SCIENTIFIC REPORT submitted to EFSA

Scientific review on African Swine Fever[1]

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Scientific reviews on Classical Swine Fever (CSF), African Swine Fever (ASF) and African Horse Sickness (AHS), and evaluation of the distribution of arthropod vectors and their potential for transmitting exotic or emerging vector-borne animal diseases and zoonoses

Scientific review on African Swine Fever

CFP/EFSA/AHAW/2007/02

CONTENTS

1. DEFINITION and DISTRIBUTION

African Swine Fever (ASF) is caused by a complex DNA virus classified in the *Asfarviridae* family, genus *Asfivirus*, which infects different species of soft ticks and wild and domestic pigs. In the vertebrate hosts, the aetiological agent replicates preferentially in monocyte and macrophage cells and causes a range of symdromes and lesions from peracute to chronic and unapparent forms of disease.

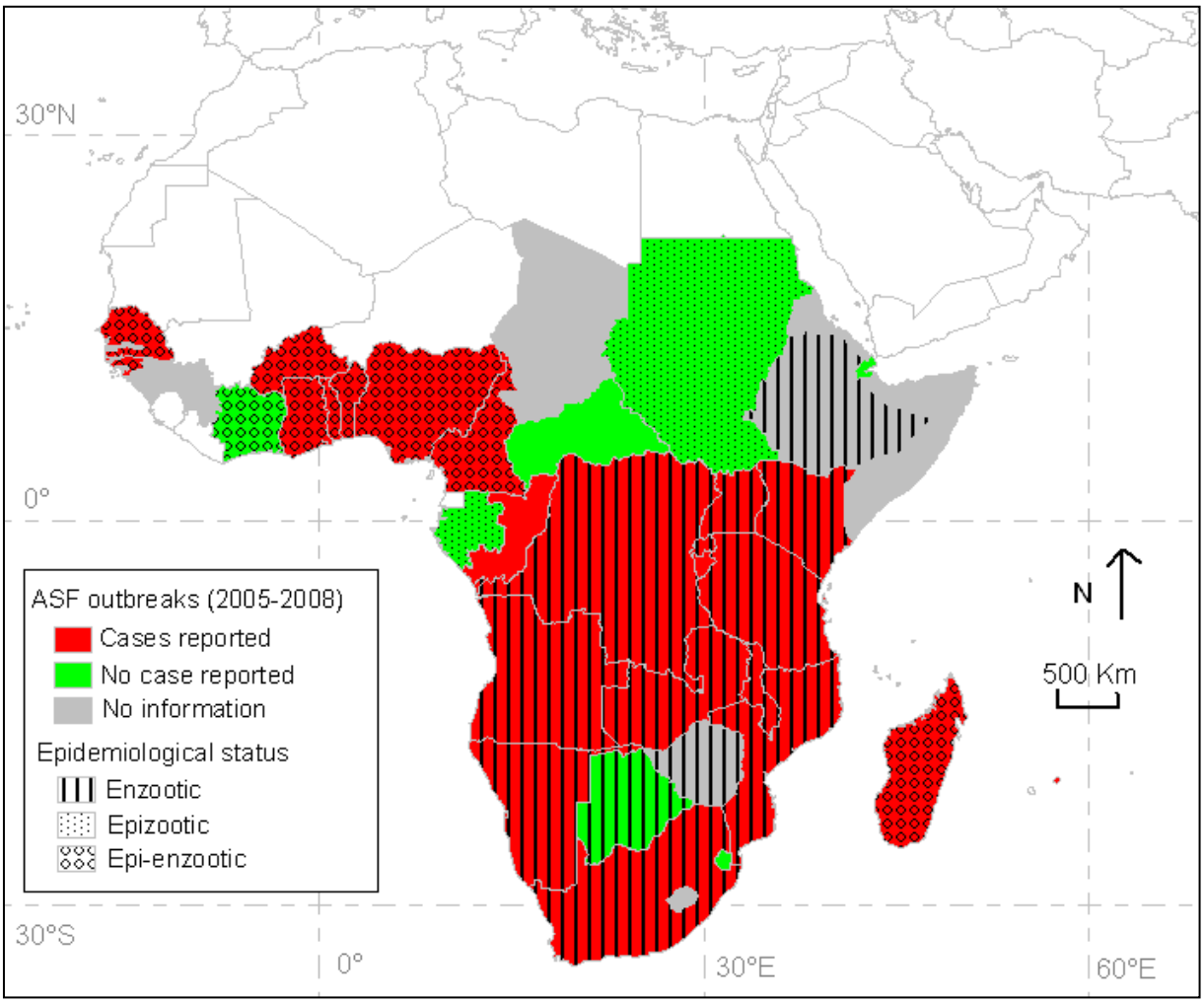
Actually, it is one of the most important diseases of domestic pigs, being of an exceptional seriousness, which can easily spread abroad and for which sanitary and socio-economical consequences have a significant impact on the national and international trade of animals and animal products. For this reason, it is listed as a Notifiable disease to the World Animal Health Organisation (OIE). At present there is no treatment or vaccine available, and control is based on rapid laboratory diagnosis and the enforcement of strict sanitary measures (Sanchez-Vizcaino, 2006). The virus has been maintained in African wild swine for a very long time: in eastern and southern Africa it is found in warthogs (*Phacochoerus aethiopicus*) and bushpigs (*Potamochoerus porcus*) also it is found in soft ticks of *Ornithodoros* genus. The wild swine in Africa can stay infected over a long period without showing any symptom of the disease -and thus can be considered as natural reservoirs of the disease, in contrast to the European wild boar (*Sus scrofa*) which appear to be highly susceptible (McVicar et al., 1988). The virus can be transmitted between *O. moubata* ticks by trans-stadial transmission as well as by sexual and trans-ovarian pathway, in contrast to *O. erraticus* in Europe where only trans-estadial transmission has been observed (Sanchez-Vizcaino, 2006). The asymptomatic wild suids and the transmission among ticks allow a cycle which can be maintained indefinitely in Africa (Parker et al., 1969).

ASF is originally indigenous to the African continent south of the Sahara. The disease made its appearance at the beginning of this century in Kenya (Montgomery, 1921) when the balance between its natural hosts and the infectious agent was altered by the introduction of domestic pigs by colonists from Europe into Africa (Scott 1965, Pini & Hurter 1975). During the first decades it

was confined in Africa until it was detected for the first time outside the African continent in Portugal in 1957, causing hyperacute disease and 100% mortality. After a silent period it reappeared in this country in 1960 (1960-1993; 1999) and was consecutively found in Spain (1960-1995), France (1964), Italy (1967, 1969, 1993), Malta (1978), Belgium (1985) and The Netherlands (1986). (Arias and Sanchez-Vizcaino, 2002a) Except for Sardinia where the disease is endemic all countries managed to eradicate ASF. Recently, the Caucasus region was affected and the infection is believed to remain in South Russia, Georgia and Armenia (2007-2008). In America, Cuba was the first country to be affected in 1971. The disease was eradicated but only after more than 400 000 pigs had died or were slaughtered. In 1978 ASF entered Brazil, spreading to the Dominican Republic (1978), Haiti (1979) and again Cuba (1980). The disease was eradicated from the western hemisphere by depopulation (Arias and Sanchez-Vizcaino, 2002b).

According to the OIE available data, it is noticeable that ASF has occurred in most of East and Southern African countries for the last 3 years (Figure 1). No case was apparently reported in Botswana, Swaziland and Sudan and no information is available for Zimbabwe, Lesotho, Ethiopia, Somalia, Eritrea and Chad. For some countries like Chad, Sudan, Somalia and Eritrea, pig production is not highly developed, leading to low risk of ASF even outbreaks have been recorded in the past. For the other countries, because they are surrounded by countries where ASF occurs regularly, such negative results must be considered more likely a lack of official reporting. In Central Africa, ASF has been confirmed in the Democratic Republic of Congo and the Republic of Congo. No case has been declared since 2005 from Central African Republic and Gabon whereas previous outbreaks were previously reported. Finally, in West Africa, ASF outbreaks have been reported in Senegal, Nigeria, Benin, Togo, Burkina Faso, Ghana and Cameroon during the last 4 years. No case was recorded from Ivory Coast and no information is available from Gambia, Guinea and Guinea-Bissau. Considering the current political instability in Ivory Coast, we may consider that no report means absence of complete information.

Figure 1: Map indicating the occurrence of ASF in African countries during the last 4 years (2005-2008) and the epidemiological status of African countries for ASF.



2. AETIOLOGY

2.1. GENOME AND VIRION ORGANIZATION

2.1.1. ASFV classification and taxonomy

The causative agent of the disease is the African swine fever virus (ASFV) a complex large icosahedral and enveloped double strand DNA virus. These features were originally responsible for the classification of ASFV within the *Iridoviridae* family. However, some unique characteristics of ASFV structure and genomic information led the international committee on Taxonomy of Viruses (ICTV) to create a new family, *Asfarviridae* (African swine fever and related viridae) into which ASFV is the unique member and is included in the new *Asfivirus* genus (Dixon et al., 2005). Like all poxviruses, ASFV replicates mainly in the cytoplasm of the infected cells. It shares with the Poxvirus a similar genomic organisation, like hairpins ends of the genome with inverted repeat sequences in terminal position (De la Vega *et al*., 1994). ASFV particles also contain the necessary machinery for both the synthesis and maturation of early viral mRNA.

The characteristics shared by ASFV with other large DNA viruses that replicate in the cell cytoplasm suggest they may have a common ancestor. These different viruses are indeed part of the *Nucleo-cytoplasmic Large DNA Virus* families which regroups *Poxviridae*, *Iridovirdae*, *Phycodnaviridae* and *Mimiviridae* (Iyer *et al*., 2006). Phylogenetic analysis however discriminate the asfarviruses into a distinct clade than the clades of poxviruses and iridoviruses (Raoult *et al*., 2004). It must be noticed that ASFV is the only known DNA virus to be an arbovirus (arthropod-borne virus, Wardley *et al*., 1983). ASFV infects soft ticks of the *Ornithodoros* genus (Plowright *et al*., 1974).

2.1.2. Virus structure

Virus particles are organized as a complex multi-layers structure (Carrascosa *et al*., 1984). Virions are composed by an 80 nm core shell containing the virus genome in a 30 nm nucleoid (forming the nucleoprotein system), surrounded by a first lipid layer (inner envelop) then by a protein layer

forming the icosahedral capsid from 170 to 190 nm in diameter. The capsid results from the assembling of 13 nm hexagonal capsomers, sort of prisms with a central hole. The distance between two capsomers is about 8 nm (from 7.4 to 8.1) and the icosahedral shape allows an assembling from 1892 to 2172 capsomers to form a mature viral particle. The extra cellular virions acquire an external membrane during the budding through the cellular plasma membrane (Rouillier et al., 1998). Their final size is comprised between 175 to 215 nm. The extra cellular virions contain the genome and ASFV enzymes and proteins that are involved in the early genes expression after the virus entry into the cell.

The analysis of the viral particle, either by electron microscopy, confocal microscopy, genetic or biochemical techniques allowed the identification of at least 54 structural proteins with a size ranging from 10,000 to 150,000 Da (Carrascosa *et al*., 1985). Some of these proteins have been localized in the virus. Attachment proteins p12 and p24 are found on the external membrane of the extracellular particles (Alcami *et al*., 1992) while p150, p37, p34 and p14 proteins (resulting from the cleavage of the polyprotein pp220) are localized in the virus core. The external envelope contains also the CD2v (EP402R), the only glycoprotein of the viral particle (Ruiz-Gonzalvo *et al*., 1996). This protein is involved in the virus haemadsorbing property. The VP72 protein (B646L gene) is the main component of the viral capsid and is surrounding the inner virus envelope acquired intracellularly by the non enveloped virions. It is not however an integral membrane protein (Cobbold and Wileman, 1998). A contrario, the most important integral membrane protein is p54 encoded by the E183L gene and localised in the outer virus envelope. The inner viral envelope is very complex since it contains also other viral proteins with transmembrane domains, including j18L (E199L), p12 (O61R) or p17 (D117L) (Alcami *et al*., 1992, Sun *et al*., 1996).

ASFV particles contain different enzymes of replication, an RNA-polymerase-DNA-dependant playing also a role in the mRNA polyadenylation, methylation and capping. Other enzymes are found in the virions like kinase, nucleoside phosphohydrolase, acid phosphatase and two deoxyribonucleases with an action on single strand DNA (Yanez *et al*., 1995).

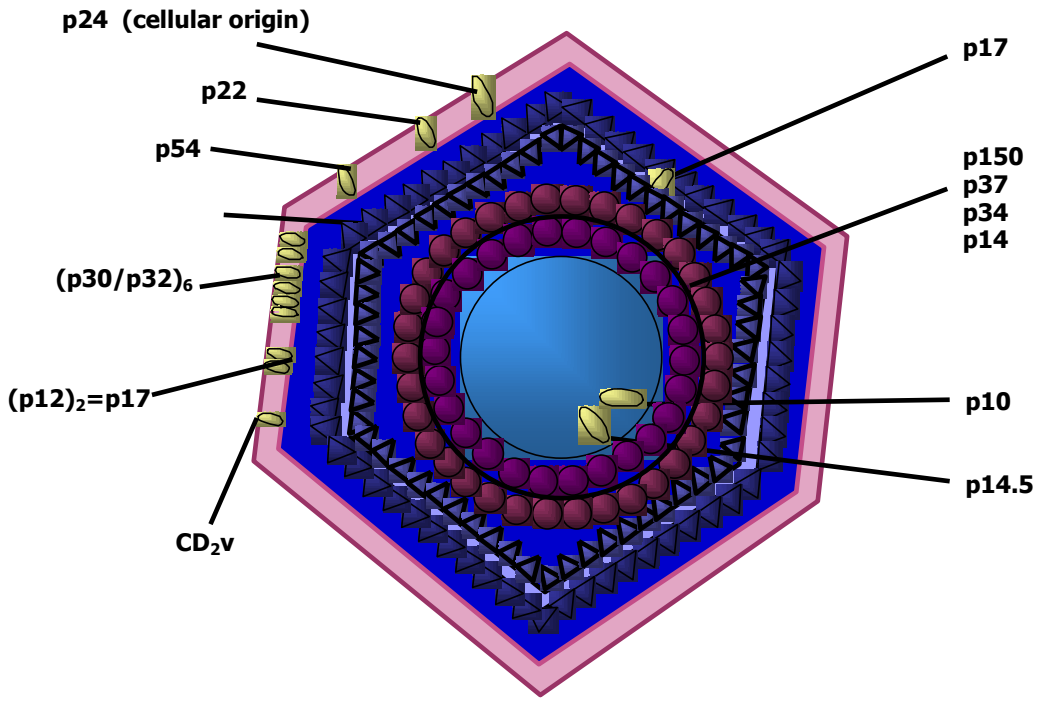


Figure 2: Schematic representation of ASFV. The virus has a core shell containing the nucleoid. The virus core is constituted by the assembly of p150, p37, p34 and p14 (resulting of pp220 cleavage) and by the p14.5 and the p10 proteins. The core shell is surrounded by a capsid composed at 35% with the VP72 protein and with the p17 protein. Then an inner membrane composed by the assembly of p22, p54, p32 (phosphoprotein inserted in a hexamer form), p12 (dimer) and the CD2v (haemadsorbtion protein) surrounds the capsid. Extra-cellular virions possess an external envelope acquired during the budding at the cell plasma membrane. Other proteins are known but remain not well localized like p49 (capsid), p35 and p15 (from the pp62 cleavage) as well as some enzymes contained in the virion.

2.2. STRAINS, GENETIC TYPING AND VIRULENCE

2.2.1. Antigenic variability

The comparison between isolates in term of genome size and enzymatic restriction profile revealed a high level of variability. This variability comes mainly from 35 kb at the 3' end of the genome and from 15 kb at the 5' end (Wesley and Tuthil, 1984, Blasco *et al*., 1989a, b). These two regions of the genome contain the multigene families (MGF) and are the principal sites of insertions and deletions, up to 9 kb (Gonzales et al., 1990; de la Vega et al., 1990; Almendar et al., 1990; Vydelingum et al., 1993; Yazawa et al.; 1994). The same characteristics have been observed in the genomes of isolates adapted on monkey fibroblast cell culture (Blasco et al., 1989a; Tabares et al., 1987). These variations are due to a change in the number of genes in the MGF, allowing a large diversity between isolates by gene homologous recombination. A diminution in the number of MGF genes seems to be associated with lower virus virulence (Tabares et al., 1987).

Comparison of the ASFV genomes showed that 85% of the encoded proteins were identical between viruses, the more variable ones belonging to the MGF. Variability is also frequently generated by a change in the number of amino-acid in tandem repeats. These repeats have been detected in 14 viral proteins among which the CD2 homologous (EP402R), the α-like DNA polymerase and the p54 protein (E183L) (Yanez et al., 1995; Rodriguez et al., 1994).

Another possible distinction between isolates is the inhibition of the haemadsorbtion properties of the virus using sera of infected animals. In fact, pigs surviving infection or pigs infected with low virulent isolates generate antibodies that inhibit virus-specific haemadsorbtion (Ruiz-Gonzalvo et al, 1993). This method allowed to distinguish three large groups: group A (Lisbon57, Funchal65 and Katanga67 isolates), group B (Lisbon60, Madrid60 and Angola72) and group C (Mozambique64). Nevertheless, some non-haemadsorbing isolates were then isolated, making this method of classification obsolete because of its lack of fine discrimination.

Antigenic variability between isolates grown both on pig macrophages and Vero cells was determined using monoclonal antibodies against structural viral proteins (Whyard *et al*., 1985). This study assigned 23 isolates from Africa, Europe and South-America into 6 antigenic groups but no discrimination according to the geographical origin of these isolates could be evidenced. Nevertheless, African isolates are more diverse, probably because of the longer period of virus circulation in this continent whereas recent European and American isolates have probably emerged from a common ancestor and have spread within only a period of 40-50 years after the initial introduction in Europe during the 50s.

Recent molecular tools based on nucleic acid amplification and sequencing, have been introduced to better differentiate virus isolates.

2.2.2. Virus genome

The ASFV genome consists of a linear double strand T-A rich DNA molecule from 170 to 190 kb of length depending on the isolates. Such difference in the genome length is resulting from the presence or absence of sequence repeats inserted in tandems in the viral genome during the virus replication. At the termini of the genome, the two DNA strands are covalentely tied by hairpin loops of 37 nucleotides with mainly T and A nucleotides incompletely base-paired (Gonzales et al., 1986). Immediately adjacent to these termini are found inverted sequence repeats of 2.1 to 2.5 kb in length. For the Ba71v isolate which was the first genome completely sequenced, the terminal repeats consist of 2134 nucleotides (nt) with different rearrangements of three different tandem repeats and two different repeats interspersed by unique sequences (Yanez et al., 1995).

The full-genome sequencing of several isolates has permitted to identify between 160 and 175 putative open reading frames (ORFs). These ORFs are located on the two strands of the DNA molecule which therefore can be read in the two directions (Dixon et al., 1994; Yanez et al., 1995). The genome contains gene-specific viral promoters which are able to drive either an early, intermediate or late expression of the genes during the virus replication cycle (Almazan et al., 1992; Almazan et al., 1993; Rodriguez et al., 1996b)

A large part of the genome is composed by five different types of multigene families (MGF): MGF 360, MGF 110, MGF 300, MGF 530 (or 505) and MGF 100. The variability in genome length results from the loss or gain of MGF genes (De la Vega et al., 1990; Yozawa et al., 1994). The central part of the inverted terminal sequence repeats contains 33 sequences of 34 nt associated in tandem-repeats. These motif repeats are also found in other positions of the ASFV genome. Two different types of internal repeats were described: small direct tandem repeats of about 10 to 50 nt localized both in the inter- and intra-genic regions of the genome and long tandem repeats of about 200 nt in the 5' terminal region of the genome (Dixon *et al*., 1994). Also, there is a central variable region of about 400 nt within a highly conserved region of 125 kb (Sumption *et al*., 1990).

The first ASFV genome completely sequenced was obtained from the Ba71v isolate, a Vero cell culture adapted virus derived from an isolate collected in Spain during the 70s (Yanez et al., 1995). The Ba71v genome is 170.101 nt long with a GC% of 38.95 and 151 putative genes. The genes nomenclature was done based on the analysis of this genome and the HindIII restriction profile. So a gene's name is the HindIII restriction fragment followed by the number of amino acid encoded and a letter indicating the orientation of the gene, left (L) or right (R) ends of the genome (Yanez et al., 1995). The adaptation of the initial isolate in cell culture has resulted in the loss of large parts of the genome at its right end. Then, another nomenclature for the genes absent of the Ba71v genome has been used based on the Malawi isolate Lil20/1 and its Sal I fragment (Dixon et al., 1994).

More recently 9 new ASFV isolates were sequenced: a European isolate (AF302811), a Western African one (AF302816) and 7 other isolates from Eastern and Southern Africa (AY261361, AY261360, AY578700, AY261363, AY578704 and AY578706). The European isolate is a low virulent virus isolated in Portugal whereas the 8 other ones are field collected viruses isolated from domestic pigs, wild swine or ticks in Africa. Their genome varies between 171.719 nt for the Portuguese OURT88/3 isolate and 193.886 nt for the Kenya50 isolate, owing to insertions and deletions in the 40 kb left-end and 25 kb right-end of the genome. The genes that are mainly

concerned are the MGF 530, 360 and 110. Some genes like KP177R coding for the p22 protein can have multiple copies in the genome (Chapman et al., 2008).

The genome variations observed between isolates are mainly insertions or deletions. However, the extent of variability in the sequence of the ASFV genome is not well defined. Sequence comparisons often show limited variations with DNA viruses compared to RNA viruses because of the higher degree of nt conservation during replication of the former. Nevertheless, some genes were actually found variable enough to discriminate isolates in different groups.

2.2.3. Genetic characterization of ASFV isolates

The partial sequencing of the B646L gene coding for the major capsid protein followed by the construction of phylogenetic trees showed a common lineage of isolates collected on domestic pigs in West Africa, Europe, Caribbean and South America. These isolates are so genetically related that they cannot be distinguished within the genotype I in spite of almost 50 years of circulation and spread over 3 continents. It must be stressed that these isolates were not established in an African-like sylvatic cycle although for instance European soft ticks are infected and wild boars are susceptible to the disease. In contrast, isolates from ticks and wild swine established in a sylvatic cycle in East and South Africa show more diversity between them, accrediting the thesis of a co-evolution of the virus in its natural reservoirs/hosts (Bastos et al., 2003; Lubisi et al., 2005). Since all domestic pig viruses isolated from East-Africa over a period of 40 years are close together, it seems that the introduction of new variants from wild life to domestic pigs is a rare event, thus supporting the idea that the domestic cycle may run independently from the sylvatic cycle (Lubisi et al., 2005).

Using partial *p72* nucleotide sequences of ASFV, Bastos et al. (2003) characterized numerous field isolates of diverse species, as well as distinct temporal and geographical origins, in order to clarify genetic relationships of ASFV (Fig. 3).

In agreement with previous genotyping grouped by restriction fragment leght polymorphism (RFLP) analysis (Blasco et al., 1989), European, Caribbean, South America and West Africa isolates are all related to genotype I, which is the largest and most homogeneous *p72* genotype, referred to as the ESAC-WA genotype (Bastos et al., 2003), which was introduced to Portugal from West Africa in 1957.

On the African continent, there are two distinct epidemiological regions are recognized:

- The Western and Central parts of Africa, from Namibia to Democratic Republic of Congo and to Senegal, where the unique genotype I is circulating. Its high homogeneity does not allow discerning the exact origin of the virus exported from Eastern Africa to Western Africa and Europe or to differentiate between outbreaks occurring as early as 1959 and as recently as 2000 in West Africa. However, it greatly suggests the absence of a diversification driven by a sylvatic cycle in West Africa although such cycle seems to exist in its original Southern African countries.
- The Eastern and Southern parts of Africa, from Uganda and Kenya to South Africa, where high levels of genetic variations are observed. A total of 22 genotypes are described, with 13 and 14 genotypes in Eastern and Southern Africa, respectively. This high genetic diversity is coherent with the presence of a sylvatic cycle in most of these countries, which plays a crucial part in the epidemiology of the disease (Lubisi et al. 2005, Boshoff et al. 2007). Zambia is particularly genotype rich with seven genotypes being identified, followed by South Africa with six, Mozambique with four, Malawi and Tanzania with three each, and Kenya and Uganda with two each (Lubisi et al. 2005, Boshoff et al. 2007).

