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REVIEW ARTICLE

African swine fever virus – the possible role of flies and other insects in virus transmission

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Abstract

African swine fever (ASF) is an acute viral haemorrhagic disease of pigs and wild boars. It presents a serious threat to pig production worldwide, and since 2007, ASF outbreaks have been recorded in the Caucasus, Eastern Europe, and the Baltic States. In 2014, the disease was detected in Poland. ASF is on the list of notifiable diseases of the World Organisation for Animal Health (OIE). Due to the lack of an available vaccine and treatment, the countermeasures against the disease consist in early detection of the virus in the pig population and control of its spread through the elimination of herds affected by disease outbreaks. Knowledge of the potential vectors of the virus and its persistence in the environment is crucial to prevent further disease spread and to understand the new epidemiology for how it compares to the previous experience in Spain gathered in the 1970s and 1980s.

Keywords: wild boar, African swine fever, *Stomoxys calcitrans*, soft ticks, vector.

Introduction

African swine fever (ASF) is an infectious and highly contagious slow-spreading viral disease of domestic pigs and wild boars. The aetiological agent is African swine fever virus (ASFV) belonging to the *Asfarviridae* family. ASFV is a large enveloped virus with icosahedral symmetry and a virion diameter of 200 nm. The viral genome is double-stranded DNA (11, 12). According to data from Pietschmann *et al.* (32), the ASF virus strains that have caused the current Eurasian epidemic are usually highly pathogenic, and they cause acute disease in experimental conditions in wild boars and domestic pigs. However, very small doses of the virus that do not cause clinical symptoms in wild boars or domestic pigs may cause asymptomatic spread and virus shedding. From the epidemiological point of view, the asymptomatic carriage of ASFV by wild boars poses an additional threat in the form of long-term disease persistence without any signs by which to be aware of the situation.

This disease was first detected in Kenya in the 1920s (27). The first case of the spread of African swine fever virus to the European continent took place in 1957, when the disease was introduced to Portugal. Then the virus penetrated into Spain, and from there it proliferated to other European countries as well as South America and the Caribbean. In the 1990s the virus was overcome, and its occurrence was territorially limited to African countries south of the Sahara, and to Sardinia in Europe (35). In 2007, the virus appeared for the second time in Europe after it was introduced to Georgia. This initiated the spread of the virus across the Russian Federation to other Eastern European countries (34). In Poland, African swine fever virus was detected in February 2014, in Lithuania in January 2014, in Latvia in June 2014, and in Estonia in September 2014. In 2017, the virus was detected in the Czech Republic and Romania, and in 2018, it was detected in Hungary for the first time (11, 29, 30). The number of ASF cases in Poland is increasing every year (Table 1), as is the number of outbreaks, although the first two years of the epidemic (2014–2015) belie the trend (Table 1).

Table 1. Number of ASF cases in Poland in 2014–2018 (9)

Year	Number of cases	Number of outbreaks
2014	30	2
2015	53	1
2016	80	20
2017	741	81
2018	2,438	109

Survival in the environment

An extremely important aspect in preventing further infection with ASFV is knowledge and awareness of the survival of the virus in the environment and in food. ASFV is characterised by high resistance to environmental factors. The virus is also very resistant to pH changes and is stable between points 4 and 10 on the scale. The virus can survive in skin fat for 300 days, the meat of infected pigs for a few months at 4°C and in salted and dried meat for 120 days. The virus can last over a year in blood, a few months in boneless meat and even a few years in frozen carcasses (6). For this reason, contaminated meat and meat products have contributed significantly to the spread of the virus.

The virus is not sensitive to the process of decomposition of carcasses. Dead wild boars often remain in the environment until they are completely decomposed. The virus from carcasses of wild boars also passes into the soil. In experimental studies, it was shown that the virus survives up to 112 days in forest soil. The probability of infection with the virus depends on the susceptibility of the wild boar and on the intensity and frequency of contact with the soil on which a wild boar carcass lay. Removal of their carcasses would seem to be important in the reduction of ASF epidemics (6).

Pathogenesis

The most common penetration route for ASFV into the body is the mucosa of the mouth cavity. Infection is also transmitted through the upper respiratory tract or damaged skin.

