as possible after their finding.
    - Report the finding of a carcass to the locally competent Regional Veterinary Administration (KVS).
    - Carcass shall be handled with gloves, rubber boots, apron, preferably using a shovel or a tool that can be disinfected.
    - Carcass shall be handed over in a closed, disinfected, tear-proof plastic bag with a minimum thickness of 200 µm or in a big bag.
    - Carcass shall be transported in a vehicle dedicated to this purpose.
    - Arrange transport of the carcass to the State Veterinary Institute in Jihlava or to a rendering plant depending on the size of the carcass and after consultation with the laboratory. Tag an examination order to the carcass.
    - Effective decontamination of the finding site (KVS or hunting ground user according to KVS instructions).
    - Disinfection of the carcass transport vehicle, including all tools and equipment used in this activity, as well as the clothing of workers who came into contact with the carcass.
3. No entry of hunters' cars into the area concerned. Cars shall be parked outside the area. Exception are vehicles designated for the disposal of hunted wild boars or carcasses.
4. Only hunters with training in basic hygiene and biosecurity principles are allowed to hunt in ASF areas.
5. Movement control of hounds.
6. Hunters shall observe the principles of personal hygiene - effective disinfectants in the recommended concentration are used to disinfect hands after hunting.
7. After return from hunting, hunters shall place their clothing and boots in plastic bags and then disinfect them.
8. All wild boars hunted in the infected area (outbreak) shall be visibly marked, sampled for ASF testing and then placed in rendering boxes. Any handling of wild boars hunted in this area, such as evisceration and taking to hunter´s home, is not allowed.

On the other hand, in the buffer zone, the hunter may dispose of the venison freely after submitting the prescribed samples, unless the emergency veterinary measures in force in the area specify that the harvested animal shall be kept for a certain period of time at a certain distance from the hunting site or that the hunting ground user shall be equipped, for example, with refrigeration facilities for storing the specimens for a specified period of time (pending the result of the ASF virus test).

Venison which tested positive for the presence of the ASF virus shall be handled in accordance with the emergency veterinary measures promulgated by the competent SVS authorities for the given area; after removal to a rendering plant, the entire area where it has been stored must be thoroughly washed and disinfected, including all utensils, work tools and the clothing of workers who have been in contact with it.

9. Upon return from the hunt, the vehicle and all items and tools that came into contact with the hunted wild boar shall be properly disinfected.
10. The hunter shall not come into contact with domestic pigs for at least 48 hours after hunting.

The critical control points for African swine fever virus (ASFV) transmission in the wild boar population, including preventive measures, are summarised in Table 2.

Table 2: Critical control points for African swine fever virus (ASFV) transmission in wild boars, including preventive measures

Biosecurity in wild boar population	
Factor	Preventive measures
Animals	
Wild boars	- Health status monitoring. - Reduction of wild boar population by shooting – only hunters. - Active search for animal carcasses and their safe disposal, including disinfection of their finding site. - Sampling of all hunted animals for ASF testing. - Handling and evisceration of hunted animals in and around the outbreak area is not allowed. - Hunted boars in the buffer zone after collecting the required samples – storage in refrigerator for a specified period (result of sample testing for ASF virus). - Baiting in and around the outbreak area is not allowed.
Hounds	- Movement control in and around the ASF outbreak area.
Companion dogs	- No entry into and movement in the ASF outbreak area.
Humans	
Hunters	- Observance of personal hygiene principles. - Hand disinfection after hunting – effective disinfectants in recommended concentrations. - Clothing and boots after hunting – store in plastic bags until they are disinfected in effective disinfectants in recommended concentration. - Contact with domestic pigs is not allowed for at least for 48 h after hunting.
Mushroom pickers, tourists, berry pickers, dog walkers	- No entry into the forest or a declared area in and around the ASF outbreak.
Vehicles	
Hunters' cars	- No cars allowed in and around the ASF outbreak area. - Parking outside and around the ASF outbreak area. - Disinfection after return from hunting.
Cars for hunted game transportation	- Access to and around the ASF outbreak area. Disinfection with efficient disinfectants in recommended concentrations.
Cars of tourists, mushroom pickers and people with dogs	- No entry into forests in and around the ASF outbreak area.
Instruments and tools	
Tools (knives, etc.)	- Disinfection after return from hunting.

Is there anything to prevent the transmission of ASF virus into a herd of domestic pigs?

The adoption of biosecurity principles on livestock farms includes a complex of measures that prevent the transmission of pathogens into the farm and reduce their spread not only within the farm premises but also between different herds (Jurado et al., 2018; Postma et al., 2016).

Naturally, some biosecurity measures may be difficult or costly for farmers to implement. Therefore, an individual biosecurity plan shall be prepared for each farm, based on an analysis of critical points and at the same time on the requirements and real possibilities of the farmer, followed by a proposal of simple, understandable measures, including a prepared roadmap for their gradual implementation and subsequent regular monitoring of their compliance.

As part of the external biosecurity assessment, it is necessary to analyse the critical control points with an emphasis on the potential risk of infection entering the farm through:

- pigs;
- people (control of access and movement of people, disinfection mats, hygiene loop, black and white operation system,...);
- vehicles (principles of movement of vehicles on the farm, disinfection entrance, loading ramps, ...);
- free living animals (farm fencing, window nets, disinfection, disinsection, rodent control, ...);
- air (general, veterinary and sanitary protection zones).
- In the internal biosecurity assessment section, attention should be paid to the analysis of critical control points with emphasis on:
- optimization of technological systems (housing, feeding and watering practices, ventilation, faeces handling, emergency systems,...);
- creating barriers (sanitation measures);
- feed and water (quantity, quality);
- health management (stress reduction, medication, vaccination, registration, health monitoring);
- product inspection (retrospective analysis of slaughterhouse findings, use of antimicrobials, monitoring of inhibitory residues, etc.).

The critical control points for the transmission of ASF virus to domestic pig herds, divided into external and internal biosecurity areas, including the proposal of preventive measures, are summarised in Table 3 (external biosecurity) and Table 4 (internal biosecurity). However, despite compliance with all biosecurity measures in domestic pig farms listed in the tables, it is necessary to take into account the issue of wild boar population dynamics when designing biosecurity measures. From an epidemiological point of view, the increasing numbers of wild boars and the associated higher population size imply an increase in the potential risk of outbreaks of highly contagious diseases and their spread. The key to combating the ASF virus is therefore strict adherence to the principles of controlling wild boar numbers based on the existing numbers in different hunting areas.

Table 3: Critical control points for the transmission of African swine fever virus to domestic pig herds

External biosecurity	
Factor	Preventive measures
Animals	
Domestic pigs Purchase of pigs Participation at exhibitions	 - Purchase of pigs from one herd with the same or better epidemiological situation. - 30-day quarantine of the purchased pigs before their introduction to the herd. -Quarantine after return from shows.
Wild boars	- Reduction of wild boar population by shooting. - Continuous undamaged fencing. - Odour fences around the farm. - Locked gateways.
Persons	
Animal caretakers	- Ban on backyard pig keeping. -Herds with a basic level of biosecurity - own working clothes and boots. - Herds with a standard level of biosecurity – farm working clothes and boots. - Herds with a high level of biosecurity – hygienic loop, farm working clothes and boots.
Veterinary surgeons Technicians for artificial insemination Consultants for nutrition, breeding Service staff	- Time period without contact with pigs from other farms. - 24 h – basic level of biosecurity – own overall and shoe covers. - 48 h – standard level of biosecurity - farm overall and shoe covers. - 72 h – high level of biosecurity – hygiene loop, farm clothes and boots.
Vehicles	
Cars for transporting pigs	- Disinfection footbath at the farm entrance. -Sanitation (cleaning, washing and disinfection) after unloading before the re-stocking of pigs.
Cars for transporting slurry and manure	- Disinfection bath/frame at the farm entrance. - No entry into the “white zone“ of the farm.
Farm workers' cars	- No entry of employees´ personal cars into the farm area –parking outside the farm area.
Visitors' cars	- No entry of visitors´ personal cars into the farm area – parking outside the farm area.
Protective zones	
Roads	Distance of the farm from: - motorway - 60 m; - 1st class roads – 25 m; - 2nd class roads – 25 m; - 3rd class roads – 18 m.
Railroads	Distance from the nearest railway – 60 m.
Power Industry	Distance of the herd from the axis of the outmost conductor of the high-voltage power line: - from 60 to 110 kV – 15 m; - from 110 to 220 kV – 20 m;

	- from 220 to 380 kV – 25 m; - from transformers – 30 m.
Veterinary protection zones	
Other pig farms	Recommended spacing distance - 1000 m.
Slaughterhouses	Recommended spacing distance: - slaughterhouse slaughtering their own pigs – 0 - 50 m; - slaughterhouse slaughtering pigs from other farms – 200 – 1000 m.

Table 4: Critical control points for African swine fever virus (ASFV) transmission within farms

Internal biosecurity	
Factor	**Preventive measures**
Optimization of technological systems - direct transmission	
Herd turnover	- Closed herd turnover – production of own gilts.
Herd technology	- All-in all-out system. - Housing of different age categories in separate facilities/sections. - Compliance with technological procedures in all segments of farm operation.
Building of barriers	
Distance between animal houses on the farm	Approximate distance between two animal facilities: - 12 - 15 m for longitudinal walls; approx. 10 m for gable walls; - no air flow from one animal facility into another.
Black and white zones on farms	White zone - buildings for animal housing. Black zone - warehouses, waste management, workshops, administrative building.
Internal barriers	Indoor fencing of the white zone inside the complex. Disinfectant mats at the entrance to animal houses/sections.
Tools and equipment	Separate tools and equipment for each age category of pigs
Disinfection	Observance of disinfection procedure for animal houses/sections between turnovers: - removal of animals from the animal house; - mechanical cleaning of the animal house/section - cleaning (faeces, feed residues,...); - soaking, washing and drying the internal surfaces of the animal house /section; - cleaning and washing of technological systems (housing, feeding and watering); - repair of technological systems; - disinfection; - testing the effectiveness of disinfection; - stocking of animals into farm.
Disinsection	-Preventing insects from entering the animal houses (nets in windows), access to feed (closed containers), regular removal of faeces. - Insect control (physical, mechanical, chemical, biological methods).
Rodent control	-Preventing rodents from entering animal facilities, preventing nesting, restricting the access to food, repelling. -Rodent control (physical, mechanical, chemical, biological methods).
Feed and water	
Feed and compound feed **- compound feed mixing plants** **-silos for storing compound feed**	Regular control of composition and quality. Sanitation of the production line, disinfection, disinsection, rodent control. Regular cleaning of silos at least twice a year.

Water - water sources - watering systems	Quality control of drinking water for humans and animals twice a year. Sanitization of the supply water pipelines between turnovers.
Health management	
Healthy pigs Prevention Prophylaxis Record keeping	1x daily - regular monitoring of pig health. Compliance with the principles of good husbandry practices. Herd health management. -Vaccination programme, including monitoring of its observance. -Regular inspection of animal husbandry and veterinary record keeping.
Diseased pigs Veterinary activities	Separate housing of pigs with altered health condition in an isolation house/section. - Disinfection of instruments, needle exchange, etc.
Product inspection	
Slaughterhouses	- Retrospective analysis of findings of veterinary inspection of market pigs, their carcasses and organs after slaughter and basic processing. - Retrospective analysis of the results of the determination of residues of inhibitory substances.

However, the implementation and effectiveness of the proposed measures depend primarily on the herd size, pig farming technologies and, above all, the strict control of compliance with the principles of good husbandry practices.

Adoption of preventive measures in all pig farms (small-scale farms, organic farms, large-scale farms) are essential to prevent the introduction of ASF virus into the farm and its subsequent spread within the farm premises.

However, the feasibility and effectiveness of preventive measures depend mainly on the farm size and pig farming technologies.

The level of biosecurity in small-scale farms, organic and alternative farms is generally lower.

