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Review

Review of the sylvatic cycle of African swine fever in sub-Saharan Africa and the Indian ocean

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ABSTRACT

African swine fever (ASF) is a major limiting factor for pig production in most of the countries in Sub-Saharan Africa and the Indian Ocean. In the absence of vaccine, a good understanding of the ecology and epidemiology of the disease is fundamental to implement effective control measures. In selected countries of Southern and East Africa, the association between *Ornithodoros moubata* ticks and warthogs has been described in detail in the literature. However, for many other countries in the region, information related to the sylvatic cycle is lacking or incomplete. In West African countries, for instance, the role of wild pigs in the epidemiology of ASF has never been demonstrated and the existence and potential impact of a sylvatic cycle involving an association between soft ticks and warthogs is questionable. In other countries, other wild pig species such as the bushpigs (*Potamochoerus* spp.) can also be asymptomatically infected by the virus but their role in the epidemiology of the disease is unclear and might differ according to geographic regions. In addition, the methods and techniques required to study the role of wild hosts in ASF virus (ASFV) epidemiology and ecology are very specific and differ from the more traditional methods to study domestic pigs or other tick species. The aim of this review is (i) to provide a descriptive list of the methodologies implemented to study the role of wild hosts in African swine fever, (ii) to compile the available knowledge about the sylvatic cycle of ASFV in different regions of Sub-Saharan Africa and the Indian Ocean in addition to the one that has been described for East and Southern Africa, and (iii) to discuss current methodologies and available knowledge in order to identify new orientations for further field and experimental surveys.

Contents

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1. Introduction

African Swine Fever (ASF), caused by a DNA virus of the Family *Asfarviridae*, is one of the most serious diseases of domestic pigs in Africa. The virus is extremely contagious and the lack of an efficient vaccine, together with the involvement of wild hosts able to maintain the virus and the existence of large free-ranging populations of domestic pigs are considered the major constraints for the control and the eradication of the disease. ASF remains an endemic problem in Madagascar and many countries in Africa and there is evidence that this represents a threat to the pig population and the rural economy of other continents and regions. It is for instance the case with the introduction and spread of ASFV from Southern Africa to Madagascar in 1998 (Bastos et al., 2003; Roger et al., 2001), to Mauritius in 2007 (Lubisi et al., 2009) and more recently into the Caucasus and Russia (Rowlands et al., 2008). In the absence of an effective vaccine, the only possibility to mitigate the transmission and spread of the disease is to implement sanitary control measures based on a solid knowledge of its epidemiology. However, this is complex and complicated by the fact that many aspects regarding the role of the wild hosts and tick vectors involved in the sylvatic cycle of the infection remain unknown. In addition, their contribution as reservoirs and vectors of the disease may vary in different continents or regions. Wild suids – warthogs (*Phacochoerus* spp.) and bushpigs (*Potamochoerus* spp.) – on one hand, and soft ticks of the genus *Ornithodoros* on the other hand, are considered the natural hosts in the sylvatic cycle in Africa and potentially in the Indian Ocean. Although the specific relationship between warthogs and soft ticks is extremely well described in the literature for East and Southern Africa (Plowright, 1981; Plowright et al., 1994; Thomson, 1985), it needs to be determined for the rest of the African continent and the Indian Ocean.

The fact that warthogs occurred in all the countries in Africa where ASF had been diagnosed in domestic pigs was noted by Thomson (1985). However, infection of warthogs with ASFV and association between *Ornithodoros moubata* complex ticks and warthogs was only established in countries in East and Southern Africa (Penrith et al., 2004b; Thomson, 1985). Findings in other countries have failed to provide convincing evidence of the warthog/tick sylvatic cycle and have demonstrated maintenance of ASFV in domestic pigs (Penrith et al., 2004b). The bushpig has been considered to be of less importance in the epidemiology of ASF than the warthog but since it is nocturnal and elusive, information on this species is much scarcer. The latter species has been suspected to be a reservoir of ASFV in areas where there are no warthogs, but where the virus is endemic (Haresnape et al., 1985). The blood virus levels in an infected bushpig are high enough to infect both the soft ticks and domestic pigs (Anderson et al., 1998). However, bushpigs do not live in burrows and therefore do not get into contact with the endophilous soft ticks naturally (Costard et al., 2009). It is reported by local inhabitants that natural interbreeding can occur between bushpigs and free ranging domestic pigs if they meet in the same areas, but scientific confirmation for this has not been provided (Jori and Bastos, 2009). It has been speculated that hybrids, if they exist, could become asymptomatic carriers among domestic pigs and thereby maintain the spread of the virus, because pure breed bushpigs do not show any clinical signs (Jori and Bastos, 2009). Bushpigs are hunted for their meat in many African countries and in Madagascar, and leftovers fed to domestic pigs could lead to infection if the virus amount in the tissues is high enough (Jori and Bastos, 2009).

In East and Southern Africa and the Indian Ocean, tick vectors of ASFV belong to the *Ornithodoros moubata* complex of species, while in North and West Africa they belong to the *Ornithodoros erraticus* group. The first group has been re-described by Walton (1962) and includes four different species: *O. moubata sensu stricto* (Fig. 1) and *O. porcinus* that are confirmed to be vectors and natural reservoirs of ASFV, and *O. compactus* and *O. apertus* that do not feed on suids. According to this author, the first two species are morphologically and ecologically distinct, although for both of them a wild strain that colonizes warthog burrows and a domestic strain colonizing pig pens and human dwellings have been described. In East Africa, the wild *O. porcinus porcinus* and the domestic *O. porcinus domesticus* were morphologically differentiated (Walton, 1962). The second group (Fig. 2) was first described as a sub-genus named *Theriodorus* (Pospelova-Strom, 1953) and includes *O. erraticus* and *O. sonrai*, which have both been found naturally infected by ASFV. *O. alactagalis* and *O. nereensis* also belong to this group but their role regarding ASF transmission is unknown. Because the systematics of both groups remains unclear in several parts of Africa, with suspected hybridization of species in sympatric zones, the classification above will be used in this paper. A recent molecular investigation of *Ornithodoros* from East and Southern Africa suggested a more parsimonious classification (Bastos et al., 2009). However, it is clear that further taxonomic investigations are needed at molecular level to arrive at a final taxonomic classification of *Ornithodoros*.

Based on investigations in East and Southern Africa, it was established that *Ornithodoros* soft ticks live in warthog burrows and transmit ASFV to immature warthogs, which develop sufficiently high levels of viremia to infect other ticks. Infected ticks would occasionally be transported by adult warthogs from natural to farmed areas where they are able to bite and infect domestic pigs, providing a pathway between sylvatic and domestic cycles (Plowright et al., 1994). Further investigations in other African and European countries have shown the existence of many diverse epidemiological situations, where soft ticks may only colonize domestic pig premises, maintaining ASFV by feeding exclusively on domestic pigs (Haresnape et al., 1985; Sanchez Botija, 1963). Equally, in the light of comparative virus investigations, it appears that the sylvatic cycle has acted as a source of new more diverse and virulent virus isolates for the domestic cycle since the greatest genetic variation, with a high number of genotypes, occurs in East and Southern Africa (Lubisi et al., 2005; Nix et al., 2006). However, the persistence of ASFV in local *Ornithodoros* ticks in Portugal and Spain demonstrates that new maintenance cycles may arise when the virus is introduced into new areas where suitable vectors are present, and this could equally occur if suitable vectors are

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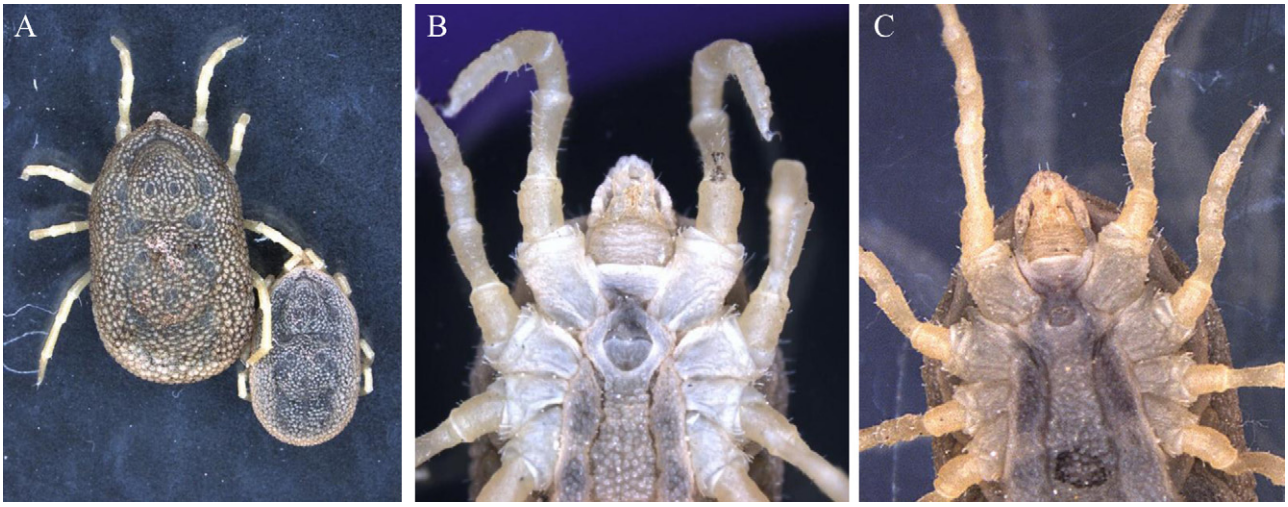

Fig. 1. *Ornithodoros erraticus*: (A) female and male (dorsal view); (B) and (C), ventral, closer view of the genital pore of a female and a male tick, respectively.

introduced into areas where the virus occurs. and There is also some evidence that transmission of ASFV from domestic pigs to warthogs is also plausible (Gallardo et al., 2011b).