The sites of initial viral replication are monocytes and macrophages of the lymph nodes located closest to where the virus enters the body. Macrophages are cells that are responsible for the development of an effective immune response in the case of an infection. Replication of the virus in macrophages leads to dysfunction of these cells (1). In the case of oral infection, the virus first multiplies in macrophages located in the tonsils and lymph nodes of the mandible. After infection, macrophages are destroyed, but before they are, the phenomenon of haemadsorption occurs, involving the attachment of erythrocytes to their surface (38). Based on this phenomenon, it can be concluded that after initial replication in macrophages the virus is transported in blood, which leads to the spread of the virus throughout the body. It also has the ability to multiply in hepatocytes and epithelial neutrophils. Viraemia or a general blood infection usually starts from four to eight days after infection. Due to the lack of neutralising antibodies in pigs, the viral load may persist for a long period.

The degree of damage to organs in the course of infection with ASFV depends on the form of the disease. ASF may have an acute, subacute, chronic, or subclinical course. The acute form of the disease is characterised by high mortality. Virus strains with moderate and weakened virulence are responsible for the subacute form of the disease, and these strains are observed in countries where the virus is endemic. The subacute form is characterised by moderate mortality and lesions are less typical (37). From the epidemiological point of view, the subacute infection is of great importance. These infections can lead to animals becoming carriers of the virus, which contributes to the constant occurrence of the virus in the environment and an increased risk of the disease spreading to further areas where the disease has not been present (13).

Transmission and vectors

Ticks. ASFV was originally associated with the ecological niche of soft ticks of the *Ornithodoros* genus and warthogs (*Phacochoerus africanus*) in sub-Saharan Africa. The warthogs have natural resistance to ASFV and they usually do not develop the full-blown disease but rather become asymptomatic carriers. It has been shown that ASFV can replicate in these ticks and can survive up to five years in the absence of transmission to a sensitive host. This contributes to an increase in the population of infected ticks, and thus to an increased risk of infection of domestic pigs and wild boars (1). In the sylvatic cycle on the African continent and in the southern part of Europe, two tick species play a role as biological and mechanical vectors: *Ornithodoros moubata* in Africa and *Ornithodoros erraticus* in Europe (12). Experimental studies have shown that other species of ticks from the *Ornithodoros* genus can participate in virus transmission. These species include *O. porcinus, O. coriaceus, O. turicata,* and *O. savignyi* (1).

Because warthogs do not disseminate the disease among themselves, the participation of ticks seems to be indispensable in maintaining the sylvatic transmission cycle. Epidemiologically significant is the presence of vertical transmission in the ticks, which may be transovarial (from female to offspring) and transstadial (between developmental stages of ticks). Transstadial, transovarial, and sexual transmission were observed in *O. moubata*, while in *O. erraticus* only transstadial transmission was observed (1).

On the European continent hard ticks belonging to the *Ixodidae* family are the most numerous. There are two species of hard ticks commonly found in Europe: the common tick (*Ixodes ricinus*) and the meadow tick (*Dermacentor reticulatus*). Scientific research shows that there is no replication of ASFV in these ticks. For this reason, neither species is a biological vector for ASFV. However, the virus can survive in these ticks for six to eight weeks. This fact makes *Ixodes ricinus* and *Dermacentor reticulatus* potential mechanical vectors (11). There are no ticks from the *Ornithodoros* genus in Poland, however, it is possible for these ticks to be introduced from endemic areas together with imported animals.

Another ASFV transmission cycle is the domestic one. The transmission of the virus among pigs occurs during direct contact between sick and healthy animals. Infected pigs are permanently infected, and the virus is present in all body fluids, secretions, and excrement, in which it can survive a few weeks (14).

In the regions of occurrence of the *Ornithodoros* ticks, a pig–tick transmission cycle is also observed. Therefore particular vigilance should be maintained even after the eradication of the disease from a specific pig population. Ticks can contribute to the long-term persistence of the virus in the environment, which may lead to new outbreaks of the disease. An example of this is the outbreak of ASF in Portugal in 1999 on a farm where ASF had already appeared in the past (5).