Small-scale pig farms focus mainly on the production of raw materials and food of animal origin for their own consumption. The pigs in small-scale farms are usually kept in authentic small pigsties, often with the possibility of free movement in the owner's paddocks and adjacent pastures, and kitchen waste is usually used for their feeding. These pigs are usually slaughtered on the farm (home slaughter), and only rarely in slaughterhouses.

In terms of biosecurity, small-scale pig farms can be characterised as farms where farmers very often do not follow the general principles of good husbandry practices. In small-scale farms, there are only limited possibilities for introducing, and in particular for complying with, the general principles of biosecurity. Consequently, small-scale farms pose a significant potential risk for the spread of ASF virus.

Organic pig farms use both technological housing systems in indoor pigsties with access to outdoor paddocks, as well as year-round housing systems in outdoor paddocks and pastures, or a combination of both. However, due to the small number of organic pig farms, the risk of introducing ASF virus into these farms is often neglected in epizootiological surveillance.

Similarly, small-scale farms and farms with small numbers of pigs, where the level of biosecurity is lower, represent a significant potential risk for the spread of ASF virus.

Large-scale pig farms

Biosecurity is generally introduced and observed, especially in large-scale pig farms, where it also reaches the highest level.

The complex relationships between specific ASF virus transmission routes, including the possibility of implementing biosecurity preventive measures depending on the pig herd size, are summarised in Table 5.

Table 5: Preventive measures depending on the transmission route and herd size

Routes of pathogen transmission	Biosecurity precautions	Backyard farms	Small-scale farms	Large-scale farms	Organic farms
Humans	Access and movement control				
	Hygienic loop				
	Protective cloths and boots				
	Disinfection mats				
	Backyard pig keeping by caretakers is not allowed				
	Active participation in chases and hunts is not allowed				
Animals	Quarantine of purchased animals				
	Isolation of diseased animals				
	Closed herd turnover				
	Mixing of pigs of different ages				
	Health monitoring				
	Vaccination programme				
	Inspection of products				
Vehicles	Disinfection entrance				
	Entry of foreign vehicles is not allowed				
	Restrictions on vehicle movements				
	Setting boundaries between the black and white zone				
	Sanitation				
Technology systems	System of operation (turnover/continuous)				
	Breeding environment				
	Maintenance of technology systems				
	Cleaning and disinfection				
Equipment, tools	Cleaning and disinfection				
Feed	Consumption monitoring				
	Quality monitoring				
	Cleaning of silos				
Water	Consumption monitoring				
	Quality monitoring				
	Cleaning and disinfection				
Bedding	Quality monitoring				
	Sealable storage				
Free living animals	Unbroken farm fencing				
Birds	Screens for windows and doors/air intakes and outlets				
Cats/dogs	Vaccination and deworming				
Insect	Disinsection				
Rodents	Rodent control				
Air	Spatial isolation of farms				
	Air filtration				

Explanation: Feasibility of implementing preventive measures

low intermediate high not monitored

11. Disinfection

Jana Prodělalová, Romana Moutelíková

Veterinary Research Institute

Pavel Novák and Gabriela Malá

Institute of Animal Science Prague

What can the African swine fever virus endure and how could it be safely eradicated?

African swine fever virus (ASFV) is extremely stable in the environment. As soon as at the time of its discovery, in 1921, ASFV infectivity was found to persist for up to three days on the surfaces of contaminated livestock houses in warm climates; the virus is infectious in faeces for up to several weeks. The findings that infectious virus was isolated from blood or serum stored at room temperature even after 18 months, and persisted for 15 weeks in decaying blood at room temperature are important (Carlson et al., 2020; Davies et al., 2017; Desmetch et al., 2021; Frant et al., 2021; Mačiulskis et al., 2020).

Under Central European conditions, ASF virus is transmitted by direct contact (i.e. contact between ill and healthy animals) and indirect contact (i.e. via mechanical vectors including humans) (Chenais et al. 2018). Ticks do not play a role here, even in the case of the presence of the virus in wild boar populations. Since a contaminated environment is a potential source of infection with the ASF virus, this risk needs to be eliminated using chemical disinfectants. The etiological agent of ASF is an enveloped virus, which is a significant advantage during its inactivation. This is because it is susceptible to the full range of disinfection agents used in commercial disinfectants, provided, of course, that the correct preparation and application procedures are followed. The choice of the active ingredient or combination of active ingredients in the disinfectant is also crucial. ASF virus is considered to be a virus which is sensitive to disinfectants containing alcohols, aldehydes, hydroxides, biguanides, halogens, peroxo compounds, ethylene oxide and some phenols and quaternary ammonium compounds (QACs) (Quinn et al., 2021).

What we tried and the end result

The effectiveness of the disinfectants was tested on carriers. The principle of the test is the action of a disinfectant on a virus dried on the non-porous surface of a polystyrene petri dish. Organic substances are added to the viral suspension to simulate biological contamination during field application of disinfectants. The laboratory strain Ba71V obtained from the European Reference Laboratory for ASF (EURL ASFV, CISA-INIA, Madrid, Spain) was used in the tests. Virucidal activity was determined using the infectious viral titre (expressed as lg TCID50). The virucidal activity value is determined as the difference between the infectious titre of the viral control Cv and the infectious titre of the test with the disinfectant t (lg TCID50 Cv minus lg TCID50 t). Only those disinfectants in which the infectious titre of the test virus decreased by at least 4 logarithmic orders of magnitude (i.e. at least 99.99%) are considered effective. An overview of the preparations used is given in Table 6. The results of testing the disinfectants are summarised in Table 7.

Table 6: Overview of the products used.

Disinfectant (main group of active ingredients)	**Active ingredients according to the manufacturer's data**
A – 1 (aldehydes)	glutaraldehyde, glyoxal, formaldehyde, QAC*
A – 2 (aldehydes)	glyoxal, glutaraldehyde, QAC*
A – 3 (aldehydes)	glutaraldehyde, QAC*
P-1 (peroxo compounds	hydrogen peroxide, QAC*
P - 2 (peroxo compounds)	bis(sulphate)-[bis-(peroxo sulphate)penta potassium]
P – 3 (peroxo compounds)	potassium hydrogen peroxosulphate
CH – 1 (halogens - chlorine)	tosylchloramide sodium
CH – 2 (halogens - chlorine)	sodium hypochlorite
J (halogens - iodine)	Iodophor
Other products tested (characteristics according to manufacturer's data)	**Active ingredients according to the manufacturer's data**
U - 1 (alkaline cleaner for pressure cleaning)	surfactants, sodium hydroxide
U – 2 (cleaning and disinfecting agent for public spaces and healthcare buildings)	QAC *
U – 3 (product for the treatment of hounds)	not given

*QAC … quaternary ammonium compounds

Table 7: Results of testing the disinfectants.

Tested disinfectant in a given concentration, ambient temperature and disinfection time	**Biological contamination**		
	serum 2% (low)	serum 10% (high)	blood 10% (high)
A-1; 1%; 30 min, + 22°C	effective	effective	effective
A-2; 1%; 30 min, + 22°C	effective	effective	effective
A-3; 0,5%; 30 min, + 22°C	effective	effective	effective
P-1; 2%; 30 min, + 22°C	effective	effective	ineffective*
P-2; x%; 30 min, + 22°C	effective	effective	ineffective*
P-3; 1%; 30 min, + 22°C	effective	effective	ineffective*
Ch-1; 1%; 30 min, + 22°C	effective	effective	effective
Ch-2; 10%; 30 min, + 22°C	effective	effective	effective
J; 1%; 30 min, + 22°C	effective	effective	ineffective*
U-1; 1%; 30 min, + 22°C	ineffective t*	ineffective*	ineffective*
U-2; %**; 30 min, + 22°C	ineffective*	ineffective*	ineffective*
U-3; undiluted; 10 min***, + 22°C	ineffective*	ineffective*	ineffective*

* 99.99% reduction was not obtained, therefore the product is classified as ineffective under the given conditions
** the test could not be performed at the concentration recommended by the manufacturer (10 %) due to the high cytotoxicity of the product
*** the product is not intended for surface disinfection and has no specified disinfection time

The product testing procedure on carriers attempts to approach the operating conditions in herds, where viruses can be expected to be coated with a protein or mucous substance and, consequently, partially

protected from the effect of disinfectants. However, this protection can be overcome relatively easily by proper mechanical cleaning, which must precede disinfection. The presence of 10% blood limits the decrease in ASF virus titre in the case of products containing peroxo compounds and iodophor.

The tested products always reduced the ASF virus titre, but not by the required 99.99% (i.e. by 3 logarithmic orders of magnitude). In the case of heavy contamination of surfaces with blood, it is therefore necessary to consider the use of a biocide with a different type of active substance. Some authors recommend disinfectants containing sodium hypochlorite, iodophor, QAC, hydrogen peroxide vapour and formaldehyde as suitable for ASF virus inactivation. QACs are always present in commercial products in a mixture with other active substances, in the spectrum of tested products, the most frequent are aldehydes (A-1 to A-3), in one case peroxo compounds (P-1). These preparations are very effective against ASF virus. In contrast, the preparation containing only QAC (U-2) did not show the desired effect. However, in this case, it was a biocide with a declared effect only against bacteria and yeasts and the observed result is therefore consistent with the manufacturer's information. A sufficient decrease in the ASF virus titre was detected neither with the use of the alkaline detergent containing tensides (U-1) nor with U-3 of unclear formulation.

What was the outcome after testing the efficacy of disinfectants against ASF virus in pig farms?

The evaluation of the efficacy of disinfectants under the conditions of farms was verified on three pig farms with indoor housing of pigs at different age categories (farrowing, piglet rearing and fattening house). The efficacy of disinfectants with three different active ingredients - peroxides, iodophores and glutaraldehyde in concentrations recommended by the manufacturers or suppliers was tested. The evaluation of the disinfection efficiency testing was in accordance with the Methodology for the implementation and evaluation of disinfection efficiency testing by the State Veterinary Administration of the Czech Republic.
The summarized results of testing the efficacy of selected disinfectants recommended for disinfection in the case ASF virus under pig farm conditions, expressed as the average values of the total number of microorganisms in swabs taken before and after disinfection, are presented in Table 8.

Table 8: Summarized results of microbiological testing of disinfection efficiency (CPM expressed in KTJ.cm^{-2} area)

Active substance	Before disinfection			After disinfection		
	average	min.	max.	average	min.	max.
Peroxy compound	1.0×10^6	1.8×10^5	2.8×10^6	2.1×10^3	3.9×10^2	7.1×10^3
Iodophore	1.1×10^6	2.0×10^5	3.0×10^6	2.3×10^3	4.3×10^2	7.6×10^3
Glutaraldehyde	1.2×10^6	2.1×10^5	3.2×10^6	2.6×10^3	4.7×10^2	8.5×10^3

For all tested disinfectants containing different active compounds, the total number of microorganisms in the samples after disinfection was reduced by 3 logarithmic orders of magnitude compared to the samples taken before disinfection. The disinfection can be considered effective if the average value of the total plate count (CPM) of the swabs taken at the selected sampling points in one animal house/section after disinfection is ≤ 5.0 10^3 KTJ.cm^{-2} area.
The highest microbial contamination was found in swabs taken before and after disinfection from feed troughs, waterers and pen floors. The lowest numbers of microorganisms before disinfection were found in swabs from the walls of the sections. From the above, it can be concluded that increased attention should be paid to mechanical cleaning of feed troughs, waterers and floors. On the other hand, the level of microbial contamination of the barrier walls in pens and section walls depended on the porosity of the material, which was higher for plastics and lower for metal.

How to properly disinfect animal pig houses?

The disinfection of animal houses is carried out in a few successive steps: removal of animals, mechanical cleaning, soaking off, pressure washing, drying, disinfection, drying, of disinfection efficiency testing, moving animals in. First of all, it is necessary to pay attention to the removal of organic material from the animal houses/section after the pigs have been removed: removal of feed residues, litter, dust, draining of semi-liquid manure sewers. organic material present in the animal houses limits the effectiveness of disinfectants and also acts as a source of nutrients for some microbes. Attention must also be paid to the removal of biofilm from waterers. The quality of mechanical cleaning determines the efficiency of disinfection, is a prerequisite for the effective action of disinfectants on disinfected surfaces, thus limiting the possibility of reducing the efficiency of the disinfectant. More than 90% of microorganisms can be removed by mechanical cleaning. It has been shown that a thorough mechanical cleaning reduces the total number of microorganisms (CPM) by 3 logarithmic orders of magnitude (i.e. 99.9%).

