



Review

Lack of evidence for long term carriers of African swine fever virus - a systematic review

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ABSTRACT

African swine fever (ASF) was first described in 1921 as a highly fatal and contagious disease which caused severe outbreaks among settlers' pigs in British East Africa. Since then the disease has expanded its geographical distribution and is currently present in large parts of Africa, Europe and Asia and considered a global threat. Although ASF is typically associated with very high case fatality rates, a certain proportion of infected animals will recover from the infection and survive. Early on it was speculated that such survivors may act as carriers of the virus, and the importance of such carriers for disease persistence and spread has since then almost become an established truth. However, the scientific basis for such a role of carriers may be questioned. With this in mind, the objective of this study was to review the available literature in a systematic way and to evaluate the available scientific evidence. The selection of publications for the review was based on a database search, followed by a stepwise screening process in order to exclude duplicates and non-relevant publications based on pre-defined exclusion criteria. By this process the number of publications finally included was reduced from the 3664 hits identified in the initial database search to 39 publications, from which data was then extracted and analysed. Based on this it was clear that a definition of an ASF virus carrier is lacking, and that in general any survivor or seropositive animal has been referred to as carrier. It was also clear that evidence of any significant role of such a carrier is absent. Two types of "survivors" could be defined: 1) pigs that do not die but develop a persistent infection, characterised by periodic viraemia and often but not always accompanied by some signs of subacute to chronic disease, and 2) pigs which clear the infection independently of virulence of the virus, and which are not persistently infected and will not present with prolonged virus excretion. There is no evidence that suggests that any of these categories of survivors can be considered as "healthy" carriers, i.e. pigs that show no sign of disease but can transmit the virus to in-contact pigs. However, localized virus persistence in lymphoid tissues may occur to some extent in any of the categories of survivors, which in theory may cause infection after oral uptake. To what extent this is relevant in reality, however, can be questioned given the virus dose generally needed for oral infection.

1. Introduction

African swine fever (ASF) is a serious viral disease of domestic pigs and Eurasian wild boar (*Sus scrofa*) caused by African swine fever virus (ASFV) and generally associated with vast socioeconomic impact in affected regions. The virus originates from Eastern and Southern Africa, where it is maintained in an ancient sylvatic cycle in which African wild suids (predominantly warthogs, *Phacochoerus africanus*) and argasid

ticks within the *Ornithodoros moubata* complex constitute the natural hosts (Plowright et al., 1994). In warthogs as well as in the other members of the wild African suids, bushpigs (*Potamochoerus larvatus*, *P. porcus*) and giant forest hogs (*Hylochoerus meinertzhageni*) infection with ASFV runs a subclinical or asymptomatic course. ASF in domestic pigs and Eurasian wild boar, on the other hand, is typically an acute to peracute haemorrhagic disease with very high case fatality rates, and death within the first few weeks post infection.

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ASF was first described by Montgomery (1921) as a highly fatal and contagious disease which caused severe outbreaks among settlers' pigs in British East Africa. Subsequently a similar scenario was described also from South Africa (Steyn, 1932; De Kock et al., 1940). Since these early years the disease has expanded its original geographical distribution and today the majority of countries in sub-Saharan Africa are considered as endemically infected (Penrith et al., 2013). ASF has also managed to spread beyond the African region on three occasions (Costard et al., 2009), with the incursion into Georgia in 2007 as the starting point for the latest episode, which has since developed into an ASF epidemic of unprecedented dimensions. The disease is currently present in large parts of Europe where it continues to spread, in particular among wild boar populations and in spite of the extensive disease control measures implemented (Gavier-Widen et al., 2015; Chenais et al., 2019). In addition, in August 2018 the disease emerged for the first time in China, the largest pig producer in the world accounting for almost half of the world's pork production, and outbreaks have since then been reported from large parts of the country (Wang et al., 2018). Moreover, outbreaks have since been reported from several countries within Southeast Asia. With these developments, ASF is currently considered a global threat.

Although ASF is typically associated with very high case fatality rates, approaching 100%, within any affected population a certain proportion of infected animals will recover from the infection and survive. Already in 1932 Steyn concluded, based on observations and results from experimental studies, that such survivors may act as carriers of the virus, and speculated that they may play an important role in the epidemiology of the disease (Steyn, 1932). Since then, the ASFV carrier has been mentioned or addressed in one or another way in many scientific publications, and its importance for disease persistence and spread has almost become an established truth (Hess, 1981; Sánchez-Botija, 1982; Wilkinson, 1984; Arias and Sanchez-Vizcaino, 2002) this to the extent that the Diagnostic manual of the World Organisation for Animal Health in the version adopted in May 2012 stated that "*recovered ASFV carrier pigs and persistently infected wild pigs constitute the biggest problems in controlling the disease*" (OIE). However, the scientific basis for such a role of the ASFV carrier may be questioned. In addition, there is no clear definition of a carrier in this context. With this in mind, the objective of this study is to review the available literature in a systematic way and to evaluate the available scientific evidence with regard to the role of the ASFV carrier. To be meaningful from an epidemiological point of view, and for the purpose of this review, such a carrier must include the long-term (i.e. beyond the duration of the acute or chronic course of the disease) ability to shed the virus and transmit the disease to susceptible animals, or in other words as suggested in FAO (1987) a carrier is "an individual that is infected by a disease agent and is capable of disseminating that disease agent but shows no sign of clinical disease".

2. Material and methods

A graphical illustration of the search and selection process can be seen in Fig. 1. A database search was initiated to locate articles relevant for the objectives of this review. The search was designed to be replicable. Three of the authors (EC, KS, SSL) identified relevant key words to be used, and performed test searches to ensure that relevant papers were included while leaving out irrelevant ones. Based on these test searches, the following final search string was generated: "african swine fever" OR asf OR asfv OR "hog cholera" AND (carrier* OR persist* OR reservoir* OR surviv* OR intermitt* OR chronic* OR subclinic* OR latent OR seropositiv* OR resist*). The search did not include any limits regarding publication date or -language. For the latter it did not seem necessary as one or more of the authors, in addition to English, were able to fully read and comprehend German, French, Italian, Spanish, Portuguese and Russian.

The search was done on the 6th or 7th of September 2018 using four

databases: PubMed, Scopus, Google Scholar and Web of Science. Together the four searches generated a total of 3664 hits (see Fig. 1) which was imported to Rayyan QCRI (Ouzzani et al., 2016) for further handling. The Google Scholar search was imported to Rayyan QCRI using the software Publish or perish (<http://www.harzing.com/pop.htm>), the other three searches could be imported directly.

In a first handling session using Rayyan QCRI, 1799 duplicates were identified and removed, leaving the remaining number of references at 1865. A first screening of titles and abstracts was performed, in parallel and blinded, by two of the authors (EC and KS) using the following exclusion criteria: not ASF; not original study or original data; referring only/mainly to ticks, warthogs or bushpigs; in vitro study; cell-level study; conference proceedings. Papers receiving conflicting decisions were screened a second time, this time unblinded, resulting in consensus decisions for all papers. Using the mentioned process and exclusion criteria, and identifying some additional duplicates, another 1775 hits were removed leaving 90 publications.