2. Specific methodologies to study the sylvatic cycle of ASF

Gathering information on the sylvatic cycle of ASF requires methodologies to assess the circulation of the virus in the various wild hosts (wild pigs and soft ticks) that are different from those currently used in the study of other ticks or diseases in domestic pig populations. The majority of available diagnostic tests have been validated for use only in domestic pigs, but they are also used in wild species. In order to assure reliable results, these assays need to be validated using samples from wild species, as the sensitivity and specificity of the assays may differ according to species.

2.1. Methodologies to study the role of wild pigs

Wild pigs other than warthogs are elusive animals. Due to their nocturnal behavior and their preference for forested habitats, they are not easy to observe, capture and study. To assess their role in the epidemiology of the disease, two types of information are important: (1) the occurrence/circulation of ASF among wild populations and (2) their potential to transmit the virus to domestic pigs.

2.1.1. Sample sources

To determine whether wild pig populations are infected, samples have to be collected from free-ranging animals. This can be done either by capturing live animals or by collecting samples from individuals killed during hunting activities.

2.1.1.1. Capture of live animals. Physical and chemical methods to capture African wild pigs are technically possible and described in specialized literature (Kock and Burroughs, 2012; La Grange, 2006; McKenzie, 1993). Warthogs have been captured by putting nets outside their burrows (Cumming, 1975). Techniques developed for wild boars in Europe such as building up baited trap boxes may work on bushpigs (McKenzie, 1993). Net bomas (net traps) are also reported to work well for the capture of wild African pigs (La Grange, 2006). In any case, wild swine are cautious animals that

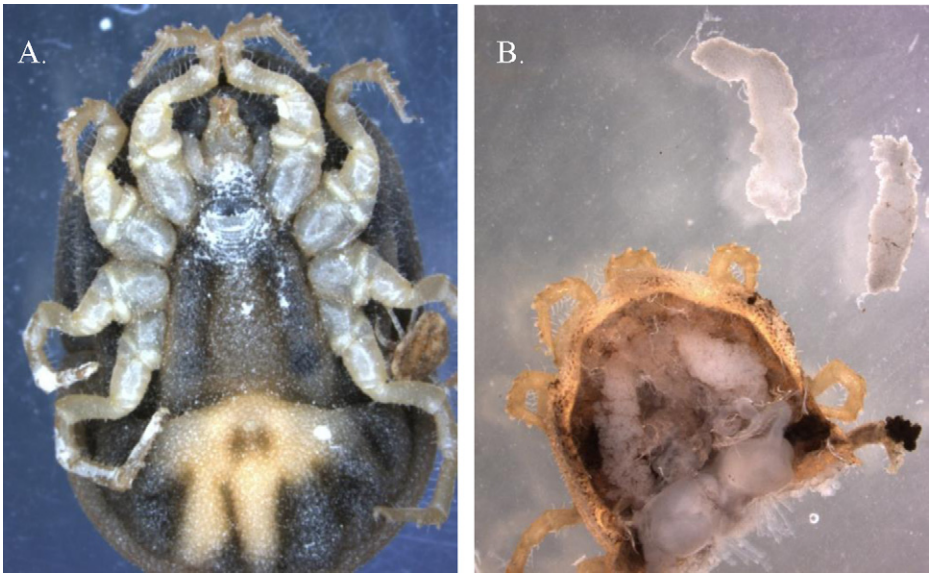

Fig. 2. *Ornithodoros moubata*: (A) female (ventral view) and nymph-1; (B) dissected *O. moubata* female showing the acinar salivary glands at both sides of the anterior body end, and a pair of removed salivary glands.

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need to be baited for a few days/weeks before they are confident to enter the traps. Once the animals are in the traps, they need to be immobilized with chemical drugs such as Zoletil or a BAM drug combination using 0.5 ml butorphanol (30 mg/ml), 0.25 ml azaperone (50 mg/ml), and 0.25 ml medetomidine (20 mg/ml) (Kock and Burroughs, 2012). However, wild pigs are prone to stress and hyperthermia and capture operations are often expensive and time consuming, and sample collection is limited to blood, while tissues would be more suitable for collection of virus.

2.1.1.2. Collaboration with hunters. Developing collaboration with hunting activities is a useful way to get samples to assess the circulation of diseases within populations of wild pigs (Ravaomanana et al., 2011; Vieira-Pinto et al., 2011). Depending on how it is organized, it can allow for the collection of different wet samples such as blood, serum, lymphatic tissue (spleen and lymph nodes) or material collected on filter papers.

2.1.2. Sample types and diagnostic methods

All sorts of samples (tissues, organs and blood) usually collected from domestic pigs can be used for ASF diagnosis in wild swine although lower viral titers were reported in bushpigs compared to samples from domestic pigs in a study in which both species were experimentally infected (Oura et al., 1998b).

The preservation of biological materials during transport between remote areas and the laboratory for initial or confirmatory diagnosis of ASFV infection usually requires the maintenance of a cold chain or the addition of preservative agents compatible with diagnostic procedures. The cold chain is less imperative with materials dried on filter papers (FPs). In the last two decades, various studies have demonstrated that samples can be stored for rather long periods on FPs at room temperature for further testing for antibody or genome presence (Behets et al., 1992; Pitcovski et al., 1999). This has been successfully applied for ASFV molecular detection (Agüero et al., 2003). FPs can even be used for ASFV detection by conventional PCR without any previous nucleic acid extraction (Michaud et al., 2007). Furthermore, blood-dried FPs could be stored up to 9 months at 4 °C or +37 °C without loss of signal and the virus could be sequenced and genotypes identified. The only identified constraint is that FPs must be well dried to avoid moisture that could then interfere with virus detection. More recently, FPs have been used for virus isolation and antibody detection (Albina et al., unpublished results).

In principle, all diagnostic tests primarily developed for ASF diagnosis in domestic pigs can be used on samples from wild pigs. Antibody detection tests based on the use of anti-swine immunoglobulins such as immunoblotting or ELISA tests can be used but competitive ELISA tests seem to provide more sensitive and consistent results (Gallardo et al., 2009). Virus isolation on pig alveolar macrophages or bone-marrow or blood derived monocytes can be achieved with samples from wild swine. The routine tests are now based on the detection of the virus genome by PCR. It should be kept in mind, however, that virus isolation in adult warthogs, which are not viremic, might not be as successful as in young individuals or domestic pigs (Gallardo et al., 2011b). Several agarose gel based or real-time PCRs have been developed with high sensitivity and specificity (Agüero et al., 2003; Fernández-Pinero et al., in press). Tests are available for the detection of ASFV antigen in domestic pigs and warthogs but they are less sensitive than molecular tests. For instance, indirect ELISAs for antigen detection had a sensitivity ranging from 200 to 100,000 PFU or HAD/ml (Hutchings and Ferris, 2006; Vidal et al., 1997), while molecular tests can consistently achieve detection as low as 100 HAD/ml (Agüero et al., 2003). It must, however, be remembered that detection of viral nucleic acid or even antigen does not necessarily indicate infectivity, and that the virus should be cultured for confirmation.

2.1.3. Breeding wild pigs and experimental infections

Warthogs and bushpigs breed well in captivity and captive collections have been successfully established in several zoological gardens. Although they are often prized for their meat in many African countries and in Madagascar, they are not known to be bred for food. Captive collections have been established for scientific purposes, in particular to study transmission of ASFV from warthogs to domestic pigs (Thomson and Gainaru, 1980), the epidemiological role of bushpigs in the transmission of ASF in Zimbabwe (Anderson et al., 1998; Oura et al., 1998a) and the potential role of bushpigs and warthogs in the maintenance of classical swine fever in South Africa (Everett et al., 2011). In all these cases the captive collections were dismantled after the scientific projects were concluded.