What factors influence the effectiveness of disinfection?

To achieve the expected effectiveness of disinfectants, factors that may influence their efficacy, namely the resistance of microorganisms, product properties, the method of product use and the characteristics of the environment, have to be taken into consideration.

Resistance of microorganisms - in terms of disinfection practices, it is necessary to respect the different resistance of various groups of microorganisms to disinfectants, which results from different morphological and biochemical properties and permeability of cell membranes. The most sensitive to disinfectants are obligate (strict) intracellular bacteria such as mycoplasmas. Less sensitive to disinfection are Gram-positive and Gram-negative bacteria, enveloped viruses and fungal spores. Resistant to disinfection are non-enveloped viruses and mycobacteria. The most resistant are bacterial endospores and protozoan oocysts. Resistant to most disinfectants are prions.

Properties of disinfectants - disinfectants that are intended to completely devitalize microorganisms are marked with the suffix -cidal, while disinfectants that limit the growth of microorganisms or prevent their reproduction are marked with the suffix -static. According to their spectrum of activity, disinfectants are classified as broad-spectrum products, limited-spectrum products and specific products.
The stability of disinfectants has an impact on their efficacy because some products undergo changes in composition during storage, reducing the content of the active ingredient or functional group. Stable agents (e.g. chloramine) are based on the product concentration, while unstable agents (e.g. chlorinated lime) are based on the active ingredient content and the optimum temperature of the working solutions - stable agents (approx. 50-60^{o}C), unstable agents ((<30^{o}C).

How to use disinfectants

Adequate concentrations of the products are a prerequisite for their efficacy. Lower concentrations of products may, in addition to reducing the disinfection efficiency, also cause the survival of less sensitive microorganisms. Disinfectants that are applied at higher concentrations (e.g. alcohols and phenols) are more affected by concentration changes, whereas those applied at lower concentrations (e.g. formaldehyde) are less sensitive to the concentration of working solutions.

Exposure time - the time required for devitalization of microorganisms depends on the product used and the resistance of the target microorganisms. Although some preparations destroy microorganisms immediately, the typical exposure time is 20-30 minutes.

Quality of application - for the product efficacy, even spread of the disinfectant over all disinfected surfaces is essential.

Water quality - high microbial contamination of water reduces the content of the active substance in the working solution. Higher concentrations of Ca^{2+} and Mg^{2+} cations in hard water used for cleaning, washing and dilution of disinfectants may reduce the efficacy of some products (e.g. quaternary ammonium compounds).

Application form of the disinfectant – the most commonly used form is a solution or foam. The amount of solution applied per 1 m^2 of surface must be in accordance with the manufacturer's or supplier's recommendation, typically 0.3 – 0.5 l per 1 m^2 of surface to be disinfected.

The powder form is only suitable for disinfection of liquids (water, urine) provided that the recommended dose of the product and its homogenization in the liquid to be disinfected is observed. In dry environments, the powder form is ineffective.

Aerosols and gases for disinfection of spaces (e.g. formaldehyde, peracetic acid, lactic acid, glycols and hydrogen peroxide) require hermetic containment of the disinfected area and dry surfaces to be disinfected; in addition, an adequate temperature (min. +15°C) and high relative humidity (min. 70%) in the disinfected area.

Characteristics of the environment

Temperature and relative humidity in the animal facility - disinfectant activity usually increases with a slight decrease in temperature, although some disinfectants are more dependent on the ambient temperature. Glutaraldehyde is effective from +5°C, while formaldehyde requires a minimum of +15°C. Persteril is effective over a wide range of ambient temperatures from 0 to +30°C.

The pH of the environment can affect the cell surface of bacteria and the action of disinfectants. Some products are more efficient in acidic environments (Persteril - pH 3.0-7.5), others in alkaline environments (QAC - quaternary ammonium compounds - pH 9-10).

Surface quality - the effectiveness of disinfectants on porous or rough surfaces (e.g. wood, concrete) is lower than on smooth surfaces (e.g. metals, plastics). The permeable surface of soft outdoor paddocks is virtually impossible to disinfect.

What are the implications?

The basic criterion for the selection of a suitable chemical disinfectant is the knowledge of the target environment and its microbial load, including the technical possibilities of application of the selected product. Disinfectants are generally less efficient on surfaces contaminated with faeces, blood, tissue debris, etc. Without prior cleaning, their efficacy is significantly reduced. Therefore, it is absolutely necessary to follow the generally recommended procedures on a long-term basis, i.e. cleaning first and then disinfection. It is also necessary to follow the instructions for the use of disinfectants; failure to comply with the recommended concentrations may again lead to a reduction in their effectiveness. The use of unapproved, non-virucidal or highly experimental products is always very risky as they usually are inefficient or their virucidal effect does not achieve the desired reduction of the virus titre, which can be a major problem because the titres of ASF virus in body fluids of infected animals are usually high.

12. Combination of biology and technology means to combat ASF in wild boar populations

Jitka Bartošová and Luděk Bartoš

Institute of Animal Science Prague

Jiří Kamler, Jakub Drimaj and Radim Plhal

Mendel University in Brno, Faculty of Forestry and Wood Technology

Jan Cukor and František Havránek

Forestry and Game Management Research Institute

Antonín Machálek and Josef Šimon

Research Institute of Agricultural Engineering, p. r. i.

What role do wild boars play in the spread of ASF?

In the population of wild boars, there are basically two routes of ASF transmission, namely direct transmission between infected and susceptible wild boars or indirect transmission between wild boars and ASF-positive carcasses (Chenais et al., 2018). Direct transmission between wild boars is dependent on many factors, including natural movement of wild boars, size of home ranges, population size, food availability or hunting intensity and disturbance of game in tourist-exposed areas. In order to minimise the transmission of the virus by live animals, it is necessary not to disturb wildlife and to restrict the movement of wild boars in the affected area and, in particular, to prevent the migration of pigs out of the affected area. In the case of indirect transmission via positive carcasses left in the countryside, the factors influencing the possibility of infection of other animals depend mainly on the stage of decomposition of the carcasses, which in turn determines the behaviour of wild boars (Cukor et al., 2020a; Probst et al., 2017). In the first days and weeks of decomposition, the carcass is probably rarely a direct source of infection for live animals, but the virus can be spread by insects or other vertebrates. Therefore, the consistent collection and disposal of carcasses is a key preventive measure for the further spread of ASF. This can be technically- and staff-intensive, as sick pigs usually withdraw to dense forest cover where they eventually die. Searching for carcasses (e.g. using thermal imaging equipment or dogs) should be organised to minimise the movement of live game, depending on the terrain and the specific conditions of the site.

The spread of the disease through natural means is relatively slow. A significant contribution to the rapid spread is due to the inconsiderate or irresponsible behaviour of people, as demonstrated during ASF occurrence in the Zlín Region, when citizens often did not respect the ban on entering the forest.

In free living populations, unlike in domestic pig farms, rapid eradication of the disease by destroying the entire farm cannot be ensured (Garcia-Jimenez et al., 2013). On the contrary, attempts to drastically reduce the size may be risky in terms of ASF spreading. Wild boar populations are thus an important factor influencing the potential spread and eradication of the disease.

Thus, when ASF is introduced into a wild boar population, it must always be taken into account that, even under ideal conditions, it will take months or years to eradicate the disease and that there is a high risk of extending the affected area (Nurmoja et al., 2017). Due to the economic risks associated with the occurrence of ASF, research attention is currently being paid to all factors that influence the spread of the disease in wild boar populations and the prospects for eradication (Jurado et al., 2018). Probably the most important factor is the generally high number of wild boars (More et al., 2018). Large populations can be expected to have a higher risk of both the emergence and spread of disease, as food competition results in more frequent contacts between pigs. Some of these pigs are also forced to inhabit less favourable areas, bringing them into close contact with potential sources of infection, e.g.

around roads. High numbers of wild boars also impose an economic burden in disposing of larger numbers of animals and prolonging the eradication period. In terms of preventing the spread of ASF and its consequences, it is therefore essential to reduce wild boar numbers to the lowest possible level before the disease is introduced into the area and then to stabilise wild boar numbers in the long term (More et al., 2018).

This entails the determination of the required annual hunting intensity and structure for specific populations, which depends on the initial size and reproduction of the population, influenced, among others, by the natural feed sources in the environment, hunting management and other factors. In the effective fight against ASF, as well as in the reduction of wild boar numbers, it is necessary to build on the knowledge of the behavioural ecology of wild boars (behaviour, life and reproductive strategies, interaction with the environment and other animal species), and start to use modern technologies, even though these practices may be perceived by the hunting community to be in disagreement with hunting tradition (use of thermal imaging equipment and night vision, group trapping devices, etc.).

Is there any way to control the number of wild boars?

The development of the wild boar population in the Czech Republic so far corresponds to the experience of many European countries (Massei et al., 2014). Updated estimates of the future population development of wild boars modelled in 2021 by Prof. Tkadlec from Palacký University in Olomouc show that in order to halt population growth, the number of hunted animals per year would have to double or triple, and the population growth could then stop around 2080.

The effectiveness of hunting does not consist in just directly reducing the number of animals in a given environment, because hunting strategies have a major impact on the social structure of the population and the reproductive strategies of animals. Both increased hunting and hunting pressure on a particular game category through changes in the composition and spatial arrangement of groups alter the impact of hunting interventions on subsequent population growth or decline.

The wild boar is a highly social species forming matrilineal groups. Multigenerational social units dominated by adult females are generally advantageous for the species to optimise the relationship between food supply and reproduction (Keuling et al., 2018; Podgórski et al., 2014; Stockley and Bro-Jorgensen, 2011). The advantage of living in such social units is the shared search for and use of food sources based on the experience of leaders, the improvement of the quality of the offspring, or, for example, the presence of "helpers", i.e. young animals that can participate in the upbringing and protection of the offspring (Kaminski et al., 2005), through adoption (Delcroix et al., 1985) etc. The suppression of reproduction in young females is part and consequence of such a socially stable system (e.g. Stockley and Bro-Jorgensen; 2011). Populations composed of stable social units are not subject to significant fluctuations in population density and compensate for any adverse effects of population pressures by social cooperation. Males of all ages are not, by the nature of the type of grouping, a stable part of this social system and thus play a completely marginal role in population dynamics.

The population dynamics, on the other hand, can be considerably influenced by human intervention into the social behaviour and structure of game, e.g. by intensive hunting. This can disrupt or completely destroy the social stability of the population, thereby removing natural barriers to the reproductive explosion.

The wild boar is a very prolific species. The oestrus period runs from November to spring and is entered first by adult females and later by younger ones. It is essential for them to reach a minimum threshold weight of around 20 kg. In the event of failing to get pregnant or mortality of piglets after birth, the estrus recurs. During estrus, almost all adults and more than 90% of gilts are mated. The assumption of multiple reproduction of pigs in one year has not been confirmed. However, in the cultivated landscape, more dams are involved in reproduction in the first year of life, thus extending the heat period until the end of winter (Gethöffer et al., 2007).