Out of the 90 remaining publications, full texts could be obtained for 80. In the next step, these 80 publications were distributed among all authors for a first full paper review. Most papers were read by two authors, apart from all the papers in Russian (4), and some of the papers in Portuguese (4), which were only read by one author. A common data collection sheet was used in this part of the review. The data collection sheet permitted easy overview of exclusion/inclusion, and served as a first screening and evaluation of the evidence presented for the included papers. In addition to the exclusion criteria mentioned earlier, one more was added in this step, namely: paper not related to carriers or persistence. 11 papers received conflicting decisions in this step. These papers were screened once more by three of the authors (EC, KS, SSL), resulting in consensus decisions for all papers. Using the mentioned process and exclusion criteria, another 45 papers were removed, leaving 35 publications. After having read these papers and screened their reference lists, three additional papers were added for screening and possible inclusion. In addition, a very recent and relevant paper was added during the reviewing process. The last additions were all included, bringing the total number of papers included in the review to 39. These 39 papers were all submitted to further reading, data extraction and analysis according to the objectives of the review.

3. Results and discussion

In total, 39 publications were finally selected and reviewed. Some dealt explicitly with the aspect of carriers/persistence while others touched on this phenomenon either in the discussion or by the results obtained, or simply as a stated fact. For a classification of the papers included by type, year and geographical origin see Table 1.

3.1. Pigs that survive ASF

To date there is no uniform definition of an ASFV carrier. Rather it has generally been assumed that either all pigs that survive the infection, or those that survive beyond a certain time post infection, are, or are likely to become, carriers. However, the results of the included studies suggest that such survivors do not constitute a homogeneous group, but that they can be divided into two main categories: (1) pigs that do not die but develop a persistent infection, characterised by periodic viraemia and often but not always accompanied by some signs of subacute to chronic disease (Sanchez-Vizcaino et al., 2015), and invariably leading to death due to a resurgence of ASF, and (2) pigs that recover fully from infection with viraemia of shorter duration and are able to lead healthy and productive lives. Differences observed in the two categories are summarised in Table 2.

Outbreaks of ASF caused by highly virulent viruses are usually characterised by severe disease in pigs of all ages and high mortality that may reach 100 percent in affected populations. The number of survivors is variable and is, as would be expected, higher with viruses of

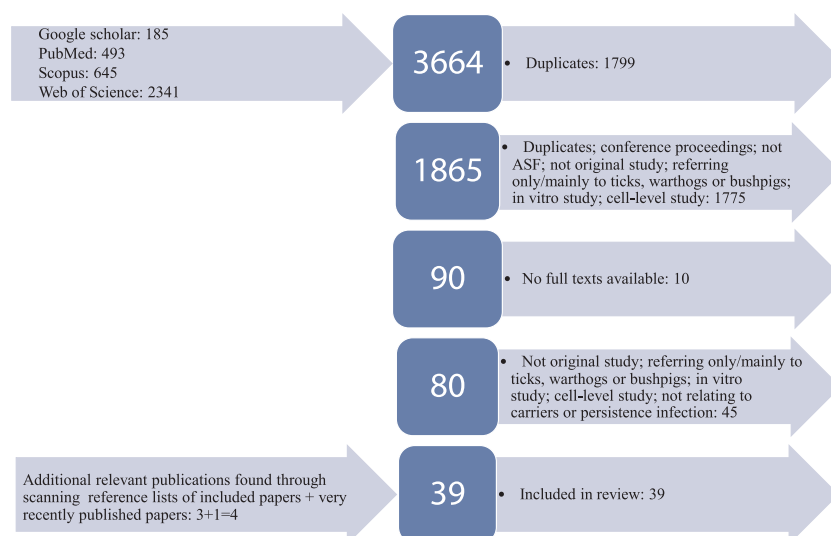


Fig. 1. Flowchart describing the search and selection process for a systematic review assessing the available evidence for long term carriers of African swine fever virus.

Table 1

Type of papers included in the study.

Paper classifications		Number of papers
Type of paper	Non peer reviewed report	4
	Epidemiological study	16
	Experimental study	20 ^b
Period of publication ^a	1921-1957	5
	1957-1995	14
	1995-2019	20
Origin of study	Africa	18
	Europe	18
	Americas	3

^a Some publications refer to studies conducted earlier.

^b One paper included both experimental and epidemiological parts.

lower virulence (Sanchez Botija, 1962; Hess, 1981). In addition, the number of survivors has been found to be much higher than expected in some populations of pigs that were infected with highly virulent viruses (Penrith et al., 2004; Okoth et al., 2013).

There is little evidence to indicate that either category of survivor is able to transmit the virus efficiently to other pigs unless by inoculating the pigs with their blood or feeding their tissues to the pigs. However, the value of keeping persistently infected pigs (i.e. category 1, above) is questionable, given the risk of resurgence of viraemia, shedding and reappearance of clinical disease. On the other hand, pigs that recover fully from ASF (category 2) and eliminate the virus in a reasonably short period of time could be an asset for pig production in resource-poor settings where even if a vaccine is developed its application may not be feasible. Investigations in an endemic area indicated that the pigs that survive may be resistant to ASF caused by infection with different viruses, suggesting an innate resistance to the effects of the virus

(Penrith et al., 2004).

The first description of a domestic pig that appeared to be fully resistant to the pathogenic effects of ASFV was by Montgomery (1921), the earliest description of ASF. At that time infection could not be confirmed, but as the pig survived inoculation with high doses of virulent viruses that killed other pigs, it is unlikely that it simply escaped infection. Subsequently, healthy pigs with antibodies to ASFV have been reported in a number of countries (see 3.4.1). However, a few populations of pigs that demonstrate a higher than expected survival rate after infection with virulent ASF viruses, that fit the definition of the second category of survivors, have been described in eastern Africa (Penrith et al., 2004; Okoth et al., 2013).

One of these populations is distributed across adjacent districts in Malawi, Zambia and Mozambique (Penrith et al., 2004) and another has been described in an endemic area of Kenya (Okoth et al., 2013). A study on a group of the pigs from Mozambique provided useful information about persistence and infectivity of virulent viruses in recovered pigs in the second category of survivors (Penrith et al., 2004). Twenty-five pigs from a number of villages in the area were purchased to be used in an investigation of natural resistance to ASF. Shortly after the pigs were assembled in a local quarantine facility an outbreak of ASF occurred, and two highly virulent viruses were isolated from the pigs that died (Bastos et al., 2004). All ten pigs that were seropositive before the outbreak and seven out of fifteen seronegative pigs survived the outbreak without developing notable clinical signs. One of the latter seven pigs died of unrelated causes before the pigs could be bled again a month after the outbreak, but the other six had all seroconverted. All 16 pigs were positive on PCR for ASFV genome but no virus could be cultured. Two months later after transfer to a quarantine facility in South Africa only one of the pigs was PCR positive and again no virus could be cultured. Nine of the sows were pregnant and gave birth to

Table 2

Summary of the differences between categories of pigs that survive ASF.