2.2. Soft ticks

2.2.1. Direct sampling

Classical direct methods for hard tick surveillance based on the capture and identification of specimens, either from vegetation (dragging method) or from animal hosts in the area sampled, do not work for soft ticks. Indeed, the specific biology of the genus *Ornithodoros* renders sampling methods more complex. As Argasids, these ticks are commonly endophilic at all stages of development (living in the nidicolous underground habitats of their vertebrate hosts) and remain attached to their hosts for a few hours or less while feeding on blood (Morel, 2003; Vial, 2009). They usually feed during the night, and then fall off when engorged and return quickly to their habitat which they occupy between blood meals. It is therefore easier to collect them directly in their underground habitat, although some authors have reported collecting immature stages (nymphs) on warthogs (Horak et al., 1983; Penrith et al., 2004b). In African and Indian Ocean countries, *Ornithodoros* soft ticks that are involved in ASFV transmission usually colonize warthog burrows, crevices/holes in pigsties, and sometimes rodent burrows that may open inside pigsties. Three different techniques have been developed to collect soft ticks in the field, depending on the structure and the organization of the habitats sampled.

2.2.1.1. Manual collection. Using a shovel, it is possible to remove the dust from crevices and holes in pigsties, as well as the content of wooden or tiled roofs, and to manually dig out the floor of pigsties. This method can also be used to examine warthog habitats. The content of the examined habitat is laid out in a white tray (Fig. 3A) and exposed to the sun to make soft ticks move and leave the sand (soft ticks are photophobic and they do not tolerate warm temperatures). Each specimen is then collected individually with flexible entomological tweezers.

2.2.1.2. Carbon dioxide trapping. Carbon dioxide gas is a good stimulant and attractant to certain species of ticks since it mimics vertebrate host breathing. Dry ice vaporizes carbon dioxide and can be used for trapping. Several types of carbon dioxide traps have been tested. The most convenient and effective device was found to be a stainless-steel tray ($30 \times 45 \times 8$ cm) carrying a polystyrene plastic cup of about 500 ml capacity filled with solid dry ice pellets (Caiado, 1990). Traps have to be placed on the ground close to potential underground habitats of soft ticks, with soil or other material covering them to their top edges, and left for a period of time. If possible, traps are set out overnight for optimal sampling.

2.2.1.3. Vacuum aspiration. This method was first described in the early 1980s (Butler and Gibbs, 1984). It has been more recently adapted for collecting *Ornithodoros sonrai* in Senegal (Vial et al., 2006a). Different adaptations have been tested in order to provide an efficient model. A petrol-mulching blower/vacuum that can be

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Fig. 3. Tick sampling methods (A) manual method and sorting tray, (B) use of vaccum aspiration to collect ticks in a wrathog burrow; (C) components of a modified vaccum aspirator device, (D) carbon dioxide trapping.

bought in any gardening shop has been used. It was operated using a petrol/oil mixture (100 ml of 2 cycle engine synthetic oil mixed with 5 l of petrol). PVC modifications and a flexible plastic tube have been added in order to examine deep burrows or cracks to collect ticks. An iron-filter has been fixed on the plastic tube within the PVC pipes in order to collect large particles of litter and live ticks. In the field, such a vacuum is used by introducing the plastic tube into burrows, cracks or holes that are expected to be inhabited by soft ticks and aspirating the content of the habitat (sand, litter, dust, *etc.*). As in the manual method, the filtered content is laid out in a sorting tray and specimens are collected individually with flexible entomological tweezers.

After collection, ticks should be kept alive or directly stored in liquid nitrogen to ensure best conservation of the virus inside ticks and to avoid DNA degradation. At the laboratory, specimens can be stored in −80 °C freezers and samples sent in dry ice in polystyrene packs (transfer must not exceed 2–3 days).

2.2.2. Indirect methods of tick detection

As for any tick bite, vertebrate hosts bitten by *Ornithodoros* soft ticks develop an immune response against tick saliva, which may be used as a biological marker of tick presence and tick exposure. The first anti-tick ELISA test was developed for *Ornithodoros erraticus* in Spain, based on the global extraction of tick salivary glands as an antigen; it was able to detect anti-tick antibodies in pigs bitten by as few as 10 ticks for up to 6 weeks after the primary bite and for up to 12 weeks after secondary bites (Canals et al., 1990). However, field evaluation showed a lack of specificity with many positive results in pig farms where the absence of soft ticks was confirmed by tick examination. Further improvements have been made to avoid such cross-reactions, especially with hard tick antigens, by purifying a more specific soluble salivary antigen extract (SGE-2) and also a deglycosylated fraction (SGE-2-P), the latteravoiding the recognition of the epitopes located on carbohydrate chains of SGE-2 by some pig serum antibodies (Oleaga-Perez et al., 1994; Pérez-Sánchez et al., 1992).

A similar test was specifically developed for the African ticks of the species complex *O. moubata sensu lato*, with apparently no cross-reaction with antigens from *O. erraticus* or any other swine ectoparasites (Baranda et al., 1997, 2000). First field evaluation of this test in Madagascar suggested a lack of specificity since most of the farms where pig sera were positive were not infested by soft ticks (Ravaomanana et al., 2010). Based on proteomic investigations in *O. moubata* and *O. erraticus*, new purified salivary proteins (TSGP1 and Oe260 for *O. moubata* and *O. erraticus*, respectively) have been recently identified as interesting candidates in recombinant form for the anti-tick ELISA test (Oleaga et al., 2007). First assays using a panel of 172 well-defined pig sera from Spain (pigs free of ticks, pigs experimentally infested with *O. moubata* or with hard ticks, and pigs from Spanish farms free of and infested with *O. erraticus*) seem promising since recombinant TSGP1 showed 100% sensitivity and 99% specificity compared to the whole *O. moubata* SGE with 100% sensitivity and 87% specificity (Díaz-Martín et al., 2011).

The protocol for obtaining the recombinant TSGP1 protein in a cost-effective way and conducting the anti-tick ELISA test is well described by Díaz-Martín et al. (2011). For the interpretation and the establishment of a common cut-off for all ELISA plates, a serological index (SI) is calculated for each optical density (OD) using the following formula: $[(NC - S)/(NC - PC)] \times 100$, where NC and PC represent the negative and the positive controls, respectively, and S stands for each sample serum (Hernández-González et al., 2008). Receiver–Operator Characteristic (ROC) analysis of the SIs obtained for the above-mentioned panel of sera allowed the cut-off value to be established in SI = 7.53%, which was the point in the ROC curve giving the highest diagnostic performance (calculated as the sum of the sensitivity and specificity divided by two).

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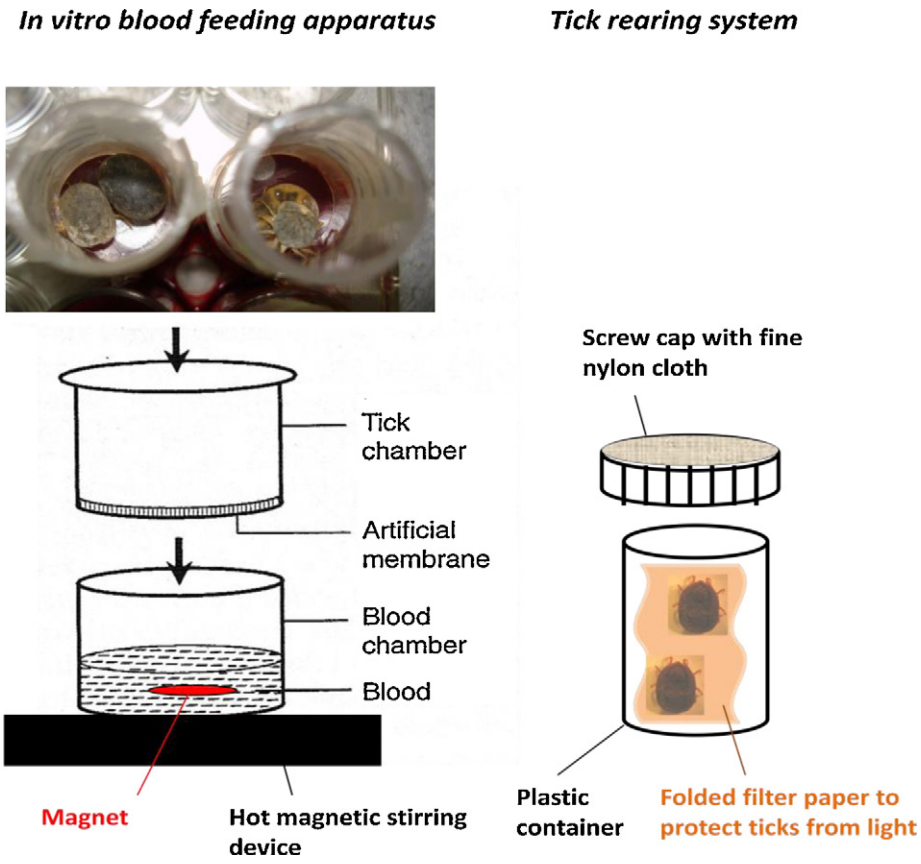

Fig. 4. Tick rearing system and artificial blood feeding apparatus.