Within this latter region, some genotypes (VIII and XIX) are extremely homogeneous and seem to be associated to pig-restricted cycles or pig-domestic tick exchanges whilst some others (V, X, XI, XII, XIII, XIV) were either isolated from domestic pigs, wild ticks or warthogs, confirming their circulation in both sylvatic and domestic cycles (Lubisi et al. 2005, Boshoff et al. 2007). Some genotypes are apparently country specific (V, VI, IX, XI, XIII, XIV, XV and XVI) while others (I, II, V, VIII, X and XII) are not restricted by national boundaries. Because of this co-circulating of

different genotypes in same locations or at same periods (for example, the homogeneous VIII and the heterogeneous XII genotypes in Malawi), it has been difficult to describe until now country- or zone-specific epidemiological patterns related to vertebrate and arthropod hosts and leading to different virulence or pathogenic effects.

However, using *p72* and other molecular markers such as *9RL* ORF, Lubisi (2005) and Boshoff (2007) showed the great interest of genetics to distinguish viruses causing outbreaks that are geographically or temporally related. For example, it was clearly shown that ASF outbreaks occurring in Uganda in 1995 were caused by two different viruses whilst the 1984 and 1990 outbreaks in Burundi were caused by identical viruses (Bastos et al. 2003, Lubisi et al. 2005). Furthermore, the outbreaks occurring in South Africa in 1995 and 1996 and believed to represent two unrelated epizootics, were in fact shown to be due to four genotypically unrelated viruses; these results contrast markedly with the recovery of a unique genotype from the temporally unrelated 1987, 1992 and 1996 outbreaks in South Africa, indicating a prolonged field presence for this virus (Boshoff et al. 2007). Finally, it was shown that the viruses recovered from the single outbreak focus in 1998 in Mozambique belonged to the two unrelated genotypes II and VIII although past outbreaks in 1994 showed the same virus in two geographically distinct regions of Mozambique (Bastos et al. 2004). Prolonged activity of both genotypes has been supported by the recovery of a genotype II virus from outbreaks in Nampula and Cabo Delgado provinces of Mozambique between 2001 and 2003, and a genotype VIII virus from an outbreak in Zambézia Province in 2001. Then, the parallel identification of the genotype II in Madagascar clearly suggested that Mozambique was the most likely source of infection for the 1998 introduction of ASFV into Madagascar (Bastos et al. 2004).

The genetic characterization of ASFV samples from Georgia (2007) carried out by the OIE reference laboratory at the Institute of Animal Health in Pirbright (UK) determined by sequence analysis of 2 genome fragments (from the *p72* and the *B602L* gene) that it belonged to the same genotype that had been isolated between 1993 and 2002 in Mozambique and Zambia, and in 1998

in Madagascar, classified as genotype II (Lubisi et al., 2005) (communication by Drs. Oura and Dixon from the IAH published in proMED post no. 20070909.1886, 9th June 2007)

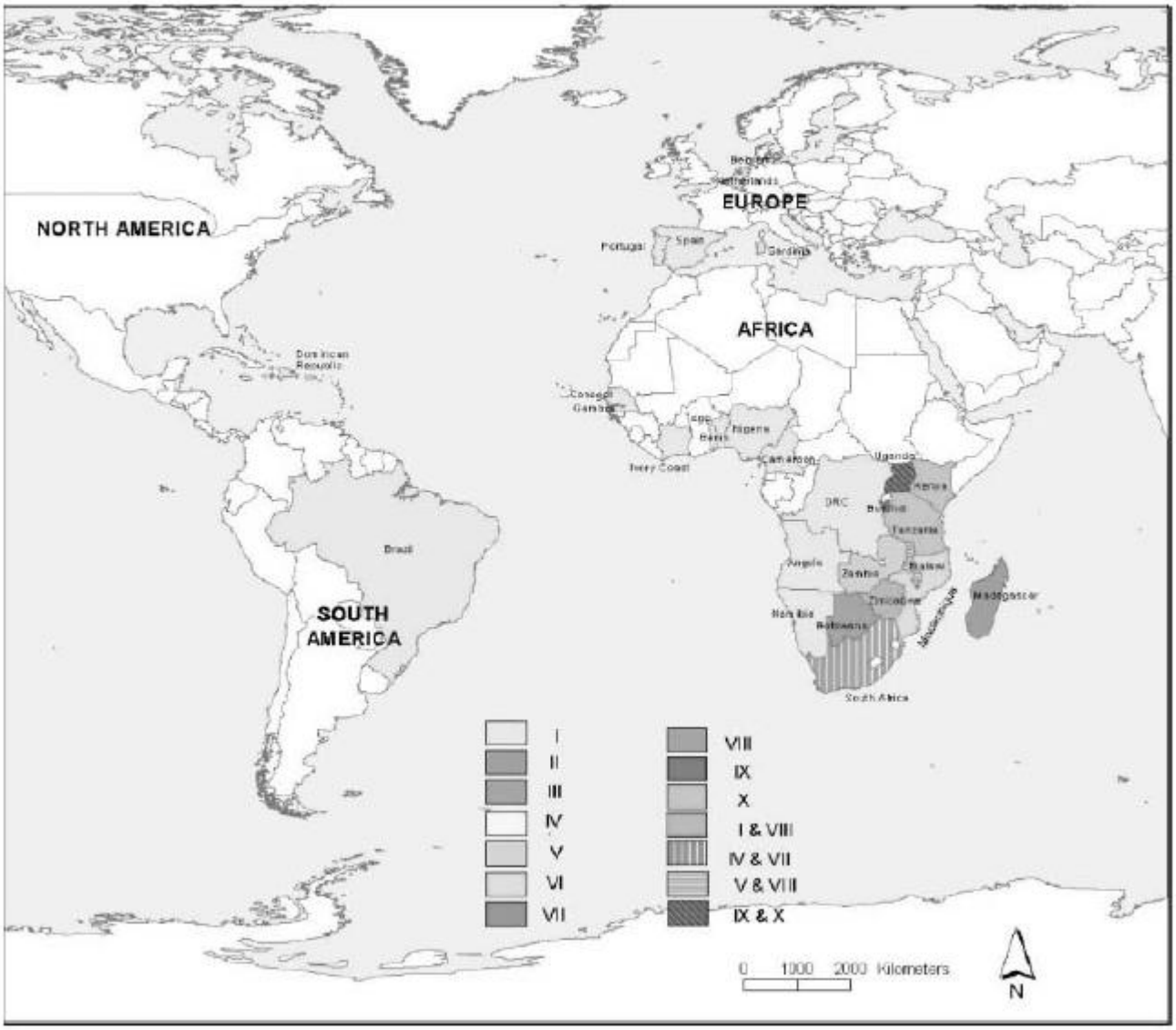


Fig. 3. Geographical distribution of ASFV p72 genotypes (Bastos et al., 2003)

2.3. VIRUS ENTRY – MRNA TRANSCRIPTION – TRANSLATION – REPLICATION

ASFV has a preferential tropism for swine monocytes and macrophages even if different susceptibility to infection can be observed according to the maturation stage of these cells (Wardley et al., 1977, Minguez et al., 1988). The virus can infect dendritic cells as well as endothelial cells, however, the main target cells for the virus replication *in vitro* and *in vivo* remains the macrophages (Rodriguez et al., 1996a, Vallee et al., 2001). At late stages of the infection, the virus can infect other types of cells like megakaryocytes, platelets, neutrophils or hepatocytes (Carrasco et al., 1992, Fernandez et al., 1992a, Fernandez et al., 1992b). Moreover, some ASFV isolates have been adapted on cell cultures like Vero or Cos cell lines. In any case, ASFV replicates in non nucleated cells because it requires a DNA synthesis in the cell nucleus (Ortin and Vinuela, 1977).

The susceptibility of a cell to ASFV infection depends on the cell surface receptor(s). Macrophages which represent the intermediate to late stage of monocyte maturation are the main target of the virus. Actually, permissive cells all express the SWC9 or the CD163 (a cell surface maturation marker, Sanchez et al., 1999, Mc Cullough et al., 1999, Sanchez-Torres et al., 2003). The virus entry involves an endocytosis receptor-mediated pathway possibly using lysosomes. It was shown that the use of drugs increasing the pH in lysosomes inhibited the virus entry into the cells (Alcami et al., 1989, Valdeira et al., 1998). The virus attachment protein is the p12. This protein contains a hydrophobic transmembrane domain anchored into the virus envelope (Angulo et al., 1993). P12 is a late protein with a very high level of conservation among the different viruses. Immediately after entry, very early genes are transcribed in mRNA by the DNA-dependent RNA-polymerase present in the virion. mRNAs are then polyadenylated and capped by other enzymes that are also packaged into the virus particle (Yanez et al., 1993). Essential factors for the early gene transcription have already been described (Salas, 1999), possibly including the proteins encoded by the homologous poxvirus genes A2L (B385R for ASFV), A7L (G1340L) and VLTF2 (B175L) (Iyer et al., 2006). Other mRNAs are produced at early or intermediate stages but they remain silent until DNA replication takes place. At last, late mRNAs are produced. Early promoters are localized at a short distance upstream from the start codon. They are rich in AT and about 50 bp long. Most of the early genes have a sequence of at least 7 T at their 3'-end although a sequence of 10 T is usually required

for the termination of transcription. This particularity may lead into a remote termination of the transcription up to a next termination signal suggesting the possibility of polycystronic mRNAs (Almazan et al., 1992; Almazan et al., 1993; Goatley et al.; 2002). The presence of early, intermediate and late mRNAs is compatible with a gene regulation in cascade.

Even if the virus replication takes place into the cytoplasm (Rojo et al., 1999), the cell nucleus seems to be necessary for DNA synthesis (Garcia-Beato et al., 1992). The replication occurs in some discrete perinuclear areas called "virus factories" (Garcia-Beato et al., 1992). The DNA synthesis required several enzymes like a virus-induced polymerase probably encoded by the G1207R gene, a homologous α-like DNA polymerase (Rodriguez et al., 1993) and three more enzymes: a thymidine kinase (Martin Hernandez and Tabares, 1990), a ribonucleotide reductase (Cunha and Costa, 1992) and a DNA ligase (Yanez and Vinuela, 1993). It is remarkable that ASFV also encodes enzymes for DNA maturation and reparation like an apurinic/apirimidic endonuclease and a β-pol type polymerase (Oliveros et al., 1997). The reason why the initiation of the replication happens close to the nucleus remains unclear to date. Nevertheless the analysis of intermediate DNA replicates showed that at early stages of infection, short DNA fragments are found into the nucleus whereas later on, longer molecules are synthesized in the cytoplasm. These intermediate short DNA molecules could be the precursors for the formation of a full viral genome in the cytoplasm (Rojo et al., 1999). But there is no real information concerning the transport of these small DNA molecules inside and outside the nucleus. However, two nuclear localisation signals have been described on the viral protein p35 and may possibly have a role in the transportation of ASFv DNA molecules into the nucleus (Eulalio et al., 2004).

In infected cells, 81 acid and 14 basic proteins have been identified with a molecular weigh ranging between 10 and 220 kDa (Carrascosa et al., 1984, Carrascosa et al., 1985, Esteves et al., 1986). Like the mRNAs described previously the proteins can be also classified in three distinct groups: the early proteins synthesised before the DNA replication, the proteins synthesised all along the replication cycle and the late proteins. Newly synthesised viral proteins can undergo post-

translation modifications. Thus, the polyproteins pp62 and pp220 are cleaved at Gly-Gly-X amino-acid consensus sequences (Lopez-Otin et al., 1989) leading to the generation of 6 structural proteins as described previously. In addition, some of the virus proteins will be myristoylated (pp220) and many of them being phophorylated (Afonso et al., 1992).

The transcription of the viral genes takes place in the cell cytoplasm and is independent of the cell RNA-polymerases. Early transcripts are needed to undergo to the later stages of the virus replication. 3- the replication of the genome takes place in the cytoplasm. The genome size varies between 150 and 190 kb. 4- expression of intermediate/late genes is dependent on the late stages of replication. Late RNAs encode structural proteins and enzymes which are packaged into the new virus particle. 5- virus assembly takes place in some discrete perinuclear areas called "virus factories". 6- the virus egress is done by budding through the plasma membrane giving to the new virion its external envelope.

Morphogenesis

ASFV morphogenesis is a very complex process which takes place in the same perinuclear "virus factories" (Andrés et al., 1997, Brookes et al., 1996). These factories are adjacent to the Golgi complex, the microtubule network and resemble aggresomes as they are surrounded by endoplasmic reticulum cisternae, mitochondria and a vimentin cage (Andrés et al., 1997, Rojo et al., 1998, Heath et al., 2001). The vimentin cage may have a cytoprotective function, preventing movement of viral components outside the viral factories and into the cytoplasm, and concentrating late structural proteins at the virus assembly site (Stephanovic et al., 2005). These sites are full of partial membrane structures, from one to six faces of the hexagons that prefigure the virus shape and are the first morphological evidences of the virion assembly. In addition, all intermediate forms of the pre-virus are observed up to the fully neo-formed virus (Rouillier et al., 1998).

The intracellular virus particles are formed by a core surrounded by two successive layers: an inner envelope and an outer capsid. The VP72 (B646L) is the major structural protein of the outer capsid. The repression of VP72 leads to the accumulation of zip structures (Garcia-Escudero et al., 1997). These zip structures consist of double envelopes derived from the endoplasmic reticulum (ER). They are assembled by the constitution of one or two protein layers made of the late polyproteins pp220 and pp62 (Andrés et al., 1997, 1998 and 2002). They can move from the virus factory to the plasma membrane and egress by budding (Epifano et al., 2006). When not repressed, the VP72 (B646) protein accumulates on the external face of the ER double envelopes and triggers the folding of the virus icosahedral capsid (Andres et al., 1997). The VP72 assembly requires the presence of the B602L chaperone protein (Cobbold *et al*., 2001). The icosahedral form of the capsid is not only due to the VP72 protein (B646L) but also the pB438L protein which repression leads to the formation of filamentous particles (Epifano et al., 2006). The inner virus envelope derives from fragments of scratched cysternae of ER. These fragments are then recruited in the virus factories where they acquired the outer VP72-capsid (Andrés et al., 1998). The translocation of these fragments into the viral factories would be endorsed by the interaction of the p54 protein (E183L) with the cell microtubule network via the light chain of dynein LC8 (Alonso et al., 2001, Rodriguez et al, 2004). The virus core maturation, including the incorporation of the virus genome, occurs concomitantly to the capsid assembly (Andrés et al., 1997). The assembly of the virus core depends on the viral cystein proteinase pS273R (Alejo et al., 2003). This protein cleaves the polyproteins pp220 and pp62. The resulting 6 major structural proteins p150, p37, p34 and p14 (deriving from the pp220) and p35 and p15 (deriving from pp62) form the core shell, between the viral DNA-containing nucleoid and the inner virus envelope (Andrés et al., 2002). It is also suspected that the polyprotein pp220 mediates the assembly of the nucleoprotein during the late stages of the virus morphogenesis, during the virus particle closure (Andrés et al., 1997).

Virus budding and egress

The neo-formed virions do not have necessarily to be transported for cell egress since the cell death by apoptosis and necrosis allow the destruction of the cell envelope and the release of the virus particles. However, after assembly, the mature virions are normally transported from the virus factories to the plasma membrane (Arzuza et al., 1992). Basically the intra-cellular material movement towards the microtubule organizing centre (MTOC) is done using the dynein-dynactin complex (Vale, 2003). The virus factories where the ASFV morphogenesis occurs are located close to these MTOC (Heath et al., 2001), and mature virus particles are found aligned all along the microtubules. This network is responsible for the virus translocation from the perinuclear sites to the cell plasma membrane (Carvalho et al., 1988; Hernaez, 2006). It was demonstrated that the p54 protein has a LC8 dynein binding motif in the C-terminal part of the protein, from amino acid Y149 to T161. LC8 dynein has a role in the motor protein multicomplex generating minus-end directed movement along the microtubule (King et al., 1996). The cell cytoplasmic LC8 monopolization by the ASFV p54 possibly alters the binding of dynein to cellular targets, promoting the virus transport. Thus the translocation of the virus particles in the two directions, meaning minus-end directed or centripetal transport depends on dynein motor activity (Alonso et al., 2001). The virus movement involves a conventional kinesin which is recruited to both virus factories and virions. This is consistent with the fact that an overexpression of the cargo-binding domain of the kinesin light chain severely affects the movement of the particles to the plasma membrane (Jouvenet et al., 2004). The mechanism whereby the virus bounds the kinesin remains to be elucidated, in particular the identification of the viral proteins involved in this process. In spite of experimental evidence the kinesin could attach the virus through an interaction with the viral protein EP120R. This protein, also named p14.5, has been previously described as a structural protein expressed later after infection (Martinez-Pomares, 1997) and has been shown to be post-translationally modified by acetylation which releases a kinesin binding site (Alfonso et al., 2004). The use of a recombinant virus in which the EP120R gene was placed under the control of an inducible promoter demonstrated that this protein was localized at the surface of the intracellular virions in interaction with the major capsid protein VP72 (B646L). Repression of the inducible promoter inhibited the transport of virus particles to the plasma membrane. However, if the EP120R protein is clearly

required for the intracellular transport of the virus, surprisingly its absence does not affect the infectivity of the virus (Andrés et al., 2001). Infection by ASFV affects the cellular microtubule network increasing the virus protein acetylation as well as preventing depolymerisation and improving stability (Jouvenet et al., 2004).

As previously mentioned, budding through the cell membrane is not essential for the acquisition of virus infectivity. Indeed, the mature intra-cellular virions are infectious and only acquire an outer envelope by budding. It has been observed that the intra-cellular virions are localized close to the inner face of the macrophage cell membrane. In addition, extra-cellular virus particles bind directly to the red blood cells (RBC) (Dixon et al., 2004). This property is due to the expression of the EP402R gene that encodes a viral analogue of the lymphocyte marker CD2. This analogue interacts with a lectin found on the red blood cell membrane and this interaction may play a role in the virus budding, attachment to RBC and virus spread in the peripheral blood.

2.4. VIRUS RESISTANCE-PERSISTANCE

ASFV is a very robust virus and its surprising stability was established for several different isolates. The virus persistence in EMEM medium or serum was shown to be:

- 6 years at 5°C in the dark
- 18 months in serum at room temperature
- up to one month at 37°C
- 3h1/2 at 56°C (notice that a serum is normally safely sterilised after 30 minutes at 60°C).

ASFV is resistant to pH changes that occur during meat maturation. Basically the virus is most stable between pH 4 to 10. But depending on the presence of organic material, virus infectivity can be still demonstrated in serum after 22h, 3 days and one week at pH 3.1, pH 3.9 and pH 13.4, respectively. In addition, the virus can resist 15 weeks in putrefied blood, 11 days in faeces held at room temperature, 18 months in pig blood at 4°C, 150 days in boned meat at 4°C and 140 days in salted dried hams. It was also reported that ASFV persisted for 150 days at 4°C and for 104 days at -4°C in skeletal muscle and for 6 months in bone marrow at -4°C (Kowalenko et al., 1965).

ASFV virus titres were determined in meat samples from infected pigs (Table 1).

Table 1: Recovery of ASFV in meat samples from four experimentally infected pigs by animal inoculation.from McKercher at al., 1978

Product	**Days after slaughter**	**Titre (heamadsorbing units 50% (Had_{50}) per g** (lower value	upper values)
Whole meat	2	$10^{3.25}$	$10^{3.75}$
Ground meat	2	$10^{3.25}$	$10^{3.75}$
Salami	3	$10^{2.0}$	$10^{2.5}$
Salami sausage	9	10^{-1}	
Pepperoni	3	$10^{3.0}$	$10^{3.25}$
Brined ham	2	$10^{2.5}$	$10^{3.75}$

In other experiments on ASFV persistence in pork products, the results of virus persistence were:

- 140 days in Iberian and white Serrano hams (Mebus et al., 1993).
- 399 days in Parma ham (McKercher et al., 1987).
- 112 days in Iberian pork loins (Mebus et al., 1993).

Persistence of ASFV in meat products have been detailed by the determination ASFV virus titres in 65 pig tissues 5 days after experimental infection (Table 2) (Farez and Morley, 1997).

Table 2: ASFV loadings in an infected pig and tissues expressed in HAD_{50} titres. The total loadings for each of the porcine tissues are calculated and the total infectivity in a carcass is estimated at 1.8 x 10^{13} HAD_{50} units. Assuming bone marrow accounts for 10% of the carcass weight, then the

infectivity in bone marrow accounts for 96% of the total carcass infectivity. A "deboned" carcass thus contains 6.4 x 10^{11} HAD_{50} units (Farez and Morley, 1997).

Tissue	Weight (kg)	HAD_{50} /g or /ml	Total loading in pig (HAD_{50})
Flare fat	1.00	$10^{5.4}$	2.5 x 10^{8}
Kidneys	0.26		
Feet	2.00		0
Head, tongue	5.00		0
Gut contents	8.40		0
Intestinal fat	0.84	$10^{5.4}$	2.1 x 10^{8}
Caul fat	0.11	$10^{5.4}$	2.7 x 10^{7}
Intestines	2.70		0
Stomach (maw)	0.55		0
Heart	0.26	$10^{5.6}$	1 x 10^{9}
Lungs	0.90		0
Trachea	0.04		0
Heart, lungs, trachea	1.20		0
Liver, gall bladder	1.50		
Pancreas	0.06		0
Spleen	0.11		0
Blood drained from carcass	3.40	$10^{7.9}$	[d]2.7 x 10^{11}
Cerebro-spinal fluid			0
Skirt	0.35		0
Hair scrapings & hooves	0.84		0
Bladder	0.04		0

Reproductive organs	0.15		0
Lymph nodes	0.04[c]	$10^{8.5}$	1.3×10^{10}
Waste	0.75		0
Bone marrow	5.464[a]	$10^{9.5}$	1.7×10^{13}
Skeletal muscle	43.712[b]	$10^{8.6}$	1.3×10^{11}
Blood in muscle	5.464[a]	$10^{7.9}$	4.3×10^{11}
Total (bone-in)	(62.0)		[d]1.8×10^{13}
Total (bone-out)	(56.6)		[d]6.4×10^{11}

[a]assumes 10% of carcass weight (54.64 kg)

[b]assumes 80% of carcass weight (54.64 kg)

[c]value for sheep

[d]model assumes only 5% of high titre blood remains in the carcass (e.g. in blood clots)

To be free of living infectious virus, ham produced from infected animals should be heated more than 3 hours at 69°C or 30 minutes at 70-75°C. Smoked and spiced sausages as well as air-dried hams required smoking to 32-49°C up to 12h and 25-30 days of drying process (Plowright et al., 1994).

In pig faeces, it was reported that ASFV could persist over 60 to 100 days (Muller, 1973, cited by Hass *et al.*, 1995). More recently, pig slurries experimentally infected with ASFV were shown to retain infectivity for at least 15 minutes at 50 and 53°C but were inactivated after 30 minutes (Turner and Williams, 1999).

Glutaraldehyde 0.2% in a 1:100 (wt/vol) ratio in heart tissue inactivated ASFV after 11 days of exposure at 22 to 26°C (Cunliffe et al., 1979). In another study, the effects on virus survival, of chlorine, iodine and quaternary ammonium in disinfectants were investigated. It was determined that chlorine was effective at concentrations of 0.03% to 0.0075%, with a dose response, iodine at concentrations of 0.015% to 0.0075% but without a dose response and quaternary ammonium compound was very effective at low concentration such as 0.003% (Shirai et al., 1999). The virus is

also very sensitive to lipid solvents and detergents, as well as oxidising agents like hypochlorite and phenol. More over, beta-propiolactone, acetyl-ethyleneimine and glycidaldehyde inactivate the virus within one hour at 37°C. Formalin (0.5%) requires 4 days. However, the virus was found to be resistant to proteases such as trypsin or pepsin and to nucleases. (Bengis 1997; Farez and Morley, 1997; Plowright and Parker, 1967; Plowright et al., 1994).