Category 1	Category 2
Infection persists for a variable length of time but apparently is usually lifelong and eventually results in death	Infectious virus does not usually persist for longer than 30 to 40 days although viral DNA may be detectable for longer
Pigs usually have clinical signs and lesions of ASF	Pigs may or may not suffer from acute clinical disease, but will recover and appear normal and healthy and with no remaining signs or lesions of ASF
The proportion of pigs that survive the acute phase of the infection depends on the virulence of the virus	It is not clear to what extent the proportion of pigs that survive the infection is related to the virulence of the virus
Virus excretion may occur periodically, in association with clinical signs.	No evidence for prolonged virus excretion.

healthy litters shortly after arrival in South Africa. A young boar that was added to the group in quarantine in Mozambique approximately eight weeks after the outbreak was seronegative on arrival in South Africa and remained so until the last time he was sampled 9 months later. During that time he had been quarantined at close quarters with the seropositive pigs recently recovered from the outbreak, and had subsequently been in daily nose-to-nose contact with the other boar and had serviced all of the sows, without ever showing any sign of disease. It was concluded that these pigs did not become long-term carriers of the virus and infective virus was not present in their blood a month after the outbreak although genomic material was detected.

3.2. The early years: 1921-1957

The disease experienced in East Africa described by [Montgomery \(1921\)](#) manifested as an acute disease of domestic pigs with close to 100 percent morbidity and mortality in affected populations. The extremely low survival rate of pigs led Montgomery to state that “the question of “carriers” among them...does not yet need consideration”. This opinion was supported by investigation of the history of outbreaks, which occurred on isolated farms with no history of introduction of new pigs, but where pigs were often not confined and warthogs were abundant. Experiments to try to determine the source of infection demonstrated that pigs became infected when kept in direct contact with pigs showing clinical signs of ASF but that asymptomatic pigs either incubating the infection or recovered from it did not appear to be infectious, and that inoculation with blood from experimentally infected warthogs could induce ASF in domestic pigs but transmission by contact with warthogs apparently did not occur. It was concluded that the carnivorous habits of pigs that could result in consumption of carcasses of warthogs, or of pigs that died of ASF, might maintain infection under natural conditions.

After the outbreaks of ASF in north-eastern South Africa, Steyn conducted experiments with two pigs that had been inoculated with blood from a warthog that had been shot in the endemic area ([Steyn, 1932](#)). The pigs developed severe clinical signs considered to be typical of ASF on the 6th day after inoculation but recovered completely. Two months after complete recovery, two domestic pigs were inoculated subcutaneously with blood from the recovered pigs. They developed typical signs of swine fever and died 11 and 12 days after being injected, indicating that the blood of the recovered pigs remained infectious for at least two months after recovery. Infection by contact was not investigated.

De Kock et al. conducted investigations into outbreaks of ‘swine fever’ that occurred in various parts of South Africa in between 1934 and 1939 ([De Kock et al., 1940](#)). The contention that recovered pigs become carriers was based on outbreaks that occurred near the coast more than 1000 km from the endemic area, and the source of the outbreaks was never established. It was assumed that at least some of the outbreaks that occurred on farms months after the end of a previous outbreak could be due to recovered pigs becoming carriers. In a natural outbreak of disease, nine pigs survived including a pregnant sow, which was isolated and gave birth to five piglets. Seven months after the outbreak, when the piglets were 4 - 5 months old, they were mixed with the other survivors and all the piglets developed ASF and died. Pigs from two neighbouring farms that had not experienced the outbreak were then mixed with the survivor pigs and they all died of ASF. Experimental infection of pigs with blood from eight of the original nine survivors from six to ten months after the outbreak resulted in acute fatal febrile disease in most cases. It is unfortunate that no experimental infections were carried out with blood from pigs involved in the endemic area outbreaks, because although it was surmised that the outbreaks in the 1930s were caused by movement of pigs, it was never conclusively proven that the outbreaks were not Classical swine fever (CSF), originating from airport or port galley waste fed as swill.

A study in Kenya between 1954 and 1956 in which 217 pigs were

exposed to ASFV yielded 13 pigs that survived the acute phase of the disease ([Detray, 1957](#)). Eight pigs in which persistent viraemia was demonstrated were kept for further studies. Pigs were considered positive for viraemia if pigs inoculated with their blood developed typical clinical signs of ASF and had typical lesions at necropsy. If the inoculated pigs did not react and were later shown to be susceptible to ASF the viraemia test was considered negative. The pig that survived the longest (456 days post exposure) gave a positive viraemia test at 440 days, although tests at 352 and 369 days were negative. The author mentioned that the pig was almost constantly exposed to ASFV during the 15 months that it survived due to 23 pigs in the same stall dying of acute ASF during that period; it was not suggested that those pigs were infected by contact with the survivor. The remaining seven survivors were considered positive for viraemia at 78 - 363 days post exposure. The conclusion was that all of the pigs had a persistent infection that accounted for a degree of immunity but indicated the existence of a carrier state. Only two of the pigs did not show clinical signs after the first inoculation but did develop viraemia. As the ongoing ASF outbreak in the same building could have caused reinfection of the 8 pigs under study, no definite conclusions about persistence can be drawn.

Blood samples from eight pigs that survived a large outbreak in Mozambique were taken two months after the outbreak and provided no evidence of presence of ASFV when inoculated onto porcine leukocyte culture ([Valadão, 1966](#)). One of the pigs was brought to the laboratory. It was not viraemic and did not develop viraemia when subsequently experimentally re-infected. Naïve pigs were inoculated with blood from the pig nine months after natural infection and again at various intervals from 2 - 120 days after the survivor pig had been inoculated with massive doses of virus. No clinical signs were observed. On the other hand, a pig inoculated with virus that had been passaged alternately in caprine and porcine leukocytes developed a severe reaction 8 days later, with illness lasting 18 days. Blood collected 24 days after the crisis (i.e. 45 days post infection) was infectious, as a pig inoculated subcutaneously developed ASF. A second sample taken 37 days later (i.e. two months after the fever abated) provided no evidence of presence of virus. The conclusion was that further studies were needed to determine the existence of a carrier state, which could be important epidemiologically.