2.3. Tick rearing and experimental infections

Experimental infections are the only method that enables confirmation of the vector and/or reservoir competence of *Ornithodoros* spp. for ASFV. Several reports of such laboratory investigations have been published over the years (De Tray, 1963a; Kleiboeker et al., 1998; Kleiboeker and Scoles, 2001). However, techniques for tick rearing and ASFV infections through either artificial or pig feeding are very diverse. In this chapter, we propose to present only the methods used most because of their practicality, reliability and representativeness of natural processes.

Ticks are kept in separate screw-capped plastic containers with a fine nylon cloth as a cover to allow equilibrium with climatic conditions of the incubator or chamber that maintains optimal humidity and temperature for tick survival and the development cycle. Depending on the tick species used and area of origin, the temperature must range from 20 to 35 °C (optimal temperature of 24 °C for *O. moubata* and *O. porcinus*, and 30 °C for *O. sonrai*) and relative humidity must vary between 50% and 95% (optimal humidity of 50% for *O. moubata*, 70% for *O. porcinus* and 80–90% for *O. sonrai*) (Vial, 2009). When resources are limited, an ancient desiccator containing a saturated KOH solution and hermetically closed may be enough to maintain adequate conditions. Because *Ornithodoros* ticks are photophobic, it is essential to maintain darkness inside the chamber (Fig. 4).

Ticks can be fed directly on pigs by pooling them in tissue bags and fixing the feeding units onto the pig's skin using neoprene glue. The pig skin must first be shaved to permit easy penetration by the ticks' mouthparts. The pigs must be immobilized throughout the feeding period using chemical or physical restraint, in order to avoid the feeding unit falling off and the ticks escaping. An alternative is the artificial feeding of ticks on pig blood through parafilm membrane using a special apparatus mimicking as much as possible natural conditions of tick feeding. This apparatus includes a tick chamber closed on top by nylon cloth to avoid tick escape and below by the parafilm membrane, a blood chamber containing a magnet, and then a hot magnetic steering device to mix and warm blood at 38–40 °C (see Fig. 4). With this method, blood can be either directly drawn from the animal and mixed with heparin (an anti-haemostatic molecule naturally secreted by ticks) or collected at slaughter and defibrinated by slow mixing and added to antibiotics for long storage (one or two weeks at +4 °C). After blood feeding by any method filter paper must be placed in the plastic rearing containers to allow ticks to attach and rest for digestion and to collect coxal fluid secreted by ticks after feeding. The next feeding can be done three to four weeks later, allowing time for molting (one or two weeks) and for complete digestion.

Experimental infections of ticks with ASFV are easier using artificial feeding since the infective dose can be controlled. By weighing ticks before and after feeding, it is possible to estimate the infective dose ingested by ticks since the ASFV titer in blood, the volume of blood and the mass volume of blood are known.

2.4. Detection of ASFV in ticks

The virus genome in infected ticks may be present in low copy number. In addition, nucleic acid extracts from ticks may contain extra PCR inhibitors compared to the usual pig-derived blood, tissue or organs. However, a highly sensitive and specific nested PCR for tick materials has been developed recently (Basto et al., 2006). With this test, it was demonstrated that ticks can host the virus for extended periods of time. Depending on the selected amplified gene, this method added to sequencing is also useful to investigate genetic diversity of virus in arthropod hosts.

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3. Review of new insights into the role of wild suids and ticks

3.1. East and Southern Africa

East and Southern Africa are without a doubt the areas of sub-Saharan Africa where the sylvatic cycle of ASF has been more extensively studied. An association between wild African pigs and African swine fever outbreaks in Kenya was suspected early and it was confirmed experimentally that both warthogs and bushpigs were susceptible to infection but did not develop clinical signs, and transmission from wild pigs to domestic pigs could only be effected by inoculation of infected blood (Montgomery, 1921). The way in which the virus was naturally transmitted from warthogs to domestic pigs was only elucidated much later, after finding evidence of the involvement of an argasid tick in maintenance and transmission of the virus was reported in Spain (Sanchez Botija, 1963).

Warthogs are widely distributed in southern Africa in tropical and subtropical savanna (Jori and Bastos, 2009). Investigations revealed that a high percentage of warthogs in Kenya and Tanzania and about 50% of warthogs in Uganda had antibodies to ASFV (Plowright et al., 1969, 1994). Serologically positive warthogs were subsequently reported in Botswana, Namibia, South Africa and Zimbabwe (Simpson and Drager, 1979; Thomson and Gainaru, 1980). In South Africa infected warthogs were detected in the north-eastern part of the country in an area comprising the Limpopo Province, the Kruger National Park and adjacent part of Mpumalanga Province, the north-eastern part of the North West Province, and the extreme northern part of Kwazulu-Natal Province. This area was proclaimed the Swine Fever Control Area in 1935 (De Kock et al., 1940). Warthogs were examined in two wildlife reserves in northern Kwazulu-Natal, South Africa and a low percentage of warthogs in the northernmost reserve, Mkuze Game Reserve (MGR), had antibodies against ASFV (Thomson, 1985). A recent study examined 98 warthog burrows for the presence of *O. porcinus* in MGRand 348 *O. porcinus* ticks were collected from 59 burrows. Despite a 29% increase in burrow infestation and a 59% increase in warthog density since 1978, no evidence of ASFV genome presence could be found in any of the ticks. Sampling was opportunistic and not exhaustive, but these results suggest that that ASFV no longer exists in MGR or that if it does the ASF infection rate is extremely low and restricted to a small number of warthog burrows (Arnot et al., 2009).

After the discovery that *Ornithodoros erraticus*, commensal in pigsties in Spain, was able to maintain and transmit ASFV to domestic pigs (Sanchez Botija, 1963), investigation in East Africa revealed the presence of ASFV in *Ornithodoros porcinus* collected from warthog burrows (Plowright et al., 1994), and demonstrated that these ticks were a competent vector in which both transovarial and sexual transmission of the virus occurred (Plowright et al., 1994). The replication of ASFV and the pathogenesis of ASF infection in *O. porcinus* have been studied in detail (Burrage et al., 2004; Kleiboeker et al., 1998, 1999; Kleiboeker and Scoles, 2001) using southern African isolates. It was demonstrated that replication of ASFV must take place in the midgut epithelium of *Ornithodoros* for infection of pigs to occur when the ticks feed on them (Kleiboeker and Scoles, 2001).

Following the recovery of infected eyeless ticks from warthog burrows in Kenya, Tanzania and Uganda, infected ticks were recovered from warthog burrows in South Africa and Namibia (Thomson, 1985; Thomson et al., 1983), Zambia (Wilkinson et al., 1988) and Zimbabwe (Hess et al., 1989). The study in Zambia determined that infected ticks were present in four areas in different parts of the country and that infection rates were sometimes fairly high, particularly in adult ticks. In all the studies, the rate of infestation of warthog burrows varied considerably, both in the number of burrows infested and the size and stages of the tick population in the burrow; in East Africa a burrow containing 30,000 ticks, mainly second and third stage nymphs, was reported, but such a high number is unusual (Penrith et al., 2004b). The number of infested burrows in an area varied from fewer than 10–100%, and the proportion of ticks infected was also variable, although generally low (0.3–1.7%), but considerably higher if only adult ticks are evaluated (Penrith et al., 2004b; Wilkinson et al., 1988).

Although there was a clear association between warthogs, ASFV and *O. porcinus*, the way in which the virus circulated between warthogs and ticks remained to be clarified, because the incidence of viremia was low in warthogs sampled and if present the amount of virus was very small (Thomson, 1985; Thomson et al., 1983). However, experimental infection of 3-month-old naïve warthogs demonstrated that in spite of showing no clinical signs of ASF the piglets developed generalized infection with marked viremia that persisted for 11 days (Thomson, 1985; Thomson et al., 1983). Comparable levels of viremia were measured in warthogs between one and two weeks old recovered from burrows and it is therefore likely that the majority of warthogs are infected in the first few weeks of life, during which time they develop viremia sufficient to infect ticks that feed on them (Plowright et al., 1994; Thomson, 1985). After this generalized infection, the virus localizes in various superficial or visceral lymph nodes, where the quantities present remain high for several days or months. Adult warthogs remain infected for life and there is no evidence to date that subsequent viraemic episodes can occur (Thomson, 1985). That this is the only way in which warthogs can become infected has been questioned in a recent study in Kenya, which found that ASFV recovered from *Ornithodoros porcinus* inhabiting warthog burrows differed at genome level from ASFV recovered from adult warthogs in the same area (Gallardo et al., 2011b). This requires further investigation, as pointed out by the authors, since the warthogs sampled could not be definitively associated with the burrows from which the ticks were collected.