3. PATHOBIOLOGY

3.1. PATHOGENESIS

Several studies on the ASF acute infection of pigs have shown that the route of viral penetration is usually via the tonsils or dorsal pharyngeal mucosa to the mandibular or retropharyngeal lymph nodes, from where the virus spreads through viremia. Occasionally, bronchial, gastrohepatic or mesenteric lymph nodes have been shown to be the first targets of infection following natural or airborne exposure (Plowright et al.1994). Following initial infection and replication of lymphoid organs, virus spreads through blood and in acute cases viremia may reach 10^8 HAD_{50}/ml. In disease caused by hemadsorbing isolates the virus in blood associates to erythrocytes which contain more than 90% of circulating virus although it is also associated with lymphocytes and neutrophils (Plowright et al 1994). Initial studies on the pathogenesis of ASF in newborn pigs show that primary viremia is identified as early as at 8 hours post-infection and secondary viremia between 15^{th} and 24^{th} hours post infection. Spleen, body lymph nodes, liver, and lungs were shown the sites of the secondary viral growth. At 30 hours, all tissues of the newborn pigs contained some virus, and maximal titers were reached as early as the 72 hours post-inoculation (Colgrove et al. 1969).

ASFV replicates primarily in monocytes and macrophages of the mononuclear phagocytic system that are the main targets for viral replication in vivo (Malmquist W.A. and Hay, D., 1960; Heuschele et al. 1966; Heushele, 1967; Colgrove, 1968; Colgrove et al. 1969; Pan, 1987; Fernandez *et al*., 1992 *a, b;* Moura Nunes et al., 1983; Oura et al., 1998b). However different authors show that ASFV infects also megakaryocytic (Colgrove et al., 1969, Edwards et al., 1985b); endothelial cells (Wilkinson and Wardley, 1978; Sierra et al. 1989; Gomez-Villamandos et al. 1995 a, b, c,), glomerulus's mesangial cells (Pan I, 1987) and epithelial cells of collector kidney tubes (Gomez-Villamandos et al., 1995a), hepatocytes (Colgrove et al., 1969; Sierra et al., 1987; Gomez-Villamandos et al., 1995d), thymus reticulum-epithelial cells (Pan I, 1987), fibroblasts and smooth muscle cells of venules and arterioles (Gomez-Villamandos et al 1995a), neutrophils (Colgrove

1968, Colgrove et al 1969; Carrasco et al 1996b) and lymphocytes (Colgrove 1968; Colgrove et al.1969;Carrasco et al. 1966a) although viral replication in this case has not been demonstrated (Carrasco et al 1966a). Infection of such a broad range of host cells is not accepted by different authors and in some of those cases, cells render infected only in advanced stages of pig infection which suggests they become susceptible for yet unknown reasons, during the evolution of infection thus depending from the initial infection of macrophages. Some authors have suggested the cause of haemorrhage to be associated with viral replication in the endothelial cells of the interstitial capillaries (Sierra et al., 1989). This hypothesis has been refused by others (Gomez-Villamandos 1995b, Carrasco et al. 1997b) who have demonstrated kidney and lymphnode haemorrhages prior to the observation of viral replication in those cells. However coinciding with those haemorrhages the authors have identified endothelial damage consisting with proliferation of lysosomes and phagocytized cell debris, increased capillary fenestration and even necrosis and loss of endothelial cells which resulted in exposure of the capillary basement membrane, to which platelets were attached (Gomez-Villamandos et al 1995b). This may be one of the causes of the disseminated intravascular coagulation (DIC) characteristic of ASF (Villeda et al., 1993,a,b), which other authors relate to the effect on endothelial cells of mediators, including prostaglandin E_2, secreted by the infected macrophages that result in the activation of the clotting cascade and disseminated intravascular coagulation (Anderson et al., 1987) and others suggest this phenomenon may be related to the release of cytokines by infected macrophages, namely to IL-1 and TNF-α (Gomez-Villamandos et al., 2003).

Thrombocytopenia is generally observed in the final phase of acute forms of disease, after haemorrhages are detected and often goes undetected due to the sudden worsening of the affected animals and it has been attributed to consumption of platelets due to coagulopathy (Villeda et al., 1993 a,b), to the direct effect of the virus on megakaryocytes (Gomez-Villamandos et al., 2003) and to various immune-mediated process involving immune complexes of ASF antigens and antibodies that cause aggregation of platelets (Edwards et al, 1985a). Nowadays it is generally accepted that the massive destruction of macrophages plays a major role in the impaired haemostasis due to the

release of active substances including cytokines, complement factors and arachidonic acid metabolites (Penrith et al., 2004)

Pigs infected with ASF generally suffer severe lymphopenia which occurs suddenly during the initial-middle phase of the disease (De Tray et al, 1957). Lymphopenia is attributed to apoptosis of lymphocytes initially suggested by different authors who described morphological features of such death mechanism in spleen of pigs with acute ASF (Konno et al., 1971; Minguez et al. 1988).) On the basis of EM observations in tissues from pigs infected with different virulent isolates, apoptosis has been observed in uninfected lymphocytes in lymph nodes and in the renal and hepatic interstitium kidney and liver tissues (Carrasco et al., 1996; Gomez-Villamandos et al., 1995a; Ramiro-Ibanez et al., 1996; Salguero et al., 2002, 2005, 2008)

Different pathogenic mechanisms have been proposed to explain the programmed cell death of lymphocytes during infection. As shown by others (Carrasco et al., 1996a), replication of ASFV as a cause of apoptosis in lymphocytes is ruled out because ASFV may infect but it does not replicate in them. More recently, production of pro-inflammatory cytokines by infected macrophages is strongly pointed to induce apoptosis on lymphocyte populations (Oura et al., 1998; Salguero et al., 2002, 2005).

Pathogenesis of ASFV chronic infections is not well characterized. Different authors suggest that these forms of disease have an auto-immune component and lesions migh result from the deposition of immune-complexes in tissues such as kidneys, lungs and skin with their subsequent binding to complement (Plowright et al., 1994).

3.2. CLINICAL SIGNS OF THE DISEASE

Distinct ASFV isolates have been reported to induce a range of syndromes varying from peracute to chronic disease, and apparently healthy virus carriers, in parallel with distinct lesions, (Martins and

Leitão, 1994). The more virulent isolates produce peracute or acute haemorrhagic disease, less virulent isolates produce mild clinical signs which can be readily confused with many other conditions in pigs, namely with classical swine fever and in some instances, low virulent isolates produce mainly subclinical and nonhaemorrhagic infection and seroconversion. The incubation period varies from 3-15 days, followed by the development of one or more of the following forms of disease (Plowright et al., 1994; Penrith et al., 2004),

Peracute

Infection with highly virulent virus isolates can result in some pigs being suddenly found dead, or close to death.

Acute (caused by highly virulent isolates)

- Fever (40.5-42°C)
- Early leucopaenia and thrombocytopaenia (48-72 hours)
- Reddening of the skin (white pigs) - tips of ears, tail, distal extremities, ventral areas of chest and abdomen
- Anorexia, listlessness, cyanosis and incoordination within 24-48 hours before death
- Increased pulse and respiratory rate
- Vomiting, diarrhoea (sometimes bloody) and eye discharges may exist
- Death within 6-13 days, or up to 20 days
- Abortion may occur in pregnant sow
- Survivors are virus carriers for life
- In domestic swine, the mortality rate often approaches 100%

Subacute (caused by moderately virulent virus)

Clinical signs resemble those of hog cholera,

- Irregular remitent fever for up one month, followed in most cases by recovery
- Duration of illness up to 30 - 45 days

- Anorexia and loss of condition
- Caughing and dyspnoea specially on exercise
- Death on forced exercise due to heart conditions
- Abortion in pregnant sows
- Death within 15-45 days
- Mortality rate is lower (e.g. 30-70%, varies widely)

Chronic (Caused by low virulent isolates)

- Various signs: loss of weight, growth retardation of growing pigs which have a long hairy coat, irregular peaks of temperature, respiratory signs
- Lameness caused by arthritis that can also become necrotic
- Animals are vulnerable to secondary infections and pneumonia
- Develops over 2-5 months
- Low mortality (less than 30%)

3.3. LESIONS

Gross lesions caused by ASFV infection depend on the form of the disease and ultimately on the virulence of the causative isolate. They can be summarized as follows:

Lesions of peracute forms of disease (Plowright et al., 1994; Penrith et al., 2004)

- Pigs that dye peracutely often do so before either clinical signs or lesions develop. However there may be a degree of skin flushing of extremities and the ventral abdomen in white-skinned pigs and a general congestion of organs, with some fluid exudation into body cavities and possibly fibrin strands on organ surfaces.

Lesions of acute forms of disease (Plowright et al., 1994; Penrith et al., 2004)
(not all lesions are seen; this depends on the isolate)

- Marked reddening to purplish cyanosis of the extremities and the ventral surface in white-skinned pigs
- Oedematous areas of cyanosis in hairless parts
- Cutaneous echymoses on the legs and abdomen
- Mucosal (ocular, oral, genital) congestion and hemorragae
- Tear marks with mucopurulent ocular exudation
- Mucopurulent nasal discharge and bloody froth at the nostrils
- At necropsy, accumulation of straw-coloured blood fluid in body cavities (pleural, pericardial and/or peritoneal) and widespread haemorrhage in organs and on parietal surfaces
- Petechiae in the mucous membranes of the larynx and bladder, and on visceral surfaces of organs
- Congestive splenomegaly. Infarcts are occasionally evident at the margins
- Petechial haemorrhages of the renal cortex, also in medulla and pelvis of kidneys
- Oedema in the mesenteric structures of the colon and adjacent to the gall bladder; also wall of gall bladder
- Lymphnodes, in particular those of the head and the gastrointestinal tract (the hepatogastric and mesenteric lymphnodes) are markedly swollen and hemorrhagic, sometimes resembling blood clots
- Lungs do not collapse when opening the thoracic cavity, are usually congested to hemorrhagic. Inrterlobular septa are prominent due to accumulation of fluid. On cut surface there is exudation of fluid and froth, and the trachea is usually filled with froth which may be blood-stained
- Gastrointestinal tract may appear normal to severely congested and haemorrhages are usually present in the gastric mucosa. Contents of the intestines are generally scant, fluid and sometimes blood-stained, and the rectum may contain bloody fluid or faeces covered with blood mucus

Lesions of subacute forms of the disease (Plowright et al., 1994; Penrith et al., 2004)

- Pneumonia is frequent and leads to serofibrinous pleuritis with pleural effusions and adhesions
- Serofibrinous pericarditis and adhesions that may result in intermandibular oedema
- Joint and tendon sheath effusions with oedema of periarticular tissues
- Lymphnodes are enlarged and hemorrhagic to fibrous

Lesions of Chronic Forms of the disease (Petisca et al., 1963; Penrith et al., 2004)

- **Skin:**

  - Local or generalized varioliform skin eruptions generally desapearing shortly after onset or that may persist due to extension of necrosis and purulent fusion with epidermis, subcutaneous conjunctive or muscle tissues.

  - Multiple abscess and sero-fibrinotic oedema localized in the groin areas, neck, teats which may progress to necrotic and purulent lesions. Skin lesions may heal.

- **Joints**

Purulent or serofibrinotic arthritis

- **Lungs and cardiac lesions**
  - Intersticial pneumonia more common in the apical and cardiac lobes of lungs which may spread. Extension of initial lesions to the entire lung was frequently observed and characterized by read and grey hepatization of lobules which may develop well limited necrosis and further purulent lesions. Necrosis of lung tissue may develop from initial phase of lung lesions.
  - Fibrinosis pericarditis
- **Lymphnodes and Spleen**
  - Lymphnodes in particular those draining affected organs are enlarged with a homogeneous firm consistency. Haemorrages are not common and if developing they localizein peripheral areas.

- Spleen may show varying lesions ranging from enlargement and haemorrhagic to normal size but with dispersed necrotic foci.

Summary as provided by the authors:

Pathogenesis mechanisms of infection by ASFV of different virulence are not yet well understood and pig immune responses against ASFV infection are complex. Antibodies *per se* do not protect against infection and more recent studies emphasize cellular and cellular based mechanisms as relevant for animal survival.

Future research identified by the authors:

Pig macrophages are the main targets for viral infection. Deeper characterization of viral interactions with these cells, and with the domestic pig as a natural host, using viral isolates well characterized at genome level (naturally obtained or experimentally manipulated) may open new insights for the manipulation of pig immune responses towards the stimulation of protective immune responses thus contributing to the development of efficient vaccines.

4. EPIDEMIOLOGY

4.1 VERTEBRATE HOSTS

ASF affects all members of the family Suidae. Wild African suids are considered the original vertebrate hosts of ASF virus in the wild. However, several species and subspecies of wild suids exist and their contribution to the epidemiology of the disease differs depending on the species, the geographic location and their potential contacts with domestic pigs.

In East and Southern Africa, warthogs (*Phacochoerus africanus*) play a demonstrated role as reservoirs of the disease, in association with soft ticks (*Ornithodoros* spp.). Despite buhspigs (*Potamochoerus*) being able to transmit ASFV to domestic pigs by direct contact (Anderson *et al.* 1998), very little information is available about their role as reservoir hosts in Africa and Madagascar. In Europe, wild boar (*Sus scrofa*) populations play some role in the maintenance of the disease and should be considered specifically in the conception of eradication strategies.

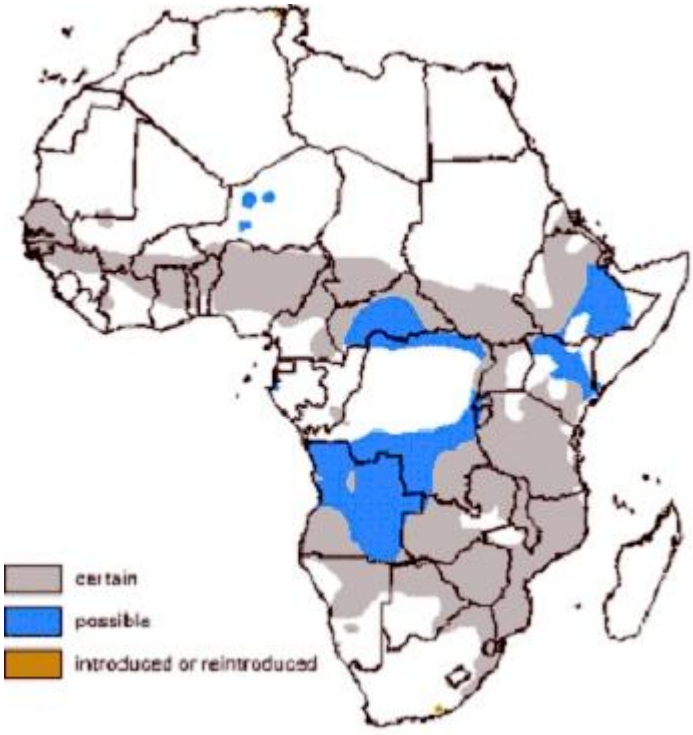
Despite ASF's progressive expansion towards Asia through the Caucasus and the Indian Ocean, important gaps of information remain about what is the real role of those wild *Suidae* regarding the maintenance of ASFV in the wild and their possible transmission to domestic pigs. The different species of wild pigs known to play a role in the epidemiology of the disease are discussed and the main information gaps and priorities for research are highlighted.

4.1.2. The Warthog (Phacochoerus africanus)

Among all wild pigs in the world, the warthog has been considered the most significant ASF vertebrate maintenance host of ASF. The warthog lives throughout Africa south of the Sahara, except in deserts, high mountains and dense forests. It is the most common of the three African *Suidae*, and it is widespread in all types of grasslands and open woodlands. Two different species are distinguished: i) The common warthog (*Phacocheorus africanus*) extending over most of the grassland/savannah ecosystem of the continent, ii) The desert warthog (*P. aethiopicus*) is confined to a small area of north-east Africa from Kenya throughout Somalia, probably extending into the extreme south-east Ethiopia. Almost all available data on this specie (behaviour, ecology and

habitat preferences) are derived from field research conducted in East or Southern Africa (Vercammen and Mason 1993). Population density ranges between 1 and 35 individuals per Km2. Water is a limiting factor and along with den holes is a major element of its home range (between 0.5 and 3.5 Km2).

Fig 5: Warthog distribution (Source: http://www.wild-about-you.com/GameWarthog.htm)



They are predominantly grazers.They spend the night in aardvark (*Orycteropus afer*) dens where the female also builds the farrowing nest. Sounders typically number 5 or less, although groups of 5 to 6 sows with offspring occasionally number up to 16 individuals.

They are seasonal breeders, farrowing lasting between 160 and 172 days when the rainy season begins. In equatorial regions, breeding is not limited to particular months. Piglets remain in the burrow, six to seven weeks and begin grazing between 2-3 weeks of age. Weaning takes place between 2 and 6 months of age, average litter size being 3 with a range of 1 – 7. Unlike other species of wild pigs, interbreeding with other suids has never been reported.

The warthog/Ornithodoros moubata cycle for ASFV

ASF is maintained in Africa by a cycle of infection between warthogs and soft ticks from which is unlikely to be ever eliminated The role of warthogs in the epidemiology of ASF is well described in the literature (Thomson 1985;Wilkinson 1988; Plowright et al. 1994). High infection rates occur in most warthog populations examined in East and Southern Africa although there are geographical differences regarding seroprevalence of ASF in warthog populations in those areas, even between geographically close locations (Wilkinson 1989). In Tanzania (Serengeti region) the prevalence of ASF antibodies was of 100%, while it was of 50% in Magadi (Heuschele and Coggins 1969). A survey in Uganda (Queen Elisabeth National Park), reported a prevalence of 58% in animals from 4 to 12 months, which decreased with age (Plowright 1981). In South Africa, ASF seroprevalence ranged from 90% to only 4% in very close geographical locations within the same area (Penrith et al. 2004).

Infected warthogs do not show signs of disease but considerable viral replication and viraemia occurs (Thomson 1980). Infection occurs basically in the burrows, where a strong symbiotic relation occurs with Argasid ticks. It characterizes by low levels of virus in the tissues, manly in the lymphatic system and low or undetectable levels of virus in blood (Plowright 1981). Young warthogs are born uninfected and become infected, when bitten by *O. moubata* in the burrow. They then develop a viraemia which lasts for 2 or three weeks. This is sufficient to infect, in turn, a proportion of ticks which feed on viraemic newborn warthogs (Thomson 1980).Viral particles in warthog blood rarely exceed 10^2 HAD_{50}/ml and progressively decrease thereafter. After this generalized phase of infection, the virus localizes in various superficial lymph nodes, with virus levels up to $10^{6.6}$ HAD_{50} and animals remain infected for life (Wilkinson 1989). The virus has a predilection for lymph nodes of the head (Plowright 1981).

Horizontal or vertical transmission does not occur in the warthog and maintenance of the virus within warthog populations is dependent on the soft tick *Ornithodoros moubata* which inhabits warthog burrows (Thomson 1980; Plowright 1981).

This warthog-*O.moubata* cycle is virtually limited to areas where Argasid ticks are distributed and has been described in most of South and East African countries (see Annex 1). Data on the

relationship between *P. aethiopicus* and *O. moubata* in the Somali ecosystem is suspected (Penrith *et al.* 2004), but has never been documented to date.

Infestation of warthog burrows -even in areas where the Argasid ticks is present-, are variable in terms of the numbers and stages of ticks found and the proportion of burrows infested, which might depend on warthog activity on those burrows.

Equally, in some areas in Central Kenya high seroprevalences of warthogs were observed but no Argasid ticks could be found in a sample of 118 burrows, suggesting that other forms of viral transmission might occur in those areas (Pierce 1974).

In West Africa, the existence of such cycle has never been demonstrated, except for a single record of *O. porcinus porcinus* in a warthog burrow in Sierra Leone (Penrith et al. 2004).

In Senegal for instance, it is likely that this relation does not occur since *O. moubata* is absent (Vial et al. 2007). That's probably also the reason why the circulation of ASF has never so far been demonstrated in warthogs outside Eastern and Southern Africa. Other ticks such as *O. sonrai* have been identified in rodent burrows in Senegal (Vial *et al.* 2006a) as being potential vectors of ASF (Vial et al. 2007). However, those could not be found in warthog burrows (Vial et al. 2006b) and it is unlikely that ASFV circulates among warthogs populations in West Africa (Taylor et al. 2007; Jori et al. 2007). This is just an example to illustrate that the warthog- *O. moubata* model cannot be extrapolated to the whole African continent.

Direct transmission of ASFV

Transmission from infected warthogs to domestic pigs has never been demonstrated (Thomson 1980; Plowright 1981). Experimental infections showed that infected warthogs lymphatic tissues contain lower quantities of virus (ranging from $10^{2.9}$ HAD_{50} to 10^{8} HAD_{50}) than those found in infected domestic pigs.

Indirect transmission of ASFV

The main role in the transmission between warthogs and possibly from warthogs to pigs is played by Argasid ticks when pigs and warthogs are sharing the same grazing areas and pigs are bitten by infected soft ticks carried by warthogs.

The hypothesis that domestic pigs can become infected by the ingestion of infected warthog tissues has never been supported by experimental data (Penrith et al. 2004).

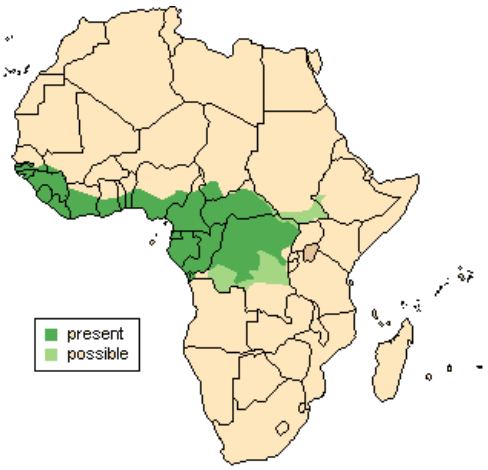
Pigs may also become infected after being bitten by soft ticks, brought to human settlements with warthog carcasses or by ingestion of infected soft ticks. This hypothesis seems more plausible since soft ticks have been in some occasions found on warthogs bodies, outside their burrows (Horak et al. 1983).

4.1.2. The Genus Potamochoerus: The Bushpig (P. larvatus) and the Red river hog (P. porcus)

The bushpig, *Potamochoerus larvatus*, and red river hog, *P. porcus*, are the two representatives of the Genus Potamochoerus. The red river hog (*Potamochoerus porcus*) is brighter in color, with a distinct white dorsal stripe and crest, long white whiskers and eartufts. It occurs only in West and Central Africa, from Senegal (Casamance region) in the extreme west, and east and south to eastern Democratic Republic of Congo. However, there has been an evident contraction in the west and extreme north of its range, due to human activity (overhunting and habitat destruction).

Fig 6: Distribution of *Potamochoerus porcus*

(Source: http://www.ultimateungulate.com/Artiodactyla/Potamochoerus_porcus.html)

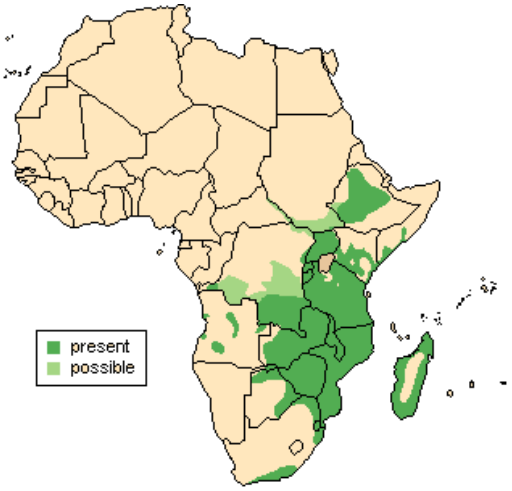


The bushpig (*Potamochoerus larvatus*) is darker in color, often without the distinct white masks and long eartufts characteristic of *P. porcus*. It has a relatively wide range, extending from and south R.D. Congo in the west, to Eastern South Africa in the south and Madagascar. In Madagascar, *P. larvatus* is considered as an invasive species suspected to have been introduced by local populations more than 2000 years ago, although the exact origin and date of arrival in the island remains unknown (Roger et al. 2001).

