REVIEW OF AFRICAN SWINE FEVER: TRANSMISSION, SPREAD AND CONTROL

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Introduction

African swine fever (ASF) is a highly fatal viral disease of domestic pigs that manifests as a haemorrhagic fever and can kill up to 100% of pigs affected (Penrith *et al.* 2004a). To date all efforts to produce a vaccine against ASF have failed. New developments in the field of molecular research have provided hope that a vaccine may be possible (Chang *et al.* 2006, Dixon *et al.* 2004, Lewis *et al.* 2000). However, alarming spread of ASF in recent years has demonstrated the immediate need for a constructive approach to prevention and control, without waiting for a vaccine to be developed. The purpose of this review is to describe events that prove the ability of ASF to spread rapidly across borders and over long distances, to examine the ways in which ASF is transmitted and the factors that facilitate its spread, and to consider the options for prevention and control, with particular reference to the South African situation. Recent developments in the search for a vaccine will be reviewed briefly.

Historical distribution and spread of ASF

ASF was first described from Kenya (Montgomery 1921) as a disease entity distinct from classical swine fever and the first description of ASF from South Africa was published in 1928 (Steyn 1928, 1932, de Kock *et al.* 1940). Angola followed in 1932 (Gago da Câmara 1932). In Kenya and South Africa it was recognised that ASF was associated with contact between warthogs (*Phacochoerus aethiopicus*) and domestic pigs, so that control measures were directed at ensuring separation between these species (Scott 1965).

ASF ceased to be a curious disease that affected the pigs of settlers in Africa when it appeared in Portugal in 1957 and again in 1960, apparently introduced from Angola (Wilkinson 1989). The second introduction into Portugal was not contained, and ASF spread rapidly to several countries in Europe including France, Italy, Malta, Belgium and The Netherlands (Penrith *et al.* 2004a). It became established in the Iberian Peninsula, which was only declared free of ASF in 1995 (Penrith *et al.* 2004a). It also established itself in Italy on the island of Sardinia, which last reported outbreaks to the OIE in the first half of 2005 (www.oie.int). In 1971 it crossed the Atlantic westwards to Cuba, from which it was eradicated with difficulty; after a resurgence in the Iberian Peninsula in 1977-78 it infected Brazil and Dominican Republic in 1978, Haiti in 1979, and Cuba again in 1980 (Penrith *et al.* 2004a). It was eradicated from these countries at considerable cost; the epic and expensive struggle over 8 years to eradicate it from Brazil has recently been described (Lyra 2006). The occurrence of ASF in European countries with highly developed pig industries sparked intense research in order to develop a vaccine (Hess 1971, Martins & Leitão 1994, Malmquist 1963, Plowright 1986, Stone & Hess 1967); some attempts to develop a vaccine had already been initiated in Angola (Mendes 1962, Mendes & Daskalos 1955). The results at that stage were probably unfortunate, as they demonstrated that it

was not possible to produce a conventional vaccine that would protect pigs without causing disease, but viruses of low virulence were apparently released in the process. These caused subacute and chronic disease, more difficult to recognise than the typical acute form of ASF, and were able to be maintained for longer in pig populations. A valuable result of the research on ASF, however, was the discovery that argasid ticks, commonly known as tampans, that inhabited pig sties, *Ornithodoros erraticus*, were able to maintain the virus and transmit it both to other tampans and to pigs (Sanchez-Botija 1963). Subsequent investigation demonstrated that related ticks of the *Ornithodoros moubata* complex, which could be found both in warthog burrows and in pig shelters in southern and eastern Africa, were important hosts of the ASF virus and the only normal means of transmitting the virus from warthogs to domestic pigs (Plowright 1977, Plowright *et al.* 1969a, b, Plowright *et al.* 1974, Plowright *et al.* 1970, Thomson 1985, Thomson *et al.* 1983, Thomson *et al.* 1980).