Straw. The source of the virus for domestic pigs may be straw obtained from areas where wild boars occur. Wild boars are the main species of free-living animals that pollute meadows and crops with their droppings. In several documented cases, the source of a new outbreak of ASF was straw harvested from nearby fields where wild boars were found. However, if the straw comes from commercial sources where appropriate drying processes have been applied, the risk of introducing ASF by this route is negligible. Other sources of virus transmission include feeding pigs with contaminated meat, swill, and contact with contaminated objects (clothing, car tyres, or hunting equipment).

Biosecurity

There are many ways of transmitting ASFV to pig and wild boar populations (Fig. 1). The main tool preventing the introduction of the ASFV onto a pig farm is well-managed biosecurity. The risk of introducing ASFV depends on the type of farm, the area in which it is located, and the general epidemiological situation of the country (3, 40). The levels of biosecurity on individual pig farms vary tremendously in Poland (19). In order to effectively protect farms against the virus, many factors should be taken into account, including the epidemiological situation of the country, virus resistance to environmental factors, and transmission routes (1, 4, 36). Preventing the introduction of ASFV onto farms involves many procedures to minimise the risk of contact between healthy pigs and contaminated objects and other animals.

An extremely important element affecting the biological safety of the farm is designation of clean and dirty areas for personnel, including changing rooms and showers. If a visit is necessary, it should be recorded in the register of visitors. In addition, if visitors enter the pig area, they should follow biosecurity measures for footwear and clothing (31). Farm staff should follow the same biosafety procedures as visitors. Before entering and leaving the pig house, all people should wash their hands (4, 31). Farm staff who have had contact with pigs from another farm should wait at least 48 h before entering their own operation (4, 7, 20). Another crucial protection for a herd against ASFV is the physical barrier. The pig house should be fenced in appropriately so as to ensure that there is no direct contact with wild boars or pigs coming from other buildings. Outdoor farms should be doubly fenced off with a margin in order to minimise contact between pigs and wild boars. The regular disinfection of agricultural equipment and vehicles located at the farm is also an essential measure (19).

Mellor *et al.* (26) demonstrated experimentally that ASFV transmission occurs with the participation of the stable fly (*Stomoxys calcitrans*). Therefore, taking into account the possible scenario of virus introduction *via* flies, sanitary measures should be used to control these insects.

Commercial farms have significantly more head of pigs than homestead-type farms, therefore ASFV infecting such a farm results in significantly higher economic losses than in the case of the smaller, non-commercial farms (16, 22).

It is also important to observe the principles of biosecurity during wild boar hunting in areas where disease cases have been recorded (4). Hunters should be trained in the basic principles of biosafety, and when participating in the hunt should avoid contact with pigs for at least 48 h afterwards (4). Animals shot should be transported in vehicles designed for this purpose and private cars should be parked outside the hunting area. In addition, hunted wild boars should be tested for the presence of ASFV.

Over the years, the way pigs are reared has changed. One of the main changes has been the move towards increasingly intensive farming and tighter control of the environmental conditions in which animals are kept. The advances that have taken place have led to better animal husbandry conditions.

Insects have also benefited from these changes. The most important insects that were favoured by them include the housefly and several other fly species: the bluebottle fly (*Calliphora vomitoria*), the common green bottle fly (*Lucilia sericata*), and *S. calcitrans*.