Data on the distributions and taxonomic variations of both of these species are sketchy and imprecise and certainly need genetic studies to clarify the systematic and distribution limits of both species.

Fig 7: Distribution of *Potamochoerus larvatus*

(Source: www.ultimateungulate.com/Artiodactyla/Potamochoerus_larvatus.html)



Very few field studies have been conducted on either of these species. Most of the information comes from studies on *P. larvatus* in South Africa (Seydack 1990;Vercammen et al. 1993). The distribution of both subspecies is apparently limited by the continuous availability of food, water and cover (Vercammen et al. 1993). Both species live in small family groups usually comprising 4-10 individuals. However in equatorial regions, groups of more than 30 individuals are reported for the red river hog, while such large herds are rare for *P. larvatus*. Usually, both species are sedentary and territorial.

During periods of inactivity they shelter in dense vegetation, and they may construct bad weather nests during cold and wet spells. Average daily movement distances were found to be 3 km, ranging between 0.5 and 5.8 km in western South Africa (Seydack 1990).

Density of *Potamochoerus* spp. in different parts of South Africa ranges between 0.35 to 0.5 individuals/ Km^2 but can go up to 3 inds/ Km^2 in tropical forest regions. However, density estimations at a local level are missing in most of its distribution range.

A distinctive aspect of *Potamochoerus* spp. is its suspected hybridization with domestic pigs and wild boars (*Sus scrofa*). This phenomenon has been repeatedly reported in different parts of Africa (Vercammen et al. 1993;Kingdon 2003) and Madagascar (Jori, pers commnunication). However, evidence for such an intergeneric hybridization and the viability of those possible hybrids have never been demonstrated and scientifically described. The reports of *Potamochoerus* spp x domestic pigs hybrids are always of free ranging female pigs being mounted by buhspigs or red river hogs, since male domestic pigs are usually chased by male bushpigs.

The bushpigs and ASFV

It is believed that bushpigs are less important than warthogs in the epidemiology of ASF, since they exist in lower numbers, are more evenly distributed and have lower infection rates (Wilkinson 1988). Despite knowing that they can get infected, there is no information available on the levels of prevalence of ASFV in buhspigs. Mansveld estimated that ASF virus in bushpigs was 10 times less frequent in warthogs (Mansvelt 1963). De Tray (1969) reported "low frequencies" of the virus in Kenya (De Tray 2008), but without supporting figures.

Information on prevalence of ASF in bushpigs is scarce (Haresnape et al. 1985; Jori et al. 2007; De Tray 2008). This is probably because bushpigs are elusive nocturnal creatures, difficult to capture. Nevertheless, it also suggests that this ASFV circulates only occasionally or at low levels (Jori *et al.* 2007).

In Madagascar, ASFV has never been detected in buhspigs since the introduction of the virus in this island in 1998. No major mortalities have ever been reported, despite the fact that when ASFV was introduced in 1998, it probably challenged a naïve population of Malagasy bushpigs. Moreover, occasional screening of bushpig samples for detection of ASFV antibodies through different methods always failed to demonstrate a possible contact with the virus (Jori et al. 2007).

Both subspecies have been reported as being naturally infected with ASFV in East and West Africa. However, only very few reports of contacts between ASFV and buhspigs in Southern Africa or

Madagascar exist. Buhspigs show no clinical signs when infected with ASFV that are pathogenic to domestic pigs (Anderson et al. 1998).

Infection in buhspigs results in low levels of viral replication and minimal pathological damage and apoptosis in lymphoid tissue, and low spread of the virus to other lymphoid tissues (Oura et al. 1998) recorded the duration of viraemia in bushpigs which lasted longer than in warthogs (35 to 91 days) and showed that ASF virus persists in the lymphatic tissues for at least 34 weeks, following primary infection (Anderson et al. 1998) at levels that ranged between 10^2 and $10^{4.9}$ HAD_{50}/ml (1).

During viraemia, transmission between experimentally infected buhspigs and domestic pigs could be demonstrated but horizontal transmission between bushpigs did not occur, suggesting that those species require higher doses of virus to become infected. Surprisingly, infected pigs which excrete large quantities of virus were not able to transmit the virus to buhspigs, suggesting that this species may not be readily infected by direct contact. However, these hypotheses have never been confirmed further. Virus can be isolated from infected bushpigs up to 34 weeks after primary infection .

Experimentally infected bushpigs were also able to infect soft ticks (*O. moubata*). However, in natural conditions, it is not likely that buhspigs maintain a close relation with soft ticks since they do not frequent burrows. Nevertheless, in areas of Africa where home ranges of *P. larvatus* overlap with those of *O. moubata* and warthogs, contacts with ticks and bushpigs could eventually occur (Anderson et al. 1998; Roger et al. 2001).

Despite the suspicion that bushpigs could play a significant role in the epidemiology of the disease (Haresnape et al. 1985; Roger et al. 2001), their epidemiological role remains unclear. However, outbreaks of ASF in domestic pigs have occurred in some areas of Malawi with presence of bushpigs and with absence of warthogs and soft ticks (Wilkinson 1984; Haresnape et al. 1985). As

buhspigs come close to communal lands attracted by crops, direct contact is possible if pigs are free ranging (Haresnape et al. 1985).

In those situations, if pigs are infected and die in the bush, the possibility exists that *Potamochoerus* can become infected by ingesting pig carcasses. Equally, buhspigs are favoured game and there are sufficient ASFV particles in infected buhspig tissues (Anderson et al. 1998), to infect domestic pigs by ingestion (Penrith et al. 2004).

If the hypothesis of hybridization between domestic pigs and *Potamochoerus* spp. is plausible, the way these hybrids would behave regarding a possible exposure to ASFV requires further investigation. Indeed, since *Potamocheorus* is an asymptomatic carrier, one could hypothesize that hybrids could become asymptomatic carrier pigs which could disseminate the disease to more susceptible pigs.

4.1.3. The Giant forest hog (Hylochoerus meinertzhangeni)

The giant forest hog (*Hylochoerus meinertzhangeni*) lives in mountain forests and adjacent grasslands below 3750 meters / 12,000 feet above sea level across Central Africa (d'Huart 2008). It has only been recorded in one instance as being infected by ASFV (Montgomery 1921). Since the distribution of this species is restricted to areas of dense forest (d'Huart 2008), where domestic pig production is not common, its role in the epidemiology of ASF can be considered negligible.

Fig 8: Distribution of the Giant Forest Hog

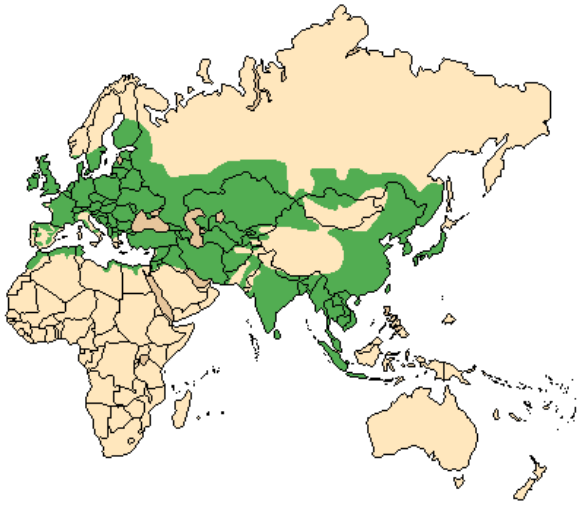
(Source:www.ultimateungulate.com/Artiodactyla/Hylochoerus_meinertzhageni.html)

4.1.4. Feral pigs and wild boars (Sus scrofa)

The Eurasian wild pig has one of the widest distributions of all terrestrial mammals and its range has been greatly expanded by human agency (Oliver et al. 1993). It is the ancestor of most of common domestic pigs. The wild boar in Africa occurs only in the North of the continent (Morocco and Tunisia). It has also been introduced in numerous African countries for hunting purposes such as South Africa, Sudan, Burkina Faso and Gabon. In the latter, the species is suspected to have interbred with *Potamochoerus porcus* (Vercammen et al. 1993).

Fig 9: Distribution of *Sus scrofa*

(Source: http://www.ultimateungulate.com/Artiodactyla/Sus_scrofa.html)



The feral pig is a domestic pig that is living in the wild, either having been released or escaped from confinement. It has been introduced by colonisation in many countries of the New World and many Islands in the Caribbean, the Indian Ocean and the Pacific.

Basically, they are the same species as the wild boar and are difficult to distinguish morphologically although there are some differences. They are able to cross between each other and the hybrids are perfectly viable almost, if not wholly, indistinguishable from purebred Eurasian wild boars (Safari Club International, 2006)

Sus scrofa and ASFV

ASF has been introduced in many areas where *Sus scrofa* is present such as Spain and Portugal (Arias and Sanchez-Vizcaino 2002a), Sardinia (Laddomada et al. 1994; Mannelli et al. 1997; Mannelli et al. 1998), Cuba (Siméon-Negrin and Frias-Lepoureau 2002), Mauritius and more recently several countries in the Caucasus (Laddomada et al. 1994; Mannelli et al. 1997; Beltran Alcrudo et al. 2008).

In the Iberian Peninsula, this species is highly susceptible to both natural and experimental infection (McVicar et al. 1981) and died massively after infection (McVicar et al. 1981; Laddomada et al. 1994; Bech-Nielsen et al. 1995; Perez et al. 1998). Depending on the virulence of circulating strains, some animals survive and positive animals have been detected during serological surveys (Perez et al. 1998). In Florida, it was observed that feral pigs were extremely susceptible to ASFV isolates from Dominican Republic (McVicar et al. 1981). This seems to be also the case in Mauritius, where feral pigs in captivity that became infected died from the disease to the same extent as domestic pigs (Jori, personal communication).

Their role in the epidemiology of the disease is unclear (Wilkinson 1989). In principle, they excrete viruses in the same quantities as domestic pigs and the epidemiological dynamic between wild boars and domestic pigs is very similar in terms of direct transmission between sick and susceptible animals (Arias and Sanchez-Vizcaino 2002a). However, they seem to be less efficient in transmitting the infection to co-specifics.

In Spain, serological monitoring of wild boars during 1990's, showed seroprevalence levels lower than 0,5% suggesting that the disease was fatal in wild boars and that very few animals survived (Bech-Nielsen et al. 1995). However, several years later the number of seropositive animals increased and was maintained in areas where domestic pigs remained infected.

4.2. INVERTEBRATE HOSTS

ASFV is the only arbovirus that has a DNA genome. The only known invertebrate reservoirs of ASFV in nature are arthropods. Within the phylum Arthropoda, subphylum Chelicerata, class Arachnida, and its dominant subclass Acari, ticks are within the order Parasitiformes that also includes mites (Sonenshine, 1991). The suborder Ixodida (also called Metastigmata) comprises three families, the Nuttalliellidae and the Ixodidae (hard ticks) and Argasidae (soft ticks).

Two ticks of the family Argasidae have been identified as ASFV reservoirs in nature. In Europe the soft tick Ornithodoros erraticus was implicated as a vector of ASF virus for domestic pigs (Sanchez Botija, 1963) whilst in Africa Ornithodoros ticks of the Moubata group were found as an ASFV reservoir (Plowright, 1969).

4.2.1. Tick-domestic pig interactions

Ornithodoros ticks readily become infected while engorging on infected swine. Using experimental infections, Plowright (Parker, 1969) showed that clean ticks acquired virus when feeding on infected swine that have a viremia ranging from 10^6 to 10^8 HAD_{50}/ml. As little as 10 to 10^2 HAD_{50}/ml of some isolates was sufficient to infect ticks by feeding, but 10- to 100-fold more viruses were necessary to establish persistent infection in 50% of ticks. Other isolates required at least 10^5 HAD_{50} to produce any persistent infection (Plowright, 1981). Concerning the involvement of *Ornithodoros* ticks in the domestic cycle, these results are compatible with virus kinetics monitored in susceptible pigs experimentally infected by ASFV isolates (Edwards, 1985; Ekue, 1989; Greig, 1970). Conversely, concerning the role of *Ornithodoros* ticks in the parallel sylvatic cycle, these figures suggest that a viremia in warthogs of at least 10^3-10^4 HAD_{50}/ml would be necessary to infect ticks, which has never been recorded in free living adult warthogs (Heuschele, 1969; Parker, 1969). However, it is suggested that new-born animals may show even higher viraemia due to immunological immaturity and mostly contribute to the contamination of soft ticks because of their constant presence in burrows (Thomson, 1980). Adult warthogs, as well as other mammalian hosts of the tick, which are able to wander freely into areas used for domestic swine farming, may act as efficient transporters of infected ticks from wild areas to domestic ones and initiate ASF outbreaks at intervals up to many months later (Parker, 1969). Although bushpigs were demonstrated to be more efficient reservoirs for ASFV (Anderson, 1998; Luther, 2007; Oura, 1998), they do not frequent burrows or caves that may be infested by *Ornithodoros* tick vectors; thus it is very unlikely that bushpigs contribute to the infection of tick vectors because of their low probability of contacts.

4.2.2 Tick-virus interactions

4.2.2.1. Kinetics of AFSV in ticks

Kleiboeker (Kleiboeker, 1998; Kleiboeker, 1999) experimentally infected different tick species of the *O. moubata* group with several virus isolates from Southern Africa and monitored the kinetics in ticks. Initial ASFV replication occurred in phagocytic digestive cells of the midgut epithelium with subsequent infection after 15 days. Generalization of virus infection from midgut to other tick tissues required 2 to 3 weeks and secondary sites of virus replication included hemocytes, connective tissue, coxal gland, salivary gland, and reproductive tissue; viral titers in salivary gland and reproductive tissue were consistently the highest detected with 10^4 to 10^6 HAD_{50}/mg (Kleiboeker, 1998). In general, only low virus titres were found in field infected ticks: the highest titre was of $10^{4.3}HAD_{50}$/tick (Basto, 2006; Boinas, 1995). In experimental studies with *O. erraticus*, ASFV infection rates generally decrease in orally infected ticks while in ticks inoculated with virus into the haemocoel it increases suggesting the existence of a gut barrier to infection (Boinas, 1995).

Persistent infection, characterized by active virus replication in ticks, has been observed for several months to several years (Greig, 1972; Plowright, 1970). In the pioneer work of Sanchez Botija (1963) in Spain, ASFV was isolated from *O. erraticus* 4 months after an outbreak of the disease and later the author reported that the virus persisted in ticks for up to 8 years after infection (Sanchez Botija, 1982). Specimens infected with the ASFV isolates were collected from pig farms that were abandoned more than 2 years and 9 months after depopulation following an outbreak in Portugal (Boinas, 1995). Some ticks were removed from a farm 2 years after an ASF outbreak and kept unfed in the laboratory for 3 years before they were assayed and infectious virus recovered (Boinas, 1995).

The adult ticks and the larger nymphal stages are the most likely to be found infected in the field collections performed as time passes (Boinas, 1995). The proportion of virus isolations in these stages have an initial increase in time up to weeks 32 post outbreak while in the smaller stages it decreases in this period (Basto, 2006). Later in time the infection rates decreased in all stages (weeks 63 post outbreak). These field findings coincide with experimental studies that report a decrease in virus titres and infection rates at weeks 41 and 61 after experimental oral infection (Basto, 2006). The rate of decrease in virus infection rates in orally-infected ticks seems to be

dependent on the initial infection titre and so the probability of persistent infection in ticks depends on the titre of the viraemia of infected pig as only the highest titres used to infect ticks in some experiments led to persistent infection in ticks (Boinas, 1995).

4.2.2.2. Prevalence of infection in tick populations

In Malawi, infection rates of 1-3% were commonly reported in Ornithodoros ticks infesting pigpens (Haresnape, 1988), which was similar to rates reported for wild ticks inhabiting warthog burrows (Plowright, 1981); however, infection rates could reach 11-24% after an ASF outbreak occurring a few months ago (Haresnape, 1989).

The reports of the prevalence of ASFV infection in *O. erraticus* populations in the field in Portugal are very diversified varying from 0.5-6.4% when the tick samples where collected up 1017 days after the ASF outbreaks or 43% when collected at the time of the outbreak of disease (Basto, 2006; Boinas, 1995).

4.2.2.3. Specificity of ASFV to tick hosts

Some authors consider ASFV and *Ornithodoros* tick as co-evolving organisms. Actually, noticeable telomeric similarities in the genomes of ASFV and *Borrelia*, the latter sharing the same *Ornithodoros* tick host in Africa and considered an original pathogen of soft ticks, suggest that ASFV is also a primary organism of *Ornithodoros* ticks and co-adapt to its tick hosts (Hinnebusch, 1991). This hypothesis could explain discrepancies concerning infection success rates noticeable between several past surveys. For example, De Tray (1963) reported a consistent establishment of the virus isolate "Uganda" in *Ornithodoros* ticks of the *O. moubata* group (34/35 were infected) whereas another isolate "Tengani" only caused persistent infection in a small proportion of ticks (2/46 were infected). More recently, Kleiboeker (Kleiboeker, 1999) compared orally and intra-hemocoelic experimental infections of *Ornithodoros* ticks collected from warthog burrows in Kruger National Park and the Northern Transvaal region of South Africa, as well as ticks from Masai Mara Reserve in Kenya, by three different viruses from South Africa, Malawi and Zimbabwe, all originally isolated from wild ticks. The oral infection conducted with the isolate from Malawi was abortive (decline of virus titers and number of ticks containing virus) while the

others persisted. Regarding the cythopathology caused by this isolate in infected ticks, the author suggested the non adaptation of the isolate to express specific genes which allow the production of large quantities of progeny virus without damaging the host cell. The reason why this virus was originally isolated from ticks could be the large opportunity for those ticks to feed on infected pigs with high viremic titers during an ASF outbreak and leakage of midgut contents into the hemocoel without tick mortality, instead of real adaptation of this virus isolate to the tick host (Kleiboeker, 1999). In 1988, Dixon analysed genomes of ASFV isolates collected over a 2 year period from ticks inhabiting warthog burrows in four regions of Zambia and observed additional sequences in the region close to the left-end terminus of the genome, which are not observed in domestic pig isolates. The author concluded that virus replication in ticks and warthogs may require additional host-specific genes not necessary for multiplication in domestic pigs and that the introduction of virus from tick/warthog sources into domestic pig populations would remove the selection pressure for maintaining these genes (Dixon, 1988). More recently, Burrage (2004) and Afonso (2004) demonstrated that ASFV multigene family 360 genes in the left variable region of the genome encode a host range determinant required for efficient replication and generalization of infection in pigs and ticks. However, no more information is yet available on specific determinants to tick/warthog hosts, as it was previously suggested. In addition, it is unknown if ASFV is able to come back from the domestic cycle to the sylvatic one although some authors suggest recombination processes during co-infections in ticks (Dixon, 1988 ; Plowright, 1976).

4.2.2.4. Diversification of ASFV in tick hosts

By sequencing the C-terminal end of the p72 gene, Bastos (2003) and Lubisi (2005) observed higher genetic variations in genotypes directly isolated from Ornithodoros ticks and warthogs or genotypes circulating in East and Southern Africa, where the sylvatic cycle plays a crucial part in the epidemiology of the ASF; some other genotypes were only found in domestic pigs and presented low genetic divergence (Lubisi, 2005). In Madagascar, using concatenated sequences of the p22 and p32 genes, Michaud (Michaud, 2007) detected relatively high genetic divergence between Malagasy virus isolates collected on domestic pigs from 1998 and 2003, compared to that observed on West African and European isolates since the 1970s. In Madagascar, it has been

suspected that the introduced virus was adapted to local bushpigs and Ornithodoros ticks, leading to its accelerated diversification. Such diversification phenomenon reported in several African countries has been previously analysed by Dixon (Dixon, 1988) on Zambian virus isolates from Ornithodoros ticks. A considerable genetic diversity was observed between virus isolates from ticks collected from same regions and even same warthog burrows; this diversity resulted from peculiar point mutations and was all along the length of the genome, instead of insertions/deletions in the region close to the left-hand terminus of the genome usually observed for host filter (Dixon, 1988). Regarding these results, ticks would be able to enhance the diversification of ASFV and the emergence of new virulent isolates to domestic pigs. However, no information is yet available on the location and the expression of genetic diversification. In addition, this process does not seem compatible with the persistence of ASFV in Iberian Ornithodoros ticks and observed genetic homogeneity of ASFV in Europe.

4.2.3. Tick-tick ASFV transmission

Transstadial transmission has been demonstrated by Hess (1989) who maintained laboratory colonies of *Ornithodoros* ticks from Zimbabwe that were already infected by ASFV and remained infected for at least 1 year; however no data are available on transmission rates between development stages.

Sexual transmission has been proved with a Ugandan isolate in *Ornithodoros* ticks of the *O. moubata* group by Plowright (Plowright, 1974), with a male-to-female transmission rate of 87.6%; this finding may explain the 4- to 6-fold increase in infection prevalence between late nymphal stages and adults observed by Plowright (Plowright, 1974).

Finally, transovarial transmission was also demonstrated by Plowright (Plowright, 1970) under field conditions on *Ornithodoros* ticks collected from warthog burrows in northern Tanzania, with a filial infection rate of 67-78%, and later by Rennie (Rennie, 2001) by laboratory experimental infections of *O. moubata* with a Zambian virus isolate originally collected from wild ticks, with a filial infection rate of 1.8-31.8%.

Regarding such modes of transmission that allow ASFV to be maintained in ticks without horizontal transmission involving swine, as well as ASFV multiplication leading to its long-term

persistence in ticks that present an extreme long life-span of 5-10 years without feeding, it is quite reasonable to consider that African *Ornithodoros* ticks act as natural reservoirs for ASFV. However, some studies do not seem to confirm this hypothesis. Although experimental infections of *Ornithodoros* ticks with ASFV do not affect their oviposition rates, progeny hatching rates and feeding rates of nymphal stages and adults, significant increase of mortality rates have been observed by some authors, especially in females (Hess, 1989; Rennie, 2000). The authors proposed this process to explain the apparent clearance of ASFV from tick populations in the field, by a partial success of tick infection according to virus titres ingested and a higher mortality of infected ticks compared to uninfected ones in the same population (Hess, 1989). Because some other experimental infection studies did not point out same increase of mortality rates (Kleiboeker, 1998; Kleiboeker, 1999), these results should remain questionable. In Europe, only transtadial has been observed in *O. erraticus* (Sanchez-Vizcaino, 2006).

4.2.4. Ecological and biological characteristics of tick hosts

4.2.4.1. Development cycle

All *Ornithodoros* species share common biological and ecological characteristics. Their life cycle is polyphasic (one host for each feeding stage) and is composed by one larval stage, several nymphal stages (from 2 to 9 according to species, feeding efficiency and climatic conditions), and one adult stage; the lifespan of this last stage may be extremely long reaching sometimes more than 5 years without feeding (Morel, 1969). *Ornithodoros* ticks commonly present two different morphologic types, the premature one and the adult one, both separated by a true metamorphosis (in opposition to nymphal moulting). Contrary to hard ticks, larvae are specifically more characteristic for morphological identification than nymphs and adults (Cooley, 1942). These ticks are hematophagous at all growing stages, apart from the larval stage of the *O. moubata* group that directly moult in nymphs without engorging (Walton, 1962). Each nymphal stage engorges once before moulting while adults may feed repeatedly on different hosts (1 to 10 blood meals). Copulation between males and females usually occur after feeding out of the host. Blood meal is necessary for pregnant females to lay eggs; the clutch size is generally low (from 20 to 300 eggs)

but several clutches (2 to 6) may be produced following one copulation provided that preliminary blood feeding (Encinas Grandes, 1993; Morel, 1969).