The litter size depends significantly on the weight of the dam, generally 6 piglets per adult sow and 4 per young sow can be expected. The contribution of young and older sows to the reproduction of the population is determined by the combination of the proportions of these two age categories of mothers in a particular population, the proportion of those in the group participating in reproduction and the number of successfully reared offspring per female. Older sows are almost all involved in reproduction, give birth to a higher number of piglets and can be expected to better care for the young which, consequently, have lower mortality after birth (Briedermann, 1971). On the other hand, there is a huge potential for females in their numbers in the first year of life, and if their numbers are not reduced during the first hunting season, the total number of piglets produced by them can even exceed the number of piglets produced by older sows. Therefore, in combination with favourable environmental conditions, young sows can have a significant impact on population growth despite the lower number of piglets born in litters and higher losses. In addition, the parturition of young dams will take place later in the spring and summer when environmental conditions are more favourable and when lower mortality can be expected due to climatic conditions that can in some years cause significant losses in contributions of older females (Orłowska et al., 2013). At the same time, later-born piglets will be immature at the start of the main hunting season in the summer months and hunting pressure will focus on the more mature ones from older dams, as hunters prefer pigs of at least 10 kg in size.

Our analyses of the number of hunted animals (data processed by the Forest Management Institute of the Ministry of Agriculture of the Czech Republic, at the district level from 1990-2019) show that the higher hunting pressure practiced in the Czech Republic in recent years has been counterproductive in terms of population consequences. The number of hunted animals increased (not necessarily from year to year, but in a long-term trend, which is crucial for the assessment of population development), but at the same time the ability of the population to reproduce increased. Population growth was virtually unaffected by the high percentage of hunted young animals (yearlings and piglets, out of the total number of animals harvested), but the percentage of sows harvested was decreased, and so the proportion of hunted sows should increase. Of the other factors, only climatic factors contributed to the modification of population dynamics.

In general, quantitative hunting (hunting of a standard number) of wild boars, as traditionally practised on our continent, cannot stop population growth, because to have an effective effect on population dynamics in terms of population control, the numbers of game hunted would have to at least double. However, this is practically unattainable under current hunting management and the unfavourable demographic development of the hunting community, and increased hunting pressure is thus increasing the reproductive potential of wild boars. The more game is hunted, the higher the reproduction rate is.

Our findings do not lead to a recommendation to stop hunting. In the case of ASF, eradication of wild boars in a designated area through rapid, intensive hunting of all animals is an effective approach. However, from the point of view of long-term management of wild boars in the Czech Republic, it is appropriate to revise the current practices. The effects of hunting should be evaluated in terms of long-term trends, not from year to year, when a number of factors other than hunting, such as climatic factors, modified management or ASF interventions, etc., may be at play.

Methods of hunting that effectively influence population development may be difficult to perform (insufficient capacity for truly intensive hunting) or problematic from an ethical and animal welfare perspective (intensive hunting of sows). Therefore, it is necessary to change the traditional hunting strategy and to use methods that have not yet been a common part of hunting management in the Czech Republic in order to achieve a significant reduction of wild boar numbers and sustainable population management.

As a convenient and effective alternative, various systems of group traps for live wild boars trapping are possible. Their main advantage is the possibility of trapping and eliminating an entire social unit of wild boars at the same time, even in numbers of several dozen. This prevents the breakdown of the stability of the social system, the loss of the mother of dependent piglets, the pigs informing each other of the danger, etc. Practical experience (e.g. in the USA) and scientific studies show the potential to eradicate

nearly 90% of the population in a given area in this way. Currently, there are collapsible, portable remote-control traps (photo traps, SMART technology) on the market. In the Czech Republic, the necessary legislation and methodology for killing trapped pigs needs to be prepared for such systems. However, their use would significantly help to reduce and maintain wild boar numbers within acceptable levels and, if necessary, eradicate wild boars, for example, in areas affected by ASF or urban agglomerations.

What role do food conditions play in this?

Pigs benefit in particular from their ability to use a wide range of food and to ensure year-round good quality nutrition, despite their inability to use ballast plant biomass (Ballari and Barrios-García, 2013). The natural diet of pigs is predominantly plant-based, with pigs being quite adaptable in their food choice and modify their diet composition substantially according to environmental and seasonal supply (Schley and Ropper, 2003). Therefore, food conditions do not limit pig populations too much (Holland et al., 2009). Although pigs are dependent on good quality food resources in the form of seeds, fruits, tubers and roots and cannot compete with ruminant herbivores in their ability to digest and utilise plant matter, they can find plenty of food in today's landscape. Pigs have generally adapted well to the changes in the current landscape and use both traditional and new food resources (Vetter et al., 2015). Probably the most important food sources for pigs are found during the growing season in the fields to which they move in sounders. Today's agricultural landscape offers them good quality food from as early as May, when they begin to consume oilseed rape and, throughout the growing season, crops appear intermittently in the fields, providing them with very abundant food and often reliable shelter. Maize plays a key role, providing food and shelter for the pigs for up to several months. Usable crop residues are often left in the fields until the following spring. The pigs are thus properly fattened during the growing season on the crops and go into winter with sufficient fat reserves. The largest pig populations are therefore found in areas where agricultural crops are adjacent to forest complexes.

Another important source of nutrients for pigs are the seeds of woody plants, especially oak trees, which have a mast year almost every year (Kamler et al., 2016). In some locations, when the harvest is abundant, pigs feed essentially only on acorns in autumn and winter, significantly reducing their visitation to bait sites and thus hunting success.

Winter is a critical period for wild boars, but under our conditions, and especially in recent decades, wild boars overcome the winter period without major losses, mainly because of the warmer temperatures and the abundant food supply in the fields and the natural fall of seeds of forest trees, as well as the feed offered to them during baiting.

Are there any technical options and tools to limit wild boar movements in the natural environment?

In the project QK1920184, means and procedures for locating both live wild boars and their carcasses were verified using thermal imaging on various carriers depending on the landscape searched (drones, thermal imaging searcher VMT-VÚZT, off-road car, electric quad bike; see the verified technology describing effective procedures for locating wild boar carcasses, for which a contract for application was concluded with the Lesy ČR, s.p. (Forests of the Czech Republic), and hounds (including a specific methodology for use in the Liberec Region in the event of an ASF outbreak. Drones of various types equipped with loudspeakers have been successfully tested for chasing wild boars to predetermined sites. A combined wild boar repeller has been developed and a utility model of a specific fence to prevent pigs from accessing has been registered. Mechanical tools are often used to direct the movement of large animals in the wild and are a common feature of grazing areas for farm animals or game park preserves. Their function is to keep animals in or out of the enclosure. The use of these mechanical tools to direct the movement of pigs is largely limited by their strength and tenacity, their dense hair, their ability to dig in the ground, but also by their considerable cognitive abilities

(intelligence) and learning capacity, which give the pigs an advantage due to gained experience (e.g. undermining fences, breaking through meshes, lifting electric fences to ensure the safe passage of piglets, etc.). Sufficiently anchored wooden or metal (stainless steel) solid fences are an effective but expensive option, especially for smaller areas. They are often used along high traffic roads and motorways, or for protection of agricultural land and crops. Solid fences have been erected as a preventive measure to avoid the spread of ASF in some EU countries (e.g. Bulgaria, Denmark, Germany). Temporary fences can also isolate a localised outbreak (e.g. ASF), as was the case in 2017 in Zlín or a year later in Belgium.

Electric fences include permanent and mobile fences, including autonomous systems powered by solar energy. The fence requires laborious installation, a regular power supply system, frequent inspection and maintenance. Most fencing systems serve as seasonal protection for relatively small plots of agricultural crops. The pulses generated cause a shock to the animal but do not pose a health risk. Voltages recommended for wild boars are 3000-4000 V with pulses generated at approximately 1-1.5 second intervals. Electric fences, in combination with odour fences, were used around the perimeter of the high-risk area to isolate African swine fever infected animals in the Zlín Region in 2017. After some time, it was shown that wild boars migrate in and out through this "barrier". The ineffectiveness of this measure might have been due to the malfunction of the power supply, interruption of the electrical circuit, inappropriate height of the conductors, reduced effectiveness due to the contact of the conductor with vegetation, insufficient earthing or low effectiveness of the odour repellent (due to insufficient replenishment, repetition of the same odour, etc.). Animals with long and thick fur or dried mud on their heads and bodies might have been insufficiently conductive. It is also known that if an animal strikes a conductor with part of its head in front of its eyes, it responds to the impulse by moving backwards. However, if contact with the wire occurs behind the eyes, the animal lunges forward, directly against the fence, and usually damage it.

Our observations confirmed that wild boars react sensitively to electric fences and, if they know about their installation, they respect them even if there is attractive feed behind it. However, wild boars often bump into newly installed fences and, when struck by electric current, they lunge forward and jump through the fence or become entangled in it, roll over it and tear it apart. We designed a modified type of fence (registered utility model) which partially eliminates the identified disadvantages.

Odour and fibre optic fences are commonly used along roads to minimize wildlife collisions with vehicles. Odour carriers consist of foam soaked with an odour concentrate of biological substances, most commonly the urine of large carnivores. Their effectiveness varies considerably depending on the distance between the carriers, weather conditions, frequency of refilling the concentrate, etc. Optical fences include reflectors that reflect light from vehicle headlights onto the surrounding road. Thus, a passing vehicle, due to a set of reflectors, creates an optical fence around the road, which discourages animals from approaching the road.

Acoustic fences are used to protect agricultural land. The sound device emits a sound at certain regular or irregular intervals to scare animals away (e.g. imitation of shooting). However, these are not useful in preventing pig migration, as pigs quickly adapt to them.

A relatively high efficiency can be achieved by a combination of the above protective measures (e.g. electric and odour fencing, as was the case in Zlín in 2017 when ASF first appeared in our territory). However, with regard to our experiments, we recommend using a combination of three measures (electric, odour and mechanical fencing) by means of an "electric fence to prevent wildlife migration" (Kamler and Drimaj, 2021: Electric fence to prevent wildlife migration. Utility model, registration number 35556), which also uses an odour and mechanical barrier to enhance the effect (Figure 15). Besides a 15 cm wire moved forward and placed 25 cm above the ground and an odour strip that is impregnated with repellent, a conductive net is also placed on the posts to act as a mechanical barrier to pig penetration. A covered cellulose sponge is placed on top of each post and the repellent is added to it at regular intervals every three weeks, as well as to the odour strip which is moved forward. The device first affects the pig with the odour from the cellulose sponge, which makes the pig alert. It then

focuses on the intense smell of the scent strip, which when touched receives a pulse that brings it back. Should the forward movement occur, it is caught by a strong and conductive net that will not let the pig move through the fence.

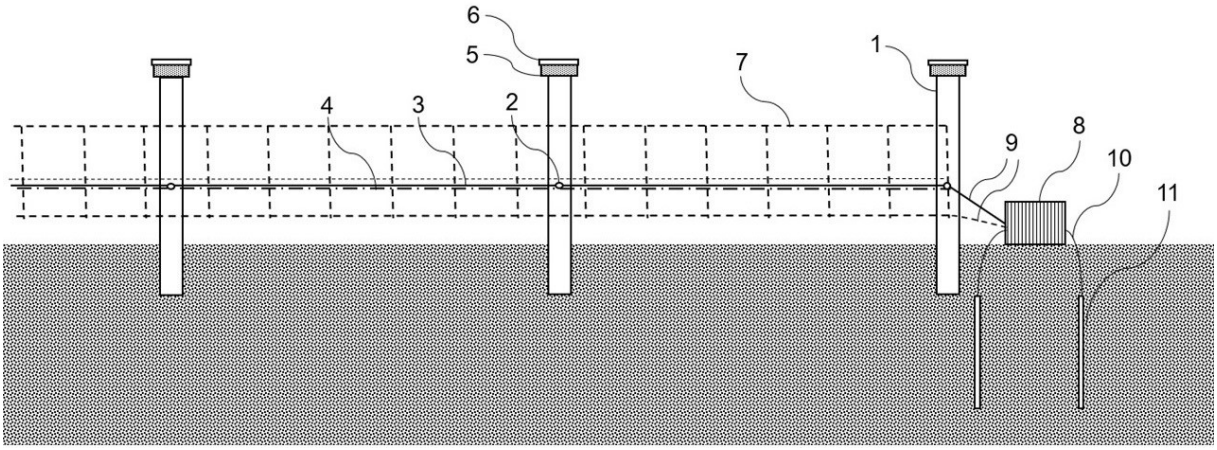


Figure 15: Diagram of the combined electric fence

Description: 1) posts hammered into the ground to a depth of 40 cm, 2) electrical insulators, 3) a 2.5 mm thick high strength wire moved forward by 15 cm in front of the fence, connected to the power supply, 4) parallel to the wire moved forward by is an odour strip with a 3 mm wick woven in, which serves as a carrier for the repellent, as well as 5) a cellulose sponge, covered with 6) a metal plate, 7) a conductive net, 8) a power supply, 9) high voltage wires connecting the power supply to the conductor which has been moved forward and the conductive net, 10) earthing with earthing rods around the power supply.