In contrast to the situation in southern and eastern Africa, warthogs were never implicated in the transmission of ASF to domestic pigs in Angola (Conceição 1949, Mendes 1994). Research in Malawi indicated that, in addition to maintenance in warthogs, ASF virus was maintained in domestic pig populations and argasid ticks in districts where warthogs did not occur (Haresnape 1984, Haresnape *et al.* 1985, 1987, Haresnape & Mamu 1986, Haresnape & Wilkinson 1988, Haresnape *et al.* 1988).

By the 1970s ASF had been reported from most countries in southern and eastern Africa where pigs were raised (Penrith *et al.* 2004a), coinciding with the distribution of warthogs and ticks of the *O. moubata* complex. Senegal, in West Africa, reported ASF to the Office International des Épizooties (World Organisation for Animal Health/OIE) for the first time in 1978, and limited studies described in an unpublished report suggested that, while the disease was apparently endemic in domestic pigs in southern Senegal, warthogs were not involved in maintaining or transmitting the virus (Sarr 1990). Ticks of the genus *Ornithodoros* are apparently absent from all but the northern parts of West Africa (Hoogstraal 1956, Leeson 1953) although there is a record of *Ornithodoros porcinus* from a warthog burrow in Sierra Leone (Walton 1964), where ASF has never been reported. In 1981 the rapidly growing pig industry in Cameroon was almost destroyed by ASF (Wilkinson 1989), which subsequently became endemic, again without the involvement of warthogs or ticks (Ekue & Wilkinson 1990). Because the virus that caused the outbreaks in Cameroon was the same as the virus that became established in Europe, it was suggested that ASF may have been introduced into West Africa from Europe, but this has never been proven (Wesley & Tuthill 1984).

Recent history of ASF

The period from 1994 to the present has witnessed a dramatic expansion of ASF in Africa, with many new countries becoming infected (Penrith *et al.* 2004a, OIE website www.oie.int). Within the "old" distribution area of ASF, two events occurred that once again focused attention on this disease. ASF was first confirmed in Mozambique in 1962 (Abreu *et al.* 1962, Mendes 1971) and from that time on had been reported from numerous localities in the northern and central parts of the country, but in 1994 it crossed the Save River for the first time and caused outbreaks in the three southern provinces, decimating a small but well developed pig industry around the capital city of Maputo (Penrith *et al.* 2007). Also in 1994, ASF broke out among commercial pigs around the city of Nairobi in Kenya, after an apparent absence from the entire country since 1963. In both cases the outbreaks were traced to movements of domestic pigs and were not linked to warthogs (Penrith *et al.* 2004a, Penrith *et al.* 2007). Far greater

expansion commenced in 1996, when Côte d'Ivoire in West Africa became infected (El-Hicheri *et al.* 1998), followed by Benin, Nigeria, and Togo in 1997, Ghana in 1999 and again in 2002 and Burkina Faso in 2003. With the exception of Côte d'Ivoire, the disease has not been eradicated and numerous outbreaks have occurred since the introductions. Counties such as Nigeria, Kenya and Zambia are still reporting disease in 2007. The islands of Santiago and Maio in the Cape Verde archipelago and Gambia, sandwiched into Senegal, also experienced severe outbreaks during the period 1998 – 2000, although the disease was not new to either country. Madagascar reported ASF for the first time in 1998. In the same period, Zambia and Tanzania experienced unprecedented outbreaks, possibly because increased pig production made larger numbers of pigs available for infection. In October 2007, the island of Mauritius reported ASF for the first time. Outside the African continent, Portugal reported a small outbreak in 1999 that was quickly contained. In June 2007, ASF was confirmed in samples from the Republic of Georgia, where great numbers of pigs were reported to have died before the diagnosis was made. Within months ASF had spread to Armenia and by the end of 2007 had also been diagnosed in wild boars in Chechnya (Russian Federation). A small outbreak occurred in Azerbaijan in January 2008 that was quickly controlled, but further outbreaks have been reported from a number of regions in Russia (www.oie.int).