The activity of the ticks depends on the conditions of temperature and humidity and the complete cycle can take only 154 days in laboratory conditions (el Shoura, 1987). In the field the activity of the tick varies with the season. In Europe, the tick is inactive in winter and activity starts when the minimal external temperatures are in the range 10-13^{o}C (inside the pens 13-15^{o}C) with peaks of activity attained in the warmest months. The complete cycle, from egg to adult male or female, after one larval stage and 3 to 5 nymphal stages, is 4 years in Spain (Fernandez Garcia, 1970) and is presumed to be between 2-3 years in the Spanish province of Salamanca (Encinas Grandes, 1993 ; Oleaga-Perez, 1990) and in Portugal (Caiado, 1990). In subtropical regions, such seasonal patterns have not been observed and complete development cycle can be done in about 150 days (Walton, 1962). This tick has a remarkable resistance to fasting, as several reports refer to maximum periods between feeding of 3 to 5 years (Fernandez Garcia, 1970; Oleaga-Perez, 1990; Boinas, 1995) and the estimated total period of life of up to 15 years (Encinas et al., 1999).

4.2.4.2. Feeding activity and host preferences

Ornithodoros ticks attach themselves to their hosts for blood feeding only for a short time, usually for less than one hour, except for larvae of some species that can stay 1-2 days on the same host (Morel, 1969). The multiplication of short parasitic periods in *Ornithodoros* ticks may have important impacts on their dynamics (predicted low dispersal capacities and low viability of restricted populations) and their parasitic functions (optimization and multiplication of blood feeding and relative host ubiquity).

It may partly explain that *O. porcinus* is able to be associated either to several mammals like warthogs when living in wild areas and to humans, fowls or pigs when living in domestic buildings, all of these situations leading to the transmission of different pathogens like ASFV for pigs or TBRF for humans (Walton, 1962; Haresnape, 1986; Haresnape, 1988). Same situation is observed for *O. erraticus* colonizing North Africa and Southern Europe. It has been essentially found in rodent and insectivore burrows in Africa (Hoogstraal, 1954) while it mainly colonizes domesticated areas in Europe, essentially pigsties (Caiado, 1988; Oleaga-Perez, 1990). Pigs are considered as the

main host and other accidental/alternative hosts include humans, rodents, sheep and chickens (Tendeiro, 1962). In Portugal, engorged ticks were found in birds nests on infested premises and birds may also act as alternative hosts for the parasite (Boinas, 1995). These ticks were frequently associated with *Borrelia hispanica* infection of humans, which was a hazard of the management of pig sties (Carvalho Dias, 1933; Gil Collado, 1948).

4.2.4.3. Endophilous status and microhabitat preferences

Because of the peculiar composition of their oilskin, African *Ornithodoros* ticks and more generally Argasidae can adapt to drier external conditions (<20%) and higher critical temperatures (63°C for *O. moubata*) than hard-body ticks, which explains their widely geographical distribution in African sub-Saharan countries (xerophilic type) (Morel, 1969). However, the absence of hard shell on soft-body ticks constraints their development and life cycle. Except *O. savignyi* that lives at ground level and is widely distributed in sub-Saharan desert areas thanks to a particularly high critical temperature up to 75°C, all *Ornithodoros* ticks colonize underground or protected habitats (endophilic type); such habitats damp out external climatic variations and delay the influence of the climate on the internal microclimate (Morel, 1969). These parasites are classified as being endophilous and nidicolous since they live in the habitat of the host (Sonenshine, 1993).

Consequently, those ticks are mainly found in burrows of small mammals and other vertebrates, dugouts and trenches infested with wild animals, caves especially those in which guano is present, litters or nests (Morel, 1969; Rodhain, 1976). Some species could adapt to domestic conditions and thus have been found in henhouses, cowshed or small ruminant buildings, pigsties and human dwellings with mud walls and floor (Walton, 1962; Rodhain, 1976).

All these different underground habitats provide optimal temperature and humidity necessary for the survival and development cycle of *Ornithodoros* ticks. As a consequence, it is reasonable to consider that habitat preference may constitute more important factors than host selection for *Ornithodoros* ticks, leading to their apparent host ubiquity. Studying distribution patterns of *O. p. domesticus* in human dwellings in East Africa, Walton (1962) noticed that ticks were either found in humid and cold highlands, humid and hot coasts and also in dry and hot lowlands. In the first area, ticks were found only in traditional buildings where the cooking fire was warming and drying the

soil. In the second and third areas, ticks were colonizing cracks where external climate was buffered to provide cool temperature and higher humidity. Regarding these results, *Ornithodoros* ticks depend in a certain limit of external climate but seem to be able to adapt to apparently unfavourable conditions by selecting underground habitats that may provide microclimatic optima. Hoogstraal (1954) observed the same patterns for *O. sonrai* in Egypt, which never colonizes dry pure sand burrows while it is found in same areas when sand-clay or silt-sand soils maintain a relative humidity from close rivers or annual rainfall.

There are no reports that the Argasid can move itself outside the buildings or its burrow; it can only move when attached to the host. Spread can be explained either by the transfer of utensils contaminated with the parasite or by the passive transfer of Argasid feeding on animals being moved. This could only be responsible for transfer over short distances, since the time of feeding is generally short, 10 to 30 minutes (Fernandez Garcia, 1970), and possibly even shorter when the animal is in movement unless trapped in a skin fold, etc.

4.2.5. Distribution patterns of tick hosts

4.2.5.1. Distribution of Ornithodoros of the moubata group in Africa

Before the 1960s, *O. moubata* was considered a unique species although numerous discrepancies were observed among field and laboratory investigations on biological and ecological characteristics, such as modes of hatching, feeding behaviors, preferences of microhabitat or optimum temperature and humidity. Studying differences in the ability of distinct geographic populations of *O. moubata* to withstand the effects of dessication, Walton (1962) proposed that *O. moubata* would be either composed of a number of biologically different populations and/or consisted of a number of distinct species. He recognized four distinct species in the O. moubata group (Walton, 1962; Walton, 1964 ; Walton, 1967):

- *O. apertus*, a rare tick known only from two localities in Kenya and exclusively associated with African porcupines (Hystrix);
- *O. compactus*, localized south of the Zambezi River and associated with several species of tortoises but never found in domestic areas;

- *O. moubata* sensu stricto, presenting a wide distribution in the xeric southern third of Africa, in South Africa with northward extensions through Mozambique to central Tanganyika in the east and through southwest Africa into Angola on the west. This species is commonly found in warthog and porcupine burrows but also presents a domestic form inhabiting human dwellings. Wild specimens of *O. moubata* are more robust and have more accentuated features than domestic forms, which may be a deme or a race of the wild species. As the known hosts of the later deme or race in African dwellings include man, dog and domestic fowl, Walton suggested that it was probably this deme which also infests domestic fowl houses in South Africa.
- *O. porcinus*, also widely distributed in the humid Central African Plateau, from central Kenya to central Mozambique, west to the eastern borders of Ruanda Urundi and upland Nvasaland. *O. porcinus* is an abundant species in the bush, inhabiting warthog and porcupine burrows, hollow baobab trees and in lairs of large animals. It has also definitely been found several times on domestic cattle and on the giant scaly anteater. A domestic form also exists and inhabits preferentially human dwellings in East Africa. Morphological and biological differences between wild and domestic populations were so consistent that Walton warranted subspecific status for each and gave the name *O. porcinus porcinus* to the wild form and the name *O. porcinus domesticus* to the domestic form.

Walton (1964) noticed that *O. porcinus* and *O. moubata* have broadly overlapping distributions in eastern Africa and have actually been found together in the same African hut in Mozambique, which explain much confusion on the taxonomy of these species.

The systematics of *O. porcinus* is all the more complicated that its domestic subspecies *O. p. domesticus* presents also several biological and ecological forms. In East Africa, many of the regions occupied by *O. p. domesticus* are very decidedly wet and cold while others are both hot and wet or hot and seasonally dry. A number of local races have apparently evolved in adaptation to the different facets of the varied geographical pattern of Eastern Africa (Walton, 1962; Walton, 1964). Races inhabiting human dwellings at high altitudes in damp cool conditions show a marked preference to feeding on man. At lower altitudes, in hot climates with long dry periods, both man and domestic fowls are bitten with equal facility by a second race. Then, in hot moist conditions,

domestic fowls become the host of choice of a third race. In addition, as soon as 1906, Wellman (Wellman, 1906) reported the presence of *Ornithodoros* ticks from the *O. moubata* group inhabiting pigsties in Angola. This frequent association with domestic pigs was later confirmed by Wilson (Wilson, 1943) and Haresnape (Haresnape, 1986; Haresnape, 1988; Haresnape, 1989) in Malawi, Bedford (Bedford, 1934) in South Africa and very recently by Quembo in Mozambique (personal communication). Finally, in Madagascar, Uilenberg (Uilenberg, 1963) also highlighted some ambiguities with one race of the *O. moubata* group that has been found mainly in pigsties. These specimens presented a specific combination of *O. p. porcinus*-like and *O. p. domesticus*-like morphological characters that did not allow including this race in one category; only recent molecular analyses by sequencing the 16S rDNA gene demonstrated its close relationship with *O. p. domesticus* (Vial, personal communication). The concept of species and subspecies in the O. moubata group is all the more ambiguous that cross-breeding experiments do not give more comprehensive results. Artificial copulations between *O. moubata ss* and both subspecies of *O. porcinus* are difficult and relatively delayed but some of them succeed, which do not support the hypothesis of distinct species. However, the production of larvae is low (from 6% to 14%) and the corresponding nymphal stages have high rates of anomalies (from 62% to 100%) (Walton, 1962). Cross-breeding between *O. p. domesticus* and *O. p. porcinus* are highly fertile (from 71% to 91%) and most hybrids are viable, except when reproductive females are O. p. porcinus (60% compared to 0% when inverse pairing) (Walton, 1962). As a consequence, Walton's classification did not receive a consensual agreement from taxonomists, for example Van der Merwe, who considered structural differences of members of the *O. moubata* complex to merit only subspecific status and thus created the name "*O. moubata porcinus*"; however, this classification is commonly use to investigate vector competence of those ticks for animal or human diseases.

Domestic forms of *O. moubata* (ZUMPT, 1961), as well as *O. p. domesticus* (Fukunaga, 2001 ; Walton, 1962), have been found naturally infected by *B. duttoni* causing human tick-borne relapsing fever in East and Southern Africa. Concerning wild ticks, specimens that were primarily collected as confirmed vectors of ASFV, mainly in warthog burrows from Kenya, Uganda and Tanzania, were all identified as *O. p. porcinus* (Pierce, 1974; Walton, 1979). However, to our

knowledge, no detailed information is available on the correct species, subspecies or race identification of *Ornithodoros* ticks transmitting ASFV from Southern Africa or East African countries located on the overlapping geographical ranges of O. moubata and O. porcinus, especially Mozambique. No specimen of the domestic form has so far been found in the bush but the wild form has been found repeatedly in the domestic environment, which may explain regular introductions of ASFV isolates from the sylvatic cycle to the domestic one and possible exchanges or cross-breeding between wild and domestic forms.

4.2.5.2. Distribution of O. erraticus in the Iberian Peninsula and North/West Africa

Same ambiguity also exists for the taxonomy of O. erraticus. The argasid ticks parasitic on pigs in the Iberian Peninsula belong to the genus *Ornithodoros* Koch (Oleaga Perez, 1989). This only existing species has been classified differently throughout the time with different species names. *O. marocanus* was used until 1930 (Gil Collado, 1948) and then *O. erraticus* by most of the authors from 1930 to 1985 (Oleaga Perez, 1989). The parasite in Spain and Morocco was reported as *O. marocanus* (large form) by Hoogstraal (1985), who also grouped the ticks found in Northern Africa as *O. erraticus* (small form) and the ones found from Tunisia to Senegal as *O. sonrai*. Electrophoretic enzyme studies of the genetic distances between the several populations revealed differences between *O. sonrai* (from Senegal) and *O. erraticus* (from Egypt) although these species have almost no morphological differences (Wallis, 1983). Both species were found in Morocco in sympatry in same rodent burrows (Baltazard, 1950; Blanc, 1951). In addition, crossbreeding experiments conducted on *O. sonrai* and *O. erraticus* between pairs of distinct species and pairs of same species but coming from distinct geographic localities have showed same ranges of fecundity failure, suggesting the existence of numerous multiple strains of ticks (Chabaud, 1954). Actually, these strains may constitute a cline of species and explain the diversity of naming for Ornithodoros ticks in North and West Africa with its extension to Europe.

In view of this confusion in classification, it was decided to use the name preferred by most of the authors- *Ornithodoros erraticus* - for the soft tick found in pig sties in the Iberian Peninsula. In the Iberian Peninsula *O. erraticus* has only been reported in the traditional type of pig herds of the Iberian/Alentejano extensive production system type. Distribution surveys using physical search or

CO2 traps (Caiado, 1990), reported the ticks in Spain in the provinces of Salamanca, Badajoz, Huelva and Caceres (Boinas, 1995; Encinas Grandes, 1993 ; Estrada-Pena, 2000) and in Portugal the distribution studies only identified the tick in the Southern Provinces of Alentejo (11 positive counties) (Caeiro, 1999) and Algarve (2 counties) (Boinas, 1995). Pigs were the only domestic animal species always present in the occupied infested premises in the surveys made in Portugal (Boinas, 1995). Ticks were only found in premises that were populated by pigs at present or in the past. Ticks were also found on farms that had certainly been empty for more than 5 years as confirmed by regular visits to the farms (Boinas, 1995).

4.3. EPIDEMIOLOGICAL PATTERNS OF ASF

4.3.1. ASF in Eastern Africa

According to past field observations and recent genetic insights, the epizootiology of ASF is clearly divisible into three distinct parts (Scott, 1965; Lubisi, 2005; Boshoff, 2007):

- The **"old enzootic cycle"** or **"sylvatic cycle"** involving wild pigs, especially warthogs, and wild *Ornithodoros* ticks with accidental transfers to domestic pigs by ticks leading to sporadic outbreaks;
- The **"intermediate enzootic cycle"** involving domestic pigs and domestic *Ornithodoros* ticks acting as vectors and reservoirs with regular contamination of domestic pigs;
- The **"new epizootic cycle"** or **"domestic cycle"** restricted to domestic pigs and characterized mainly by direct transmission modes via pig movements and contacts, contaminated fomites or infected meat and leading to typical extensive outbreaks with rapid eradication by global slaughtering. As it has been observed in some European and West African countries, ASF may become **"enzootic and domestic"** if viruses adapt to domestic pigs and their feral descendants, which become the major carriers for ASFV through chronic or asymptomatic forms (Mebus, 1980).

This classification should be considered only for the introduction process for the first time in a domestic pig herd whereas ASFV spread is then usually taken over by direct transmission modes from infected pigs to susceptible ones. Regarding this classification, it is possible to re-interpret past

epidemiological descriptions of ASF outbreaks in East and Southern Africa, in order to extract main information on their spatial and temporal dynamics. Such descriptions are actually rare because of the contagiousness of the disease and the difficulty to monitor outbreaks since their beginning. However, Heuschele (1965) and Pini (1975) gave details on ASF outbreaks related to the "old enzootic cycle" in Kenya and South Africa while Haresnape (Haresnape, 1986; Haresnape, 1988; Haresnape, 1989) provided a good description of ASF outbreaks caused by the "intermediate enzootic cycle" in Malawi.

In Kenya, the outbreak monitored by Heuschele (1965) broke out in April 1964 in on a farm near Kitale. The authors were asked by Kenya Veterinary Department authorities to examine specimen materials from this outbreak for the presence of ASFV, as well as to collect additional samples. They also travelled to the farm to obtain a full history of the outbreak and other epizootiological data. In South Africa, Pini (Pini, 1975) firstly summarized the successive ASF outbreaks officially reported in the total country from 1926 to 1972 and then detailed 18 outbreaks occurring from May 1973 to March 1974 in the controlled area in non approved piggeries that are allowed to keep pigs only for local consumption. Both studies described sporadic emergence of ASF (Kenya: emergence in 1964 after 6 years of silence since the latest 1958 outbreak; South Africa: 3 active periods of disease in 1926-1938, 1951-1962 and 1973-1974 and 2 silent periods of 11 and 10 years, respectively), suggesting the introduction of viruses in domestic pigs from an enzootic sylvatic cycle. Pini (Pini, 1975) could detail temporal and spatial dynamics of the 18 outbreaks monitored in 1973-74 in South Africa and confirm the main wild source of infection. The outbreaks could apparently be grouped into 6 primary foci of infection. The first was reported on May 1973 on a farm in the eastern part of Letaba District. The origin of the infection was attributed to a warthog that was found and killed on the farm and the meat used for human consumption. The second case of disease occurred a month later on another farm in the same district approximately 35 km from the previous outbreak. The origin of this infection was not established but was not related to the first one because of the difference of hemadsorbing effect of both isolates. A third focus was recorded in Pietersburg District. The source of the infection was not traced but it was suggested that infection

came from meat scraps obtained from a local butcher who had probably purchased meat from infected farms. The fourth focus of infection occurred in September-October 1973 in the western part of Letaba Distric and reported outbreaks may all have been caused by movement of affected pig products. The fifth focus of disease was recorded in White River District in December 1973-January 1974. All the farms affected were closed and wild pigs were found in the area. In the same time, investigation of ASF in the western Transvaal demonstrated that *O. moubata* infected by ASFV was present in the burrows used by the warthogs. In Kenya, same sources of first introduction are described by Heuschele (1965) although spread is then caused by contagious pigs. Several of concerned pig holders or pig keepers were actually found hunting wild pigs for food and their most recent known hunt occurred three months prior to the outbreak; investigations in the wild confirmed the natural infection of warthogs and their abundance near infected pig farms (Heuschele, 1965). All these observations are coherent with the probable genotypes causing these outbreaks. In Kenya, genotypes I and X, which have been isolated from pigs, ticks, warthogs and bushpigs, were both circulating during the described outbreak. In South Africa, genotype XX may be responsible of the 1973-74 outbreaks but no investigations could confirm its presence in wild pigs or ticks. Finally, as it is agreed for ASFV isolates originated from wild natural reservoirs, both authors reported a mortality rate of 100% in domestic pigs.

In Malawi, Haresnape (1984) assessed the occurrence of ASF through detailed field surveys based on verbal enquiries and a questionnaire to pig owners in selected areas in the Central Region of Malawi since August 1981. It was clear that the number of cases of ASF confirmed in the laboratory during 1981 and 1982 represented a gross underestimate of the real incidence of the disease. Several outbreaks were reported in Dedza, Lilongwe, Mchinji, Dowa and Ntchisi districts, mostly from April to December 1981, whereas no outbreak was officially recorded in Northern and Southern Malawi during the same period. In Lilongwe, outbreaks were distributed through 3 distinct foci, without any affected areas between these localised foci, suggesting that pigs were contaminated locally in their pigpens. Mortality rates of 86-100% were reported and several pigs with antibodies to ASFV have been found in the west of the district although there was no report of

any recent outbreaks in this area (Haresnape, 1984). Such results suggest that pigs which survive infection become inapparent carrier animals and may play a role as a local source of infection in Malawi. Furthermore, investigations on *Ornithodoros* tick presence in pigpens and houses, as well as their natural infection with ASFV, were conducted through a four-phase survey from 1982 to 1985. Whereas ticks occurred over a wide area of Malawi, they colonized more likely houses than pigpens and the areas where ticks highly infested pigpens were almost exclusively located within the Central ASF enzootic zone (Haresnape, 1986). ASFV infected ticks were found in pigpens and houses from Mchinji district which is in the centre of the ASF enzootic area and no ticks were found infected outside this zone; in all villages where infected ticks were collected, deaths of pigs from ASF had been reported shortly beforehand (Haresnape, 1988). The overall infection rate was approximately 3% but was much higher (24%) just after an ASF outbreak; the proportion of infected ticks decreased with the passage of time but infected ticks were still present in all villages several months after outbreaks, which greatly suggest the role of domestic ticks as vectors and reservoirs (Haresnape, 1989). Finally, concerning wild areas, the unique warthog habitat in which ticks were found during the study was in Lilonde National Park in the Southern region of Malawi, which is well outside the ASF enzootic area; none of the ticks collected there were infected, although further south in Lengwe National Park, five of six warthog sera were seropositive for ASFV (Haresnape, 1988). At this period, only genotypes VIII and XII were circulating in Malawi (Lubisi, 2005); the first genotype was only isolated from domestic pigs and the second one from domestic pigs and *Ornithodoros* ticks, which also confirm a typical "intermediate enzootic cycle" involving only domestic pigs and domestic *Ornithodoros* ticks in Malawi in the 1980s.

More recently, field investigations were conducted in 3 three different areas of Madagascar (Marovoay region in the North, Ambatondrazaka in the West and Arivonimamo region in the Centre near Tananarive), in order to assess the epidemiological factors explaining the enzootic persistence of the disease since its introduction in 1998. While 15-42% of ASF suspicions or confirmed cases were reported from pig farmers, cross-sectional surveys detected 0.3% of seropositive pigs and 3% of pigs infected by ASFV among the 3 different regions. Except during

the first spread of the disease in Madagascar, low mortality rates are usually declared by pig owners and ASF outbreaks emerge regularly every year (Costard, unpublished data). Further investigations on wild reservoirs could not detect any natural infection in bushpigs. Numerous examinations in pigpens and also wild areas showed the absence of *Ornithodoros* ticks within the 3 different regions. Ticks were only found in the Central region of Madagascar, in a single farm of Mahitsy, where their presence had been reported 8 years before; natural infection by ASFV was detected in those ticks whilst no pigs had been introduced in the pigpen for at least 4 years, confirming the role of these ticks as natural vectors and reservoirs for ASFV (Ravaomanana, unpublished data). All these results may reflect a **"peculiar enzootic domestic cycle"** in Madagascar, which mainly involves pig carriers and viral isolates of peculiar low levels of antibodies and virus, as it is observed in East Africa. *Ornithodoros* ticks may play a role, as they are naturally and persistently infected, but their actual restricted distribution makes their involvement very unlikely.

4.3.2. ASF in West Africa

We have very limited information on the history of ASF in West Africa before the 90's. The first outbreak of ASF is known to have occurred in Senegal in 1959, originating from Ginea-Bissau via Casamance region (Sarr, 1990). Nigeria reported its first outbreak in 1973 and Cameroon in 1982 (Lefevre, 1998; Lefevre, 1998) considers that the disease in Nigeria then probably became enzootic although underreported. In Cameroon despite the implementation of control measures no eradication could be achieved and outbreaks occurred every year (OIE, 2008).

Since 1996, the disease occurs for the first time in some West African countries: Ivory Cost (1996), Benin (1997), Togo (1997), Ghana (1999) and Burkina Faso (2003). It seems in 2008 that no or very few countries remained free of disease. Until 2004 the diseases had never been reported in Guinea. Since 2005 they are no data available but the situation in the neighbouring countries might suggest that outbreacks could have occurred. Similarly no data is available for Liberia and Sierra Leone because of the politic situation in these countries.

The description of the different outbreaks of ASF in West Africa lets us distinguish two epidemiological patterns.

Although the indirect transmission via Ornithodoros ticks has been shown in Madagascar, East and South Africa, the epidemiological situation might be different in West Africa.

According to the results of a study carried on in Senegal between April and August 2006, the epidemiological scheme could be different from the pattern warthogs - ticks – pigs (Le Glaunec, 2006). During this study, investigations were carried on in the Sine-Saloum region of the Senegal: soft ticks were found in 43.6% of the farms (or around, near the pig rest zone). The farm prevalence had a 95% confidence interval of [33.89% - 53.23%]. Nevertheless no statistical link has been found between the presence of soft ticks in (or around) the farm and cases (or suspicions) of ASF outbreaks during the 3 years before the investigation. Furthermore on these sites contact between warthogs and pigs might be highly improbable.

