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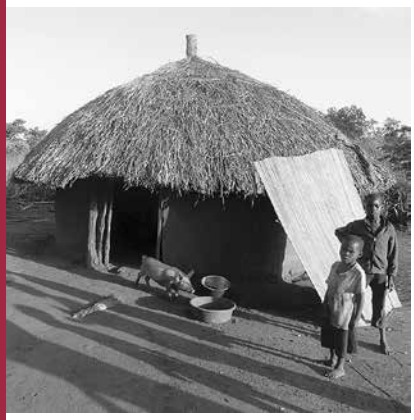

manual

AFRICAN SWINE FEVER: DETECTION AND DIAGNOSIS

A manual for veterinarians

Cover photographs:
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AFRICAN SWINE FEVER: DETECTION AND DIAGNOSIS

A manual for veterinarians

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Acronyms

ADR	International Carriage of Dangerous Goods by Road
ASF	African swine fever
ASFV	African swine fever virus
AU-IBAR	African Union Inter-African Bureau for Animal Resource
AWB	Air waybill
CISA	Center for Research on Animal Health
CSF	Classical swine fever
DGR	Dangerous Goods Regulation
DBS	Dried blood spot
EDTA	Ethylenediaminetetraacetic acid
EFSA	European Food Safety Authority
ELISA	Enzyme-linked immunosorbent assay
EMPRES-i	Global Animal Disease Information System
EuFMD	European Commission for the Control of Foot-and-Mouth Disease
FAO	Food and Agriculture Organization of the United Nations
FAOSTAT	FAO Statistical database
FAT	Fluorescent antibody test
FMD	Foot-and-mouth disease
GEMP	Good emergency management practices
HAD	Haemadsorption reaction
HAI	Haemadsorption inhibition assay
IATA	International Air Transport Association
IAEA	International Atomic Energy Agency
IB	Immunoblotting
IFA	Indirect fluorescence antibody test
INIA	National Institute for Agricultural and Food Research and Technology
IPT	Immunoperoxidase test
NGO	Non-governmental organization
OIE	World Organisation for Animal Health
PCR	Polymerase chain reaction
PDNS	Porcine dermatitis and nephropathy syndrome
PRRS	Porcine reproductive and respiratory syndrome
SOPs	Standard operating procedures
TAD	Transboundary animal disease
WAHIS	World Animal Health Information System
WHO	World Health Organization

Introduction

The purpose of the manual is to provide veterinary professionals, para-professionals, and laboratory diagnosticians with the information they need to promptly diagnose and react to an outbreak or case of ASF. Pig farmers, hunters and forest managers will also benefit. Any statement made in this manual is intended to provide guidance and should not be treated as a prescription.

The manual provides general information on the disease and its causes, including epidemiology, transmission pathways and geographic distribution. It then follows chronologically with the detection and diagnosis of ASF, from field diagnosis (clinical signs, postmortem findings and differential diagnosis) to laboratory confirmation (i.e. all main techniques for the detection of both virus and antibodies). Included are recommendations on how to sample, pack and transport specimens from the field to the laboratory, and the immediate actions required at farm level when an outbreak is suspected. Although in less detail, the manual also covers ASF awareness-raising, prevention and control. Finally, sources of assistance are recommended, together with suggestions for further reading.

African swine fever (ASF) is a contagious viral disease that affects pigs of all ages, inducing a haemorrhagic fever. It can appear in a variety of forms ranging from peracute, acute, subacute, to chronic and unapparent. It is most often recognized in the acute form with an associated lethality of up to 100 percent.

African swine fever is a severe threat to pig production systems. It not only threatens food security and challenges the livelihoods of pig producers and other actors in the supply chain, but may also have major consequences on international trade as a result of trade restrictions.

Feral pigs and European wild boar (*Sus scrofa ferus*) are equally susceptible to ASF. Although African wild suids do not show clinical signs of infection, they are, together with *Ornithodoros* soft ticks, the natural hosts and reservoir of the virus, while domestic pigs are accidental hosts. In domestic pigs, ASF is transmitted mainly through direct contact, via the oro-nasal route, through excretions from infected pigs, or from ingestion of pork or other contaminated products containing the virus (e.g. swill, waste, carcasses, etc.). Further transmission pathways are indirect contact through fomites or vector-borne transmission through bites from infected *Ornithodoros* soft ticks, where present. The disease is not a zoonosis, i.e. it does not infect humans.