Are there technical options to locate wild boars in the natural environment?

In nature, it is very difficult to find a universal procedure that would guarantee the successful search and chase of animals. The nature and ruggedness of the landscape, forest cover, the area of individual crops, the season and the experience of hunters and unmanned aerial vehicles UAV operators play a major role. The advantage is that hunters know the places where game is most often found, and in these places, observation and hunting posts or pulpits are built. Wild boars are known to be found in fields mainly during the ripening of crops and in forests mainly in winter when they feed on tree seeds. During the summer months, they often withdraw from the fields for the day to higher vegetation where they can find shade and cool off. If the crop cover is sufficiently high and dense (maize, oilseed rape), they may also stay during the day, especially if there is sufficient rainfall. Furthermore, they can then create wallows and can often get access to groundwater by digging holes. In such conditions, they can stay for months, with correspondingly huge crop damage.

The success of the hunt can be significantly increased by locating wild boar sounders using drones and thermal imaging and by targeted driving them towards shooters. The project has successfully tested the possibility of using drones with thermal imaging and loudspeakers to drive wild boars deliberately to shooters or trapping areas or traps. Due to the fact that wild boars are nocturnal animals and hide in dense vegetation during the day, where it is impossible to find them also with regard to the density and temperature of the surroundings using thermal imaging on drones, we focused on searching and chasing at night, when wild boars are active and go out to the fields to feed. However, night flying of drones is prohibited and an aerial work permit for flights at night must be obtained from the Civil Aviation Authority. Recordings of barking dogs chasing pigs and squealing pigs held by dogs were used for testing. For searching by thermal imaging, it is important that the surface temperature of the pig's body on the back is more than 3°C higher than the surface temperature of the surrounding area. The body surface

temperature of a wild boar on its back is relatively constant at around 16-25°C. It follows that the best time is during the night hours, preferably after midnight, and in the colder months. It is not recommended to search in sunshine, in rain and in tropical nights, unless dew falls and cools the ground surface. Before flying, the Suitability can be verified by experimenting with a dog left lying in the vegetation and a drone with thermal imaging is launched to a height of about 30 m. If the dog is visible on screen, then the wild boar will also be visible. Dense vegetation that completely covers the surface of wild boars, such as dense deciduous forest at the time of leafing, coniferous forest and too dense and tall rapeseed stands, are not suitable for searching. It has been verified by experiments that it is possible to search in stands of cereals, fodder crops, rapeseed and maize. Ideal conditions for searching are in stubble fields after harvest (Figure 16), but driving of animals to shooters is more difficult there because the animals usually run to the nearest cover, i.e. places with taller vegetation where they feel safer. In tall vegetation they are better directed as they try to run in the direction away from the source of the sound of barking dogs, gunshots or squeals.

During the growing season, when field crops are taller than 60 cm, the only effective way to search is with drones with thermal imaging cameras. If the vegetation is low or it is after harvest, it is possible to search by driving through the hunting area in a car with a thermal imaging monocular mounted on the roof of the car with a mechanism to control the rotation and tilting of the thermal imaging camera. The ideal solution is remote control of the rotation and wireless transmission of the image to a tablet placed in the car. This allows the fellow-passenger to monitor the surroundings of the road at night, and if wild boars are encountered, the drone with thermal imaging and loudspeaker can then be used to drive the boars to shooters or trapping areas or traps.

Figure 16: Group of wild boars detected by thermal imaging

Can thermal imaging be used to search for wild boar carcasses?

Knowledge of the behaviour of wild boars towards conspecific carcasses is still very limited, so much attention has been paid to extending the information in this area. The behaviour of pigs was initially very cautious in relation to carcasses. Behaviour varied considerably, particularly according to the stage of carcass decomposition, and also depending on other factors such as the local abundance of the wild

boar population or the location of its placement. The first wild boars were observed at the carcass no earlier than four days after its placement in the landscape and no later than 20 days depending on the site. Thus, the average time from placement of a carcass to its first visit by wild boars in hunting grounds of the Central Bohemian Region with a relatively high wild boar population was approximately 9 days, but was quite variable. On average, snout contact with the carcass or treading the carcass occurred after 22 days, with direct contact recorded no earlier than 11 and no later than 36 days after the carcass placement. Direct contact occurred by 'poking' or 'digging' on the remains of the exposed wild boar. Indirect transmission between wild boars and carcasses of dead ASF-positive animals can occur at this stage, as defined by the ”wild boar-habitat“ transmission cycle (Chenais et al., 2018).

Depending on climatic factors, the muscles are rapidly decomposed by the activity of necrobiotic insects during the growing season. In the case of the summer months, the muscles can be completely removed within three weeks of carcass placement. The decomposition of the muscles was followed by further stages of direct contact, when the wild boars wallowed in the remains of skin and bones or chewed these remains. This stage, which occurs approximately one month after death or placement of the carcass in summer, is probably the most risky in terms of possible transmission of ASF to healthy animals, as also reported in a German study (Probst et al., 2019).

The findings shed light on the key role of carcasses in the indirect transmission of ASF virus within the "wild boar-habitat" cycle. The results of the monitoring of wild boar behaviour towards exposed wild boar carcasses clearly confirm the suitability of the protective measures selected by the State Veterinary Administration and the Ministry of Agriculture in the area of ASF outbreak in the Zlín Region in 2017-2018. At the time of the ASF virus outbreak, the area was repeatedly and very carefully searched. The carcasses found were removed from the risk area and the finding sites were properly disinfected. This chosen procedure was undoubtedly one of the factors that helped to stop the transmission of ASF in the Zlín Region and successfully eradicate the disease.

Similar measures, focusing on thorough field searches and removal of carcasses, were also implemented in Belgium, where the disease was successfully eradicated and Belgium was officially declared an ASF-free country, similar to the Czech Republic. Risk areas were systematically searched in Belgium. Carcasses found were removed from the countryside and transported under strict rules to minimise the possibility of spreading the disease. The surroundings of the carcasses were thoroughly disinfected to avoid contamination of the environment with the ASF virus (Boklund et al., 2018).

On the basis of the described behaviour of wild boars towards carcasses, the results can be applied in relation to the intensity of searches in affected areas. In the case of the summer, systematic searches with regard to the first contacts of wild boars with carcasses can be planned at approximately three-week intervals (the average time until first direct contact was 22.2 days in the summer). However, during the growing season, increased efforts should be made due to the lush vegetation and the potential for more difficult searches in the landscape (Boklund et al., 2018). In the case of the off-growing season with low temperatures and slow decomposition of carcasses, when complete preservation may occur at sub-zero temperatures, systematic searches in the landscape at approximately four-week intervals may be chosen on the basis of behavioural analysis. However, this period is very risky from the point of view of virus transmission, particularly because of the possible death of positive animals in the autumn months. Carcasses are preserved during the winter and infected muscles can then be consumed in the spring, as was the case in the experiments carried out, when demonstrable cannibalism occurred in April in carcasses exposed in January. Periods of sub-zero temperatures are also problematic for the preservation of the virus, which can survive for up to 1000 days in frozen muscles (EFSA Journal, 2010). Even a single carcass that is not removed from the infected area during the winter months can cause the re-emergence and subsequent spread of an outbreak of African swine fever.

However, consistent and systematic searching in the areas affected by African Swine Fever has to be coordinated with other measures to limit increased movement of wild boars, which could lead to direct transmission between infected and susceptible animals (Chenais et al., 2018). For these reasons, it is advisable to coordinate the search for carcasses on clearly defined dates, when as large an area of the

infested area as possible is searched on a one-time basis and in great detail. This is followed by another rest period, with the avoidance of disturbing wild boars and a ban on entering the infected area. The spread of the ASF virus is also restricted by prohibiting feeding and supplemental feeding of game (Desmecht et al., 2021), which can also lead to direct infection of animals, but also to the death of positive wild boars near feeding sites or baiting sites and subsequent passive spread through contact with carcasses.

The use of thermal imaging techniques to locate wild boar carcasses is one of the effective methods to prevent the spread of African swine fever (ASF) virus. Early detection, removal of the carcasses and disinfection of the finding site prevents transmission of the virus to healthy wild boars due to direct contact with infected carcasses. Infected carcasses play a key role in the spread of ASF. The ASF virus can survive for many months in carcasses of dead pigs, depending on climatic conditions, and for years in frozen carcasses. Transmission can occur indirectly through insects inhabiting infected carcasses, through contact of healthy animals with contaminated soil during engraving and foraging, or through direct contact of healthy animals with infected carcasses. Direct contact occurs by burrowing into the carcass out of curiosity, wallowing in decomposed remains, chewing on bones, and in some cases cannibalism and consumption of the muscle tissue has been documented.

The rate of decomposition depends mainly on climatic conditions affecting the activity of aerobic and anaerobic bacteria and the presence of necrophagous insects. In summer, complete decomposition of the muscles occurs within one month, whereas carcasses of animals that die in autumn are preserved by low outdoor temperatures until late spring.

The sooner the carcass is found, removed and the site disinfected, the better in terms of ASF prevention. The identification of wild boar carcasses by thermal imaging takes advantage of the different surface temperatures of the carcass and the surrounding area. The use of thermal imaging cameras in wildlife surveys is relatively well known (Cilulko et al., 2013; Ditchkoff et al., 2008; Havens and Sharp, 2016). For example, the use of thermal cameras in the determination of the time of death on large-scale farms of domestic pigs has also been described. The use of thermal camera in searching for wild boar carcasses was practically verified in experiments carried out by the project team of the Institute of Animal Science Prague (IAS) in the Sedlice game preserve, which specializes in wild boar keeping. The verification was carried out both on the basis of the difference between the rectal temperature of the carcass and the air temperature and the theoretical visibility of the carcass determined by thermal imaging, and directly on the basis of the temperature emitted by the carcass using various means of thermal imaging technology, such as handheld hunting or industrial thermal cameras or thermal imaging drones (Machálek et al., 2018; Šimon et al., 2019).

Depending on the environmental conditions and financial and operational possibilities, various types of equipment can be used to search for carcasses, be it handheld thermal cameras, a telescopic thermal camera, a drone with thermal imaging or a thermal imaging camera mounted on an off-road vehicle. The best time of day to search for carcasses with thermal imaging camera begins at sunset and ends at sunrise. As the sun's rays begin to hit the vicinity of the carcass and heat it, a number of sun-heated areas can be seen in the thermal camera's scope, which may have a temperature very similar to the carcass or even higher, especially if the carcass is older and decomposition processes are not as vigorous.

However, if exposed to the sun rays, the carcass warms up as well. This can be used for thermal imaging in autumn, winter and spring, when the carcass heats up faster than the cold ground when exposed to the sun. However, searching for carcasses by thermal imaging during the day cannot be generally recommended because of the number of "false alarms" due to other areas heated by the sun. Searching for carcasses is also complicated by fog or an immediately preceding rainfall event, not to mention snow cover.

In the outbreak area, where carcasses are searched for daily, thermal imaging camera can also be used to search for carcasses outside the ideal hours, especially in winter when the sun is weaker, but also in summer, e.g. on cloudy days or in shading vegetation.