Analysis of ticks collected in the same areas in January 2006 demonstrated for the first time that *O. sonrai* is naturally infected with ASFV (Vial, 2007).

These different results could explain that if ticks could eventually be in contact with pigs and sustain ASF in the farms, they might not play an important role in the emergence and spread of the disease in and between the farms. *O. sonrai* might contain the ASF virus but its ecology may not explain that these ticks are reservoir and vector of the disease.

Information on the existence of a sylvatic cycle is scarce and it seems that there might be no or very limited connection with the domestic cycle. There is no evidence of the presence of the virus neither in warthogs nor in "sylvatic" ticks. But there is also no evidence of its absence.

As a matter of fact we can distinguish two main epidemiological patterns in West Africa:

- The "epizootic domestic cycle" characterized by regular introduction of the virus due to commercial exchanges between regions. Thus, the origin of sporadic outbreaks with high mortality in West Africa such as Benin, Togo(1997), Ghana (1999) and Burkina-Faso (2003) could be mainly due to importation of infected pigs or infected meat (OIE, 2008; Lefevre, 1998). Transmission occurs most of the time by direct contact between pigs, but could also occur through human passive transport between farms. Usually the spread of the disease stops because of high pig population mortality with or without control measures.

- The "enzootic domestic cycle": the explanation of the enzootisation of the ASF disease in West Africa such as Senegal, Gambia, Guinea Bissau, Nigeria and Cameroon, could be the presence of symptom free carrier pigs in local breeds (Haresnape, 1987). In these animals in which the disease survives we could find modification of the virus with apparition of low pathogenic strains. These low pathogenic strains could reverse their viral capacity in stress situations.

In Senegal, Sarr (1990) considered the disease had become enzootic in Casamance during the 80's. Recently several studies clarified the ASF situation particularly in Senegal. In the regions where most of pig farms are found, a first study collecting suspicions and notified cases gave us a rough estimate of the ASF farm prevalence. This first approach encompassing around 400 farms in three regions determined ASF risk factors. A logistic regression on risk factors presenting significant odd ratios revealed 3 main risk factors in the farms: free ranging, possibility for other breeders to enter into the farm and presence of cases or suspected cases in the surrounding area.

The scheme of the domestic biological cycle for ASF adapted to Senegal was described by factorial analysis. The disease could be due to circulating virus in the neighbouring area. Its introduction in farms could be due on one hand to free ranging pigs which could be in contact with infected pigs (reproduction, snout contacts…) or with people carrying passively the virus, or in the other hand to other farmers entering the farm for trade or to alert to the presence of the disease (passive carrying). Indeed, farms do not have bio-security measures except modern farms that represent less than 2% of the total. Furthermore traditional farms are use to have several breeding behaviours not statistically risky but which increase the spread of the disease. For example inefficiency of the quarantine of incoming animals, burying pig carcass around the farm, lack of treatment and veterinary inspection are moving animals via public transport. This lack of knowledge is also important when measures are to be decided when ASF is suspected in the farm. 57% of the farmers will respond in first instance by selling their animals healthy or ill in order to avoid the sanitary decisions, especially in the region of Fatick and Kolda. Depopulating periods is not a usual practice (19% of the farmers) and there are only few declarations of suspicion to the veterinary services. On the contrary, in Ziguinchor region, 73% of the

breeders react when there are ASF cases in the surrounding area. They enclose their animals to limit the free ranging, food is under control and troughs are separated from other animals.

A second survey was conducted to sample farms in the same regions and permitted to confirm these data. The farm prevalence is respectively 31.75% [20.3% - 43.2%] in Kolda region, 45.24% [30.2% – 60.3%] in Fatick region and 65.71% [54.6% - 76.8%] in Ziguinchor region. These results disclosed on a first hand a significant difference between Zinguinchor region and both other regions. On a second hand they disclosed that farmers really under estimate the disease prevalence, they only suspect the disease and sometimes notify it when they see clinical signs or mortality. This is why we can question the presence of asymptomatic carriers or if a lot of animals could recover keeping signs of the infection (IgG). This reality may reflect the importance of chronic disease which could be explained by the majority of pigs being of local breed and thus more resistant to ASF. Farmers are not aware of this chronic form of the disease characterized with respiratory disorders and drop in the growth and production. The increase of the prevalence in Fatick region (between 9% and 18.7%) compared to the one given by the Senegalese Institute for Agronomical Research (Sarr, 1990) reflects the important spread of the disease from south to north. This could be due to farmers moving to avoid the enzootic form of ASF and its constraints in these southern regions. This phenomenon is enhanced by a constant and increasing commercial exchange with this Casamance area. Furthermore there is a hudge lack of knowledge about this disease in the regions of Fatick and Kolda, respectively 20% and 25% of the breeders do not have any idea about the existence of ASF.

4.3.3. ASF in Europe

ASF remained endemic in Portugal and Spain for more than 30 years, until 1993 and 1995 respectively when the disease was finally combated as a result of an intensive eradication programme (Sanchez-Vizcaino, 2006). The Italian island of Sardinia was the only European area to remain in an endemic situation. Recently, in 2007, the Caucasian countries have become affected, and in many areas there are scenarios which can lead to endemicity. There are 3 distinct scenarios:

1. *The domestic-domestic scenario without ticks:*

Direct transmission by contact between among sick and healthy animals is the most common route of transmission (Sanchez-Vizcaino, 2006). This scenario occurred in Portugal and Spain and spread

was mainly due to the presence of carriers and contaminated transport. Spain managed to eradicate the disease from this scenario in 1989, after four years of strict enforcement of biosecurity measures, including the scrict banning of feeding swine with infected raw meat or pork products. Once ASF in established in domestic pigs, carrier pigs become an important source of virus, and their role in the epidemiology of the disease is a major consideration in designing a strategy for ASF eradication. The serological recognition of carrier pigs was an important aspect in the successful eradication of ASF in Spain (Arias and Sanchez Vizcaino, 2002).

2. *The domestic-domestic scenario with ticks:*

This scenario also happened in Portugal and Spain, where the extensive production of Iberian pigs challenged the eradication efforts, which lasted until 1993 and 1995, respectively. *O. erraticus* mainly colonized domesticated areas, essentially pigsties in outdoor pig productions in Spain and Portugal (Caiado, 1988; Oleaga-Perez, 1990). Ticks were usually found hidden in the cracks and crevices of the traditional old buildings and very rarely in modern buildings or outside the sties, in rabbit burrows within a limited range (less than 300m) of infested buildings (Oleaga Perez, 1989; Oleaga-Perez, 1990). To determine in which on which farms O. erraticus was present, serological tests were developed to detect anti-O.erraticus antibodies on swine (Canals et al., 1990). Because of the ability of the virus to persist in tick populations for long periods this has been referred by the field veterinarians as one of the major reasons for abandoning *O. erraticus* infested pig farms after an ASF outbreak (Boinas, 1995).

3. *The free-ranging with/without wild boar and/or vector scenario:*

This is the prevailing scenario in Sardinia and the Caucausus. In Sardinia, intensive and confined production systems represent a low percentage of total pig breeding systems, which are majoritarily constituted by backyard pigs (>90%) (Rutili, 2006). In the Caucasus the majority of pig breeding is seasonal and in backyard holdings. In Armenia there are some semi-professional farms under full confinement in specialized premises, however high biosecurity standards are not frequently met (Beltran-Alcrudo et al., 2008). Free-ranging pigs share communal lands in Sardinia (Montirano, 2007, in the EU Standing Committee on the Food Chain and Animal Health document:

http://ec.europa.eu/food/committees/regulatory/scfcah/animal_health/asf_67112007_sa.pdf, last accessed October 2008). In affected areas of Georgia, Armenia and Azerbaijan, backyard pigs often share communal lands, there is lack of continuous containment of pigs and free roaming and scavenging are widely practised (Beltran-Alcrudo et al., 2008). This allows contacts between domestic pigs and wild boars, in which infection patterns are similar. European wild boars are susceptible to ASF infection, with clinical signs and mortality rates similar to those observed in naturally infected domesticated pigs in Spain, Portugal and Sardinia (Italy) (Contini et al., 1983; Sánchez Botija, 1982).

It has been observed that the virus tends to disappear from wild boar populations if there are no subsequent re-infections (Laddomada et al. 1994) through contacts with free-ranging infected pigs and therefore, that wild boars do not play a major role as a virus reservoir in the absence of free ranging infected domestic pigs (Laddomada et al. 1994; Perez et al. 1998; Ruiz-Fons et al. 2008).

However, in areas where the disease is actively circulating and contacts with free ranging pigs occur, they can represent a serious challenge as disseminators of the virus across different territories (Beltran Alcrudo et al. 2008). Following the recent introduction of ASFV to Georgia in June, 2007, the disease has been spreading within the country and wild boars would have become infected through contacts with free ranging pigs. Wild boars have been reported infected in the Russian Republic of Chechnya, bordering Georgia and are equally suspected to have spread the disease to Azerbaijan and Armenia. It is feared that the infection in the wild boar population could complicate the short and long term control.

The ways of transmission between domestic and wild pigs are likely to be through ingestion of infected carcasses or by direct contact. Information regarding the way the virus is transmitted between both species is unclear, but in any case requires contact between wild and free ranging domestic pigs.

In the case of the European wild boar, as it occurs with the buhspig, contacts with infected ticks in their natural environment are probably unlikely since they do not live in burrows. So far, contacts between soft ticks (*Ornithodoros erraticus*) and wild boars could never be demonstrated (Louza et al. 1989; Laddomada et al. 1994). However, this situation could exceptionally occur if they share

common home ranges with domestic pigs when pig premises are infested. It is not known whether there are Ornithodorus in the Caucasian positive areas in and around pig pens or whether they will be vectors in case of presence. In Sardinia, there are no biological vectors but an endemic situation has been reached through the contacts among free-ranging, feral and wild swine.

4.4. RECENT EPIDEMIOLOGICAL TOOLS SUPPORTING CONTROL AND MANAGEMENT STRATEGIES

ASF is one of the most complex animal diseases, as different epidemiological cycles, involving domestic and wild swine and ticks as biological vectors in multiple combinations can happen. The existence of reservoirs, the lack of vaccine or treatment and the underdevelopment of pig rearing in endemic areas makes it an expensive disease to eradicate. There are no ASF models developed to aid decision making policies in respect of the best control strategy. The different scenarios and cycles increase modelling difficulty and uncertainties. Also, consequences other than economical, like the psycho-social or the environmental impact are hard to quantify. The identification of risk factors for the spread of ASF has been performed by several authors in several areas. The main risk identified is the free-ranging of pigs (Edelsten et al.1995, Allaway et. al, 1995 in Malawi; Manelli et al., 1997 in Sardinia), although insufficient veterinary resources and inadequate dissemination of information has also been recongnised (Edelsten et al., 1995 in Malawi; el Hicheri et al., 1998 in Cote d'Ivoire; Penrith et al., 2007 in Mozambique), and in Madagascar wild reservoirs and ticks seem to play a central role for the mainte. More recently, spatio-temporal analysis has proven useful in Sardinia to identify high risk areas in the province of Nuoro (hot-spot area in number of outbreaks), allowing to target resources (Mannelli et. al, 1998). Socio-economic impact has been dealt with by Samui et al. (1996) in Zambia, by Lyra (2006) in Brazil and by Babalobi et al. (2007) in Nigeria. The decade of 2000 is markedly moving to utilise epidemiological tools to prevent and early detect infectious diseases, as has been pointed out by the FAO and the OIE in several occasions (Roeder et al., 1999; Domenech et al., 2006). Epidemic investigation has evolved predominantly at the molecular level, and the different strains circulating in Africa have been genotyped revealing hypothesis regarding spread (Bastos et al., 2003; Bastos et al., 2004; Lubisi et al. 2005; Nix et al, 2006; Wambura et al., 2006).

Summary as provided by the authors:

ASFV replicates in swine and in soft ticks of the genus Ornithodorus. ASF can be transmitted directly from diseased or carrier pigs to healthy swine (wild or domestic) whenever contact is possible; or indirectly through fomites, ingestion of raw infected pork or pork products, or by biological vectors like Ornithodorus ticks. However, there are different epidemiological cycles or scenarios depending on the specific circumstances in each geographical area regarding virus strain, host susceptibility, biological vector presence and/or vector interaction with susceptible hosts. In East Africa, for example, ASFV is maintained mainly in a sylvatic cycle involving *O.moubata* vectors and warthogs, complicating control efforts. Conversely, in the Iberian Peninsula, *O.erraticus* was mainly associated to domestic rather than wild suids habitats, and the disease was effectively controlled, among other measures, replacing the old pig sties by modern structures. In Sardinia and some African countries, the disease is maintained by free range/backyard production systems that have recovered from infection and can act as carriers. In the Caucasus, contacts between diseased wild boars and free ranging pigs seem to play an important role in the spread of ASF. In Western Africa, one of the predominant patterns has been transmission between domestic pigs due to uncontrolled movements.

Future research identified by the authors:

Several international projects are being carried out to elucidate some of the current challenges in the epidemiology of ASF in Africa and Europe. In the Caucasus, it remains to be established if wild boars could have a reservoir rol or are only infected in areas where there are ongoing outbreaks in domestic pigs, and if there are biological vectors involved in which case it would be necessary to investigate their vectorial capacity or biting habits. In Sardinia and in Africa, attention is focused on the evolution of the circulating strains, from the molecular and the biological point of view an in the mechanisms of virus maintenance. Also, modelling of the best control options and risk mapping to aid in a targetted surveillance/ control are to be developed, together with an assessment of the risk of introduction of the disease into Europe.

5. DIAGNOSIS

ASF may be clinically undistinguisible from other pig diseases such as CSF, erysipelas, Salmonellosis, Pasteurellosis or other septicaemic conditions. For this reason it is essential to send samples for laboratory examination. Two main approaches have classically been carried out in order to detect ASF: 1) the direct identification of the virus and 2) the detection of antibodies against the virus (OIE Manual of diagnostic Tests and Vaccines for Terrestrial Animals 2008). Tests are applicable both in domestic as well as in wild life populations.

Reference experts and laboratories are shown in the following table 3.

Table 3. Reference experts and laboratories for ASF

Expert	Laboratory	Contact
Dr J.M. Sánchez-Vizcaíno	Facultad de Veterinaria, Laboratorio de Vigilancia Sanitaria (VISAVET),	HCV Planta sótano, Universidad Complutense Avda. Puerta de Hierro s/n, 28040 Madrid SPAIN Tel: (34.91) 394.39.75 Fax: (34.91) 394.39.08 Email: jmvizcaíno@vet.ucm.es or visavet@vet.ucm.es
Dr Chris Oura	Institute for Animal Health, Pirbright Laboratory	Ash Road, Pirbright, Woking, Surrey GU24 ONF, UNITED KINGDOM, Tel: (44.1483) 23.24.41 Fax: (44.1483) 23.24.48 Email: chris.oura@bbsrc.ac.uk
Ms Alison Lubisi	Onderstepoort Veterinary Institute, Exotic Diseases Division	Private Bag X5, Onderstepoort 0110, SOUTH AFRICA Tel: (27.12) 529.95.60 Fax: (27.12) 529.95.95 Email: Lubisia@arc.agric.za

5.1 AGENT DETECTION

The OIE Manual includes precise instructions on which samples are suitable for laboratory testing and how to preserve them during their transportation to the reference laboratory. Virus can be isolated, particularly from lymphatic tissues.

Hemadsoption test (HAD)

The haemadsorption test (Malmqvist & Hay, 1960) is based on the capacity of pig erythrocytes to adhere to the surface of pig monocyte or macrophage cells infected with ASFV. Most A isolates produce this phenomenon of haemadsorption. A positive result in the HAD test is definitive for ASF diagnosis. Some 'nonhaemadsorbing' isolates have been reported. Most of them are avirulent, but some do produce typical acute ASF.

Fluorescent Antibody Test (FAT)

The FAT assay (Bool et al., 1969) is used as an additional method to detect antigen in tissues of suspect pigs in the field or those inoculated at the laboratory. Positive FAT results, together with clinical signs and appropriate lesions, can provide a presumptive diagnosis of ASF. It can also be used to detect ASFV antigen in leukocyte cultures in which no HAD is observed and can thus identify nonhaemadsorbing strains of virus. It also distinguishes between the CPE produced by ASFV and that produced by other viruses, such as Aujeszky's disease virus or a cytotoxic inoculum. However, it is important to note that in subacute and chronic disease, FAT has a significantly decreased sensitivity. This reduction in sensitivity may be related to the formation of antigen-antibody complexes in the tissues of infected pigs which block the interaction between the ASFV antigen and ASF conjugate (Sánchez-Vizcaíno, 2006; OIE Manual 2008).

Polymerase Chain Reaction (PCR)

Highly conserved region of the genome are amplified by PCR techniques using specific primers for these genomic regions. This method allows the detection and identification of a wide range of isolates belonging to all the known virus genotypes, including both nonhaemadsorbing viruses and isolates of low virulence. This technique provides high sensitivity and specificity and is specially recommended for the identification of ASFV DNA in pig tissues that have undergone putrefaction, or when samples may have been inactivated during transportation to the laboratory. Four validated PCR procedures have been included in the OIE Manual: a highly sensitive gel-based PCR assay for

the detection of ASFV (Agüero et al., 2003), a PCR test that allow the differentiation of ASFV from CSFV (Agüero et al., 2004) and two real-time PCR assays (Zsak et al., 1995; King et al., 2003)

Pig Inoculation

Pig inoculation has classically allowed the differentiation between CSF and ASF, as these diseases produce indistinguishable clinical signs. However, this technique is currently not in use, since alternative laboratory tests that give reliable results for both ASF and CSF are available. The pig inoculation test is slow, expensive and difficult to perform and results in acute distress for the animals involved, which raises serious animal welfare concerns. It is therefore no longer recommended for use.

5.2 ANTIBODY DETECTION

Serological tests are recommended where the disease is endemic or where a primary outbreak is caused by a strain of low virulence or avirulent. Some of these tests have been validated and are used for laboratory diagnostics and can be used for large-scale screening of sera (Arias and Sánchez-Vizcaíno et al., 1992; Escribano et al., 1990; Pastor et al., 1990; Sánchez-Vizcaíno, 1987; Pan et al., 1972). The most commonly used serological test is the ELISA –Enzyme-linked Immunosorbent Assay- The OIE Manual recommends the confirmation of suspected cases of disease by using a standard serological test (ELISA), combined with an alternative serological test (IFA). In warthogs, contacts with ASF virus can be detected serologically with different available tests such as Indirect ELISA (Hamblin et al. 1990), Western Blott or recombinant p30 proteins serological tests. However, in some cases, the percentage of detection has found to be very low (below 50%) (Perez-Filgueira et al. 2006). In bushpigs, many authors have failed to detect antibody circulation, and the validation of serology as a useful tool to detect infected bushpigs is not reported in the literature.

ELISA

The ELISA (Arias and Sánchez-Vizcaíno et al., 1992; Pastor et al., 1990) test can detect antibodies to ASFV in pigs that have been infected with virulent and avirulent isolates. This is the great advantage of ELISA.

Although ELISA sensitivity is very high, it can drop when the samples are poorly preserved. To solve this problem, several new ELISAs based on the use of ASFV recombinant proteins are now being validated (Gallardo et al., 2006). Carrying out a second confirmatory test such as the immunoblotting test or the IFA test described below is recommended in the case of a doubtful result or a positive result when sera are suspected to be poorly preserved.

Indirect Fluorescent Antibody Test

This test (Pan et al., 1974) is recommended as a confirmatory test in ASF-free countries where positive results in the ELISA test are found, and for sera from endemic areas that give an inconclusive result in the ELISA (OIE Manual 2008).

Immunoblotting

This test is recommended by the OIE as an alternative to the IFA test to confirm equivocal results with individual sera. The immunoblotting test is based in the same antigen-antibody binding principle. It is very specific and enables easy and objective interpretation of the results and a better recognition of weak-positive samples. Viral proteins that induce specific antibodies in pigs have been determined. These polypeptides have been placed on antigen strips and have been shown in the immunoblotting test to react with specific antibodies from 9 days post-infection.

Counter Immunoelectrophoresis

This test (Pan et al., 1972) provides results in only 30 minutes, but due to its low sensitivity it is recommended for the screening of pools of sera, but not individual samples. Counter Immunoelectrophoresis is a cheap technique that only requires the use of electrophoresis equipment and a constant-current power supply.

The Commission Decision 2003/422/EC approves a diagnostic manual for ASF in the European Union.

Summary as provided by the authors:

Laboratory tests are essential to establish a definitive diagnosis of ASF and there are many techniques available that allow a rapid diagnosis which aids in the control of ASF in the absence of treatment or vaccine.

The isolation of the agent is recommended when a suspension of a disease occurrence arises in countries free from ASF but suspecting its presence and there are four methods: (1) inoculation of pig leukocyte or bone marrow cultures (cytopathic and hemadsortion effects); (2) antigen detection by direct fluorescent antibody test (FAT) in smears or cryostat sections of tissue; (3) detection of virus genome by PCR; (4) Negative results on viral isolation on leukocyte cultures or bone marrow cultures are confirmed. **Cells from negative cultures are examined for antigen** by FAT and subinoculation into fresh leukocyte cultures. If tissues are unsuitable for virus isolation and antigen detection, PCR is recommended. In doubtful cases, the material is passaged and the procedures are repeated.

Serological tests are recommended where the disease is endemic or where a primary outbreak is caused by a strain of low virulence or avirulent. There are four methods: (1) tests for specific antibody detection in serum or extracts of tissue by ELISA (which is the prescribed test in the OIE manual for international trade); (2) Indirect fluorescenct antibody test; (3) Immunoblotting (confirmatory test); (4) Counter immunoelectrophoresis test (only for screening of large groups). There are also type-specific test but they are not available for routine use.

Future research identified by the authors:

Research is focused on the development of tests for virus strain typing, in the absence of neutralising antibodies that would allow serotype differentiation. Also, there is an ongoing evaluation of the current diagnostic tests to investigate the behaviour of the African isolates.

6. PREVENTION, CONTROL AND ERADICATION

There is no vaccine available against ASFV so the control strategy largely relies on early detection through rapid diagnosis, implementation of strict biosecurity measures, and stamping out of infected and/or exposed swine (Sanchez-Vizcaino, 2006). The exponential intensification of animal movements and product exchanges enhances the risk of ASF introduction in a free country. Actually, the virus was recently introduced into Eastern Europe. It was initially reported in Georgia (June 2007), then the neighbouring Armenia (August 2007), Azerbaijan (January 2008) and Russia (June 2008). These countries are developing trade with the new eastern member states of EU, thus, ASF is an important issue for the next future. Considering this threat, an effective vaccine would help to control on of the major pig disease in Africa –providing an alternative to mass slaughter of animals and preventing the spread of the ASF in both Africa and Europe after an outbreak.

Nevertheless, it has been possible to successfully control and even to eradicate ASF in many countries even in the absence of an available treatment or vaccine. Other countries, however, are still fighting against it. Challenges to the control and eradication include the existence of free-range production systems, contacts with *Ornithodorus* ticks and/or wild suids, and endemicity involving asymptomatic carriers of the disease. As an example, in just 4 years (1985-89) Spain managed to eradicate the disease in most of its territory (areas rearing intensive pigs); while in those areas with outdoor production systems and *O. erraticus*, eradication efforts totalled 10 years (1985-1995) (Ministerio de Agricultura, Pesca y Alimentación, 1996). Success is highly determined by a good communication among all parties involved in an outbreak (diagnostic laboratories, farmers, field and official veterinarians, disease crisis centres, media). Improvements in pig housing to avoid tick and wild animal contacts have proved to be very efficient in order to minimize infection to eradication levels and should not be forgotten. A cost-benefit analysis should determine in each case if contingency efforts will be directed towards control or towards eradication.

Because ASF is a costly disease and because there are no effective vaccines for its control, it is especially important that ASF-free areas are kept free by preventing ASFV introduction (Sanchez Vizcaino, 2006).