In summary, these studies provide no evidence for a true carrier state in recovered pigs. In the first place there were a number of limitations to these studies. In the earlier studies the only way to demonstrate that virus was present was to inoculate pigs with suspected infectious material, usually blood but occasionally tissues of pigs suspected to be infected with ASFV. Diagnosis depended on clinical signs and pathology, neither of which can provide a definitive diagnosis due to their resemblance to a range of other diseases ([Oura, 2013](#)). Inoculation of whole blood from shot warthogs in particular could potentially give rise to other septicaemic conditions that clinically and pathologically resemble ASF ([Steyn, 1932](#)). In the case of the study by De Kock et al. the blood used for experimental infections was obtained from pigs that died in outbreaks very far from the endemic area whose origin was highly speculative ([De Kock et al., 1940](#)). In the study by DeTray the pigs were kept in an infected environment and it was uncertain whether the observed lengthy persistence was due to a single infection or whether reinfection occurred ([Detray, 1957](#)). In the second place, all of the pigs in which prolonged persistence of viraemia was demonstrated were either derived from a small number of survivors of outbreaks with high mortality or, in one case, was inoculated with a virus that had been manipulated by passage in caprine and porcine cells. However, the blood of the latter pig, investigated in the 1960s when virus isolation in cell culture had become available, was free of virus two months after the outbreak ([Valadão, 1966](#)).

3.3. The first escapes: 1957-1995

ASF escaped the African region for the first and second time in 1957

and 1960, respectively. The disease became established on the Iberian Peninsula and spread further within Europe and to the Americas. Already a few years after the introduction and establishment of ASF on the Iberian Peninsula, a gradual change in the disease presentation attributed to attenuation of some circulating strains was described, and which has been associated with the vaccination studies based on live attenuated ASFV strains performed on the peninsula in the 1960s (Sanchez-Vizcaino et al., 2015). Whereas all outbreaks initially had caused case fatality rates approaching 100%, affected establishments with higher proportion of survivors, including animals with a prolonged chronic course of the disease with unspecific and rather mild signs, started to appear (Sanchez Botija, 1962). Such survivors were early on referred to as virus carriers and considered as important for disease spread between herds (Sanchez Botija and Polo Jover, 1964; Ordas et al., 1983a; Ordas et al., 1983b; Vigario et al., 1983b).

3.3.1. Epidemiological studies

An effort to better understand virus persistence in carrier pigs is described in a study by Sanchez Botija in which five pigs which had recovered from ASF were used (Sanchez Botija, 1962). Blood was drawn from the pigs at different points of time between two- and eight-months post-infection and was then inoculated into naïve pigs to demonstrate presence of live virus. In none of the experiments did virus transmission occur. One of the five pigs was re-infected by inoculation three months after the initial infection. This pig had a protective immunity from the initial infection and did not develop clinical disease after the inoculation. However, a long-term infection was demonstrated by inoculation of blood into a naïve pig six months after the second infection.

Ordas et al. described a serological survey of > 26 000 apparently healthy pigs sampled in abattoirs and on farms, in which presence of antibodies was used as evidence for a carrier status, and aimed at assessing the incidence and distribution of virus carriers in Spain (Ordas et al., 1983b). Based on the results, the authors concluded that the proportion of carriers was low over all ($\approx 1.3\%$), but that the proportion of carriers within positive herds can be much higher. A similar study was carried out in Portugal, in which 25 000 samples collected from apparently healthy pigs at abattoirs were screened for presence of antibodies (used as evidence for a carrier status) (Vigario et al., 1983a). The proportion of seropositive pigs found was 0.9%.

Based on serological, virological and epidemiological data collected during the eradication of ASF from Spain, Pujols Romeu et al. and Bech-Nielsen et al. concluded that the presence and the epidemiological role of virus carriers within the studied populations was limited (Pujols Romeu et al., 1991a; Pujols Romeu et al., 1991b; Bech-Nielsen et al., 1993). Virus could only be recovered through isolation from blood and tissues from 0 and 4.4% out of 198 and 1549 seropositive pigs, respectively. Repeated serological testing over a 40–50 days period of 615 seropositive pigs in 91 herds showed a reduction in the proportion of antibody positive animals as well as mean antibody titres, and no recurrence of disease, thus failing to demonstrate virus circulation from seropositive animals (Pujols Romeu et al., 1991a). Also, the lack of recurrence of the disease in areas where clinically affected farms had been depopulated, in spite of continuous presence of seropositive survivors in the area in question, suggested a limited role for carriers in the maintenance of the disease in the population (Bech-Nielsen et al., 1993).

3.3.2. Experimental studies

To assess the persistence of virus in carriers, Ordas et al. furthermore examined surviving pigs from two previously infected farms for presence of virus using virus isolation, with positive results after 45 days, but not after five and ten months (Ordas et al., 1983b). Moreover, three surviving pigs which were kept together with four naïve sentinel pigs for three months, six to nine months after diagnosis, did not transmit the infection. Similarly, forty-seven pigs which had recovered

from outbreaks in Portugal were used to investigate excretion patterns from carriers (Vigario et al., 1983b). The surviving pigs were kept together with four naïve sentinel pigs for one year. Eight months into the study three of the surviving pigs died within a period of 20 days from acute ASF, associated with resurgence of viraemia and reappearance of clinical disease. In spite of this, none of the sentinel pigs developed disease or seroconverted. Thus, the study could not demonstrate any virus excretion from the surviving pigs considered as carriers. To investigate virus persistence, eight of the survivors chosen at random were slaughtered. No gross pathological lesions were found, and virus isolation failed to demonstrate virus presence in any of the numerous tested tissues.

Wilkinson described experimental infections with the Malta/78 isolate, carried out at Pirbright (United Kingdom) (Wilkinson, 1984). In one experiment, virus was detected for up to six months in tissues from infected pigs. In another, two pigs that recovered after acute signs of infection developed fever and viraemia 11 and 12 months post infection. Virus titres of 10^4 and 10^6 HAD₅₀/ml were detected and then decreased below detection level at day 11 and 25, respectively. An experiment to provoke recurring symptoms in recovered pigs by corticosteroid administration 9–31 weeks after infection was also described. At nine weeks, viraemia of 10^6 HAD₅₀/ml was detected in two pigs and one pig treated at 26 weeks had a virus titre of $10^{4.5}$ HAD₅₀/ml. The pigs treated at nine weeks could transmit virus to in-contact pigs while the other pig could not. The author concluded that 1) virus persists and could be reactivated by corticosteroids at least up to six months after infection in surviving pigs and 2) ASF persistence in a pig population could occur either due to infection being reactivated in surviving pigs some months after recovery or due to surviving pigs being re-infected and excreting virus without showing clinical signs.

Gusmão Vasco described studies carried out on survivors from an outbreak in a research facility in Portugal. In this outbreak, 47 of 148 pigs survived the infection, some recovered from the acute stage of the disease while others did not show any clinical signs (Gusmão Vasco, 1984; Gusmão Vasco, 1991). They did not transmit the infection to four naïve pigs introduced into the group. The studies also included attempts to obtain pigs with innate resistance to ASF by inbreeding. Among the inbred animals that displayed reduced susceptibility to ASF, 90 were slaughtered with no visible lesions and no virus detection by leukocyte blood culture.