In a survey carried out in Malawi, *Ornithodoros* ticks were collected from houses and pig shelters in villages in the endemically infected area as well as from a warthog habitat outside the endemic area (Haresnape and Mamu, 1986). Overall in the endemic area 0.3% of ticks collected from pig shelters were infected with ASFV, but in individual villages the infection rate was as high as 12% (Haresnape et al., 1988). The ticks were collected by hand by a number of different individuals and infestation rates were not specified, but the ticks were reported to be numerous in some villages and absent from others (Haresnape and Mamu, 1986; Haresnape and Wilkinson, 1989). The warthog habitat investigated was infested with *Ornithodoros* but a sample of 1400 specimens proved negative for ASFV. Collection of *Ornithodoros* from three villages in Malawi shortly after an outbreak of ASF revealed an overall infection rate in ticks of 24%; this declined over time but eight months after the outbreaks infected ticks were still present (Haresnape and Wilkinson, 1989). It has since been demonstrated that virus can persist for several years in quiescent ticks that had no opportunity to feed on infected pigs (Basto et al., 2006). The ASF endemic area of Malawi forms part of a larger endemic area that includes adjacent districts in Eastern Province in Zambia and Tete Province in Mozambique characterized by frequent outbreaks of ASF with lower than expected mortality (Haresnape et al., 1985; Penrith et al., 2004b). Earlier investigators interpreted the infection pattern as the result of infection with viruses of lower virulence, but a study in the Angónia District in Mozambique, adjacent to the endemic area in Malawi, indicated that a high proportion of serologically negative pigs survived natural infection with highly virulent virus, suggesting that the pigs have adapted to the virus rather than the virus to the pigs (Penrith et al., 2004a). The mechanism

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for survival has not been elucidated as only one of the 105 offspring of serologically positive pigs imported from Mozambique survived experimental infection with the same viruses (Penrith et al., 2004a).

The existence of a cycle of maintenance between domestic pigs and *Ornithodoros* in Malawi raises the question of whether this association exists elsewhere in Africa (Bastos et al., 2009). Haresnape and Mamu (1986) summarized published information about the distribution of the '*Ornithodoros moubata* complex' ticks in Africa and quoted references to its occurrence in pigsties or in association with pigs in Angola, Congo (DRC), Malawi, Rwanda-Burundi, South Africa and Zimbabwe.

In Portuguese speaking countries of Southern Africa (Mozambique and Angola), the association between warthogs, *Ornithodoros* and ASF was never investigated, but the patterns of ASF described by different authors are strongly suggestive of involvement of ticks in maintenance and transmission (Mendes, 1994; Penrith et al., 2007, 2004a). Wilkinson et al. (1988) did not find ticks in pig shelters in Zambia adjacent to the endemic area in Malawi and the inhabitants of the area in Zambia did not recognize the ticks; Haresnape and Mamu (1986) described sites in Malawi where no ticks were found and only the older people recognized the ticks. In a survey carried out in two districts in a non-endemic area in southern Malawi after severe outbreaks occurred there in 1989, *Ornithodoros* ticks were found in only one village and proved to be negative for ASF virus (Allaway et al., 1995). The survival rate of pigs in that area was considerably lower than the rates recorded in the endemic area, suggesting that ASF was epidemic in the districts investigated, as would be expected in an area where ASF was epidemic.

Apart from the role of warthogs in ASFV sylvatic cycle, bushpigs have also been suspected to be natural reservoirs of ASFV. They have a similar distribution in Africa but a different habitat preference (Anderson et al., 1998). Two species are recognized, *Potamochoerus larvatus* in eastern and southern Africa and *P. porcus* in West and Central Africa (Vercammen et al., 1993). Investigations of ASF in bushpigs have been much more limited than in warthogs. In Kenya, apart from an experimental infection reported by Montgomery (1921), ASFV was recovered from tissues of several bushpigs but 46 bushpigs proved serologically negative for antibodies (De Tray, 1963a,b). Apart from spleen of one bushpig, the tissues tested in this study were not specified (Anderson et al., 1998). In Malawi lymph nodes from three bushpigs and spleen and sera from 11 bushpigs proved negative for evidence of infection with ASFV (Haresnape et al., 1985) and in Zimbabwe sera of 10 bushpigs had no antibodies to ASFV (Anderson et al., 1998). In South Africa, the recovery of ASFV from bushpigs in the control area was reported to be ten times lower than the level of infection observed in warthogs from the same region (Mansveld, 1963).

These results seem to suggest that the frequency and prevalence of infection in bushpigs is lower than in warthogs and that antibody detection may not be an ideal way to evaluate infection rates in bushpigs (Jori and Bastos, 2009). The only detection of antibodies against ASF in bushpigs found in the literature has been reported recently in Uganda. In 2010, 4 bushpigs were captured, sampled for blood and equipped with GPS radio collars in the Lake Mburo region. Serum was tested for the presence of antibodies against ASFV with the P72 blocking ELISA (Ingenasa, Madrid, Spain) and two of them were positive (Björnheden, 2011). This finding, together with the fact that monitored bushpigs were found to move in the surroundings of villages and feed on the crops grown by farmers, suggests that bushpigs can become infected naturally and must be considered as a possible reservoir and source of transmission of ASFV (Björnheden, 2011) in Uganda and other countries in the region.

3.2. West Africa

Information about the sylvatic cycle in West Africa has been scarce for many years. In Nigeria, where the disease is endemic, a serological survey undertaken more than 30 years ago in warthogs and bushpigs (Taylor et al., 1977) did not manage to detect antibodies in those wild pig species. More recently, a bushpig from a zoological collection from Plateau State was confirmed to be infected based on molecular detection of viral genomic DNA (Luther et al., 2007a) and lately characterized as being genotype I (Owolodun et al., 2010), confirming the susceptibility of *Potamocheorus porcus* to ASFV. A young hunted warthog from Adamawa State, Nigeria was also found positive for ASFV on PCR (Luther et al., 2007b), and constitutes the only available evidence that ASFV may be able to circulate among warthog populations in West Africa. However, these are isolated incidents and to date there have not been large scale studies on wild hosts in West Africa, except for the one in Senegal described here. In this coastal West African country, the pig sector plays an important economic role in regions hosting the majority of the non-Muslim populations (basically located in the Southern Casamance Region and close to coastal and main cities popular with tourists such as Dakar and Saint Louis), and consists mainly of small scale traditional free-range farming systems in which pigs are not confined for most of the year (Etter et al., 2011). Since the first description of the disease in 1959, Senegal has experienced regular re-emergence of ASF outbreaks compatible with a permanent source of virus originating from domestic pigs but also with the possible existence of a sylvatic cycle, although no

Fig. 5. Type of rodent burrows opening in pig pens and infested by *O. sonrai* in Senegal.

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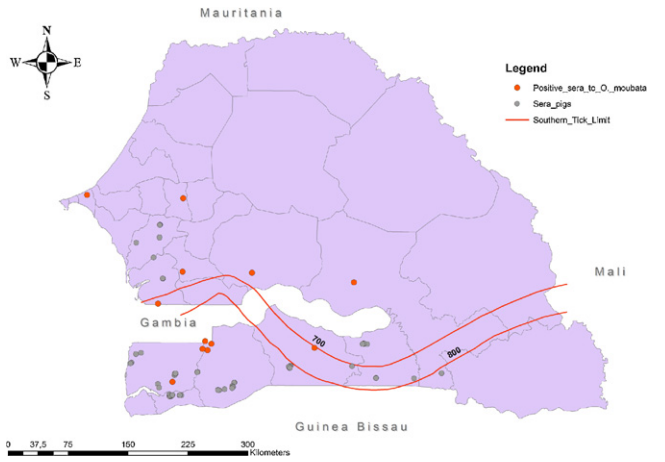

Fig. 6. Map of Senegal showing locations where pigs were sampled and tested for detection of anti-tick antibodies against Ornithodoros erraticus (positive pigs sera shown in red dots). The red line represents the rainfall curve of 800 mm, considered the natural southern limit of the distribution of O. sonrai in 2001.

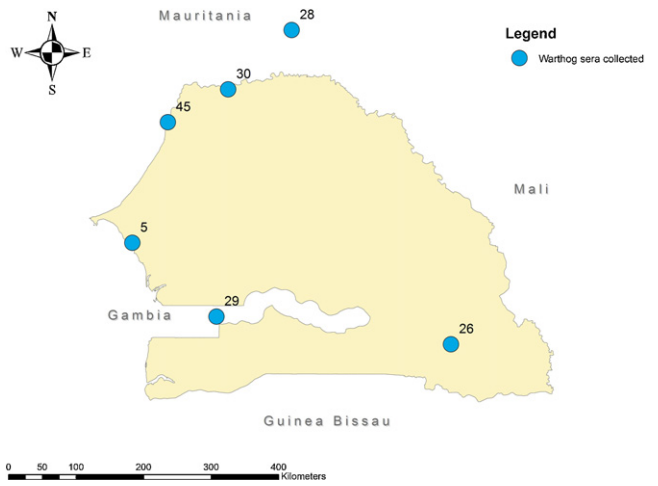

Fig. 7. Sampling spots of warthog populations in Senegal and neighboring countries (Gambia and Southern Mauritania). Figures of the number of animals samples are indicated.

such cycle has been established so far. In addition, the presence of warthogs in many protected areas of the country is common although over-hunting has seriously depleted their populations in some regions. Similarly bushpigs (*P. porcus*), once common in the south of the country, are now almost extinct. The soft tick present in Senegal is *Ornithodoros sonrai*, which is closely related to *O. erraticus* and the *O. moubata* complex, and has been found in rodent burrows within or close to human settlements and pigsties (Fig. 5), with an infestation rate ranging from 8 to 36% in Central Senegal (Vial et al., 2006b). The southern limit of the geographic distribution of that tick species is reported to be below the 750 mm isohyets (Trape et al., 1996) (≈13°30′N in Senegal; Fig. 6). Some remnants of ASFV DNA have been detected in ticks collected within or near pig buildings, which suggests the possible existence of an epidemiological cycle in which ticks act as a reservoir at least in domestic areas (Vial et al., 2007). Only experimental infections of *O. sonrai* by ASFV could confirm such a hypothesis and its role as vector and reservoir of ASFV. However, in wild areas, those ticks were only found in rodent burrows and despite an active search, not a single tick was found after inspection of 48 warthog burrows in the same geographic area (Vial et al., 2007). In the absence of *Ornithodoros* ticks in warthog burrows, it is highly unlikely that warthog populations from Senegal can become infected with ASFV, particularly in the absence of contact with domestic pigs (Penrith et al., 2004b; Plowright et al., 1994).