6.1. EUROPEAN EXPERIENCES AND SCENARIOS

The European Union (EU) successfully eradicated ASF from its territory, with the exception of the Italian island of Sardinia which remains endemic. The last EU countries to eradicate ASF were Portugal (1993) and Spain (1995). The EU regulations that deal with the control of ASF are collected under Council Directive 92/119/EEC, in which general measures for the control of certain animal diseases, like ASF, are laid (notification, zoning, surveillance, restriction/ban of movements and trade, stand-still, cleaning and disinfection, tracing back and forward, disposal of carcasses, restocking, reference laboratories and contingency planning); and Council Directive 2002/60/EC, with specific measures for the control of ASF, like the processing of all swine-derived products and waste, specification of the general measures of CD 92/119/EEC, control with insecticides, measures for the control and eradication of ASF in feral pigs, and measures to prevent the spread of ASFV by (biological) vectors and to retrieve vectors.

Eradication and control measures are relatively easy to implement on intensive industrialised productions. Complications however arise if the vector is involved, if the swine population implied is wild or if there are free-ranging pigs. Spain, Sardinia and the Caucasus represent three examples of distinct epidemiological conditions which have been addressed in different ways:

1) The example of Spain. Spain was endemic of ASF for more than 30 years (1960-1995). The Spanish pig production experienced huge changes going from family breeding to industrialised production in a few years. The increase in production from 1960 to 1989 was estimated to be of 178.3% (Bech-Nielsen et al., 1995). Export restriction was however hampering the pig industry development. In 1985 the Spanish Coordinated Eradication Programme was implemented. The main measures taken included: (1) detection of ASF positives and carriers (diagnosis network by mobile veterinary field teams; serologic control of breeders, reference and regional laboratories;

elimination of outbreaks; identification, slaughter and compensation of carriers; zoning for up to 3 months; presence of 10-20% of sentinels for 1-3 months previous to restocking; no restocking if *O. erraticus* on site); (2) strict enforcement of sanitary measures (improvements of sanitary infrastructures of holdings with funds and credits; enhanced biosecurity; investigation of contacts; establishment of protection and surveillance zones; control of movements, individual identification for animals moved for fattening/breeding; regionalisation); (3) active participation of farmers (publicity campaign and media cover; associations for sanitary defense, the members of which received aids from the administration to check health status). Ninety six per cent of the Spanish territory stopped experiencing outbreaks since 1987 (indoor production system), however, the southwest area continued to be infected. Infected area was maintained until 1993 because of unsanitary pigpens, infected *O. erraticus* and uncontrolled wild boars. Obtaining samples from wildlife populations with the collaboration of hunter's associations has proved to be an efficient method of monitoring the disease in wildlife populations (Perez *et al.* 1998; Arias and Sanchez-Vizcaino 2002b). From 1993 serological screening programme carried out different criteria depending on type of area (free, surveillance or infected) but mainly on wild boars and breeding sows, and all pigs in areas of *O.erraticus*; unsane pigpens destroyed and metal fences were constructed around good pigpens to avoid entrance of animals. Last outbreaks in Portugal had been recorded in the border with Spain, so from 1994 until 1996 there was a Coordinated Programme with joint efforts with Portugal and partly funded by the EC to eradicate ASF in the remaining infected areas. In 1995 Spain was declared free, having successfully eradicated ASF even from endemic areas without essentially needing a vaccine. (Arias and Sanchez-Vizcaino, 2002b)

2) The example of Sardinia. Sardinia is a Mediterranean island belonging to Italy and self-sufficient regarding pig production. ASF has been in Sardinia since 1978. The epidemiological scenario that prevails in Sardinia is traditional pig rearing with close contact with wild pigs but no presence of the biological vector. There was an eradication plan that was abandoned in 1998. The high risk factors for the endemic situation in Sardinia have been pointed out to be: illegal movements; deliberate auto-infection; free-ranging pigs; poor collaboration from pig owners; civic

use of commumal lands permitted by a law but lack of an extensive pig breeding regulation. (From the EU: http://ec.europa.eu/food/committees/regulatory/scfcah/animal_health/asf_67112007_sa.pdf). Endemicity is also attributed to the prevailing practice of private house slaughter without veterinary inspection (ProMED mail post 20021128.5910). After a recrudescence of ASF in 2004-05, a new EU eradication plan was approved in March 2005 based on the correction of specific weak points in the monitoring and control of the disease; the division of the island into zones with different rules according to their ASF status (Infected, High risk, and Surveillance); marking of pig meat and pig products from Sardinia; and follow-up of the control measures. (Record of the Standing Committee on the Food and Animal Health held in Brussels on 2nd March 2005; http://ec.europa.eu/food/committees/regulatory/scfcah/animal_health/summary36_en.pdf). Commission Decision 2005/362/EC and amendments deal with the approval of ASF eradication in feral pigs in Sardinia.

The delay between suspicion and culling has been estimated to be 6 days. The update on 1st November 2007 (http://ec.europa.eu/food/committees/regulatory/scfcah/animal_health/asf_67112007_sa.pdf) stated there were no active outbreaks, but still 3 active surveillance zones, and no positive holdings in wild boar-infected zones. An electronic epidemiosurveillance system was put in place. Management orders in 2006 included:

- revise compensation for owners
- familiar breeding regulation
- sanitary education for owners
- duty for communal authorities to regulate the pig breeding in their lands
- regional legal and technical support for municipalities
- 3 million euros for financing high risk area projects → work in progress (fences, water, etc). So far (Dec07-Apr08), finishing works in 5 municipalities
- Oncoming meetings with order forces and forest guards.

3) The example of the Caucasus. Georgia, Armenia, and Azerbaijan (non-EU territory) together with areas in Russia boundering Georgia have been recently affected by ASF for the first time (2007-08). The situation in Georgia is marked by the difficult political situation, which does not favour the regional coordinated ASF control efforts, a high percentage of undeveloped pig industry (small backyard holdings, free roaming and scavenging) and presence of wild boars. In Armenia, the situation is similar regarding backyard pig production and presence of wild boar, although there are also some full-confinement specialized premises (http://www.fao.org/docs/eims/upload/242232/EW_caucasus_apr08.pdf).

Azerbaijan had a reduced pig population (the majority of country is Muslim) the whole of which was culled to control the disease (ProMED mail post 20080202.0416).

Both Georgia and Armenia received international help and assessment to aid with the control of ASF. Georgia and Armenia received recommendations on the control program and on the epidemiological investigation from a joint mission EC/FAO/OIE in June 2007 (ProMED mail post 20070627.2066, http://www.fao.org/newsroom/en/news/2007/1000612/index.html), from the Swiss Agency for Development and Cooperation in July (ProMED mail post 20070920.3131; full report can be found at http://www.safoso.ch/activities/ongoing_activities/index.html). Armenia also received a visit from FAO in October 2007 (ProMED mail post 20071004.3275), as their assistance was needed for compensation schemes, improved diagnosis and surveillance, and training of farmers. Finally, the FAO Technical Cooperation Programme has provided Armenia and Georgia each with US$ 500 000 for emergency assistance against ASF (TCP/ARM/3102, available online at http://www.fao.org/world/Regional/reu/projects/TCP_ARM_3102%20(E)_en.pdf; TCP/GEO/3103, available online at http://www.fao.org/world/Regional/reu/projects/TCP_GEO_3103%20(E)_en.pdf).

ASF was late detected in Georgia as it was misdiagnosed as Postweaning Multisystemic Wasting Syndrome (PMWS) (ProMED mail 20070607.1845) in May 2007, which was confirmed to be ASF

by the OIE reference laboratory (IAH, Pirbright) in June 2007. The start date of the outbreak was estimated to have happened in April 2007 (WAHID report http://www.oie.int/wahid-prod/public.php?page=single_report&pop=1&reportid=5720). The Caucasus area lacked PCR diagnostic capability until June 2007 when it was established in Georgia and Azerbaijan (ProMED mail post 20070615.1954).

6.2. AFRICAN EXPERIENCES AND SCENARIOS

6.2.1. Production systems in Africa

Pig farming in countries like Mozambique and in many other African countries can be characterized by commercial farming, basically found in urban and periurban areas, and small scale farming found basically in rural areas. Commercial farmers usually apply rigorous and adequate sanitary measures to protect their herds from ASF. In contrast, in rural areas, traditionally reared pigs are allowed to roam freely, allowing contacts with wild pigs and soft ticks. Very often, those farmers ignore the ways of transmission of the disease and the measures they need to apply to prevent it.

The source of infection for commercially farmed pigs appears to be the movement of infected pigs from rural to urban areas and in that sense, the existence of a small scale family sector, represents a major permanent risk for the commercial sector. Unfortunately, the occurrence of disease in commercial farms results in substantial economic losses (Samui, 1996). This results in negative perceptions among development agencies that are reluctant to invest in pig production in most of sub-Saharan Africa.

Therefore, adequate measures of protection are necessary for both sectors to be able to co-exist with each other. Despite high frequency of outbreaks in many countries in Southern Africa, pigs remain a species of considerable socio-economic importance. As a result, pig farming in rural areas is considered a worthwhile occupation in many countries since it provides a source of protein and income. This has allowed, in addition to the two categories of pig farmers stated above, the emergence of farmers in rural and peri-urban areas, that are investing in feed to support better production and are receptive to suggestions to improve their productivity and to implement

preventive measures. In rural and peri-urban areas, education programmes of pig farmers associations, community leaders or rural extension officers implementing knowledge about prevention and control of ASF are perceived as a useful method to achieve producer-based systems of surveillance and control of ASF (Penright et al. 2004a & b).

6.2.2. Control measures

6.2.2.1. Areas with existence of a sylvatic cycle and limited pig tradition

In the absence of vaccine and considering the implication of the sylvatic cycle in the epidemiology of the disease in Southern and East Africa, it seems logical that the separation between domestic pigs and wild hosts and treatment of pig premises with acaricides (Pérez-Sánchez, 1992; Plowright, 1994) where tick infected by ASFV occurs, should be useful methods of ASF control. The physical separation between domestic pigs and wildlife has given good results in controlling the disease, even in areas where the virus was circulating among natural populations of infected warthogs (Plowright 1981;Wilkinson 1984)

Based on the presence of the above epidemiologically significant factors and occurrence of outbreaks, South Africa has designated, since 1935, ASF control areas that mainly constitute the North West, Limpopo, Mpumalanga and northern KwaZulu-Natal. In these areas commercial pig farming is discouraged and where it occurs, strict requirements such as pig proof double fenced in paddocks have to be adhered, to ensure that pigs do not come in contact with wild pigs or ticks. Movement of pigs and pig products from these areas is restricted to movement permits. Animal slaughter and area quarantine are the only methods of control for the disease. These measures were also quite successfully applied in Kenya between 1964 and 1994, providing good results (Penrith et al. 2004a). In South Africa, eight outbreaks with a total of 260 cases of pig infections were reported, mainly in Limpopo Province between 2000 and 2005. These are largely rural, mostly poverty stricken areas where pig- production is the major source of income with some commercial producers. Most outbreaks in the ASF control areas occur within free ranging herds. Control has otherwise been successful, that a system of accreditation of farms with high health status has been implemented.

Finally, for some Southern and Eastern African countries such as RSA, Zimbabwe, Botswana, Namibia and Tanzania, control is facilitated by lack of tradition in pig farming.

6.2.2.2. Control in countries with only a domestic cycle and widespread pig tradition

The situation is different in other areas of Africa, such as Mozambique, Angola, Malawi, or many West African countries were keeping pigs is widespread. In the cases where only a domestic cycle is present, as seems to be the case in West Africa, the disease is maintained by "rolling epidemics" (Penrith et al. 2004a), transmitted from one pig population to the other, as new susceptible populations of pigs become available. In those situations, control can be achieved as long as the pigs are confined and protected from the exposure of other pigs and fomites. This situation can be easily achieved by commercial farmers or farmers that have sufficient means to feed their pigs in captivity.

However, in many developing countries, and particularly in Africa, poverty conditions condemn pig owners to let their pigs in free ranging conditions to scavenge for food. In those, circumstances the disease is incontrollable. This situation is aggravated by lack of infrastructures and resources from the veterinary services to confirm the diagnosis and react promptly, and lack of provisions from the governments to compensate pig owners for eventual implementation of stamping out operations, which would have catastrophic socio-economic implications and are clearly inapplicable in that context. This has devastating effects in the economies of rural people in developing countries and the potential spread of the disease to other geographic locations above the African continent such as the introductions in Madagascar (Roger, 2001), and more recently in Mauritius (Defra 2007) or the Caucasus (Penrith et al. 2004a).

In that context, a suitable and potentially viable alternative would be to achieve the participation/cooperation of pig producers in developing countries (Penrith et al., 2007). In those cases, education and awareness among the producers associations of how the disease is transmitted and about preventive and control methods have proved to be useful, to detect the disease promptly

and to control it, in those cases where pigs can be kept indoors. In systems of traditional pig production, where feeding pigs indoors in uneconomical, exploitation of natural resistance of pigs might be an option (Penrith et al. 2004a), that has not been so far sufficiently explored (Thomson et al. 1999).

Ideally, the control measures to be implemented in the case of a localized outbreak are the following ones:

- Early warning and reporting
- Quarantine of infected herds and movement control
- Immediate slaughter of all pigs and compensation of the owners
- Burial and treatment or burning of carcasses in burial sites for carcasses as close as possible from the infected site. Avoid moving carcasses over distances.
- Wholesale destruction of edible meat, or alternatively boiling of the meat from non infected pigs for 30 minutes, before transport and consumption.
- Cleaning and disinfection of infected premises with 2% sodium hypochlorite, sodium hydroxide or commercial viricidals
- Treatment of the premises with acaricides.
- Keep premises empty before restocking. The period of keeping them empty is variable: OIE recommends 40 days (International Animal Health Code Comission 2001). However, much shorter periods can be considered under tropical conditions (Plowright et al. 1994).
- A population of sentinel pigs, fully susceptible and serologically negative should be introduced in the premises and monitored during six weeks without clinical signs before restocking (Penrith et al. 2004a).

6.3. VECTOR CONTROL

Control of *O.erraticus* is very difficult due to its long life, resistance to fasting, the possibility of alternative hosts other than pigs and the possibility of hiding deeply in the fissures of the buildings where it is difficult to spray with acaricides. Its eradication from the old buildings has generally been very unsuccessful. Suggestions for control include inoculation of the pig hosts with avermectins or

chlorpyrifos, use of fumigation with methylene bromide associated with a spray application of a product type carbaryl (Endris, 1992). No vaccine against the ticks exists yet, but studies have been undertaken to evaluate several salivary glands extracts and "concealed" gut antigenic extracts (Manzano-Roman, 2007; Manzano-Roman, 2006; Astigarraga, 1997; Astigarraga, 1995).

From the observations made in the field, no effective method for the long-term control of ticks exists and the various alternatives need further investigation. At present the only practical measure is not to house pigs in old, infested, buildings and prevent their access to them when located within the area of free ranging of a pig herd. There are tests that identify the presence of ticks by detecting salivary antigens of ticks in swine (Canals et al., 1990).

6.4. VACCINES

Since the first attempt to develop a vaccine in Portugal in 1963, a satisfactory vaccine immunisation has been not achieved. Live-attenuated, inactivated, proteins or recombinant vaccines have been tried unsuccessfully. The reasons are essentially the ASFV lack of induction of neutralising antibodies and ASFV's variability.

Inactivated vaccine does not produce any protection. Live-attenuated vaccine protects some pigs against challenge with the homologous strain of virus, but the possibility of some of these pigs becoming carriers and developing chronic lesions exists and increases when a large number of pigs are vaccinated (Manso Ribeiro e al. 1963; Sanchez Botija 1963). Other studies have shown that serum from pigs resistant to homologous and some heterologous strain of ASFV inhibits (in vitro) infection of cells with different, but related, heterologous strains (Ruiz Gonzalvo et al., 1986). The analysis of the complete nucleotide sequence of ASFV (Yanez et al. 1995) has opened new opportunities to explore immune mechanisms of protection and roles of various ASF virus genes. However, the eradication of ASF from Portugal and Spain, after more than 20 years of endemicity, proved that vaccine is not essential in the eradication of this complex disease (Sanchez Vizcaino, 2006).

6.4.1. Immunology

Studies on porcine protective immune responses against ASFV have been of the major goals of researchers since the disease was first diagnosed in Africa (Montgomery, 1921). The difficulty in inducing effective immunity may be related to the great variability observed among ASFV isolates or to the fact that ASFV replicates in some cells typically involved in the immune response, like monocytes and macrophages. ASFV is highly antigenic and high levels of specific antibodies are produced during ASFV infection, which are detectable for a long time after initial exposure (Sanchez-Vizcaino, 2006). Initial research strategies aimed at identifying the role of antibodies in protection. Depending on the virulence of the isolate, ASFV infected pigs produce antibodies detectable at the beginning of the disease (Malmquist, 1963; Coggins, 1974; Hamdy e Dardiri, 1984), but viraemia develops in parallel to the high level of antibodies (de Tray, 1957; De Boer *et al.,* 1969). Moreover pigs surviving natural infection with virulent and/or attenuated isolates resist challenge inoculation with the homologous and with the originally virulent isolates respectively, although with non-detectable neutralising anti-ASFV antibodies (Mendes, 1954; Mendes e Daskalos, 1955; Mendes, 1962; Malmquist, 1963; Manso Ribeiro *et al*., 1963; De Boer 1967). Passive transfer of humoral immunity by the administration of anti-ASFV serum from pigs and other infected animals doesn't interrupt the course of infection (De Boer, 1967; De Boer *et al.*, 1969). The incapacity of the immune sera to neutralize the pathogenic capacity of ASFV was also identified in studies in which immune serum was administrated to convalescent animals in conjunction to ASFV (Montgomery, 1921; Steyn, 1932; Baptista and Mendes, 1954; De Tray, 1963). To the present, no clear evidences show that specific anti-ASFV antibodies block the lethal effect of the viral infection. Nevertheless, studies have demonstrated that antibodies can interfere with the development of the disease when sera or colostrum obtained from pigs surviving the infection were experimentally inoculated in pigs together in with ASFV, in which a delay in onset and the development of the disease, a reduction of viral titres and increase in the surviving rate to viral infection was observed (Schlafer *et al.*, 1984a, b; Wardley *et al.*, 1985).

The role of anti-ASFV antibodies in the complement mediated cell lyses and in ADCC based on *in vitro* studies (Norley and Wardley., 1982, 1983a) suggests these mechanisms may play a role on the protection by antibodies described above.

As previously mentioned although the neutralising capacity of ASFV by specific antibodies has not been demonstrated, pigs surviving natural or experimental infection with virulent isolates survive challenge with homologous virus and pigs inoculated with attenuated ASFV survive infections with the parental virulent virus. These facts point for potential role of cellular and/or cellular based mechanisms in the survival of the pigs against ASFV infection as first reported by the development of hypersensitivity reactions to antigens (Shimizu et al., 1977), by the development of NK activity (Norley and Wardley 1993b) and by cytotoxicity activity of leukocytes (Norley et al., 1984) identified in experimentally infected pigs.

Other studies on the functional integrity of swine immune system during ASFV infection were developed based on measurement of peripheral blood leukocyte numbers, quantification of T and B lymphocytes and assessment of lymphoproliferative responses to mitogens and to virus or viral antigens. Some authors did not observe major changes in blood components of swine inoculated with some ASFV isolates (Knudsen et al., 1987; Genovesi et al., 1988; Wardley and Wilkinson, 1980), while lymphocytopenia observed by others during early post swine infection has been attributed mainly to B lymphocytes (Wardley and Wilkinson, 1980), or to a decrease on T lymphocytes (Sanchez-Vizcaino, 1981). Others observed no evidence of replication of ASFV in either T-cells and B-cells (Gomez-Villamandos, Hervas et al., 1995; Minguez et al., 1988).

Assessment of lymphocyte functional capacities, in experimentally ASFV inoculated swine, through the study of lymphoproliferative responses to mitogens has also shown conflicting results depending on the model of infection used (Martins and Leitão, 1984). The study of specific cellular effector mechanisms potentially involved in swine protection against ASFV infection has been based on experimental models in which pigs immunized with naturally occurring or attenuated low virulent isolates have shown to survive infection with highly virulent isolates (Escribano et al., 1993). Among others the non-haemadsorbing, naturally occurring low-virulent ASFV/NHV/P68 (NHV) isolate has been used to protect animals against ASFV/L60 (L60) allowing comparative studies of swine immune responses induced by each isolate and after challenge inoculation (Martins and Leitão, 1994).

Following development of research on different cellular immune mechanisms in viral infections and in particular regarding the identification of MHC restricted T cell cytotoxicity (Zinkernagel et al., 1974), later on the 80's and early 90's research efforts started to focus on cellular and cellular based immune responses towards ASFV infection.

Relevant on those studies, activity of ASFV specific cytotoxic T lymphocytes was evaluated in SLA "inbred" pigs experimentally infected with the ASFV/NH/P68 isolate (NHV) ; Cytotoxic assays were conducted *in vitro* using mononuclear leucocytes from infected pigs as effector cells and singeneic and halogeneic macrophages infected in vitro with different ASFV, as target cells. This model allowed for the first time the identification of ASFV specific $CD8^+$ lymphocytes that lyse macrophages infected with different ASFV isolates in the context of SLA Class I (Martins et al., 1988 e 1993). The *in vitro* re-stimulation of effector lymphocytes with homologous virus induced the production of IL2 and the development of LAK cells (limphokine activated killer) that have lytic activity over the infected macrophages (Scholl et al., 1989).

The identification of the CTL activity opened new insights for the characterization of some viral antigens recognized by these cells, namely:

- VP32 expressed in macrophages infected by the "Vaccinia" recombinant virus using the experimental infection model E-75a, attenuated by passages in cultured Vero cells (Alonso et al., 1997);

- VP72 expressed in macrophages treated with the protein extract of external membranes from recombinant clones in the form of fusion proteins with the lipoprotein of the membrane of *Pseudomonas aeruginosa* (OprI) (Leitão et al., 1998), using the NH/P68 infection model. The use of the expression vectors above mentioned allowed the obtention of a recombinant clone named A2 (Leitão et al., 2000) with the capacity to stimulate the LCT specific activity for ASFV *in vitro*. This protein demonstrated to have 99% of identity with the ORF of the gene G1340L of the isolate BA71V. LMN from pigs inoculated with preparations from the outer membrane of A2 reduced in 99.6% the titles of ASFV, when *in vitro* incubated with cultures of autologous macrophages. Nevertheless, after inoculation with the virulent virus these pigs developed ASF acute and fatal. The above-mentioned studies suggest that different viral components are able to contribute to the CTL

activity, thus rendering difficult the identification of dominant CTL epitopes in the different ASFV isolates or attribute their exclusive role in protection against natural infection. However, the relevant role of ASFV specific CTL activity on protection has been recently confirmed by showing that i*n vivo* depletion of $CD8^+$ T lymphocytes abrogates protective immunity to ASFV (Oura et al., 2005)

The role of NK cells in protection against ASFV has been pointed in studies using pigs inoculated with the NHV isolate (Leitão *et al*., 2001) in which two patterns of infection are observed: pigs that remain assymptomatic and pigs that develop chronic lesions of ASF. In pigs remaining assymptomatic the viraemia was rarely observed in late stages of infection, the levels of serum Igs were unchanged, the concentration of anti-ASFV specific antibodies observed was relatively low, but the levels of the NK activity were very high. These animals survived challenge with the highly virulent L60. In pigs with chronic forms of disease no changes where observed in the levels of NK cells (in relation to control pigs), but developed fever and viraemia after 14 days of infection and high levels of specific anti-ASFV antibodies with marked hipergamaglobulinemia involving IgG1, IgG2, IgM and IgA. These results point to the importance of the role of the NK cells in the survival of the pigs to infection.

