

New insights into the role of ticks in African swine fever epidemiology

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Summary

African swine fever (ASF), one of the most important diseases of swine, is present in many African countries, as well as in eastern Europe, Russia and Sardinia. It is caused by a complex virus, ASF virus (ASFV), for which neither vaccine nor treatment is available. ASFV affects swine of all breeds and ages, and also replicates in soft ticks of the genus *Ornithodoros*, facilitating ASFV persistence and recurrence of disease. Depending on the involvement of these ticks, and the presence or not of sylvatic asymptomatic animals, several epidemiological cycles have been identified. The disease persists in East and southern African countries in a sylvatic cycle between *O. porcinus* (of the *O. moubata* species complex) and common warthogs. In some countries a domestic pig–tick cycle exists, whereas in other regions, notably West Africa, the role of soft ticks has not been demonstrated, and ASFV is transmitted between domestic pigs in the absence of tick vectors. Even in several East and Central African countries which have the sylvatic or domestic cycle, the majority of outbreaks are not associated with ticks or wild suids. In Europe, *O. erraticus* was detected and identified as a crucial vector for ASF maintenance in outdoor pig production on the Iberian Peninsula. However, in most parts of Europe, there is a lack of information about the distribution and role of *Ornithodoros* ticks in ASF persistence, particularly in eastern regions.

This article reviews ASF epidemiology and its main characteristics, with a special focus on the distribution and role of soft ticks in ASF persistence in different settings. Information about tick detection, control measures and future directions for research is also included.

Keywords

African swine fever – Epidemiology – *Ornithodoros* – Pig – Reservoir – Risk factor – Swine – Tick.

Aetiological agent

African swine fever (ASF) is one of the major threats confronting pig production all over the world. It is caused by a large, complex virus (around 200 nm in diameter), the only member of the *Asfarviridae* family (1). It has a double-stranded DNA genome that ranges from 170 to 193 kilobase pairs (kbp), depending on the viral isolate (2), and encodes more than 150 infection proteins (3), of which more than 50 are immunogenic.

African swine fever virus (ASFV) infection induces a high-intensity immune response, including partial neutralising antibodies against some of the viral proteins (4, 5). However, these antibodies are not able to completely neutralise ASFV infection (6). Consequently, the classification of ASFV isolates is based on genetic characterisation. To date, 22 genotypes have been described, based on partial sequences of the p72 gene, the major structural protein (7, 8). All 22 are present in Africa, whereas only genotypes I and II are present outside this continent.

The virus replicates in monocytes and macrophages of the mononuclear phagocyte system of swine, although infection has also been demonstrated in hepatocytes, and endothelial and renal tubular cells. Replication was also demonstrated in soft ticks of the genus *Ornithodoros*, which can act as reservoirs of the disease (9) and have an important role in several epidemiological scenarios.

Epidemiology and modes of transmission

Africa

In eastern and southern Africa, ASFV is maintained in an ancient sylvatic cycle in which the common warthog, *Phacochoerus africanus*, and argasid ticks of the *Ornithodoros moubata* complex maintain the infection without showing clinical signs of disease (10). (The taxonomy of the *O. moubata* complex is unresolved and so, for the purposes of this article, the name *O. porcinus* is used for the ticks that transmit ASFV in this region.) Neonatal warthogs become infected in the first weeks of life when they are fed on by the ticks that live in the natal burrows (11). Although the young warthogs remain apparently healthy, they develop sufficiently high viraemia to infect naive ticks. The argasid ticks are true biological vectors in which the virus is transmitted transovarially, transstadially and also sexually from males to females (12). In endemic areas, almost all warthogs become infected at this stage and develop antibodies against the virus. Although ASFV may be detected in the lymphoid organs of warthogs, older warthogs do not develop sufficient viraemia to cause shedding of the virus, and transmission to domestic pigs occurs only when infected ticks feed on them (10). Other wild African suids not associated with *Ornithodoros* are also resistant to the pathogenic effects of ASFV but their specific role in ASF epidemiology, if any, has not yet been demonstrated (13).

The introduction of domestic pigs into endemic areas has provided a highly susceptible host for the virus as well as new ways for the virus to be transmitted. This is because the virus is shed in large quantities in secretions and excretions during the viraemic phase of the disease, including up to 48 h before clinical signs appear (14), and is present in infective quantities in the tissues of pigs that die of ASF. The spread of the virus to West Africa and parts of Central Africa, where evidence for the sylvatic cycle is lacking, has principally occurred through the movement of infected pork (15), in which the virus can remain viable for several months (16). Pigs that recover from ASF may shed the virus for at least 30 days post infection and viable virus can persist in the tissues for longer (17). Traditional

free-range pig-farming systems prevail in sub-Saharan Africa and enable contact with wild hosts as well as with infected pigs and their remains, while viral circulation can be maintained almost indefinitely in large high-contact pig populations (15).