Mebus and Dardini described experiments carried out at Plum Island (USA). They used pigs that had recovered from previous experimental infection with either a Brazilian or a Dominican Republic isolate of ASFV to test if they could transmit the infection to contact pigs (Mebus and Dardini, 1980). No transmission occurred via direct contact and no virus could be detected in the recovered pigs post mortem. However, feeding naïve pigs tissue material from the recovered pigs, slaughtered up to five months after inoculation, resulted in clinical infection. The authors compared the results to what could occur if recovered pigs, that show no ante or post mortem signs of ASF are slaughtered and offal is fed to other pigs, demonstrating a risk of ASF spread via this route.

Also at Plum Island, McVicar et al. used a moderately virulent strain from the Dominican Republic (DR79) to inoculate pigs oronasally (McVicar, 1984). Virus excretion could be detected up to 8 days in nasal mucus and saliva and up to 11 days in rectal swabs, the highest amount of virus was excreted in faeces. Viraemia, with decreasing titres, lasted up to eight weeks, while virus could be detected in lymph nodes and tonsils up to 13 weeks after infection.

In later experiments at Plum Island, Carrillo et al. used three groups of experimentally infected pigs to examine the persistence of virus in blood after recovery from the acute infection (Carrillo et al., 1994). One group was challenged with the virulent E75-L7 isolate and treated with anti-ASFV immunoglobulins, the second group was inoculated oronasally by the attenuated E75-CV1 isolate and subsequently challenged twice with the virulent E75-L7, and the third group was

inoculated with E75-CV1 and challenged once with E75-L7. In the first group, viraemia was detected for 30 days post infection. In the other two groups, a low-level viraemia was detected for 30 days after the initial inoculation but not after subsequent challenges. In contrast, viral DNA could be detected by PCR at more than 500 days post inoculation.

To investigate virus carrier state including possible transmission from seropositive pigs, Badaev et al. carried out experiments in the People's republic of Congo (current Republic of Congo) (Badaev et al., 1992). Seronegative pigs of local as well as improved breeds from endemic and free areas were exposed to seropositive pigs. At the start of the experiment ASFV was isolated from blood of some of the seropositive pigs. The number of seropositive animals as well as the level of antibodies in the already seropositive pigs increased during the 4.5 months long experiment. In addition, clinical signs and mortality were recorded in some previously exposed and all naïve pigs of improved breed. ASFV was isolated from the dead pigs. Based on this, the authors concluded that seropositive pigs are virus carriers. However, the fact that two seropositive pigs were viraemic already at the start of the experiment, suggests that these pigs may have been chronically infected (i.e. belonging to category 1 of survivors, Table 2). Possibly the seronegative pigs thus became infected subsequent to resurgence of viraemia associated with shedding in the chronically infected pigs. Furthermore, the experiment was carried out in field conditions (in a private farm) in an endemically infected region. Thus, the exposure from other sources (including infected ticks) cannot be excluded (no control group).

In summary, all the epidemiological studies included from this period were designed based on the concept that seropositive pigs were carriers, but none of the studies found any evidence of an epidemiological role for such pigs. In the experimental studies that were able to show natural transmission from surviving pigs, the transmission was always associated with resurgence of viraemia and reappearance of clinical signs of the disease including death of the chronically infected animals (Table 3). The viraemic period following the acute infection and in absence of clinical signs was limited, but virus could be isolated from tissues up to several months post infection. It was also demonstrated that localized virus persistence in lymphoid tissues may occur, which may cause infection after oral uptake.

3.4. The modern Era: 1995-2018

3.4.1. Epidemiological studies

Of the papers from this period included in this review, eleven are epidemiological field studies and included in this section. Despite the ongoing epidemic in Europe and Asia, all these studies are from Africa. They comprise three studies from Kenya, one from border areas

between Kenya and Uganda, three from Uganda, two from Nigeria and two from Tanzania, and are based on historical (Nigeria 1997-2005) and more recent data.

Several of the studies (Owolodun et al., 2010; Gallardo et al., 2011; Muwonge et al., 2012; Atuhaire et al., 2013; Thomas et al., 2016; Abworo et al., 2017) include sampling of pigs at slaughter. The study by Atuhaire et al. includes analysis of presence of ASF antibodies and ASFV genome from blood and organ samples collected bi-monthly during one year at the pig slaughter house in Kampala (Uganda), and presence of ASF antibodies in blood samples collected from live pigs in ten different locations (Atuhaire et al., 2013). The prevalence of ASFV genome in organs samples was 11.5%. The prevalence of ASFV-antibodies from pigs at the Kampala slaughterhouse and from the districts was in both cases surprisingly high compared to other studies from the region (above 50%). Muwonge et al. describe data from ante- and post mortem examinations as well as analysis of serum samples taken from pigs intended for slaughter at different slaughter slabs in one geographical region of Uganda (Muwonge et al., 2012). Few pigs with pathological lesions indicating ASF infection (3.8%) were found, and 0.2% of sera from these pigs were seropositive for antibodies against ASF, indicating recent infections. The study carried out in western Kenya by Abworo et al. comprises several parts: cross sectional and longitudinal monitoring for prevalence of ASF antibodies and ASFV genome as well as testing tissues and sera of a sub sample of sacrificed pigs from the cross-sectional part of the study (apparently healthy pigs bought by the project and subsequently slaughtered), and from pigs intended for slaughter sampled at slaughter slabs for ASFV genome prevalence (Abworo et al., 2017). No ASFV genome-positive and only one antibody-positive pig were found in blood samples from the 1107 live pigs sampled in the cross-sectional part of the study. Around 11% of organ samples from the sub-sample of 28 sacrificed pigs, and from 25% of 16 apparently healthy pigs intended for slaughter, tested positive for ASFV genome with PCR, while blood samples from these pigs were all ASFV negative. These results indicate that live pig populations and pigs intended for slaughter in the kind of setting that prevails in the study area should be treated as separate populations while drawing epidemiological conclusions. In the study by Gallardo et al. samples were collected from warthogs and soft ticks in the field in Kenya, and from domestic pigs at a Nairobi slaughterhouse (Gallardo et al., 2011). The pigs for slaughter were examined ante- and post mortem, and blood samples tested for presence of ASF antibodies and ASFV genome. Virus isolation was performed on ASFV genome-positive samples. Around half of the blood samples taken from apparently healthy pigs were positive for ASFV- genome with PCR, out of which 59% were also positive on virus isolation. None of the pigs were positive for ASF antibodies. In the study by Thomas et al (2016) pigs slaughtered at 26 different village

Table 3

Summary of information on transmission of ASFV by surviving pigs; studies that did not enable observation of ASFV transmission to in-contact pigs not included.