Transmission and spread of ASF virus

ASF virus is transmitted directly during contact between infected and susceptible pigs, by consumption of the meat from infected pigs, by the bites of infected tampans (*Ornithodoros* spp.), and by contact with material or objects (bedding, feed, equipment, clothes and footwear, vehicles) contaminated by virus-containing matter such as blood, faeces, urine or saliva from infected pigs. Although warthogs are natural hosts of the ASF virus, it has been well demonstrated that they are unable to transmit the virus directly to domestic pigs (Thomson 1985). The role of other African wild suids (bush pigs of the genus *Potamochoerus* and the giant forest hog, *Hylochoerus meinertzhageni*), if any, in the epidemiology of ASF has not been clarified, but bush pigs were able to transmit virus to domestic pigs under experimental conditions (Anderson *et al.* 1998). Experimental studies have shown that ASF virus can only be airborne over short distances, not much more than 2 metres (Wilkinson *et al*. 1977). Apart from tampans, the only other arthropods that have been shown to be capable of maintaining ASF virus for a reasonable period (up to 48 hours) and transmitting it to pigs are stable flies (*Stomoxys* spp.) (Mellor *et al*. 1987). Since these are small flies, they would transmit the virus within rather than between herds unless they were inadvertently transported to another farm. Other potential sources of ASF virus that have been suggested but never proven and appear to be extremely unlikely are water (the virus is rapidly diluted and therefore unlikely to be present in infective doses, which are quite high for ASF), rodents and birds (Penrith *et al.* 2004a). There is no reliable evidence for transmission from sows to foetuses during pregnancy (Penrith *et al.* 2004a). Sexual transmission in pigs has also not been documented, but ASF virus is shed in genital secretions and therefore the Terrestrial Animal Health Code (OIE 2006, www.oie.int) provides guidelines to ensure that semen is free of ASF virus.

In South Africa, since 1951, outbreaks of ASF have only been recorded in or very close to the ASF control zone (a single small outbreak occurred just outside the zone in Limpopo Province in 1996), which was delimited according to the known distribution of infected warthogs and accompanying tampans. This area is situated in the north-eastern part of South Africa and includes most of Limpopo Province as well as parts of North West Province, Mpumalanga and Kwazulu-Natal. These outbreaks are invariably caused by contact between improperly

confined pigs and warthogs that results in infected tampans biting the pigs. Regulations for pig farming in the control area state that facilities in which pigs are kept must be surrounded by a pig-proof barrier, usually double fencing. Producers who farm under these conditions do not experience ASF and have a proud record of freedom from infection.

The vast majority of recent outbreaks in African countries have not been linked to contact between domestic pigs and warthogs or other wild pigs, but resulted from the movement of infected pigs or pig products. It is relatively easy to find links to wild pigs, and when these are not found they can be excluded as a source of infection. On the other hand, it is usually difficult if not impossible to trace with certainty the exact source of a pig-related outbreak, since the movements involved are usually illegal. Over the last decade advances in molecular genetic characterization of ASF viruses have contributed considerably to our understanding of where outbreak viruses may have originated and therefore how they might have spread. The example of the virus that caused the outbreaks in Cameroon was mentioned above. Subsequent studies using more sophisticated techniques have demonstrated that all the outbreak viruses from West Africa and those that caused the outbreaks in Europe, the Caribbean and Brazil belong to a single genotype (Bastos *et al*. 2003, Ekue & Wilkinson 2000, Nix *et al.* 2005, Otesile *et al.* 2005, Phologane *et al.* 2005), while in southern and eastern Africa there are many genotypes (Lubisi *et al.* 2005, 2007, Boshoff *et al*. 2007). The virus that infected Madagascar proved likely to have originated in Mozambique (Bastos *et al.* 2004, Gonzague *et al.* 2001, Lubisi *et al.* 2005), and the same genotype has now been confirmed as the cause of the outbreaks in Mauritius and in the Republics of Georgia and Armenia. It was possible to demonstrate that outbreaks that occurred in different parts of Tanzania in 2001, 2003 and 2004 were caused by unrelated viruses and therefore represented three separate introductions (Wambura *et al.* 2006). While this is extremely useful information, it is often not possible to determine the exact source and route of infection.