In the cold season, an intensive search for carcasses is needed in outbreak areas as soon as sunny conditions allow, and the carcasses should ideally be found within 48 hours of death at an ambient temperature of 5°C or less, when post-mortem cooling and preservation occur. Carcasses preserved at low temperatures can last almost intact until the spring season, when at temperatures around 15°C, intense decomposition accompanied by high heat production are renewed and ideal conditions for searching with a thermal imaging camera arise. In summer, the ideal conditions for searching for wild boar carcasses by thermal imaging last for about one month after death, when intense decomposition processes producing large amounts of heat take place. The time of search in summer should be adapted to the sunny conditions and especially during bright sunny days the search should be performed in the night hours. Depending on the climatic conditions, especially outside the periods of extreme temperatures, the decomposition of carcasses can take place slowly over several months. Carcasses that decompose slowly are then identifiable by thermal imaging all the time until decomposition is completed, although their visibility may not be as clear as during the first few days after the death.

What are the implications?

Recommendations for practice can be divided according to their purpose into long-term recommendations (to address the trends of the so far unstoppable increase in the size of the wild boar population in the Czech Republic) and short-term or local recommendations (applicable e.g. to the eradication of wild boars in outbreaks of African swine fever). Obviously, there is no single ideal, universally applicable strategy, so a specific combination of possible measures should always be chosen, taking into account the objective, the environment, the time horizon and the technical and personnel capacity of the area. In view of the acute situation, whether it is the need for local eradication of wild boars due to, for example, ASF, or overpopulation in general, we recommend the use of effective tools, irrespective of whether or not they are part of the established hunting tradition in the Czech Republic.

It is necessary to continuously support lifelong education of game managers and to motivate hunting ground users to stabilise the number of wild boars, while not supporting such an intensity and structure of hunting that is not sufficient to hunt the increased numbers of wild boars or even leads to further increase in the number of wild boars. Any incentive instruments should therefore be set to ensure the necessary level and structure of the animals hunted.

Baiting should only be used as part of an effective hunting or trapping strategy (hunting of selected animals, hunting or trapping an entire group). Strictly limit the amount of feed that otherwise serves as an important food source for pigs (up to 5 kg of feed per baiting site per day is sufficient, preferably up to 0.2 kg per pig per day).

Combined electric fencing should be used to protect the defined area. Drones with thermal cameras can be used to detect wild boar carcasses in field hunting areas, handheld thermal imaging monoculars in field and forest hunting areas and specially trained dogs in a difficult landscape.

13. Use of electric fences to limit the movement of wild pigs

Miloš Ježek, Václav Silovský and Monika Faltusová

Czech University of Life Sciences Prague, Faculty of Forestry and Wood Sciences

Is it possible to restrict movement of wild boars in their natural environment?

The possibility of movement and migration radius control of wild animals has been tested for many years. The most effective in this respect are fixed stationary barriers - fences. These have been successfully used to protect roads (e.g. motorways, motorway-type roads, high-speed railways, etc.) or to protect buildings in built-up areas. Unfortunately, they have several drawbacks. They are long term barriers to movement intended for preventing animal migration and at the same time their construction is difficult both from an administrative point of view and in terms of the money spent on its accomplishment. Therefore, its accomplishment is carefully considered, and it is always necessary to assess the situation thoroughly.

Simple temporary fence-like structures are used to restrict movement for a short time, usually supplemented by an electric fence to increase their effectiveness. These have the advantage of speed of accomplishment (several kilometres can be built in one day) and, as they are temporary structures, are significantly less administratively demanding than standard fences. They are very successfully used in livestock farming (cows, sheep, horses, etc.), but their use in wildlife management is not very common. They are increasingly used to protect agricultural crops (especially maize, wheat, rape, etc.) or to protect a property in the open countryside (e.g. golf courses). They also have the advantage that they can be partially selective, and can be used as a barrier for e.g. larger ungulates, while allowing smaller species to move freely through the landscape. They are also increasingly used as a measure to minimise the risk of farm animals being attacked by large carnivores (especially wolves).

In recent years, electric fences have been used in several cases to possibly prevent the migration of wild boars during the process of African swine fever eradication. The first case of use was in the Czech Republic, where electric fencing was installed at the most likely wild boar crossing points and an area of approximately 5 000 ha was 'fenced'. In view of the fact that together with other measures in the area, this important disease has been eradicated, electric fencing has also been applied in other countries where African swine disease has occurred (e.g. Belgium, Germany).

Can electric fences be used to protect large areas?

Unfortunately, there is still a lack of studies that would verify the functionality of this measure on larger territorial units (areas of thousands of hectares). The data published so far demonstrate functionality only on small areas (usually in the order of units or tens of hectares). Therefore, in the course of the project QK1910462, an experiment was carried out to verify the functionality of these measures in a real open landscape environment.

The research was carried out on the grounds of the Czech University of Life Sciences Prague in the School Forest Enterprise in Kostelec nad Černými lesy. The area is located east of Prague (about 20 km) and is a forest complex widely used by people for tourist activities and with a permanent presence of wild boars (density of 4-8 animals/1 km^2).

Data from tracking wild boars using GPS and BioLogging technology were used to verify the functionality of the electric fence. Wild boars were captured in trapping devices, immobilized and fitted with a tracking collar. The tracking collar recorded the GPS location every 30 minutes. It is also equipped with a highly sensitive accelerometer and magnetometer. The accelerometer records the acceleration of the animal in three axes and the magnetometer records its position relative to the cardinal points. Both

operate at a recording frequency of 10 Hz. Using these sensors, we can determine the behaviour of the tagged animal and also reconstruct its path of movement between two GPS points. Thus, we obtain continuous information about its movement. In total, data from 17 wild boars were used in the experiment.

A standard electric fence was chosen for testing. The electric fence consisted of an electric generator and a fence set up with posts and wires. The electric generator supplied the fence line with current pulses of high voltage (4,000 V) and very short duration (less than 0.0003 sec.) As a conductor, we used plastic string with metal fibres on fibreglass or on wooden poles with insulators (Figure 17).

Figure 17: Installed electric fencing

The electric fence was installed in two sections (Figures 18 and 19). They were chosen to intersect home ranges and movement locations of wild boars tagged with GPS collars in the last month of monitoring. The first section was the electric fence between the villages of Zvánovice and Struhařov in a length of 2.5 km and the second was a fence located near the village of Louňovice in the Voděradské bučiny in a length of 2.6 km. The fence was placed along maintained gravel forest roads. The crossing with other roads was always done by installing a sleeve along the minor road (about 15 m) so that the road remained passable but animal movement was minimised. This system is commonly used on game preserves.

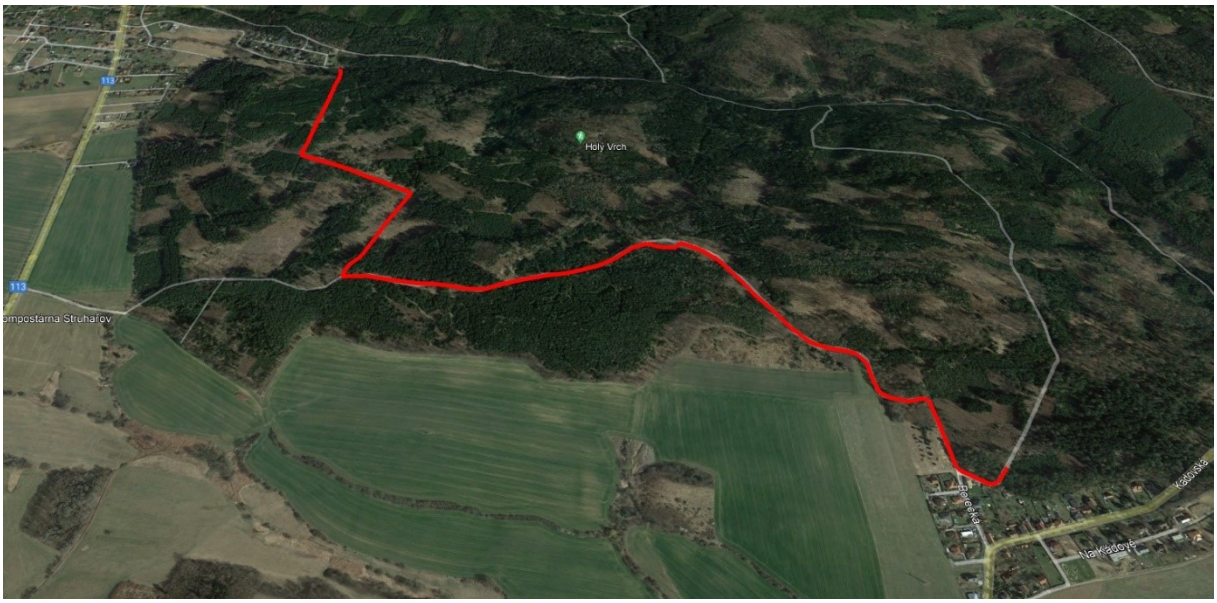


Figure 18: Section 1 of the electric fence

Figure 19: Section 2 of the electric fence

Electric fences were installed during these periods:

		Periods
Control period prior to the installation:	13 April – 12 May	K 1
Installed electric fence:	13 May – 10 June	Installation
Post-installation control period:	11 June – 10 July	K2

And what did we discover?

In section 1, there were 3 wild boars, all marked with GPS collars. A total of 158 electric fence crossings occurred during the K1 control period. During installation, there were only 11 crossings and after removal of the fence there were also only 11 crossings (period K2, Table 8).

Table 8: Number of fence crossings in section 1 in the three monitoring periods

	Pig 1	Pig 2	Pig 3	Total
K1	74	56	28	158
Installation	6	2	3	11
K2	6	5	0	11

The restriction of movement of individual pigs is even better demonstrable by their locating them in the landscape using GPS tracking collars (Figure 20).

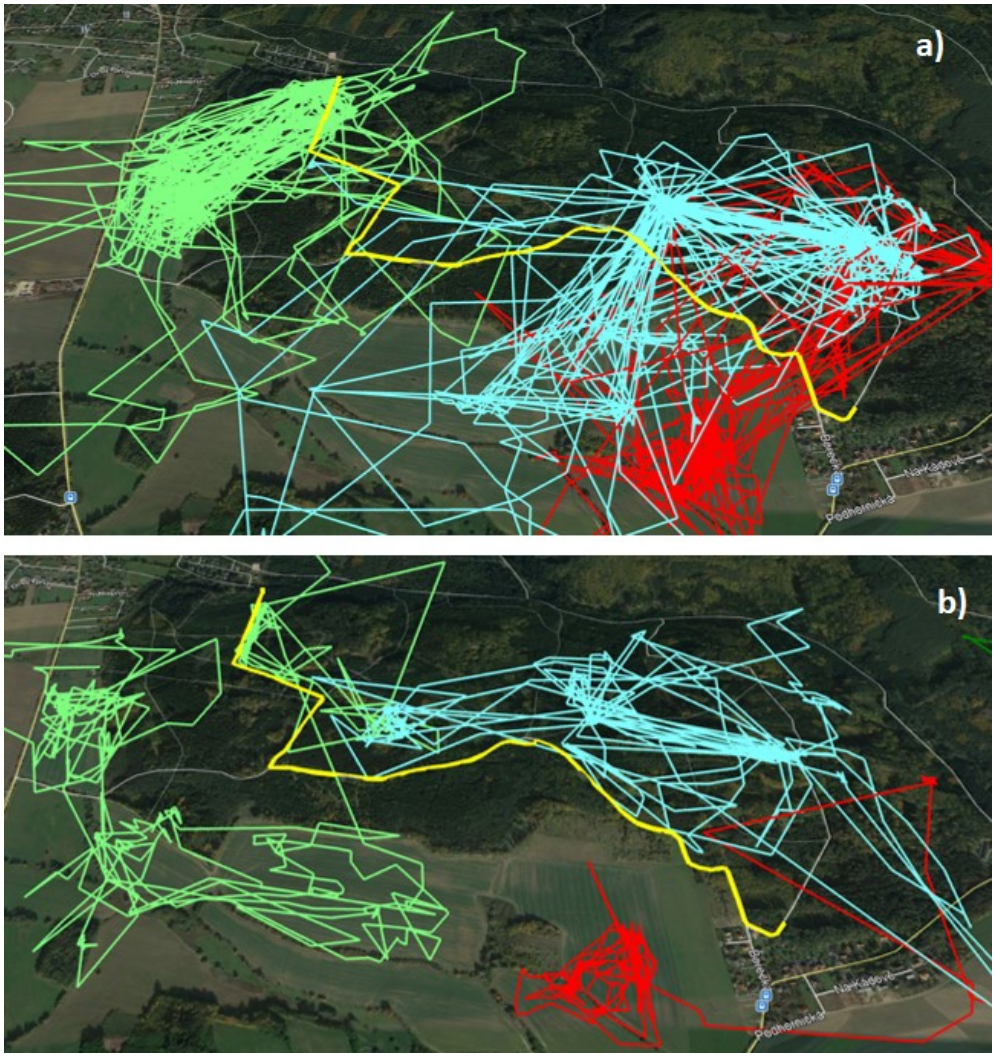


Figure 20: Movement of wild boars in the electric fence experiment (yellow line shows the electric fence; a) control period 1; b) experiment

At the same time, this part of the experiment demonstrated the high intelligence of wild boars and their spatial orientation. In Figure 18, the movements of individual pigs are shown in different colours. The movement from one side of the fence to the other was interesting in terms of one wild sow, which is highlighted in red. Even though in previous observations she had always used for movement exclusively the part that was fenced off by the pen, after its installation she walked around one of the villages and reached the other side. It is important to note, however, that she had never gone that way before. So her motivation was strong, and her goal was to get permanently to the other side of the fence. Once she got to the other side, she stayed there until the end of the experiment.