In order to assess the circulation of ASFV in warthog populations in the Senegalese territory, a serum bank of 162 warthog samples collected between 2003 and 2006 in different hunting camps and nature reserves from six different areas in Senegal and neighboring countries (Fig. 7) was tested with an in-house p72 competitive ELISA test against ASFV in the national laboratory of Dakar. All the results were negative. A pool of 73 of those sera (34 from northern Senegal and 39 from Southern Senegal) were sent to the INIA reference laboratory to be tested with the VP73 Blocking ELISA (Ingenasa) and a p30 recombinant in-house ELISA using positive and negative control sera from warthogs provided by the ARC-OVI regional reference laboratory in South Africa. All the sera from warthogs were again found to be negative for ASFV antibodies with both ELISA tests, despite the warthog control sera being confirmed as being positive and negative for both tests. This suggested that the 163 negative results obtained in the other surveys in 6 different locations in the region can be considered as valid, which supports the hypothesis that the disease does not circulate in natural warthogs populations in that region. However, it needs to be acknowledged that a proper sampling strategy designed to demonstrate the absence of infection could not be implemented and therefore the absence of ASFV circulation cannot be confirmed with sound and complete epidemiological data.

In order to assess contacts between soft ticks and warthogs, 79 sera from warthogs and 129 sera from domestic pigs were tested with an anti-tick ELISA test developed for *O. erraticus* All sera from warthogs were negative with this test, despite the fact that anti-tick antibodies were detected in 9.3% of the domestic pig sera. Moreover, 42% of the pigs were within the defined boundaries for tick distribution and the remaining 58% were at an average distance of 50 km south of that boundary (see Fig. 6).

These results provide further support to the hypothesis that, as suggested by Vial et al. (2007), *O. sonrai* is not found in warthog burrows and as a result, warthogs in Senegal and neighboring countries rarely become infected with ASFV. The fact that the ELISA test against *O. erraticus* salivary glands is able to cross react with antibodies against *O. sonrai* in domestic pigs, provides an additional indication of the genetic and antigenic proximity between *O. erraticus* and *O. sonrai*.

3.3. Indian Ocean

Two islands of the Indian Ocean – Madagascar and Mauritius – have received incursions of AFSV with a different degree of involvement of potential wild hosts of the disease.

3.3.1. Madagascar

ASFV arrived on the southern coast of Madagascar (most probably Fort Dauphin) in 1997–1998 in provenance from Mozambique (Gonzague et al., 2001), devastating more than half of the domestic pig population (Roger et al., 2001). It then evolved into an enzootic disease. One of the questions raised by this situation is whether wild hosts play a role in the persistence of the virus. Indeed, soft ticks of the *Ornithodoros moubata* complex had been described in the island since the 18th century but previous reports indicated their progressive disappearance from north-western areas (Rhodain and Fontenille, 1989; Roger et al., 2001). In addition, *Potamochoerus larvatus* occur in large numbers in the dry and tropical forest areas of the island and they are widely hunted and used as a source of meat and income (Andrianjakarivelo et al., 2003).

In a field survey, 35 tissue samples and 27 sera collected from bushpigs captured and slaughtered by local hunters in the north-western part of the country were screened for the presence of

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Fig. 8. Type of pig shelters (outside and inside) infested by *O. porcinus* in Madagascar.

ASFV and ASFV antibodies in two separate reference laboratories in Europe (Ravaomanana et al., 2011). It should be noted that this sampling was opportunistic and it is not known how representative the sample is of the target population because the total population of buhspigs in the study area has never been quantified. Sera were screened with two different common ELISA tests and one immunoblotting technique (Perez-Filgueira et al., 2006). All samples from bushpigs (sera and spleen) were found negative for both antibodies ($n = 27$) and virus ($n = 35$). Our results suggest that prevalence of ASF virus in the sampled population of Madagascan bushpigs may be low with a sporadic pattern or non-existent, supporting the hypothesis that up to the period of the study, bushpigs have played a minor or insignificant role in the maintenance and dissemination of ASF in Madagascar.

In addition, serum samples from the 27 bushpigs were tested for the presence of antibodies against saliva antigens from *Ornithodoros moubata* (Oleaga-Perez et al., 1994). They were all found negative, suggesting no contact between bushpigs and ticks. Conversely, seven of 126 serum samples collected from domestic pigs in an area close to where the bushpigs were captured (5.6% of the sample), originating from seven different farms, were positive for anti-tick antibodies. However, direct tick searches in those farms did not confirm the presence of ticks and likely suggested a lack of specificity of the anti-tick ELISA test or possibly the existence of confounding factors because pig serum sampling and tick searches were delayed for periods from several months to one year (Ravaomanana et al., 2010). Tick searches were also conducted in two other pig production regions of Madagascar where ASF outbreaks have been regularly reported, the Central region near Antananarivo and the eastern region near Ambatondrazaka. Among 105 farms visited, *Ornithodoros* soft ticks were found in only one farm from Mahitsy, 150 km from Antananarivo, where the presence of ticks had been reported ten years previously (Ravaomanana et al., 2010; Roger et al., 2001). Among 182 ticks tested, 2 out of 21 ticks collected in 2000 (9.52%) and 11 out of 161 ticks collected in 2007–2008 (6.83%) were found naturally infected with ASFV (Ravaomanana et al., 2010). Enquiries to farmers indicated that no pigs that could have acted as a possible source of ASFV had been held within the infested buildings for at least 4 years. The structure of the buildings where *O. moubata* was found can be seen in Fig. 8. These results suggest that local *O. porcinus* ticks may be possible vectors and long-term natural reservoirs of AFSV in Madagascar but their epidemiological role is considered limited considering their restricted distribution range in that country.

3.3.2. Mauritius

The first cases of ASF ever detected in Mauritius occurred in Roche Bois, close to Port Louis, in June 2007 and the disease was officially confirmed in October 2007. During the first half of 2007 several outbreaks occurred in different countries in the eastern coastal region of Africa (Madagascar, Mozambique and Kenya), which could have acted as a potential source of the introduction into Mauritius. Genetic characterization indicated that the 2007 and 2008 ASF outbreak strains from Mauritius grouped with other genotype II viruses which caused outbreaks in Mozambique and Madagascar from at least 1998 to 2007, with 100% nucleotide similarity (Lubisi et al., 2009). Drastic control measures such as targeted stamping out, biosecurity measures, movement control of pigs and pig products and closure of the abattoir were implemented until March 2008 and about 13,000 pigs were culled (Lubisi et al., 2009; Penrith et al., in this issue). The last outbreak was reported in July 2008 in Olivia. Since then, no further cases have occurred. However, there was some concern that potential wild hosts present in the island could maintain the disease and allow it to re-emerge. Therefore, some information was collected on the hypothetical role of potential wild hosts that could maintain ASFV to perform an assessment of the risk of re-emergence of the disease (Etter et al., 2010). The 'wild pigs' of Mauritius are in fact feral domestic pigs (*Sus scrofa*) that were introduced by sailors in the beginning of the XVIth century (Oliver and Brisbin, 1993). They are therefore highly susceptible to ASF, excreting virus in similar quantities to domestic pig (McVicar, 1984; McVicar et al., 1981). However, some authors have suggested that, at least in the case of wild boars, they are less efficient than the domestic pigs in transmitting the infection to other conspecifics (Laddomada et al., 1994) due to the fact that densities are lower than in the case of captive domestic pigs.