In parts of eastern and Central Africa, the shelters that pigs occupy at night provide habitats for *O. porcinus*, which also inhabits human dwellings. A domestic cycle between *O. porcinus* and domestic pigs has been demonstrated in Malawi (18). Such a cycle is also likely to be important in neighbouring countries where suitable conditions exist. Pigs in these areas have higher survival rates after infection with virulent ASFV, with up to 50% of pigs showing no signs of disease, although antibodies against ASFV indicate that they have been infected. The basis for this resistance has not yet been determined but does not appear to be simply inherited (19).

Europe

African swine fever virus genotype I was first introduced into Europe in 1957 through contaminated waste that entered Portugal. After its rapid control, a second introduction of the same genotype occurred in 1960, again in Portugal, resulting in rapid spread through the whole Iberian Peninsula (Portugal and Spain), where ASF persisted for more than 30 years. From this area, several escapes of ASFV occurred to both American and European countries. However, all these ASF outbreaks were efficiently controlled, except on the island of Sardinia, where ASF has been present since 1978 (20). The last introduction of ASFV to Europe occurred in 2007, when ASFV genotype II entered Georgia, probably through contaminated waste from boats (21, 22). From Georgia, ASF rapidly spread to neighbouring countries, such as Armenia, Azerbaijan and the Russian Federation, where the virus continued its spread, affecting Ukraine, Belarus and even reaching countries within the European Union (Lithuania, Latvia, Estonia and Poland) (23, 24).

In Europe, ASFV infection affects both domestic and wild boar populations. The main transmission routes were related to direct contact between infected and healthy animals, as well as to contaminated materials, including meat products, vehicles and other fomites (20). Studies conducted in Spain and Sardinia suggested that the role of the wild boar in maintaining ASF epidemiology was not as significant as that of domestic pigs, imported products and fomites, at least when there were no other sources of infection (25, 26, 27, 28, 29). However, the final cases of ASF reported from Lithuania, Latvia, Estonia and Poland (23, 24), demonstrated that wild boar could nonetheless have an important part in disease spread.

In contrast, the important role of *O. erraticus* in disease maintenance was clearly confirmed (9, 30) in those areas

where its presence has been demonstrated (Portugal and Spain). Viable ASFV can persist in infected soft ticks for at least five years (31). Therefore, control measures in these areas should consider the potential presence of ticks, and consequently extend clearance periods to avoid the risk of ASF reappearance. The presence of this tick was pointed out (32) as one of the reasons for the longer persistence of the disease in south-west Spain, where extensively farmed pigs could potentially come into contact with *O. erraticus*.

However, in other affected European territories, *Ornithodoros* has not yet been implicated in the ASF cycle nor has its presence been clearly demonstrated. Some field studies were carried out in Sardinia during the 1980s in search of the tick (in 357 holdings from 20 different districts in the province of Nuoro). However, no positive results were found in any of the holdings studied (33). In the Caucasus region, other species of *Ornithodoros* were reported during the 1970s, all of them belonging to the *O. erraticus* complex. Considering that all *Ornithodoros* species tested in the laboratory so far seem able to transmit ASFV (*O. erraticus*, *O. moubata*, *O. porcinus*, *O. coriaceus* and *O. savignyi*), it is highly probable that other congeners potentially present in those territories (*O. asperus*, *O. lahorensis* and potentially *O. tartakovskiyi*) would also be efficient vectors for ASFV (34). Indeed, an earlier study confirmed the ability of ASFV strain Georgia 2007/1 to replicate in *O. erraticus* ticks (35).

Preliminary studies recently performed in Sardinia and the Russian Federation have evaluated the presence of antibodies against *Ornithodoros* ticks in the sera of backyard pigs, using a previously described enzyme-linked immunosorbent assay (ELISA) technique (36, 37). Very strong positive results were found in sera collected from backyard pigs in the southern regions of the Russian Federation, whereas no Sardinian samples returned positive results (L. Mur, personal communication). These results need to be confirmed by collecting ticks in the field, as confirming the presence and competence of these *Ornithodoros* species would certainly have important implications for ASF control policies in these areas.