Section	Study	Type of study	CT ¹	Observation
3.1	Penrith et al., 2004	Experimental	No ²	Sentinel pig/survivors
3.2	Montgomery, 1921	Experimental, field	No	Laboratory and field observations
	De Kock et al., 1940	Experimental	Yes	Pathological lesions suggesting chronic infection; virus identity unclear
3.3.2	Ordas et al., 1983b	Experimental	No	Sentinel pigs/survivors
	Vigario et al., 1983b	Experimental	No	Sentinel pigs/survivors
	Wilkinson, 1984	Experimental	Yes	Resurgence of viraemia and reappearance of clinical signs in survivor pigs, induced by corticosteroid treatment
	Gusmao Vasco, 1984;1991	Experimental	No	Sentinel pigs/survivors
	Mebus and Dardiri, 1980	Experimental	No	Infection only by feeding tissues from dead pigs
	Badaev et al., 1992	On-farm experimental	Yes	Source of infection uncertain; possibly resurgence of viraemia in category 1 survivor
3.4.2	Gallardo et al., 2015	Experimental	Yes	Category 1 survivor, chronic infection
	Nurmoja et al., 2017	Experimental	No	Sentinel pigs/survivor
	Petrov et al., 2018	Experimental	No	Sentinel pig/survivors
	Gallardo et al., 2018	Experimental	No	Sentinel pigs/survivors
	Eblé et al., 2019	Experimental	Yes	Study design does not allow assessment of the long-term ability to shed the virus and transmit the disease

¹ CT = contact transmission.

² Contact transmission not part of the experiment but did not occur when a naïve pig was added to recently exposed seropositive group of pigs.

slaughter slabs in Kenya were briefly examined and blood samples taken before slaughter. Around half of the samples were positive for ASFV genome with PCR. Owolodun et al. present a study that includes data on ante mortem clinical scores, post mortem examinations and presence of ASFV-genome from pigs slaughtered at slaughter slabs in five geographical regions of Nigeria (Owolodun et al., 2010). Approximately 15% of apparently healthy pigs were ASFV-genome positive at slaughter. Ongoing outbreaks in the study areas are mentioned, possibly explaining these results.

In resource-poor settings with no or very limited governmental financial compensation at disease outbreaks, slaughter and trade with diseased or contact animals are to be expected. Other studies have shown that these types of coping mechanisms are widespread (Fasina et al., 2010; Dione et al., 2014; Lichoti et al., 2016; Chenais et al., 2017a; Chenais et al., 2017b). Even if the local situations and pig farming practices might differ slightly between countries and study settings, the included studies were all carried out in low-income countries dominated by small holder pig farming. Coping mechanisms are thus most probably similar in many aspects. Therefore, we can assume that slaughterhouse samples will, despite ante-mortem examination carried out routinely at the larger slaughter houses or as part of the study set up as in the studies of Gallardo et al. (2011) and Owolodun et al. (2010), include samples from pigs in the incubation or latent phases of ASF. Pigs slaughtered at village slaughter slabs, as in the study by Thomas et al. (2016), will in many cases not be subjected to any ante-mortem or meat inspection, and clinically diseased pigs will thus occasionally be slaughtered (Dione et al., 2014; Lichoti et al., 2016; Chenais et al., 2017a; Chenais et al., 2017b). Any claims to generalise results from samples taken from pigs intended for slaughter to the “live pig population” must therefore be taken with great caution. Still, the studies by Gallardo et al. and Thomas et al. indicate similar and very high proportion of blood samples from pigs being ASFV genome positive, which should warrant attention and further studies (Gallardo et al., 2011; Thomas et al., 2016).

Okoth et al. investigated blood samples from wild and domestic pigs in two locations in Kenya and found a high proportion of ASFV genome-positive blood samples from healthy pigs from one region (28% of in total 143 different pigs sampled at two separate occasions) (Okoth et al., 2013). The authors discuss a possible association between farms with ASFV genome-positive pigs, sightings of bushpigs in the farm vicinity, and distance to a national park. The authors further mention that such a high proportion of ASFV genome-positive pigs could indicate that the circulating virus is of low to medium virulence (compared to the highly virulent strains usually described from the region), or pigs being genetically resistant to ASFV-infection. The study does not provide evidence for either of these hypotheses. Further genetic investigation found that these pigs clustered more closely with European wild boar than other pigs sampled in Kenya (Mujibi et al., 2018). Braae et al. tested blood from 127 pigs from two regions in Tanzania and found three samples positive for ASFV genome with PCR (Braae et al., 2015). The pigs were apparently healthy, but the study reports outbreaks occurring in the area at the time of sampling, influencing how the status of these samples can be interpreted. In a follow-up study, Uttenthal et al. sampled pigs from the same two regions, and tested them for presence of ASF antibodies (Uttenthal et al., 2013). Between 1–16% of piglets were found antibody positive, with the highest proportion in pigs under six months of age. Based on a reference from a study on classical swine fever virus (Rangelova et al., 2012) the authors concluded that these positive results could not be due to maternal antibodies, but rather stemmed from infections transmitted by pigs surviving the outbreaks described by Braae et al. (2015). Penrith et al. however, showed that piglets born from seropositive sows, conceived in temporal connection with an outbreak, remained seropositive with maternal antibodies for at least 4 months and some up to 6 months (Penrith et al., 2004). The seropositive piglets in the study by Uttenthal et al. were conceived rather soon after an outbreak and could according

to those results still have had maternal antibodies (Uttenthal et al., 2013). This underlines the importance of not extrapolating evidence obtained from studies on different viruses. Olugasa et al. tested tissue samples from 162 clinical cases in Nigeria for presence of ASF antibodies and ASFV genome and found a high proportion of positive samples (it remains unclear if the results refer to antibody- or ASFV genome-positivity) (Olugasa and Ijagbone, 2007). These results are conveyed by the authors as a “continuous presence of recovered pigs in the population”. This statement is not supported by the presented data, which rather shows that pigs presenting with clinical signs resembling ASF mostly tested positive for either ASF antibodies or ASFV genome.

Out of the reviewed studies investigating the situation in the live pig population, only the study of Muhangi et al. was designed with the intention to investigate the existence of long-term ASF-carriers. In that study more than 700 pigs from 241 farms in Uganda were blood sampled twice with an approximately six-month interval and tested for the presence of ASF antibodies and ASFV genome. The only pigs testing positive for ASFV genome in blood (three) were pigs sampled in temporal connection with outbreaks (Muhangi et al., 2015).

With different objectives, study design and methodologies, the results of these papers are of varying relevance for this review. Importantly, however, these papers are all cross-sectional field studies (or a combination of cross-sectional, longitudinal or experimental studies), and none of them actually investigates the capacities for included pigs to transmit infection. Despite this, some of the papers claim to have proven carrier- or persistent infection status. Some of the papers are also quite often referred to in exactly this regard.