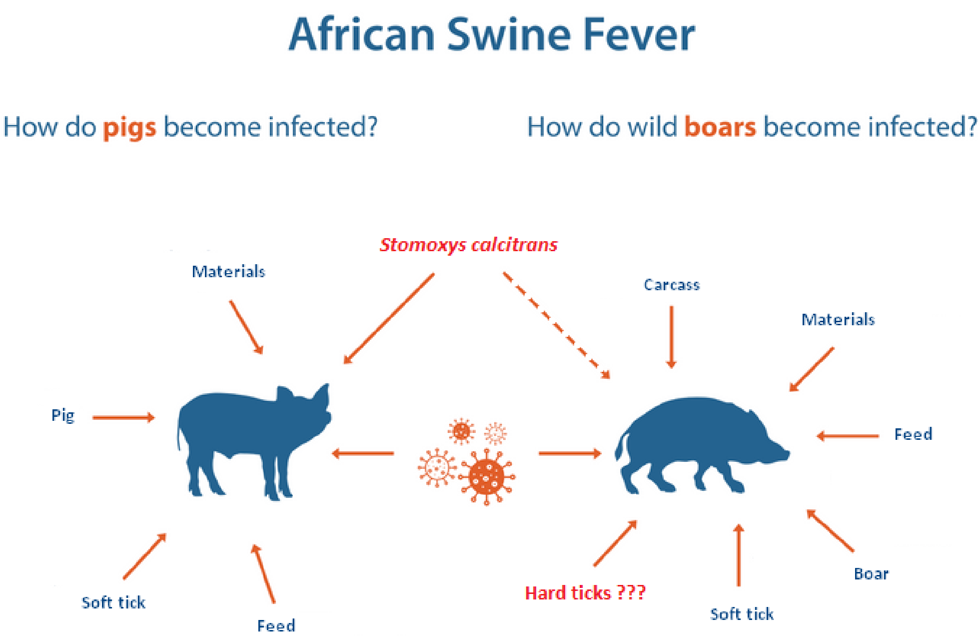

Fig. 1. Probable routes of ASFV transmission to pigs and wild boars. Flies as vectors of pathogenic microorganisms for pigs

In many cases, flies can be a significant problem in livestock breeding, so knowledge of the life cycles of these insects seems to be necessary for control on farms keeping pigs and other farm animals. Flies are common insects in farm buildings where animals are kept. These insects have access to contaminated materials such as dead pigs and secretions and excretions of diseased pigs, and therefore they can be an important risk factor in the spread of many infectious pig diseases (24, 33, 41).

It has been shown that these insects often move between swine farms (most often over a distance of up to 2–3 km), especially in periods of stronger winds, and thus can contribute to the spread of many diseases as mechanical vectors (25). However, cases of much longer displacement of up to 30 km have been documented (28). Flies that do not feed on blood can carry pathogens mechanically (on the outer layers of the body) or with faeces and vomit. In the case of flies with a stabbing mouthpiece feeding on blood, pathogens may be transmitted along with blood (25).

The housefly

During millions of years of evolution, insects have adapted to life in all environments. Among insects in close proximity to humans and animals, some species are dangerous. The most dangerous scenario is the transmission of pathogens that threaten the health and lives of people and animals, and the housefly (*Musca domestica*) is one such insect that can transmit many pathogens (24). The speed of the fly's progress through the life cycle depends to a large extent on factors such as the freshness of the fertiliser and usually lasts from several days to two months. The life cycle of a housefly also largely depends on the ambient temperature in which the larvae live. The higher the temperature, the shorter the development time. For example, at 25°C development takes about two weeks. It should also be remembered that in faeces and various types of organic waste, the temperature is higher than the ambient temperature and allows the development of flies in unfavourable environmental conditions, for example during winter months.

Current literature confirms that the housefly can transmit bacterial, viral, and fungal pathogens that affect both humans and animals (8, 10, 18, 21, 23, 39). For example, one disease transmitted by a housefly is porcine reproductive and respiratory syndrome (PRRS) (33). It has been shown that the housefly can carry pathogens on the mouth apparatus, hair covering the body, and legs. An important route of insect pathogen transmission demonstrated by the housefly is with faeces, saliva or vomit (10). Besides the housefly, *Lucilia sericata* and *Calliphora vomitoria* are also found on farms.

S. calcitrans as a mechanical vector of ASFV

Many outbreaks of ASF have been detected on farms with high biosecurity standards. One of the possible reasons was the straw obtained from areas inhabited by wild boars. In addition to this source, insects as ASFV vectors may be a cause, possibly in certain seasons particularly, because the biology of various groups of insects may be associated with strong seasonality in the occurrence of ASF outbreaks on pig farms. The highest number of outbreaks is observed in June, July, and August.

S. calcitrans, like the housefly, is a cosmopolitan species of great economic impact on animal husbandry. It is grey with dark stripes on the thorax and has a stiff, protruding mouthpiece with stinging bristles. The entire developmental cycle from egg to mature fly takes from 22 to 57 days (26). Females usually lay eggs in five to seven clutches. Faeces of other animals or decaying plant waste are the preferred environments for oviposition. *S. calcitrans* may also establish breeding sites on livestock farms because of the existence of favourable conditions for the life cycle, thus gaining easy access to hosts (15).