Clinical signs

African swine fever virus can cause different forms of the disease, depending on the virulence of the isolate, the route and dose of infection, and the characteristics of the host (e.g. age, breed, immunity). The clinical courses more frequently observed in the field are the hyperacute form, in which animals die before the appearance of any signs other than high fever and death, the acute form and the subacute form. In these last two forms, the infected animals survive longer, and it is possible to observe the presence of haemorrhagic lesions on the skin, as well as haemorrhagic

excretions (epistaxis, melena, etc.). The haemorrhagic lesions in internal organs are also more evident in the acute and subacute form and are found mainly in the lymph nodes, spleen and kidneys.

When ASF was present on the Iberian Peninsula, another form of the disease was reported, characterised by necrotic lesions on the skin and arthritis (38). However, this clinical form has not been described in any of the territories where ASFV has been present for longer periods of time (e.g. Africa and Sardinia). Therefore, it has been hypothesised that this chronic form could have originated through the natural evolution of the ASFV isolates employed in the vaccination studies conducted on the Iberian Peninsula during the 1960s. To confirm this, several molecular studies are in progress to elucidate the similarity between chronic and vaccine isolates (J.M. Sánchez-Vizcaíno, personal communication).

During necropsy, haemorrhages, oedema and infarcts in the lymph nodes are the most commonly observed lesions. These are also seen in the spleen, which is frequently enlarged and dark in colour. Other organs are frequently affected, such as the kidneys, liver, gall bladder, stomach or lungs, frequently with petechiae and haemorrhages (39).

Diagnosis

The laboratory diagnosis of ASF is well developed, including validated techniques that perfectly detect the 22 existing genotypes (40). As no vaccine is currently available, the presence of antibodies always indicates previous infection. Therefore, serology is a valuable tool for the surveillance of ASF in affected countries. However, it should always be performed in parallel to antigen detection, as antibodies take some time to appear and many animals die before their antibodies can be detected (41).

For antigen detection, the most frequently used techniques are the haemadsorption (HAD) test, direct immunofluorescence (DIF) and polymerase chain reaction (PCR). The HAD test is the gold standard technique. However, as it is a time-consuming and complex process, it is only performed in reference laboratories and during the first detection of the disease in a previously disease-free territory. DIF is a rapid test that is easy to perform and only requires a smear of target tissue and a fluorescent conjugate. It should be used in acute forms of the disease or in parallel with other serological techniques, as the presence of antibodies could interfere by forming immunocomplexes.

Polymerase chain reaction is the most widely employed test for nucleic acid detection and several protocols are currently approved by the World Organisation for Animal

Health (OIE), including conventional PCR (42) as well as multiplex and real-time tests (43). In addition, other recently developed protocols include PCRs using Universal Probe libraries, loop amplification assays (LAMPs), linear-after-the-exponential PCR (LATE-PCR) and a range of novel, real-time PCR methods (44). Two PCR protocols have also been established to detect ASFV in European and sylvatic cycle *Ornithodoros* ticks (45, 46).

In terms of serological assays, ELISA is the most widely used test, especially for large-scale screening purposes. In addition to the OIE-recommended ELISA based on a semi-purified antigen (47), there are several commercial kits currently available. Other serological tests are recommended to confirm the ELISA's results, including immunoblotting tests (48), indirect immunofluorescence (49) or immunoperoxidase (50).

An ELISA test based on the extract of salivary glands of *O. erraticus* was widely employed during the eradication period in Spain to detect pigs exposed to the bites of *Ornithodoros* ticks (30, 36), and this is still in use in several areas of Europe. Further assays have been developed to improve the test's specificity (37). However, in contrast to *O. moubata*, for which several cloned antigens are available for diagnosis (51, 52), none of the antigens of *O. erraticus* has been cloned. Therefore, salivary gland extracts of *O. erraticus* remain in use. Both tests have proven very useful for estimating in which areas pig-feeding ticks are potentially located, constituting the first step in tick detection (53).

Control methods

Biosecurity

Biosecurity is currently the only option that farmers have to protect their pigs against ASF. In areas where ticks are not involved, since the virus is transmitted directly by contact with infected pigs or material via the oronasal route, which requires a relatively high dose of virus, simple biosecurity measures provide adequate protection. These consist of: keeping pigs permanently confined in pig-proof structures; restricting access to the pigs; not feeding swill that could contain uncooked or under-cooked pork; and either keeping a closed herd or only buying pigs or using boars from herds known to be uninfected. When people must have access to the pigs, a change of footwear is essential. Disinfecting footwear from which all extraneous material has been removed by thorough scrubbing, using a disinfectant that is effective against ASFV at the recommended concentration, can be employed but, in the long term, maintaining a fresh supply of boots or even rubber flip-flops is likely to be more economical and reliable than ensuring that the disinfection

process is effective. If there is no alternative to feeding swill, the swill should be boiled, with constant stirring, for 30 min and cooled prior to feeding. If possible, newly introduced pigs should first be isolated from the herd for up to 14 days and observed for any signs of disease, particularly in areas where ASF is present.