Free-ranging pigs are easily exposed to infection with ASF virus by contact with pigs from various sources and due to their scavenging habits, which can include feeding on the carcasses of pigs that have died of ASF and on garbage that contains remains of infected pigs. It is recognised, however, that people play an extremely important role in spread of ASF virus. Not only are they responsible for moving pigs and their products, often over long distances, but they also act as transmitters of the virus by moving from contaminated to uncontaminated premises without any hygienic precautions. The possible role of injections using needles contaminated with the blood of infected pigs has been documented (Penrith *et al*. 2004a).

Infected pigs are most dangerous during the incubation period of the disease, when they may shed infective quantities of virus for up to 48 hours before developing clinical signs of disease, and during the clinical stage of disease, when enormous amounts of virus are present in blood, secretions and excretions. Pigs that recover may shed virus for up to a month after the disappearance of clinical signs. There is no evidence that recovered pigs can become long-term carriers of the virus (Penrith *et al.* 2004b, Valadão 1969), but in large pig populations ASF virus can be maintained for long periods owing to the availability of a constant supply of susceptible pigs (Penrith *et al.* 2004b, Penrith *et al.* 2007). The ability to maintain and circulate the virus is enhanced in pig populations that have developed a degree of resistance to ASF virus. Lower mortality means that the population is not reduced during an outbreak to the same extent as a fully susceptible population, and the outbreak is therefore not self-limiting (Penrith *et al.* 2004b, Penrith *et al.* 2007). Such populations, which probably are fairly widespread have been identified in Malawi, Mozambique, eastern Zambia, and

probably Angola (Conceição 1949, Haresnape *et al.* 1985, 1987, Mendes 1994, Penrith *et al.* 2004b, Penrith *et al.* 2007, Wilkinson *et al.* 1988), and serve as a constant source of infection for other areas.

ASF virus has a remarkable ability to survive for long periods in a protein environment, and therefore meat from pigs slaughtered in the infective stages of ASF or that die naturally of the disease provides a good source of virus. The virus is quite resistant to high temperatures, requiring exposure to a temperature of 60°C for at least 20 minutes for inactivation, so that not only fresh and frozen pork but also smoked, salted and dried pork may contain infective quantities of virus (McKercher *et al.* 1978, Mebus *et al.* 1997, Plowright *et al.* 1994). ASF virus is also able to survive for long periods in some tissues, for example bone marrow, in spite of putrefaction. This probably plays a role in infecting scavenging pigs in areas where the disease is endemic.

Control

ASF in South Africa has for more than half a century been well controlled by applying the existing legislation in the ASF control area. However, recent studies into the distribution of tampans and their ASF infection status have indicated that tampans are present at least 100km south of the current control zone in Gauteng and ASF virus genomic material could be detected using the polymerase chain reaction (unpublished data from the Exotic Diseases Division, Onderstepoort Veterinary Institute). The potential impact of these findings and of the fact that live virus could not be isolated from the tampans, still needs to be determined. The potential impact of changing climatic conditions on the distribution of tampans in southern Africa also needs to be investigated. The control measures are aimed at preventing contact between warthogs and domestic pigs, and ensuring that infected pigs and material do not leave the area. However, many of the recent ASF events have been linked to international movement and trade. This demonstrates that we cannot be complacent about the safety of the rest of the country from ASF, since the South African ASF control area is not the only possible source of virus. The risk is underlined by events such as the introduction of porcine respiratory and reproductive syndrome (PRRS) and classical swine fever (CSF) into the southern coastal area of South Africa in 2004 and 2005 and Type O foot and mouth disease into Kwazulu-Natal in 2000.