In conclusion, and with the exception of the study by Okoth et al. (2013), the proportion or prevalence of ASFV genome-positive pigs found if examining blood samples from apparently healthy pigs intended to live, and without connection to ongoing outbreaks is very low or zero. In connection with outbreaks (temporal and geographical), higher proportion of pigs intended to live have detectable ASFV genome in the blood. Important to note in this regard, in resource poor settings few outbreaks are investigated or reported (Chenais et al., 2015), and absence of official notification can thus not be used to define outbreak status of an area. The proportion of pigs testing positive for ASFV genome using samples taken at slaughter are higher than in samples from the field (and with higher prevalence in organ samples than in blood). However, none of the reviewed papers provide any evidence that the findings of ASFV genome are manifestations of a persistent infection- or carrier status. The evidence rather points towards slaughter and trade as frequently used coping mechanisms. Apparently healthy pigs being positive for ASFV genome in tissues or blood can be in the incubation, latent or recuperation phases of ASF, and the design of most of the reviewed studies does not allow defining the time of infection. Further, none of the studies show any results indicating that ASFV genome-positive pigs can transmit the infection, or that they are chronically infected. Higher prevalence in organs than in blood further indicate the limited possibility of PCR positive pigs to transmit the infection, unless slaughtered and fed to susceptible pigs

3.4.2. Experimental studies

Over the last years, several studies have been conducted addressing or including the issue of virus persistence in animals surviving ASFV infection. One of the studies that carries persistent infection already in the title, is the publication by de Carvalho Ferreira et al. (2012). The group of authors investigated excretion dynamics of animals in the later stages of ASFV infection, i.e. over a period of 70 days. Though not explicitly stated, the definition of persistence used is apparently that the animals survived the initial stages of infection, i.e. 30 days post infection. In a nutshell, it was shown that dose or infection route (intranasal inoculation or contact infection) did not influence the overall excretion pattern and that nasal, ocular and vaginal excretions had lowest viral loads. Viral DNA was consistently present in oropharyngeal swabs for up to 70 days, but virus isolation (VI) positive results were seen mainly

within the first 15–20 days post infection. Infectious virus was found up to 66 dpi. In faeces, virus was only occasionally present but sometimes with high titres. Viral DNA persisted in blood for up to 70 days. In more detail, the researchers used three different, moderately virulent ASFV strains, i.e. Brazil'78, Malta'78 (at two doses), and Netherlands'86. Animals infected with ASFV Brazil'78 all succumbed to the disease and thus no persistence was established. Animals of the ASFV Malta'78 groups showed more variable outcomes. In the group with high dose inoculation, only one directly inoculated animal succumbed to infection at day 9 post inoculation. The others survived until the end of the trial at day 70. One animal showed a second peak of clinical signs from 45 dpi. In the group with the lower inoculation dose, all inoculated animals survived while five out of seven naturally infected animals (animals commingled with the experimentally inoculated pigs from 24 h post infection to mimic natural infection) died between days 13 and 19 post initial inoculation of the group. Again, one surviving animal showed a second peak of clinical signs from 50 dpi. In the group of animals inoculated with ASFV Netherlands'86, all inoculated animals survived infection while six out of seven naturally infected animals died between days 25 and 28 post initial inoculation. Again, one animal showed a reappearance of clinical signs.

In a follow-up study, *de Carvalho Ferreira et al. (2013)* estimated transmission parameters for ASFV based on the experimental results presented in the earlier publication to provide a quantitative insight into the epidemiology of the disease. Among other things they estimated the basic reproductive ratio, R_0 , assuming a “minimum infectious period”, including only the acute phase of the disease, vs. a “maximum infectious period”, which also included the period of prolonged shedding (referred to as chronic or carrier phase). The values for R_0 given a “maximum infectious period”, which could be interpreted as the potential efficiency of disease spread for “carriers”, were thus estimated as comparably high. However, this was a very theoretical exercise where the authors assume equal infectiousness during the “carrier” phase as the acute phase. As demonstrated in several studies, however, both levels of viraemia and virus shedding decrease after the acute phase, and thus this assumption has no scientific support. Therefore, as also stated by the authors, the R_0 based on the acute phase is more likely to be closer to the true value.

In a very recent study, the same research group investigated whether pigs that had survived and recovered from the acute phase of infection with the same ASFV Netherlands '86, could transmit the disease to naïve pigs by direct contact transmission (*Eblé et al., 2019*). Briefly, six clinically healthy survivor pigs were commingled one-to-one with naïve contact pigs in two periods of two weeks i.e. 28–41 dpi and 42–55 dpi. Two of the twelve contact pigs, both in the second contact period, developed an acute ASFV infection at 42 and 44 dpi, respectively, and were euthanized at 47 dpi. Based on these results, the authors conclude that transmission of ASFV via carrier pigs does occur. However, the trial ended at 55 dpi and thus this result, albeit very interesting and relevant, does not provide evidence of a long-term ability to shed the virus and transmit the disease.

Gallardo et al. (2015) performed a study with a low virulent, non-haemadsorbing ASFV strain that is known to induce chronic disease (*Leitao et al., 2001*). In this study, transmission from survivors to sentinel pigs was shown up to three months after primary inoculation (*Gallardo et al., 2015*). Briefly, four hybrid pigs were inoculated intramuscularly (10^5 TCID₅₀) and 72 days later, two additional pigs were exposed to the remaining animals ($n = 2$). Clinical signs in most inoculated animals were indicative for a more chronic course of the disease. In this study, the animals were slaughtered at days 35, 65, 99, and 134. One of the in-contact pigs developed signs of chronic ASF from 32 days post exposure while the other showed only a fever peak at 45 days post exposure. These pigs were euthanized at 42 and 62 days post exposure, respectively, and showed mainly lesions in the respiratory tract. Both animals showed viraemia and seroconverted.

Another study that was performed to assess the risk of chronic

infections and carriers upon low dose infection of young wild boar and domestic pigs with a recent genotype II strain did not result in any survivors (and thus possible carriers) (*Pietschmann et al., 2015*).

A study that was performed to directly assess the issue of long-term persistence was reported by *Petrov et al. (2018)*. Due to the fact that the high virulence of recent genotype II strains impedes large scale studies on long-term carriers and fate of survivors in general, the study was done with the moderately virulent strain Netherlands'86, the same strain that was mentioned above. In brief, the study comprised 30 fattening pigs that were oro-nasally inoculated. Twenty out of the 30 pigs recovered after acute to subacute disease and long-term detectability of viral genome (up to 91 days). The surviving animals were subsequently commingled with six sentinel pigs of the same age for approximately two months. No transmission occurred and by the end of the study (day 165 post initial inoculation), all animals were negative for ASFV by virus isolation.

Recently, an Estonian ASFV strain was reported that showed an attenuated phenotype, especially in domestic pigs (*Zani et al., 2018*). In initial studies with this virus (*Nurmoja et al., 2017*), high virulence was observed that resulted in acute lethal infection of nine out of ten young wild boar. However, one animal recovered completely after an acute disease and was commingled with sentinels from day 50 to 96 post initial inoculation. At this time, the animal was still positive by PCR. The sentinels remained healthy and ASFV- and seronegative throughout the experiment and the survivor was negative for ASFV in all tested tissues at the end of the study. A virus that was recovered from the survivor in the acute phase of the disease was used for additional inoculations of potbelly-type minipigs and domestic pigs (*Zani et al., 2018*). In these animals, acute transient infection occurred with only mild clinical signs. Tests to directly assess the potential carrier state were not performed.