In areas where there is a cycle between warthogs and ticks, keeping the pigs in pig-proof premises, surrounded by a fence or wall that extends below the surface of the ground for at least 0.5 m, has proven effective. Double fencing, with a distance of at least 2 m between the fences, is recommended to prevent the incursion of ticks, but the necessity for this depends upon how the pigs are kept within the fence. Double fences are essential if the pigs are free-ranging but not if they are housed and the pens are at some distance from the fence.

In areas where ticks are involved, either in a domestic or sylvatic cycle, housing for pigs should be constructed so as not to offer shelter for ticks. Earth floors and walls constructed of packed stones, wood or material such as overlapping corrugated iron sheets offer hiding places for *Ornithodoros* and the use of acaricides is generally ineffective. Floors and walls should therefore be solid and well built so that cracks do not develop in which the ticks could find shelter.

Apart from *Ornithodoros* ticks, the only other arthropod that has proven capable of maintaining and mechanically transmitting viable ASFV for up to 48 h is the stable fly, *Stomoxys calcitrans* (54). In spite of anecdotes, there is no scientific evidence that rodents, birds or other animals can assist in the transmission of ASFV. However, controlling flies by removing wastes and preventing access to feed by birds and rodents are part of good hygiene, prevent a variety of other diseases and should always be practised on pig farms.

Vaccine

At present, there is no vaccine available for preventing and controlling ASF infection. The highly complex nature of ASFV and its genetic variability, together with multiple virulence factors that facilitate its evasion of the immune response and the lack of total neutralising antibodies, are probably mainly responsible for the lack of vaccines. Many attempts have been made since the early entry of the disease into Europe. Specifically, the first experiments in Europe were performed with live attenuated ASFV in Portugal and Spain during the 1960s. These vaccines protected against homologous infections, but led to the appearance of unacceptable chronic lesions (skin, joints, etc.) (55).

Consequently, other strategies were investigated to try to reduce the virulence of ASFV, achieving an adequate level

of protection without the secondary problems of the live attenuated viruses. The deletion of specific genes has been assayed several times (56, 57, 58) but more information on ASFV pathogenicity is required to guide the selection of appropriate virulence genes for deletion. Attempts to create replication-deficient vaccines that contain all the external particles of the virus but are not infectious are also in progress.

Other approaches, such as subunit vaccines and DNA vaccines, were investigated after their success as vaccines for other animal diseases. These studies were essential for elucidating several aspects of ASFV, such as the fact that ASFV is a highly immunogenic virus and its infection produces a large number of antibodies. Some of these antibodies are able to partially neutralise ASFV antigens. However, such antibodies are not able to completely neutralise ASFV infection. Nevertheless, a humoral response is essential but not enough for protection against ASFV. Some studies clearly demonstrate that T-cells play an important role in ASF protection, even in the absence of specific antibodies (59). Therefore, we can conclude that, based on the experiments performed so far, a potential vaccine for ASF should activate both humoral and cellular responses, as well as satisfying safety requirements.

Vaccines are currently being investigated, not only for ASFV infection, but also for controlling tick infestation. The control of *Ornithodoros* ticks has always been based on the use of chemical acaricides, despite their low efficacy due

to difficulties in the product reaching tick refuges (53). These products also present other problems, including the occurrence of drug resistance, toxicity and environmental and animal product contamination. Therefore, the best solution for control would be the development of an efficient vaccine against *Ornithodoros*.

Several strategies have been studied for this purpose, including the use of gut membrane extracts (60), salivary antigens (61) and gene deletions (62). In the case of *O. moubata*, one antigen has demonstrated its efficacy (Om44) (61), whereas for *O. erraticus* variable results have been observed, including positive reactions with salivary gland extracts. Although these vaccines are still under development, the current availability of new proteome methodologies has accelerated these studies, and it is expected that some antigens with protective properties against *Ornithodoros* ticks will be found.