Similar results to section 1 were obtained in section 2 (Table 9.)

Table 9: Number of fence crossings in section 2 in the three monitoring periods

	Pig 4	Pig 5	Pig 6	Pig 7	Pig 8	Total
K1	0	62	34	96	192	384
Installation	2	26	7	35	68	138
K2	6	8	25	39	72	150

What are the implications?

The testing carried out clearly shows that the installation of electric fences can reduce the spatial activity of wild boars. The electric fences significantly restricted the movement of wild boars, and they chose on which side of the fence to continue to move on within a few days of installation, and followed the fence line. Significant crossings and, therefore, breaking of fences occurred most frequently in the first three days after installation. Thereafter, the breaking of fences was very rare and only a few cases occurred.

From the point of view of limiting spatial activity to limit the contact of wild boars from two areas and thus limit the spread of the disease, we can install electric fences as a long-term effective and efficient measure. Although the measure cannot prevent 100% of the migration, it can significantly reduce it. At the same time, it is a measure that can be installed very quickly, its administrative burden is not so significant, and it does not affect the movement and life of other wild animals.

14. Conclusion

The aim of this handbook was to provide a summary of the available information on African swine fever. The document shows that we can recognise the disease, we can diagnose it. However, we do not know how to treat it, nor do we have a vaccine that could protect pig farms. The only 'weapon' in the fight against this disease is to prevent it from entering this country. If, after all, the virus is detected in this country, the most important thing will be to comply with the biosecurity principles of individual farms. These principles are the prerequisites for protecting animal and human health and for ensuring adequate conditions for the production of biologically safe raw materials and foodstuffs of animal origin, which, of course, in addition to the economic benefits for farmers, also include environmental protection aspects.

Basic biosecurity precautions that can be implemented not only on large-scale farms but also on small-scale farms which, if properly and strictly followed, are effective in minimizing the risk of introduction and spread of ASF virus, include:

- Monitoring of the health status of wild boars in the immediate vicinity of the farm and the disease situation in cooperation with hunting associations and the authorities of the State Veterinary Administration of the Czech Republic.

- Strict control of the wild boar population in hunting grounds and, in regions with ASF virus occurrence, strict adherence to specific up-to-date veterinary administration guidelines on wild boar management in order to minimise the spread of ASF for a given locality.
- Use of mechanical or other technical measures to control the movement of wild boars where appropriate.
- Maintained compact farm fencing to prevent access of wild animals, including wild boars, to the farm premises, housing, feed stores, etc. and regular inspection of its condition.
- Purchase of pigs from trusted and verified sources (commercial farms).
- Pig housing in closed buildings.
- Prevention of direct contact of pigs on farms with persons who could have been in contact with wild boars (e.g. hunters, tourists moving in the wild with a higher frequency of wild boars), - a minimum 48-hour interval for hunters between hunting in an area with ASF in wild boars and contact with domestic pigs on the farm.
- No unauthorized persons are allowed to enter the pig houses; only persons responsible for the care or treatment of the animals may enter the pig houses.
- All workers who are in contact with pigs must follow the basic rules of the "black and white system", always enter the farm through the hygiene loop (i.e. dirty loop room for storing civilian clothes and shoes - shower - clean loop room for storing farm clothes; when treating pigs, use only farm clothes and boots which must not leave the farm premises); they should wash their hands with soap before entering the animal houses and, of course, also after completing their work on the farm or when moving between the animal facilities on the farm; furthermore, they cannot bring their own food containing pork to the farm.
- All staff who come into contact with pigs (caretakers) or have access to the animal houses must not raise pigs in the backyard at home and must avoid visiting other pig farms.
- Ensure prevention of feed and water sources contamination (secretions, excretions, faeces) by wild animals.
- Ban on the feeding of fresh green fodder harvested in areas at risk of ASF virus.
- All feed and bedding purchased must come from verified sources.
- Grain crops produced and harvested in the area of the declared outbreak of ASF and its immediate surroundings should be stored away from the reach of wild boars and domestic pigs for a minimum of 30 days and, if possible, treated during that period to inactivate the potentially present ASF virus.
- Straw harvested in an area with an increased risk of ASF to be used as bedding for domestic pigs must be stored away from wild pigs for a minimum of 90 days before use.
- Strict compliance with the principles of good husbandry practice regarding hygiene measures, consisting in regular sanitation, i.e. cleaning, washing, disinfection, disinsection and rodent control in all animal houses including their accessories and vehicles using effective products.
- Disinfection mats with effective disinfectants placed at the entrance to the farm, in animal houses, but also at the different sections.
- Preventing the entry of crawling and flying insects into animal houses (repair of windows, installation of window nets, etc.), regular preventive and repressive disinsection aimed at the developmental stages of insects and adults.
- Prevention of rodent entry into the farm and animal houses by repairing all holes that allow rodents to enter the houses, or their nesting and habitation, continuous preventive and repressive extermination depending on the intensity of rodent presence on the farm.
- Proper disposal of carcasses or their parts in accordance with current legislation to prevent the spread of pathogens from this potentially infectious material and their transfer to a closed rendering box located at the farm border so that they cannot come into contact with wild animals.

In the fight against the disease, it is only logical that mutual cooperation among all parties concerned - livestock farmers and other agricultural entities, hunters, veterinarians, local administration - will be necessary, as was the case with the creation of this monograph involving experts from as many as eight institutions.....

15. References

Ballari, S.A., Barrios-García, M.N., 2013. A review of wild boar Sus scrofa diet and factors affecting food selection in native and introduced ranges. Mammal. Rev., 44: 124–134.

Barasona, J.A., Gallardo, C., Cadenas-Fernández, E., Jurado, C., Rivera, B., Rodríguez-Bertos, A., Arias, M., Sánchez-Vizcaíno, J.M., 2019. First oral vaccination of eurasian wild boar against African swine fever virus genotype II. Front. Vet. Sci., 6: 137.

Beltrán-Alcrudo, D., Arias, M., Gallardo, C., Kramer, S., Penrith, M.L., 2017. African swine fever: detection and diagnosis – A manual for veterinarians. FAO Ańimal Production and Health Manual No.19. Rome. Food and Agriculture Organization of the United Nations (FAO).

Blome, S., Gabriel, C., Beer, M., 2013. Pathogenesis of African swine fever in domestic pigs and European wild boar. Virus Res., 173(1):122-130.

Boklund, A., Cay, B., Depner, K., Zsolt, F., Guberti, V., Masiulis, M., Spiridon, M., Stahl, K., Miteva, A., More, S., Olsevskis, E., Satr, P., Thulke, H., Viltrop, A., Wozniakowski, G., Broglia, A., Abrahantes, C., Gogin, A., Verdonck, F., Amato, L., 2018. Epidemiological analyses of African swine fever in the European Union (November 2017 until November 2018).

Briedermann, L., 1971. Ermittlungen zur Aktivitätsperiodik des mitteleuropaischen Wildschweines (Sus scrofa L.). Der Zoologische Garten, 40: 302–327.

Carlson, J., Fischer, M., Zani, L., Eschbaumer, M., Fuchs, W., Mettenleiter, T., Beer, M., Blome, S., 2020. Stability of African swine fever virus in soil and options to mitigate the potential transmission risk. Pathogens, 9: 977.

Chenais, E., Ståhl, K., Guberti, V., Depner, K., 2018. Identification of wild boar-habitat epidemiologic cycle in African swine fever epizootic. Emerg. Infect. Dis., 24: 810–812.

Cilulko, J., Janiszewski, P., Bogdaszewski, M., Szczygielska, E., 2013. Infrared thermal imaging in studies of wild animals. Eur. J. Wildl. Res., 59: 17.

Costard, S., Wieland, B., de Glanville, W., Jori, F., Rowlands, R., Vosloo, W., Roger, F., Pfeiffer, D.U., Dixon, L.K., 2009. African swine fever: how can global spread be prevented? Philos. Trans. R. Soc. Lond. B Biol. Sci., 364(1530): 2683-2696.

Criscuolo, E., Caputo, V., Diotti, R.A., Sautto, G.A., Kirchenbaum, G.A., Clementi, N., 2019. Alternative methods of vaccine delivery: An overview of edible and intradermal vaccines. J. Immunol. Res., article ID 8303648.

Cukor, J., Linda, R., Václavek, P., Mahlerová, K., Šatrán, P., Havránek, F., 2020. Confirmed cannibalism in wild boar and its possible role in African swine fever transmission. Transbound. Emerg. Dis., 67(3): 1068-1073.

Davies, K., Goatley, L.C., Guinat, C., Netherton, C.L., Gubbins, S., Dixon, L.K., Reis, A.L., 2017. Survival of African swine fever virus in excretions from pigs experimentally infected with the Georgia 2007/1 Isolate. Transbound. Emerg. Dis., 64: 425–431.

De La Torre, A., Bosch, J., Iglesias, I., Muñoz, M.J., Mur, L., Martínez-Lopéz, B., Martínez, M., Sanzéz-Vizcaíno, J.M., 2015. Assessing the risk of African swine fever introduction into the European Union by wild boar. Transbound Emerg Dis., 62: 272–279.

Delcroix, I., Signoret, J. P. & Mauget, R., 1985. Communal rearing of the young within the social group of European wild boars. Ann. Zootech. 34, 369-369.

Desmecht, D., Gerbier, G., Gortázar Schmidt, C., Grigaliuniene, V., Helyes, G., Kantere, M., Korytarova, D., Linden, A., Miteva, A., Neghirla, I., Olsevskis, E., Ostojic, S., Petit, T., Staubach, C., Thulke, H., Viltrop, A., Richard, W., Wozniakowski, G., Cortiñas, J.A., Broglia, A., Dhollander, S., Lima, E., Papanikolaou, A., Van Der Stede, Y., Ståhl, K., 2021. Epidemiological analysis of African swine fever in the European Union (September 2019 to August 2020). EFSA J. 19.

Ditchkoff, S. S., Raglin, J. B., Smith, J. M., Collier, B. A., 2005. From the field: capture of white-tailed deer fawns using thermal imaging technology. Wildl Soc Bull. 33(3):1164–1168.

Drimaj, J., Plhal, R., Kolibáč, P., 2015. Prase divoké a jeho životní projevy v kulturní krajině. (The wild boar and its life manifestation in the cultural landscape.) Ochrana přírody, 70(3): 6–10

Eblé, P.L., Hagenaars, T.J., Weesendorp, E., Quak, S., Moonen-Leusen, H.W., Loeffen, W.L.A., 2019. Transmission of African swine fever virus via carrier (survivor) pigs does occur. Vet. Microbiol., 237: 108345.