Relevant on the ASFV pathogenesis as above mentioned, the virus preferentially infects pig monocytes and macrophages, which are the main targets for the *in vivo* viral replication. In accordance to this, studies *in vitro* have shown that ASFV of different virulence infect and induce lyses of blood derived macrophages (Casal et al, 1984; Enjuanes et al., 1977; Malmquist and Hay, 1960), bone marrow and alveolar macrophages (Carrascosa et al.,1982; Malmquist and Hay, 1960). Macrophage infection by ASFV is of foremost relevance on the pathogenesis infection taking in consideration that macrophages play important roles both on innate and acquired immune responses. Relevant in the role of macrophages as orchestrators of immune responses they synthesize cytokines that have an impact in the development of the inflammatory responses (pro-inflammatory cytokines) and cytokines that participate in the development of the specific immune mechanisms (immunoregulatory cytokines) through the activation of Th1 and Th2 responses (Murtaugh et al., 1996).

Among others, initial studies have shown that ASFV inhibits phagocytosis, antibody mediated phagocytosis and chemotaxis in porcine macrophages cultivated *in vitro* although no changes were observed in the expression of Fc receptors, neither in the capacity of the induction of the antibody dependent citotoxicity (ADCC) (Martins et al., 1988). In identical circumstances, the expression of SLA antigens was not changed on those cells (Gonzalez-Juarrero et al.1992).

More recently, research towards the characterization on the impact of ASFV infection on the expression of relevant cytokines at mRNA levels in porcine blood derived macrophages infected in vitro with the two ASFV isolates of different virulence, L60 and NHV, demonstrated a particular effect of the infection by this isolate in that significantly increased levels of transcripts for TNFα, IL6, IL12 and IL15 were identified at 6 hours post infection in contrast to the effect of infection with L60 (Gil et al.,2003). Extended studies, recently published (Gil et al, 2008), on the impact of the infection of porcine macrophages by the two above mentioned ASFV isolates in the expression of IFNα, TNFα, IL12p40 (mRNA and protein) and TGFβ (mRNA) at different times post infection (2, 4 and 6 hours), confirm the differential expression of those cytokines on macrophages infected with either ASFV isolates and reinforce the role of the NHV as capable to induce cellular based responses in the natural host such as the previously described ASFV-specific CTL activity (Martins et al., 1993) and NK activity (Leitão 2001), thus supporting the relevance of Th1 responses in the host, towards activation of protective cellular immune responses against ASFV infection which may be relevant for the development of efficient vaccines.

6.4.2. ASFV genes involved in immune-evasion

Like other large DNA viruses such as Herpes, Pox or Adeno viruses, ASFV has developed a large range of defence mechanisms to escape from the immune host responses. *In vivo*, the virus replicates mainly and preferentially in macrophages which are a key player of the innate immunity, able to react very quickly and with a large range of responses to infection. They are indeed responsible for the secretion of pro-inflammatory cytokines. The main strategy used by the virus to evade host defences is to modulate the signalling pathway of infected macrophages in order to interfere with the expression of certain genes including those playing a role in the innate and acquired immunity (Dixon et al., 2004).

Some of the virus proteins have a direct impact on the host immune responses. The A238L gene encodes a protein playing a crucial role in the control of the immune responses. This protein has an IκB-like domain that inhibits the host transcription factor NFκB (Yanez et al., 1995). The NFκB pathway is part of the transcriptional activator of genes encoding pro-inflammatory cytokines, chemokines and anti-apoptotic proteins like IAP, Bcl2 and Bcl-IX (Ghosh et al, 1998). The IκB family members bind the NFκB transcription factor resulting in the inactivation of gene transcription (Baeuerle and Henkel, 1994; Ghosh and Karin, 2002). Actually, A238L attach the NFκB transcription factor thus inhibiting the induction of the pro-inflammatory cytokine responses (Powell et al., 1996; Revilla et al., 1998). A238L protein has also a PxIxIxT domain at the C-terminal end of the protein that binds the calcineurin catalytic subunit – a serine-threonin phosphatase – (Miskin et al., 1998, Miskin et al., 2000). Calcineurin has a wide range of action, like activation of transcription factors, modulation of the receptor activity and regulation of the cell apoptosis by the pro-apoptotic Bad protein activation (Clipstone and Crabtree, 1992; Macian et al., 2001; Hamalainen et al., 2002; Crabtree and Olson, 2002; Graef et al., 2003). To some extent, the activity of A238L is similar to that induced by cyclosporine A, an immunosuppressive drug that also binds calcineurin and inhibits its phosphatase activity (Jin and Harrison, 2002). Blocking the activation of macrophages result in the down-regulation of NFAT (nuclear factor of activated T cells) and/or Elk1 transcription factors and consequently prevents the lymphocyte activation. In addition, the expression of A238L protein in infected macrophages reduces the expression of cyclooxigenase-2 and the production of prostaglandin E2 (Granja et al., 2004).

ASFV modulates the cell apoptosis. Rapid apoptosis of infected cell would block the replication of the virus and prevent the dispersion of new virions. Numerous viruses have thus developed mechanisms to by pass this defence mechanism by encoding genes to prevent cell apoptosis (Benedict et al., 2002; Hay and Kannourakis, 2002). ASFV encodes two proteins similar to host anti-apoptosis proteins. The A224L protein shows similarity to the IAP family of apoptosis inhibitor. Bounds to the caspase-3, it inhibits its protease activity as well as the cell death (Nogal et

al., 2001). It must be noticed that the A224L protein is packaged into the virion which could indicates an early action after the cell infection. The second protein is the ASFV A179L which is a Bcl-2 homologue. This viral protein contains a domain in which any mutation consequently stops its anti-apoptotic activity (Neilan et al., 1993; Afonso et al., 1996: Revilla et al., 1997). A third protein was described to have anti-apoptotic effect: the EP153R whose action is not clear but which was described to reduce the transactivating activity of the cellular p53 protein (Hurtado et al., 2004).

After the identification of several genes involved in host immune defence or apoptosis evasion, deleted mutants were generated. Virus deleted for A238L, A224L (IAP homologue) and EP153R gene (C-type lectin) were constructed but their inoculation in pigs did not show any difference in terms of virulence comparing to the virulent wild type isolate (Neilan et al., 1997a, 1997b, 1999). This may result from the fact that the virus may have other subsidiary genes sharing the same functions or able to overcome the loss of these genes.

MGF encoded proteins have been demonstrated to decrease the transcription of the type I interferon-encoding gene and indirectly the transcription cascade activated by this gene and responsible for a major innate response of the immune system to virus infection (Afonso et al., 2004). Other viral proteins can interfere with the cell signalling pathway. j4R protein binds to the α-NAC (α chain of nascent polypeptide associated complex) which is supposed to play a role in the translocation of proteins to the secreting system (Wiedmann et al., 1994;Goatley et al., 2002) as well as in c-jun trans-activation responsible for the transcription of many immunomodulatory genes (Yotov et al., 1998). An ubiquitin-coupled enzyme encoded by the virus has been also demonstrated to have a role in the regulation of other host genes transcription pathway by binding the host nuclear protein SMCy (Bulimo et al., 2000).

The CD2v, encoded by EP402R gene, has a peptide signal and a single transmembrane domain. Its extracellular domain is similar to the host CD2 adhesion protein and contains two Ig-like domains.

CD2v supports the adsorption of RBC to the infected cells (Rodriguez et al., 1993; Borca et al., 1994). The virus particle itself acquired the CD2v by budding through the outer cell envelope containing the CD2v (Ruiz-Gonzalvo et al., 1996). Pig infection using a CD2v deleted virus delays the viremia by slowing down the virus dissemination by the systemic route (Borca et al., 1998). One of the major ASFV characteristic is also to reduce both *in vivo* and *in vitro* the lymphocyte proliferation, the CD2v being responsible for a competitive inhibition of the interaction between the lymphocyte/CD2 and the macrophage/LFA3 (Borca et al., 1998). It was also reported that internal cytoplasmic part of the CD2v protein attaches to the actin binding adaptator SH3P7 that has possibly a major role in the protein translocation to, through and from the Golgi and thus modulates the protein trafficking within the infected cell (Kay-Jackson et al., 2004; Warren et al., 2002, Mise-Omata et al., 2003).

Selective or spontaneous gene deletion in ASFV isolates has allowed the identification of critical genes in terms of virus virulence in domestic pigs, cell tropism and capability to the virus to replicate in ticks (Tulman and Rock, 2001). The virus replication in pig macrophages *in vitro* is altered if some genes encoding for enzymes involved in the nucleotide metabolism are deleted, like DNA repair enzymes (Oliveros et al., 1997) or dUTPase homologues (Yanez et al., 1995). A DNA molecule can contain uracil after incorporation of dUMP during the DNA synthesis or after a spontaneous deamination of cytosine residues, leading to dU-dA and dU-dG base pair, respectively (Oliveros et al., 1999). These two base pair motifs are from highly mutagen to lethal for normal cells (Ingraham et al., 1986; Impellizzeri et al., 1991). To be repaired DNA-containing deoxyuridines is first excises by a DNA-uracil glycosylase (Barnes et al., 1993) and the use of dUTP instead of TTP to fill-in the gap is avoided by an enzyme: the deoxy-uridine phosphatase (dUTPase) which requires a certain dUTP/TTP ratio to achieve this substitution (Curtin et al., 1993). This enzyme eliminates dUTP from the dNTPs pool but generates also some dUMP, precursor of TMP synthesised by the thymidylate kinases (Kornberg et al., 1992). Macrophages are highly differentiated and non-dividing cells which consequently have a limited pool of available nucleotides, particularly a low rate of TTP and dCTP, and do not have the capacity to synthesise

nucleotides (Terai et al., 1991). The presence of the viral dUTPase in the infected cell cytoplasm at both early and late stage of the infection is supposed to allow a high TTP/dUTP ratio in order to minimize the mis-incorporation of uracil in the viral DNA. Despite the absence of the viral enzyme in the cell nucleus its early presence in the cytoplasm is supposed to be efficient enough to maintain the integrity of the viral DNA synthesised early after the infection (Garcia-Beato et al., 1992). Thus, if the deletion of the dUTPase encoding gene reduces the virus replication in the macrophages, the deletion of the thymidine kinase generates a low virulent isolate in pigs (Moore et al., 1998; Oliveros et al., 1999).

The deletion of the 9GL gene (B119L) involved in the virus morphogenesis as part of the redox chain, reduces the virus replication in macrophages (100-fold reduction) and also the virulence in pigs (Lewis et al., 2000). In addition, the elimination of MGFs 530 and 360 has been demonstrated to reduce the virus replication in ticks as well as the mortality of infected macrophages, suggesting a role of the encoded protein in the cell survival (Zack et al., 2001; Burrage et al., 2004). The deletion of the DP96R in the E70 isolate, if it did not impact the growth of the virus on macrophages reduced the viraemia in infected pigs from 100 to 1000-fold (Zack et al., 1998).

6.4.3. Recent strategies

African wild swine survive to infection with highly virulent isolates and domestic pigs could also recover from infection with low to moderately virulent strains (Leitao et al., 2001) or with closely related strains (Boinas et al. 2004; Leitao et al., 2001), it is sensible that defining immunological targets and the mechanisms involved in these models of protection may allow the development of an efficient vaccine. However, immunization has not yet been achieved.

To develop a vaccine some elements must be taken in account: first the high number of targets and immunological events observed in infected animals and the fact that only few antigens (p32, p72 and p54) or attenuated viruses have led experimentally to partial protection (Borca et al. 1994; Gomez-Puertas et al. 1998). A putative vaccine may consequently stimulate the response of both CD4 and CD8 T lymphocytes and B cells. Second, as it was described above ASFV can modulate

the host immune system by expressing proteins involved in the immune evasion suggesting that the gene encoding for these proteins are crucial for the virus survival in the host.

Consequently, three different strategies have been defined to develop a vaccine against ASFV

1. Generation of a recombinant and infectious Aujezsky virus targeting the macrophages and encoding for viral proteins that stimulate both production of neutralizing antibodies and cellular response.
2. Delivery of recombinant proteins of the same virus antigens and including a fragment of an antibody recognizing porcine MHC class 2 to pig antigen presenting cells.
3. Generation infectious but attenuated recombinant virus deleted specifically for genes of virulence and immune system evasion or deficient to replicates in cells.

The first and second strategies were chosen because first an Aujezsky's virus vaccine is already accepted and used (van Oirschot GD 1990) and some studies demonstrated the role of neutralizing antibodies in the animal protection (Zsak et al. 1993; Borca et al. 1994; Onisk et al. 1994; Gomez-Puertas et al. 1997). However, recently it has been shown that neutralizing antibodies to ASF proteins p30, p54 and p72 are not sufficient for antibody-mediated protection (Neilan et al. 2004). It has been demonstrated that CD8 T cells of multiple antigenic specificities are activated after ASFV infection in pigs (Gomez-Puertas et al. 1997; Jenson et al. 2000) explaining why immunization using only one epitope have failed. The use of a mix of recombinant proteins containing multiple epitopes is so pertinent and could allow a better protection.

The third strategy may ensure that almost all the virus proteins would be expressed in order to allow the recognition by the immune system of most of the immune virus target to able a cross-protection between not too closely realted isolates. It can be possible using the same approach to develop recombinant ASFV defective enough to be unable to persist in pigs after one replication cycle (Andres et al. 2002). ASFV encodes for about 60 genes that are not essential for the virus replication. These genes play a role in the host immune system evasion or virulence and must be targeted. As examples: the CD2v is an adhesion protein that mediate the haemadsorbtion on the red blood cells accelerating the virus propagation in the animal by the systemic pathway ; it's deletion

delay the onset of the disease (Borca et al. 1998). A238L gene plays a major role in the modulation of the immune response by interfering on one hand with the induction of pro-inflammatory cytokines (innate immune response) (Tait et al. 2000) and on the other hand by inhibiting the NFAT transcription factor controlling the activation of the lymphocytes (acquired response) Miskin (1998, 2000) Also, the j4R protein or a virus –encoded ubiquitin conjugating enzyme are known to have a role in the modulation of the host gene transcription pathway (Goatley et al. 2002; Bulimo et al. 2000). At last, a lot of genes belonging to the different MGF are known to be genes of virus virulence (Tulman and Rock 2001). Deleting selectively several of these genes in combination may lead to generate an attenuated virus which can be an efficient vaccine.

6.3. RISK ASSESSMENT- POSSIBLE SOURCES OF INTRODUCTION AND SPREAD

Risk assessment of the introduction of ASF may enable to target surveillance efforts and an epidemiological analysis may help to identify specific risk factors that enable a better control. The identification of possible ways of introduction is based on past experiences and highlights the importance of active and passive surveillance as well as biosecurity measures.

The information available about the sources of introduction and spread of ASF is compiled in Table 4. This information may be interesting for policy makers and it could be used for future risk assessments

Table 4. Suspected sources of introduction or spread of ASF

Date	Country	Suspected source of introduction or spread	Reference
1989	Zambia	Bush area clerks fed sandwich leftovers to other clerks' pigs	ProMED 20010924.2327
1960	Portugal	Imported meat products	Neitz, 1963
1978	Brazil	Raw waste at international airport	McDaniel, 1986
1978	Brazil	Trade and tourism between Spain, Portugal and Brazil	Lyra, 2006
1978	Rep. Checa	Raw waste at international airport	McDaniel, 1986
1978	Malta	Raw waste at a sea port	McDaniel, 1986
1978	Sardinia	Raw waste at a sea port	McDaniel, 1986
1980	Cuba	Live pigs/pork products imports	McDaniel, 1986
1983	Italia	Import of pig products	McDaniel, 1986
1985	Belgium	Import of pork	Bironf et al. 1987
1985	The Netherlads	Illegal feed swill from hospitals, hotels and restaurants	Terpstra et al, 1986
1995	Malawi	Introduction from endemic area in Malawi. Factors contributing to spread: scavenging pig husbandry, lack of mobility for vets to ensure observance of restrictions.	Edelsten et al. 1995
1998	Nigeria	Media advising not to eat meat from infected pigs (might have influenced people to give it to their animals?).	ProMED 19980916.1866
1998	Nigeria	Infected neighbouring country (Benin?)	ProMED 19980709.1282
1998	Togo	Infected neighbouring country	ProMED

			19980615.1128
1998	Madagascar	Late diagnosis (misdiagnosed as csf or taschen disease), no test for asf available, lack of surveillance or alert system, limited economic resources, *O. moubata porcinus* presence, *Potamochoerus larvatus* possibly contact with domestics	Rousset et al. 2001
1999	Botswana	Pigs had broken through fence and mixed with warthogs	ProMED 19990804.1339
2001	Zambia	Neighbouring abattoir, spread through movement of animals	ProMED 20080209.0527
2001	Kenya	Ugandan infected pigs brought to slaughter → pigs fed with abattoirs offals; or pigs brought to slaughter then sold alive instead.	ProMED 20010927.2356
2001	South Africa	Direct contact domestic pig-warthog	ProMED 20010810.1893
2004	Tanzania (endemic)	Traded pigs introduced from neighbouring country into refugee camps	ProMED 20040426.1157
2004	Namibia	Tick bites: inadequate separation domestic/wild; feeding with offals from hunted warthogs (through the warthogs skin with ticks or directly by spleen and lymphnodes)	ProMED 20050109.0072
2005	Nigeria	Reoccurence by fomites	ProMED 20050815.2387
2006	Uganda (endemic)	Residents blamed for failing to carry out proactive measures	ProMED 20060109.0076
2007	Burkina faso	Illegal movement of animals	ProMED 20070728.2429

2007	Kenya	Illegal movement of animals and uncooked swill feeding	ProMED 20070505.1456
2008	Tanzania (endemic)	Introduction of live susceptible pigs	ProMED 20080307.0924

Summary as provided by the authors:

There is no vaccine available against ASFV so the control strategy largely relies on early detection through rapid diagnosis, implementation of strict biosecurity measures, and stamping out of infected and/or exposed swine.

Some of the common failures to control the disease once it is spread are listed below:

- Late detection
- Insufficient resources or political unstability = poor disease control and surveillance system, no culling possible, inadequate communication schemes, null or low compensation to farmers, lab technology missing, insufficient vet mobility to observe compliance, insufficient vet or social workers training, rural pig housing that allow the presence of the vector or access to/from wild animals.
- Scavenging pig husbandry or free-roaming animals.
- Uncooked swill feed.
- Illegal movements.
- Ticks and wild pigs as reservoirs.
- Co-circulation of several genotypes with different characteristics.
- Failure to identify risk factors.

Prevention is by far the best possible measure to avoid the disastrous consequences that an incursion of ASF may provoke, considering the high costs to control its spread due to the lack of an effective vaccine or treatment. Risk assessment tools should be applied in each area attending to the specific epidemiological conditions, such as sanitary condition in neighbouring or trading countries; quantity and type of imports; pig husbandry systems and practices; sanitary condition of pig industry; presence of vector, presence of wild boars; social-political-religious factors; networks among pig premises; or veterinary efficacy. The assessment can highlight where and whether it would be possible to experience a sylvatic cycle, involving ticks and boars that could be clinically inapparent; a sylvatic-domestic, where enhanced biosecurity measures should be applied to outdoor

pig production systems; or domestic, in which case standard measures against Notifiable diseases could be applied if resources are not limiting. Planning in advance is therefore essential, even regarding budget allowances, so that credits and international assistance can also be prepared to be readily available.

Early warning systems and a good epidemiosurveillance network are the next step to avoid an uncontrolled spread. Unfortunately, in many cases ASF has been the scapegoat to build an efficient disease control system.

Future research identified by the authors:

Identification and definition of the mechanisms of virus encoded “evasion” genes which interfere with host defences and immune responses and/or affect virus productivity and virulence, will improve basic knowledge for better understanding viral-host interactions. Pig macrophages are the main targets for viral infection. Deeper characterization of viral interactions with these cells, and with the domestic pig as a natural host, namely at the level of cytokine and chemokine protein translation and consequent activation of relevant cellular protective mechanisms, using viral isolates well characterized at genome level (naturally obtained or experimentally manipulated), may open new insights for the manipulation of immune responses towards the stimulation of protective mechanisms thus contributing to the development of efficient vaccines.

Epidemiological models could help to choose the best control strategy and to identify risk areas for targeted surveillance, improving prevention, or to optimize resource allocation for control purposes.

Annex 1. Date, location and brief description of historical ASF introductions from East and Southern African countries (according to scientific papers published and official reports from FAO and OIE)

Country	Date	Description	Source
Guinea-Bissau	1958	No information on the first introduction but enzootic situation suspected since this date	Sarr 1990
Senegal	1959	Introduction in Dakar in traditional farms - suspected source: Guinea-Bissau by pig trade from Casamance	Sarr 1990
	Since 1959	No official reports but spread to all pig production areas - chronic form with pig carriers	Gilbert & Memery (nd)
	1978	First official report but already present	FAO 1998a
	1986-1989	Numerous outbreaks annually in Ziguinchor, Fatick, Thies and Dakar	Sarr 1990
	1996-2005	1-5 outbreaks/year reported in Casamance, Sine-Saloum and Thies regions, Official report only when infected commercial farms	FAO 1998a
Nigeria	1973	First report	FAO 1998a
	1997	Introduction in Lagos and Ogun states - source: Benin	FAO 1998a
	1998	Spread to Benue region in big commercial farms (<60000 pigs died)	FAO 1998d
	2001	Outbreak at the University's Teaching and Research Farm and other parts of Ibadan city – Source unknown or re-emergence?	Babalobi 2003
Sudan	1978	2 outbreaks	Sanchez Botija 1982
Sao Tome	1979	1 outbreak in a farm close to farms receiving pork meat from	Sanchez

& Principe		Angola (7000 pigs died or slaughtered) – no information on potential spread	Botija 1982
Cape Verde	1980 1985 1998	First report in Santiago island Introduction and then enzootic in at least islands of Maio and Santiago with peaks of morbidity/mortality twice a year in spring and winter Peak particularly devastating	FAO 1998b FAO 1998c FAO 1998c
Cameroon	1982	50% of pig population died, stamping out policy leading to pig breeding given up for small pig holders who never restock - suspected source: Europe - spread to become enzootic with sporadic outbreaks annually	FAO 1998a
Chad	1983-1985	Outbreaks related to Cameroon	Sanchez Botija 1982
Ivory Coast	1996	Agban escarpements and then spread to traditional farms in Adidjan - source: infected meat from Agban - 22 000 pigs died & 100 000 pigs slaughtered (=25%)	FAO 1997
Benin	1997-1999 2000 2001	Introduction in the Hindé neighborhood of the Nokoué lake and in the international market of Dantokpa in Cotonou, 1781 outbreaks mainly in southern and central areas of Atlantique, Mono, Ouémé and Zou (350 000 pigs died, 42 000 pigs slaughtered = 50%) Reemergence with 51 outbreaks in the same 4 departments Spread to Natitingou and Parakou departments with 47 outbreaks	FAO 1998a, Ayissiwebe 2004 Ayissiwebe 2004 Ayissiwebe 2004
Togo	1997	Introduction from the border of Benin and spread to southern areas (4000-5000 pigs died, 2500 pigs slaughtered)	FAO 1998a

	1998 2002	Spread to Lomé and Kara (crossroad for Benin, Ghana and Burkina Faso) 1 suspected outbreak in Bassar district near the boarder with Ghana	FAO 1998a
Ghana	1999 2002	Introduction and spread to Dangme East district in Greater Accra region, Awutu Efutu Senya district in Central region & Ho and South Tongu in Volta region (600 pigs died 6927 pigs slaughtered) Reemergence in Zabzugu district - suspected source: Togo - virus extremely virulent causing complete depopulation in pigs	FAO 2000 FAO 2002
Gambia	2000	Outbreaks in Greater Banjul area and Western Division & spread to North Bank and Lower and Upper River Divisions (8511 pigs died out of 10 291 cases in 38 foci throughout the country except Lower River Division)	FAO 2001
Burkina Faso	2003-2005	Kompienga region near the boarder with Togo and Benin, spread to the Cantral region in Kadiogo district (90% of pigs died in Kadiogo), spread to Southern-Central region and Central escarpments	Rey-Herme 2004, OIE 2005
Madagascar	1997 Since 1998	First introduction from the eastern coast of the African continent to the southern part of the island and spread to the other regions except the north and the west Several outbreaks reported in the whole country suggesting enzootic situation but large under-reporting	Rousset 2001

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