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Éclairages nouveaux sur le rôle des tiques dans l'épidémiologie de la peste porcine africaine

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Résumé

La peste porcine africaine est l'une des principales maladies des porcins, présente dans de nombreux pays d'Afrique ainsi qu'en Europe orientale, en Russie et en Sardaigne. Elle est causée par un virus complexe, le virus de la peste porcine africaine contre lequel il n'existe aucun vaccin ni traitement. Le virus de la peste porcine africaine affecte les porcins de toutes les races et classes d'âge ; la réplication virale a également lieu dans l'organisme des tiques molles du genre *Ornithodoros*, ce qui contribue à la persistance du virus et à la réémergence de la maladie. Plusieurs cycles épidémiologiques ont été décrits dont les traits distinctifs sont le degré de participation de ces tiques et la présence ou l'absence d'animaux sauvages asymptomatiques. La persistance de la maladie dans les pays d'Afrique orientale et australe est associée à un cycle sylvatique impliquant *O. porcinus* (du complexe d'espèces *O. moubata*) et le phacochère commun. Si dans certains pays le cycle de transmission entre le porc domestique et les tiques est avéré, dans d'autres régions le rôle des tiques molles n'a pas été démontré

(en particular en África de l'Ouest) et le virus se transmet d'un porc domestique à l'autre sans l'intervention de tiques vectrices. Même lorsque l'existence d'un cycle sylvatique ou domestique est démontrée, par exemple dans nombre de pays d'Afrique de l'Est et Centrale, une majorité de foyers surviennent sans intervention des tiques ni des suidés sauvages. En Europe, la tique *O. erraticus* a été détectée et son rôle en tant que vecteur responsable de la persistance de la peste porcine africaine dans les élevages de porcs en plein air de la péninsule Ibérique a été démontré. Toutefois, nous manquons d'informations sur la distribution et le rôle des tiques *Ornithodoros* dans la persistance de la peste porcine africaine dans la plupart des régions d'Europe, en particulier les régions orientales.

Les auteurs font le point sur l'épidémiologie de la peste porcine africaine et sur ses principales caractéristiques, en mettant l'accent sur la distribution et le rôle des tiques molles dans la persistance de la maladie dans différents contextes. Ils présentent également des informations sur la détection des tiques, les mesures de contrôle appliquées et les orientations futures de la recherche.

Mots-clés

Épidémiologie – Facteur de risque – *Ornithodoros* – Peste porcine africaine – Porc – Porcin – Réservoir – Tique.



Nuevos datos sobre la función de las garrapatas en la epidemiología de la peste porcina africana

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Resumen

La peste porcina africana (PPA), que es una de las más importantes enfermedades porcinas, está presente en muchos países africanos, así como en Europa Oriental, Rusia y Cerdeña. Su agente causal es un virus complejo contra el que no existe ni vacuna ni tratamiento. El virus de la PPA afecta a porcinos de todas las razas y edades, y también se replica en garrapatas blandas del género *Ornithodoros*, lo que facilita la persistencia del virus y la reaparición de la enfermedad. Hay distintos ciclos epidemiológicos descritos, que dependen de la intervención de las mencionadas garrapatas y de la presencia o ausencia de animales silvestres asintomáticos. La enfermedad persiste en países del este y el sur de África en un ciclo silvestre que discurre entre *O. porcinus* (del complejo de especies *O. moubata*) y el facocero común. En algunos países existe un ciclo entre el cerdo doméstico y la garrapata, mientras que en otras regiones, especialmente en el África Occidental, no está demostrada la intervención de garrapatas blandas, y el virus se transmite entre cerdos domésticos sin garrapatas que ejerzan de vector. Incluso en ciertos países del África Oriental y Central donde se dan el ciclo silvestre o el doméstico, los brotes no suelen venir asociados a garrapatas o a suidos salvajes. En Europa, concretamente en la Península Ibérica, se ha detectado y descrito la intervención de *O. erraticus* como vector indispensable para el mantenimiento de la PPA en la producción porcina al aire libre. Sin embargo, en la mayor parte del territorio europeo falta información sobre la distribución de las garrapatas *Ornithodoros* y la función que cumplen en la persistencia de la PPA, especialmente en las regiones orientales.

Los autores pasan revista a la epidemiología y las principales características de la PPA, deteniéndose especialmente en la distribución de las garrapatas

blandas y su función en la persistencia de la PPA en diferentes circunstancias. También ofrecen información sobre la detección de garrapatas, la lucha contra la enfermedad y las líneas de investigación de cara al futuro.

Palabras clave

Cerdo – Epidemiología – Factor de riesgo – Garrapata – *Ornithodoros* – Peste porcina africana – Porcino – Reservorio.



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