At the time of the outbreak, the population of feral pigs in the island was estimated at 6000 heads, but real figures were difficult to assess. These animals roam freely on hunting estates in mountainous areas of the island at low densities, but the actual numbers are very uncertain due to the large size of the estates (several hundred hectares) and the thick forest vegetation. A limited number of farms have occasionally maintained feral pigs in feedlots. At the time of the introduction of ASF, only two estates rearing feral pigs

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in feedlots remained. Animals were kept in small paddocks of 2.5 ha and had no contact with domestic pigs. Nevertheless, some fatalities due to ASF occurred in those populations, reaching up to 50% in some of the paddocks. It seems very plausible that the feral pigs in those feedlots were infected by consumption of infected swill obtained from the harbor. Swill feeding was a very common practice in Mauritius and is suspected to be the most likely route of introduction of ASF into Mauritius (Lubisi et al., 2009).

According to the information collected during a survey in 2009, there were reports of free-ranging animals in some hunting estates being affected by the disease and dying. They could have been infected through contact with feral pigs in feedlots that were contaminated by swill. The disease in the free-ranging population of feral pigs was reported as not lasting long, probably due to the low densities of the population which were unable to maintain the virus. However, those reports could not be confirmed in the laboratory, and should be considered with caution, since the animals could have died of other causes.

Subsequent surveillance of the remaining feral pigs in Mauritius did not reveal any clinical signs of the disease during the last 12 months following the last outbreak. A random sample of 12 feral pigs within the age class 6–12 months was sampled in the two remaining farms. No positive results with PCR or with Blocking Elisa provided any virological or serological evidence of ASFV in the sampled feral pig population. Young pigs born after the last outbreak were sampled and were also found negative. Despite the fact that the sampling undertaken in feral pigs was limited and opportunistic and therefore not necessarily representative of the total population, no new cases occurred after the disease was controlled in the domestic pig chain, suggesting that, as reported in wild boars (Laddomada et al., 1994; Perez et al., 1998), in the absence of recurrent infection from domestic pigs ASFV is not able to persist in natural feral pig populations.

The presence of ticks in Mauritius has not been documented. Therefore it was suspected that they do not occur on the island. The limited information collected from medical doctors regarding the occurrence of human relapsing fever (borreliosis) in Mauritius, which relies on the same vector host as ASF (Vial et al., 2006a), also seemed to suggest that *Ornithodoros* is not present in Mauritius. However, to confirm their absence the samples of 12 feral pigs and 336 domestic pigs chosen randomly across the national territory were tested for the presence of antibodies against the salivary antigens of *O. moubata* and *O. erraticus*. The results of the anti-tick ELISA tests, using the SGE and the deglycosylated SGE to eliminate false positive reactions, confirmed the absence of antibodies to salivary antigens of *Ornithodoros* spp. These results provide convincing evidence of the absence of *Ornithodoros* ticks in Mauritius and allowed us to conclude that the risk of the re-emergence ASF after its eradication due to a hypothetical sylvatic cycle was negligible (Etter et al., 2010).

4. Discussion

4.1. Limitations of field sampling and diagnosis methods

As described earlier, the study of wild hosts of ASFV is often challenging and requires specific sampling techniques that are different from those employed in the study of diseases in domestic pigs or hard ticks. Those methods have, however, their weaknesses. The conservation and quality of the samples collected by hunters is often a challenge. Another important issue when sampling wild populations is the lack of basic demographic and epidemiological parameters that are needed to design and calculate sample sizes or to interpret or extrapolate the results at population level. In wild pig populations, as in most other free-ranging wildlife, the actual population size and distribution in the study area is often unknown, as is the disease distribution and the distribution of the samples collected. Sample collection is often opportunistic and prone to several biases such as the age of the animals or sampling being limited to a particular area or season (Duncan et al., 2008; Seo et al., in press). It is important to be aware of those biases in order to minimize and quantify them when possible.

A second type of bias, called measurement bias, relates to the actual test that is used to detect disease in wild species. In ASF studies, common diagnostic tests for domestic pigs are often used in wild pigs despite never having been validated in the sub-Saharan African species. This can lead to over- or underestimation of ASFV burdens within wild pig populations under study (Wobeser, 2006). In the case of warthogs, some specific ELISA tests have been compared (Gallardo et al., 2009) and the comparative serology results using recombinant protein-based ELISAs and OIE-ELISA strongly suggest that the pB602L and p54-based ELISAs are more sensitive than the OIE-ELISA test, or the well-known antigenic protein p30 (Perez-Filgueira et al., 2006). Despite the fact that validation requires an extensive number of samples and test replication, it is critically needed in order to be able to correctly interpret the results from screening surveys in wild pig populations such as warthogs.

In terms of diagnostics, an approach that has been insufficiently studied is the possibility of detecting ASF antigens or antibodies in fecal material. This method, which has provided very good results in the study of viruses in wild primates (Neel et al., 2010) and more recently, promising prospects for the detection of classical swine fever virus antibodies in fecal samples of wild boars (Seo et al., in press), would offer huge advantages in terms of sampling large numbers of wild individuals without the constraint of having to physically capture the animals.

When sampling *Ornithodoros* ticks, considering the variability of reported infestation rates and type of infested habitats in wild and domestic areas in the African and Indian Ocean regions, tick sampling methods must be adapted to optimize success of examination. The manual method of tick collection (Fig. 3A) has proven to be laborious, time consuming (30 min–3 h for each manual collection session) and awkward in the case of large-scale studies. Conversely, carbon dioxide trapping or vacuum aspiration allows the collection of large numbers of ticks each time within short periods. In addition, they also allow for the collection of ticks at different stages of maturation, including small larval and nymphal stages that are difficult to detect by the manual method. It has been found that trapping sensitivity is 7 times higher with carbon dioxide trapping than with the manual method (C. Martins, personal communication). When planning tick trapping activities in a study area, it is preferable to use at least one of the methods that have been used elsewhere in the region, so that results can be comparable. Another criterion for tick sampling method selection is the type of habitat examined. The manual and carbon dioxide trapping methods are more convenient for sampling ticks in pig buildings since ticks can be removed from crevices or holes with a shovel and are attracted by dry ice. In deeper habitats like warthog or rodent burrows in the wild, aspirating may be better with the use of the plastic tube extension (see Fig. 3B). Access to material according to local conditions is also an important factor. Although traps are very easily handled and assembled and the material is inexpensive, dry ice may be difficult to find in some countries and it cannot be stored for more than 3 days at room temperature (gas production ceases). Conversely, the manual and aspiration methods can be used in any field conditions since sampling material is easy to find (except perhaps the petrol-mulching blower/vacuum) and inexpensive. The vacuum operates with petrol and is totally autonomous. Finally, some methods may be more or less hazardous for collectors. Because traps can be left on site without the presence of the operator, this method greatly reduces the exposure of the operator to tick bites, unlike manual and aspiration methods

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(important to wear protective clothes). Aspiration also exposes the operator to harmful petrol fumes (important to use protective mask) and precautions should be taken to ensure that the warthog burrows are not inhabited by potentially dangerous animals.

Despite the fact that ASFV has been detected in ticks since the 1960s, more efficient molecular techniques such as the nested PCR have been developed (Basto et al., 2006). However, this method presents high risk of contamination and new very sensitive detection tools like real-time PCR should be developed. Another perspective is the enhancement of tick rearing and experimental infection since they are essential tools to assess epidemiological or ecological parameters that can be used in tick distribution or risk assessment models and they are the only way to study and confirm the competence of ticks as vectors or reservoirs of ASFV. In the past, many studies investigated the ability of *O. moubata sensu lato* and *O. erraticus* to transmit and maintain ASFV (Boinas et al., 2011; Kleiboeker et al., 1998, 1999; Kleiboeker and Scoles, 2001; Rennie et al., 2000). This was also the case for potential soft tick vector species present in the Caribbean islands and the United States during the 1980s (Endris et al., 1991; Groocock et al., 1980; Hess et al., 1987; Mellor and Wilkinson, 1985). Such studies showed that an association, whether occasional or permanent, between pigs and ticks of the genus *Ornithodoros* in any country where ASFV was introduced could result in the maintenance of the virus in the vector population, as occurred in Portugal and Spain with *O. erraticus*. Investigations on the potential of various other *Ornithodoros* species to maintain and transmit ASFV in the absence of no prior exposure concluded that all members of the genus investigated could become competent vectors. Nowadays, tick rearing is rarely done because maintaining tick colonies is laborious and time consuming. Only three main tick colonies are currently still maintained: in the UK (Institute of Animal Health, Pirbright), in France (CIRAD, Montpellier) for vector competence investigations, and in Spain (IRNASA, Salamanca) for anti-tick ELISA test development.

The recent introduction of ASFV into the Caucasus where *Ornithodoros* tick species are present should justify new experimental trials to assess the potential competence of new tick candidate species as vectors of ASFV. However, experimental infection methods need to be standardized to allow meta-analyses of results and general comparison between tick species and tick-virus associations, which is not possible with available historical data. Currently, there is no consensus among scientists to determine common indices to draw conclusions about vector or reservoir competence.