EFSA Journal, 2010. Scientific Opinion on African Swine Fever. EFSA J. 8.

EFSA, 2014. Evaluation of possible mitigation measures to prevent introduction and spread of African swine fever virus through wild boar. Parma, Italy; EFSA Journal, 12(3): 3616.

EFSA AHAW Panel, 2015. Scientific opinion on African swine fever. EFSA J., 13: 4163.

FAO, OIE and EC, 2019. African swine fever in wild boar ecology and biosecurity. FAO Animal Production and Health Manual No. 22.

Frant, P.M., Gal-Cisoń, B., Bocian, Ł., Ziętek-Barszcz, A., Niemczuk, K., Woźniakowski, G., Szczotka-Bochniarz, A., 2021. African swine fever in wild boar (Poland 2020): Passive and active surveillance analysis and further perspectives. Pathogens, 10: 1219.

Garcia-Jimenez, W.L., Fernández-Llario, P., Benítez-Medina, J.M., Cerrato, R., Cuesta, J., García-Sánchez, A., Gonçalves, P., Martínez, R., Risco, D., Salguero, F.J., Serrano, E., Gómez, L., Hermoso-De-Mendoza, J., 2013. Reducing Eurasian wild boar (Sus scrofa) population density as a measure for bovine tuberculosis control: effects in wild boar and a sympatric fallow deer (Dama dama) population in Central Spain. Prev. Vet. Med., 110(3-4): 435–446.

Gethöffer, F., Sodeikat, G., Pohlmayer, K., 2007. Reproductive parameters of wild boar (Sus scrofa) in three different parts of Germany. Eur. J. Wildl. Res., 53: 287–297.

Golding, J.P., Goatley, L., Goodbourn, S., Dixon, L.K., Taylor, G., Netherton, C.L., 2016. Sensitivity of African swine fever virus to type I interferon is linked to genes with in multigene families 360 and 505. Virology, 493, 154–161.

Guinat, C., Gogin, A., Blome, S., Keil, G., Pollin, R., Pfeiffer, D.U., Dixon, L., 2016. Transmission routes of African swine fever virus to domestic pigs: current knowledge and future research directions. Vet. Rec., 178(11): 262-267.

Havens, K. J., Sharp, E. J., 2016. Thermal Imaging Techniques to Survey and Monitor Animals in the Wild: A Methodology.

Holland, P.E., Burrow, J.F., Dytham, C., Aegerter, J.A., 2009. Modelling with uncertainty: Introducing a probabilistic framework to predict animal population dynamics. Ecol. Modell., 220(9): 1203–1217.

Jurado, C., Martínez-Avilés, M., De La Torre, A., Štukelj, M., De Carvalho Ferreira, H.C., Cerioli, M., Sánchez-Vizcaíno, J.M., Bellini, S., 2018. Relevant Measures to Prevent the Spread of African Swine Fever in the European Union Domestic Pig Sector. Front. Vet. Sci., 16(5): 77.

Kaminski, G., Brandt, S., Baubet, E. & Baudoin, C., 2005. Life-history patterns in female wild boars (Sus scrofa): mother–daughter postweaning associations. Can. J. Zool. 83, 474-480.

Kamler, J., Dobrovolný, L., Drimaj, J., Kadavý, J., Kneifl, M., Adamec, Z., Knott, R., Martiník, A., Plhal, R., Zeman, J., Hrbek, J., 2016. The impact of seed predation and browsing on natural sessile oak regeneration under different light conditions in an over-aged coppice stand. iForest, 9: 569–576.

Keuling, O., T. Podgórski, A. Monaco, M. Melletti, D. Merta, M. Albrycht, P. V. Genov, F. Gethöffer, S. G. Vetter, F. Jori, R. Scalera A J. Gongora. 2018. Eurasian Wild Boar Sus scrofa (Linnaeus, 1758). In M. Melletti a E. Meijaard eds. Ecology, Conservation and Management of Wild Pigs and Peccaries. Cambridge: Cambridge Univ Press, str. 202-233.

Lewerin, S.S., Österberg, J., Alenius, S., Elvander, M., Fellström, C., Travén, M., Wallgren, P., Waller, K.P., Jacobson, M., 2015. Risk assessment as a tool for improving external biosecurity at farm level. BMC Vet. Res., 11: 171.

Mačiulskis, P., Masiulis, M., Pridotkas, G., Buitkuvienė, J., Jurgelevičius, V., Jacevičienė, I., Zagrabskaitė, R., Zani, L., Pilevičienė, S., 2020. The African swine fever epidemic in wild boar (*Sus scrofa*) in Lithuania (2014–2018). Vet. Sci., 7: 15.

Machálek, A., Šimon, J., Procházka, J., 2018. Vyhledávání kadáverů divokých prasat pomocí termografické kamery. Agritech Science [online], 12 (3): 1-6.

Massei, G., Kindberg, J., Licoppe, A., Gačić, D., Sprem, N., Kamler, J., Baubet, E., Hohmann, U., Monaco, A., Ozoliņš, J., Cellina, S., Podgórski, T., Fonseca, C., Markov, N., Pokorny, B., Rosell, C., Náhlik, A., 2015. Wild boar populations up, numbers of hunters down? A review of trends and implications for Europe. Pest Manag. Sci., 71: 492–500.

More, S., Miranda, M.A., Bicout, D., Bøtner, A., Butterworth, A., Calistri, P., Edwards, S., Garin-Bastuji, B., Good, M., Michel, V., Raj, M., Saxmose Nielsen, S., Sihvonen, L., Spoolder, H., Stegeman, J.A., Velarde, A., Willeberg, P., Guberti, V., Masiulis, M., Olsevskis, E., Satran, P., Spiridon, M., Thulke, H.-H., Viltrop, A., Wozniakowski, G., Bau, A., Broglia, A., Cortiñas Abrahantes, J., Dhollander, S., Gogin, A., Muñoz Gajardo, I., Verdonck, F., Amato, L., Gortázar Schmidt, C., 2018. Scientific Opinion on the African swine fever in wild boar. EFSA J., 16: 5344–5378.

Nurmoja, I., Petrov, A., Breidenstein, C., Zani, L., Forth, J.H., Beer, M., Kristian, M., Viltrop, A., Blome S., 2017. Biological characterization of African swine fever virus genotype II strains from north-eastern Estonia in European wild boar. Transbound. Emerg. Dis., 64: 2034–2041.

Olesen, A.S., Lohse, L., Boklund, A., Halasa, T., Gallardo, C., Pejsak, Z., Belsham, G.J., Rasmussen, T.B., Botner, A., 2017. Transmission of African swine fever virus from infected pigs by direct contact and aerosol routes. Vet. Microbiol., 211: 92-102.

Orłowska, L., Rembacz, W., Florek, C., 2013. Carcass weight, condition and reproduction of wild boar harvested in north-western Poland. Pest Manag. Sci., 3: 367–370.

Plowright, W., 1981. African swine fever. In *Infectious Disease of Wild Mammals,* 2nd ed.; Davis, J.W., Karstand, L.H., Trainer, D.O., Eds.; Iowa State University Press: Ames, IA, USA, 1981; pp.178–190.

Podgórski, T., Śmietanka, K., 2018. Do wild boar movements drive the spread of African Swine Fever? Transbound. Emerg. Dis., 65: 1588–1596.

Podgórski, T., Lusseau, D., Sonnichsen, L., Jedrzejewska, A B., 2014. Long-lasting, kin-directed female interactions in a spatially structured wild boar social network. Plos One, 9, 11.

Postma, M., Backhans, A., Collineau, L., Loesken, S., Sjölund, M., Belloc, C., Emanuelson, U., Beilage, E.G., Nielsen, E.O., Stark, K.D.C., Dewulf, J., MiINAPIG consortium, 2016. Evaluation of the relationship between the biosecurity status, production parameters, herd characteristics and antimicrobial usage in farrow-to-finish pig production in four EU countries. Porcine Health Manag., 2: 9.

Probst, C., Globig, A., Knoll, B., Conraths, F.J., Depner, K., 2017. Behaviour of free ranging wild boar towards their dead fellows: Potential implications for the transmission of African swine fever. R. Soc. Open. Sci., 4: 170054.

Probst, C., Gethmann, J., Amler, S., Globig, A., Knoll, B., Conraths, F.J., 2019. The potential role of scavengers in spreading African swine fever among wild boar. Sci. Rep., 9: 11450

Quinn, P.J., Markey, B.K., Finola, C.L., FitzPatrick, E.S., 2021. Disinfection and biosecurity in the prevention and control of disease in veterinary medicine. In Block's Disinfection, sterilization, and preservation, 6th ed.; McDonnell, G. and Hansen, J., Eds., Wolters Kluwer: Philadelphia, PA, USA, 2021; pp. 1034–1106

Sánches-Cordón, P.J., Chapman, D., Jabbar, T., Reis, A.L., Goatley, L., Netherton, C.L., Taylor, G., Monotoya, M., Dixon, L., 2017. Different routes and doses influence protection in pigs immunised with the naturally attenuated African swine fever virus isolate OURT88/3. Antivir. Res., 138, 1-8.

Sánches-Cordón, P.J., Jabbar, T., Chapman, D., Dixon, L.K., Montoya, M., 2020. Absence of long-term protection in domestic pigs immunized with attenuated African swine fever virus isolate OURT88/3 or Benin_MGF correlates with increased levels of regulatory T cells and interleukin-10. J. Virol., 94 (14): e00350-20.

Sánchez-Vizcaíno, J.M., Laddomada, A., Arias, M.L., 2019. African Swine Fever. In *Diseases of Swine*, 11th ed.; Zimmerman, J.J., Karriker, L.A., Ramirez, A., Schwartz, K.J.; Stevenson, G.W., Zhang, J. Eds.; John Wiley and Sons, Inc.: Hoboken, NY, USA, 2019; pp. 443-452.

Schley, L., Roper, T.J., 2003. Diet of wild boar Sus scrofa in Western Europe, with particular reference to consumption of agricultural crops. Mammal. Rev., 33: 43–56.

Ståhl, K., Sternberg-Lewerin, S., Blome, S., Viltrop, A., Penrith, M.L., Chenais, E., 2019. Lack of evidence for long term carriers of African swine fever virus - a systematic review. Virus. Res., 272: 197725.

Stockley, P. A J. Bro-Jorgensen. 2011. Female competition and its evolutionary consequences in mammals. Biological Reviews, 86, 341-366.

Šimon, J., Machálek, A., Procházka, J., 2019. Vliv klimatických podmínek na vyzařovanou teplotu kadáveru prasete divokého. (Effect of climatic conditions on the heat emitted from wild boar carcasses.) Agritech Science [online], 13 (3): 1-6.

Tkadlec, E. 2021. Meze růstu populace prasete divokého na dohled? (Are limits to wild boar population growth in sight?) Svět Myslivosti, 22, 8-11.

Urbano, A.C., Ferreira, F., 2022. African swine fever control and prevention: an update on vaccine development. Microbes Infect., 11(1): 2021-2033.

Vetter, S.G., Ruf, T., Bieber, C., Arnold, W., 2015. What is a mild winter? Regional differences in within-species responses to climate change. PLoS ONE, 10: e0132178.

Xie, Z., Liu, Y., Di, D., Liu, J., Gong, L., Chen, Z., Li, Y., Yu, W., Lv, L., Zhong, Q., Song, Y., Liao, X., Song, Q., Wang, H., Chen, H., 2022. Protection evaluation of a five-gene-deleted African swine fever virus vaccine candidate against homologous challenge. Front. Microbiol., 13: 902932.

AFRICAN SWINE FEVER – RESEARCH RESULTS IN THE CZECH REPUBLIC
Published by the Veterinary Research Institute
Hudcova 296/70, 621 00 Brno, Czech Republic
Graphic design: Andrea Ďurišová
Text and photos: Team of authors
Phone: +420 5 3333 1111
E-mail: vri@vri.cz
www.vri.cz111
Email: vri@vri.cz

www.vri.cz