Because ASF virus is not transmitted over long distances without human assistance, it is possible to prevent it by implementing strict biosecurity. This involves applying rules that, if rigorously observed, will prevent not only ASF but also other unwanted diseases. These rules include limiting access for people and vehicles to the area where the pigs are kept, ensuring that workers and other essential visitors such as veterinarians are disinfected before entering the premises, preferably by supplying them with protective clothing that does not leave the premises with them, and not feeding swill that could contain pork. The last includes inadvertent feeding of leftovers, and therefore no human food should be allowed into the pig facility. Disinfectant foot baths are generally not highly effective in terms of destroying the virus and should therefore not be relied upon as the only precautionary measure, but should certainly be included as part of the biosecurity plan for the farm. The concept of compartmentalisation, recently added to the OIE options for obtaining recognition of freedom from disease, is highly applicable to ASF, and many of the principles have been already pioneered in the ASF control zone. The guidelines for compartments are still under development, but are based upon maintaining strict biosecurity throughout the whole production chain to prevent the entry of specific diseases, taking into account all the ways in which they are transmitted.

Reliance on animal health authorities to ensure safety from ASF and other diseases can result in disappointment, not because they are not doing the best that they can do to protect the national herd from diseases, but because human beings show infinite ingenuity in circumventing regulations, particularly when the motive is profit. It is therefore crucial for control of diseases like ASF that all stakeholders in the pig industry understand the disease and know what they themselves must do to prevent it in the sphere in which they operate.

In the future, vaccines may be added to the control options for ASF. Current research into vaccines is based on the observation that certain strains of ASF virus of low virulence protect pigs from challenge with closely related virulent isolates (Boinas *et al.* 2004; Leitão *et al.* 2001) and the immunological targets and mechanisms involved in these models of protection are used for the development of vaccines to prevent this disease. One strategy is to develop attenuated ASF viruses by sequentially deleting genes known to be involved in evasion of the host immune system, virulence genes and genes required for replication in tick vectors as well as pig macrophages from a virulent virus whilst testing the potential vaccines at different stages to ensure that sufficient immunity for protection against infection and disease is maintained.

Recent studies have refuted earlier reports that neutralising antibodies do not play an important role in ASF protection. One such study demonstrated that passive transfer of anti-ASF virus serum could protect pigs against lethal challenge (Onisk *et al*. 1994), while neutralising antibodies to a number of proteins such as p30, p54 and p72 can significantly influence the outcome of disease and in some cases provide full protection against homologous and heterologous challenge (Gomez-Puertas *et al*. 1996, 1998, Barderas *et al*. 2001). However, one recent study was in contrast to these findings by demonstrating that neutralising antibodies to these three proteins were unable to mediate protection (Neilan *et al*. 2004). To improve on previous results, research is ongoing to express these proteins using recombinant Aujeszky's viruses, which offers the advantage of intracellular expression and can thus stimulate a range of lymphocytes (both CD4 and CD8 T cells as well as B cells) responsible for the immune response. Developments in the field of recombinant technology offer great promise for success in providing an effective vaccine in the future, but such a vaccine is not yet available.

It must, however, be remembered that vaccines do not render other precautions unnecessary. On the contrary, biosecurity remains the cornerstone of protecting animals against disease.

Conclusion

ASF continues to pose a severe threat to pig production worldwide. Expansion of the distribution area of the disease since 1994 has underlined the ability of ASF virus to traverse long distances rapidly, perhaps best illustrated by the unexpected appearance of an eastern African genotype of ASF virus in the Republic of Georgia in 2007. Outbreaks since 1994 in new areas have almost all been associated with movement of domestic pigs and their products rather than contact with the wild pigs that are natural hosts of the virus. In South Africa, it is necessary to be aware that the African swine fever control area may not be the only possible source of infection for areas that are currently free from ASF. The responsibility for control of serious transboundary diseases such as ASF will always officially belong to the veterinary authorities, but there is no doubt that breakdowns will occur unless pig producers understand how ASF is transmitted

and take all the necessary precautions to ensure that their own herds will not become infected.

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