Finally, for indirect tick detection or to study potential tick-wild suid interactions, *O. erraticus* and *O. moubata* SGEs can be considered suitable antigens for serological surveillance of these ticks by ELISA tests, but they have some drawbacks. Firstly, its collection is time-consuming and difficult to standardize, and its composition is poorly known and may include non-specific antigens, giving rise to unexpected cross-reactivity. Deglycosylation of these extracts can eliminate some false positive reactions but the more promising tools are the new purified salivary antigens TSGP1 for *O. moubata* and Oe260 for *O. erraticus*. The recombinant TSGP1 from *O. moubata* has been obtained and proved to be more specific than the whole *O. moubata* SGE in experimental conditions (Díaz-Martín et al., 2011) and this recombinant did not react with any sera from wild and domestic pigs taken in Senegal, where *O. moubata* has been never reported. This indicates a high specificity for the TSGP1-ELISA, although further validation of this test will be needed, using a broader panel of well-defined positive and negative sera obtained from wild and domestic African pigs. Another issue of the use of any anti-tick ELISA test in wild suid-soft tick interaction surveys is the relatively short duration of the pig immune reaction against tick saliva. In his experiments, Canals et al. (1990) mentioned an average 3-month period and no other information is available concerning this critical period. In Madagascar, apart from intrinsic lack of specificity of the test used, another hypothesis for the observed discrepancy between tick examinations and the ELISA test results was the delay of one month to one year between taking the pig blood samples and examining the sties for ticks, with possible disinfection of pig premises between both campaigns or loss of pigs because of pig movements for national ceremonies (Ravaomanana et al., 2010). Another approach to investigate tick-pig interactions and to obtain answers about tick and suid exchanges would be the use of highly sensitive population genetic tools to assess gene flows between ticks and wild and/or domestic pigs or to investigate hypothetical hybridization between bushpigs and domestic pigs.

4.2. General results and perspectives for further epidemiological investigations

Results available to date suggest the possibility of epidemiological cycles of ASF additional to those already described. The epidemiology of ASF can differ substantially in each country, depending on the ecology of the different available hosts. In the absence of data, extrapolation from other regions can be misleading and complicate the control of the disease. This review aimed to update the information on different Sub-Saharan African and the Indian Ocean regions and countries and provide some new data on a diversity of situations and countries in that region.

However, important information gaps remain for many Central African countries. Despite the fact that ASF is known to occur and cause substantial losses in several countries such as Angola, Cameroon (Ekue et al., 1989), Congo (Gallardo et al., 2011a) and Democratic Republic of Congo, information on the role of potential hosts such as *Ornithodoros* ticks or the local species of bushpig have been insufficiently explored to date, except for very occasional reports (Ekue and Wilkinson, 1990).

In West Africa and in Madagascar, the roles of *O. sonrai* and *O. p. domesticus* respectively are being tested by experimental infections. Further investigations will be also be conducted to better understand the impact of some extrinsic and intrinsic determinants of vector competence, in order to predict possible variation in competence according to the tick's environment. Another important challenge is to understand the role of ticks in genetic diversification of ASFV, as has been suggested by results obtained in East and Southern Africa where an ancient sylvatic cycle is clearly confirmed. Such investigations would need to be based on tick experimental infections and their long-term monitoring over decades, including the assessment of the virulence of such new tick virus isolates in domestic pigs. This diversification process has not only been postulated for ticks but also for a possible joint effect of ticks and wild suids, which may complicate experimental trials.

The role of bushpigs in the epidemiology of ASF and its potential transmission to domestic pigs, if any, still needs to be elucidated. Unlike warthogs, bushpigs have been demonstrated experimentally to be able to infect in-contact domestic pigs while in the acute phase of ASF, although this was not achieved in all the experiments (Anderson et al., 1998). The fact that they are not known to be associated with *Ornithodoros* ticks therefore does not entirely preclude a possible role for them. Despite the fact that their suspected role as reservoirs is based on the fact that virus has been isolated from them on many occasions in different countries in East and Southern Africa, there are very few instances in which seroconversion has been observed except for one specific case in Uganda (Björnheden, 2011). In the other serological screenings undertaken on that species all the results were negative (Jori and Bastos, 2009). However, it is not known at this stage whether seroconversion in bushpigs is unusual, whether common methods of antibody detection do not perform well enough in that species or whether prevalences of ASFV under natural conditions are too low to be detected in small samples (Ravaomanana et al., 2011).

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Bushpigs are a common source of meat and income in many parts of Sub-Saharan Africa (Nielsen, 2006) and in Madagascar (Andrianjakarivelo et al., 2003; Jenkins et al., 2011). Feeding domestic pigs with offal derived from the carcasses of infected bushpigs has never been explored as a potential transmission route and deserves further investigation (Jori and Bastos, 2009). Equally, bushpigs are occasionally reported to cross-breed with free-ranging domestic pigs in many parts of Africa but this has never been proven. The possibility that they can produce *Potamochoerus* spp x domestic pig hybrids should be tested in captive conditions, since such hybrids could become an additional reservoir host (Jori and Bastos, 2009).

Nowadays, *in silico* models such as those developed in the case of wild boar and classical swine fever can provide interesting simulations to assess the transmission and spread of pig viruses among populations of wild pigs, allowing one to develop scenarios on management strategies (Kramer-Schadt et al., 2007) and the economic impact of those measures (Boklund et al., 2008). However, while those type of models have the potential to be applied in the case of wild hosts and ASFV, their development requires basic epidemiological and ecological information on species abundance, population structure (Kern et al., 1999), spatial distribution of natural populations (Acevedo et al., 2007), and prevalence of disease and intra- or interspecies transmission parameters (Boklund et al., 2008), many of which are still currently unavailable for ASFV and the wild pigs concerned in the majority of countries and situations in Africa and Madagascar. While some of these parameters can be estimated, the quantity and quality of information and data remains very poor for the outputs of those models to be reliable. However, the iterative process of construction of such models can be a useful tool to identify and direct future research to provide key information parameters and data. Another type of information that would be necessary in potential spread models and that has been insufficiently studied to date is the likelihood of potential contacts between domestic and wild pigs in interface areas. Several methods to estimate those contacts between wild and domestic animals can be applied, such as the use of questionnaires (Brahmbhatt et al., 2012; Jori et al., 2011), the application of telemetry to assess home range overlapping (Proffitt et al., 2010) or the use of fecal bacteria such as *E. coli* as biological indicators of contact between two animal populations (Rwego et al., 2008a,b). Since few of these methods have been applied to the study of contacts between domestic and wild pigs (Wu et al., in press), they would be potentially useful in Africa and the Indian Ocean and deserve further investigation and application.

5. Future prospects

The role of wild hosts in the maintenance and spread of ASF is very variable depending on the livestock systems of a given country or region. Indeed, in some areas where the disease is endemic and widespread in domestic pigs, the impact of occasional introductions of ASFV from wild reservoirs to the domestic pig chain might be low or negligible. In other cases, populations of wild swine species might represent a serious challenge for controlling disease spread, as has been suggested for wild boars and their role in the spread of the disease in the Caucasus (Blome et al., 2012) and Central Asia (Rahimi et al., 2010). Circulation of ASFV in natural wild boar populations has been detected repeatedly in several countries in the Caucasus region since its incursion into Georgia in 2007. The high virulence of the ASFV circulating strains in wild boars suggests that it is highly unlikely that the disease will become endemic in the wild boar population (Gabriel et al., 2011). However, wild boars have been suspected to contribute to the dissemination of the disease across different countries in the Caucasus in some instances (Beltran Alcrudo et al., 2008). The presence of local ticks of the genus *Ornithodoros* and the suggestion that any species of *Ornithodoros* seems to be more or less competent as a vector for ASFV are also risk factors for the long-term persistence of ASFV, as has been clearly shown in Spain and Portugal. Therefore, evaluating the presence and role of wild reservoirs is fundamental in order to assess the economic and technical feasibility of a potential eradication plan of the disease (Penrith et al., in this issue).

However, a major constraint for producing further knowledge in this field is obviously the difficulty of obtaining biological samples from many of those wild host species. To address that constraint, additional consideration should be given in the future to the use of non-invasive techniques such as the detection of ASFV DNA, antigens and/or antibodies in fecal samples from wild pigs, which could also be used for genetic purposes. Genetic tools can also play a role in addressing major questions such as assessing the magnitude of gene flows or exchanges between wild host populations.

The development of statistical tools such as Bayesian models can also be a way to evaluate the performance of diagnostic tests in the field, compensating for lack of data, small sample sizes or sample biases (Ravaomanana et al., 2011). Experimental infections in wild hosts appear to be essential to assess and understand host competence for tick and pig species and to monitor the evolution of potential new host-virus interactions (Anderson et al., 1998; Oura et al., 1998a). Data and parameters obtained from observational and experimental studies could provide the basis for developing mathematical models that can predict and simulate the introduction and spread of ASF within and between different populations of wild hosts. Simulation can replace experimental studies when ethical issues constrain experimental approaches. Probabilistic methods including risk assessment could combine in a coherent whole the available information and could target the gaps of information to be addressed by future research. Finally, models containing epidemiological and ecological information from wild pigs can be used to test control scenarios in a cost-effective way.

Thomson and Gainaru (1980).

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