Painful bites can lead to loss of blood and discomfort, which may result in lower productivity of bitten livestock. These flies tend to accumulate in places where animals remain for relatively long periods. It has been observed that the number of flies decreases as the distance from these places increases. This suggests that the distribution of the *S. calcitrans* population is significantly affected by the distribution of livestock (17). The key aspect for the survival of *S. calcitrans* is the link between the species and its hosts.

Besides *S. calcitrans*, several other flies also feed on the blood of animals, including *Stomoxys niger*, *Stomoxys sitiens*, and *Stomoxys indicus*. There are 18 species named within the *Stomoxys* genus. Flies belonging to the genus are mechanical vectors of pathogens present in the blood of animals, especially farm animals. They can also be mechanical vectors of human pathogens. The viruses transmitted by *S. calcitrans* may include equine infectious anaemia virus (EIAV), West Nile fever virus (WNFV), Rift Valley fever virus (RVFV), lumpy skin disease virus (LSDV), bovine herpes virus (BHV), bovine leukosis virus (BLV) and vesicular stomatitis virus (VSV). In addition, *S. calcitrans* can carry bacteria such as *Bacillus anthracis*, *Pasteurella multocida*, *Erysipelothrix rhusiopathiae*, *Francisella tularensis*, and *Enterobacter sakazakii*. There are also indications that *S. calcitrans* may be a mechanical vector for ASFV (3).

Reports of successful experimental transmission of African swine fever virus by *S. calcitrans* appeared in 1987. The flies were infected with ASFV by feeding on blood taken directly from infected pigs or from swabs soaked with blood containing the virus. The virus was present in the blood up to two days after infection. This result suggests that virus transmission is possible for at least this time (26).

Studies on ASF transmission *via S. calcitrans* were also performed by Olesen *et al*. (29). Flies were infected by feeding on blood containing ASFV and the presence of the virus in flies was confirmed by polymerase chain reaction (PCR). Pigs were fed infected flies as a homogenate of 20 flies per pig, and after a few days some pigs showed signs of the disease (29).

Conclusion

ASF is a serious socio-economic problem. Goods are imported and exported on a massive scale, creating the risk of introducing potential viral vectors along with the transported goods to areas where they have not previously been present. In the case of ticks from the *Ornithodoros* genus, this can lead to the virus becoming established in the environment for a long period. In addition, the results of experimental studies carried out so far seem to confirm the possibility of transmission of ASFV by *S. calcitrans*, therefore studies of insects near farms are extremely important. Considering the biology of *Stomoxys* flies, it seems that they may be involved in ASF transmission over short distances (for example, within a farm). A significant threat in terms of virus transmission may be in the sanguivorous flies of the *Tabanidae* family, because they are larger than *S. calcitrans* and can fly over long distances. These flies can be mechanical vectors for many viral pathogens (2). Unlike *S. calcitrans*, these flies do not breed on farms where animals are kept and therefore can have easier contact with wild boars living in the forests. In addition, these flies consume up to five times as much blood during feeding. This leads to a larger infectious dose per fly. When considering the infectious dose required for an animal to develop the disease, the animal's health should be considered – weak or diseased animals will be more susceptible to infection than healthy animals due to a weaker immune system response. Therefore, the dose for the weakened animal will be lower than for a healthy animal. The development of full-blown disease in such an animal will result in further transmission between animals living on the same farm. Their greater size and the environment in which they can live and reproduce gives flies from the *Tabanidae* family an important role in virus transmission as well as *Musca domestica*, *Calliphora vomitoria*, *Lucilia sericata*, and *S. calcitrans* flies and *Ixodidae* family and *Ornithodoros* genus ticks. The transmission of ASFV by insects is an additional biosecurity challenge for farms producing and breeding pigs. Research into the identification of new potential ASFV vectors is extremely important, because the discovery of new biological and mechanical vectors will allow a more accurate understanding of the virus transmission pathways, more effective disease control, stronger protection of pig holdings, and greater reduction of disease spread.

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