Another study (*Gallardo et al., 2018*) compared two ASFV strains from Southern Estonia, one from Valga county (ES15/WB-Valga-6), and one from Tartu county (ES15/WB-Tartu-14). These strains represented two variant strains with different sequence patterns in the central variable region (CVR) of the genome (GII-CVR1 and GII-CVR2), and the respective animals had shown high (Valga) and low (Tartu) antibody titres, respectively. The study comprised three parts. In trial 1, intramuscular inoculation of two pigs was performed for each of the variants (10^5 HAU₅₀) and the inoculated animals were co-housed with four contact animals per group. Four recovered animals were then commingled in trial 2 with seeder pigs inoculated with the homologous virus, and additional contact animals (domestic pigs and a European wild boar) were added. In trial 3, survivors of trial 1 were co-housed with naïve sentinels from 135 dpi (59 days post challenge) for a period of more than 100 days. Under the conditions of trial 1, the ASFV from Valga county induced variable clinical signs and two contact animals recovered after mild, remittent clinical signs and long-term detectability of viral genome beyond two months post infection. The recovering animals showed mainly skin cyanosis, joint swelling, and respiratory distress. All animals seroconverted. The virus from Tartu county induced acute lethal disease in the inoculated pigs and one contact animal. The other contact animals showed variable clinical signs with cyanosis and respiratory signs prevailing. One of the pigs died after a subacute course at 36 days post exposure. Necropsy of this animal revealed pneumonia, fibrinous pericarditis and enlarged, partly haemorrhagic lymph nodes. The other animals recovered. In trial 2, both seeder pigs showed severe clinical signs and died or were euthanized after an acute lethal disease course with characteristic clinical signs. The course of the disease in the naïve contact animals was also acute-lethal. The recovered animals survived homologous challenge with short, transient viraemia. One animal showed clinical signs upon challenge: swollen joints, erythema, inguinal lymphadenitis, and cyanosis of the ears. Increased antibody titres could be observed in all animals. No transmission was observed from the survivors to sentinel pigs, and at the end of the trial, no infectious virus could be isolated

from these animals (trial 3).

Assessing the studies reported above, it becomes clear that most of the recent trials showed quite comparable results in terms of excretion pattern. However, the interpretation of results and definition of persistence or carrier state was different. As an example, the conclusion drawn by Petrov et al. (2018) that no evidence for a carrier state is seen, seems to contrast the conclusions drawn from the study using the same virus by de Carvalho Ferreira et al. (2012), in which survivors are referred to as persistently infected animals. However, it has to be kept in mind that the study design and length was different. In general, long-term detectability of both virus and genome was quite similar in the studies mentioned above. It should also be pointed out in this context, that a positive PCR analysis only demonstrates presence of nucleic acids, which does not necessarily mean presence of infectious virus (Oura et al., 2013). Inagaki et al. (2016) demonstrated that for influenza virus H1N1, the duration of shedding as detected by PCR went far beyond the window of transmission. Based on this, the authors suggest that the infectious period in general is grossly overestimated when PCR is used to assess infectivity. It seems reasonable to assume that this also holds for ASFV.

The findings and conclusions drawn by Petrov et al. (2018) are also in contrast to those in the recently published study by Eblé et al. (2019), again using the same virus. In the latter, contact transmission occurred after the acute phase of infection but still in a rather short period after initial infection and in a phase that one could still call recovery phase. In Petrov et al. (2018) on the other hand, animals that recovered completely from infection seroconverted and did not transmit the virus to sentinel pigs after complete recovery and clearance of infectious virus. Still however, these animals showed ASFV genome in blood and oro-pharyngeal swabs for over three months (91 days). This can explain apparently healthy animals with positive genome detection at slaughterhouses or in the hunting bag (category 2, Table 2). It is a matter of discussion whether one should speak of persistency in these cases. Organ samples taken from these animals under the above-mentioned experimental conditions were negative for virus but in some cases, a few genome copies remained even up to 165 dpi. There is no evidence in these studies however, that resurgence of viraemia and shedding can occur in such animals, which is supported by observations from the field of the apparent extinction of the genotype II deletion mutant reported by Zani et al. (2018). The virus was only found for a very limited time in a rather confined area suggesting that virus circulation died out in spite of the presence of recovered seropositive animals, thus supporting the lack of any significant epidemiological role.

A completely different issue are animals surviving for a longer time but showing signs of chronic disease (category 1, Table 2). In these animals virus seems to persist in amounts that are transmissible.

4. Concluding remarks

Based on our systematic review of available literature, we can conclude the following:

- There is no general definition of an ASF virus carrier. Rather, in most papers (except for some of the recent experimental studies) any survivor or seropositive animal has been referred to as carrier. There is no evidence in the papers of any significant role in the epidemiology of the disease of such carriers.
- There are two types of “survivors”: 1) chronically infected pigs which eventually will succumb to the disease, and which may excrete virus in association with resurgence of viraemia and, in most cases, reappearance of clinical signs of the disease. These infections have generally been associated with low virulent, often non-haemadsorbing viruses. 2) pigs which clear the infection independently of virulence of the virus, and which possibly are more common in some pig populations. These pigs are not persistently infected and will not present with prolonged virus excretion beyond 30 to 40

days in the majority of cases.

- None of the categories of survivors can be considered as “healthy” carriers, i.e. pigs that show no sign but with the the long-term ability to shed the virus and transmit the disease to susceptible animals.
- Localized virus persistence in lymphoid tissues may occur to some extent in any of the categories of survivors, which in theory may cause infection after oral uptake. To what extent this is relevant in reality, however, can be questioned given the high virus dose generally needed for oral infection. The dose needed for oral infection is usually 10^4 HAU₅₀ (McVicar 1984) and thus 140,000 times higher than the dose needed for parenteral infection. However, with repeated exposure to contaminated liquids or involvement of weak or immunocompromised animals, the dose can be much lower (Niederwerder et al., 2019; Pietschmann et al., 2015).
- The appearance of animals with a prolonged chronic course on the Iberian Peninsula has been associated with the vaccination studies based on live attenuated ASFV strains performed during the 1960s. Given the current situation in South East Asia, with an unprecedented speed of spread of the disease, there are concerns that a vaccine will be released on the market and used massively before it's been tested enough to make sure that it's safe. It can't be excluded that this would drive virus evolution and disease dynamics towards chronic forms again. However, in our opinion, there is a difference between a virus carrier (i.e. an animal that is infected by a disease agent and capable of natural dissemination -shedding- of that disease agent, but which itself shows no sign of clinical disease), and a chronically infected animal, which normally presents with clinical signs and eventually will succumb to the disease.

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Declaration of Competing Interest

None

Appendix A. Supplementary data

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