Today, the disease is considered endemic in sub-Saharan Africa, the Italian Mediterranean island of Sardinia, and parts of the Caucasus and Eastern Europe. The extremely high potential for transboundary spread of ASF was demonstrated by its arrival in the Caucasus in 2007 and its progressive advance through the Russian Federation into Eastern Europe, where it now seems established. Already endemic in some of these regions, it is gaining increased attention from governments and international organizations. A serious risk exists of further spread of ASF from these areas given the extensive transboundary movements of individuals, pork products, fomites, and infected wild boar. Any country

with a pig sector is at risk of ASF. The backyard sector, with its low biosecurity, is particularly vulnerable.

Since there is currently no effective vaccine or treatment, the best strategy against ASF for countries/zones that are still free of the disease is preventing the entry of the virus through improved border control, proper awareness-raising, and improved biosecurity. Prevention through limitation of wild boar movements is much more challenging, so early detection is the best approach here. For infected countries, awareness and improved biosecurity also apply, together with quick control of outbreaks though movement restrictions and stamping-out policies. Given the threat the disease poses to global agriculture and trade, ASF must be reported to the World Organisation for Animal Health (OIE).

ASF – An overview

THE PIG SECTOR

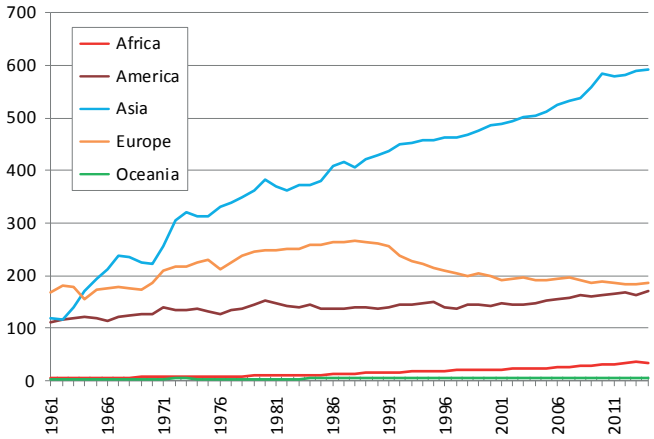
Within global livestock production, the pig sector plays a key role as a source of animal protein. Largely due to the increase in worldwide demand for meat, pigs have become a crucial food source due to their fast growth, efficient feed conversion, quick turnover, and prolificacy. Pork is the most consumed meat from terrestrial animals, accounting for over 37 percent of global meat intake, followed closely by chicken (35.2%) and beef (21.6%) (FAO, 2013).

The pig sector has grown steadily over the past decades (Figure 1), but the increase has been uneven around the globe. Large populations occur in China and parts of Southeast Asia such as Viet Nam, in Western Europe, central and eastern areas of the United States, Central America, and southern Brazil. In Africa, where ASF is endemic, pig numbers are growing steadily, reflecting the increased adoption of pig husbandry in a continent where ruminants are by far the dominant livestock species. The distribution of pigs is largely influenced by religious and cultural factors – there are few or no pigs in largely Muslim countries (Figure 2).

The sector is characterized by a deep divide between traditional, small-scale, subsistence production on the one hand, and industrialized pig farming with increasing vertical integration on the other. Of course, there is a whole range of intermediate systems in between.

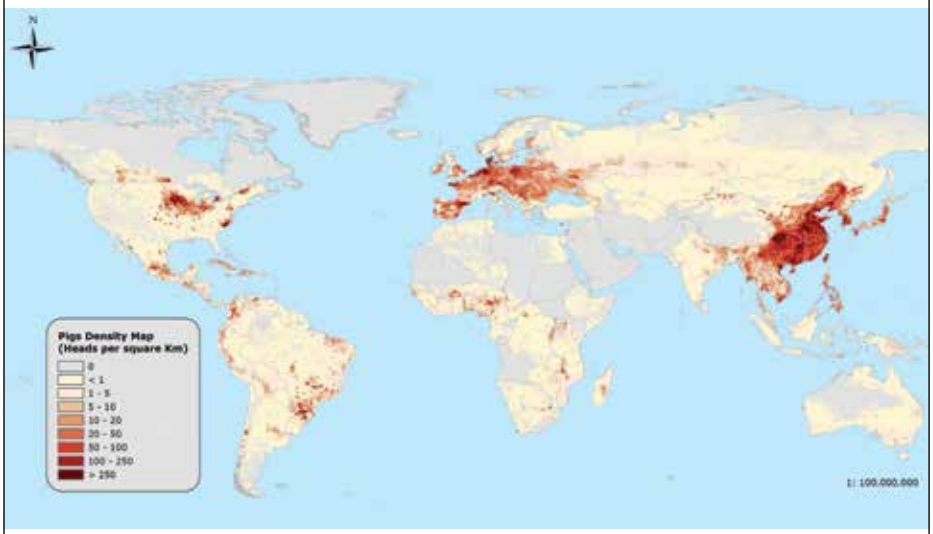
Commercial pig production has intensified significantly in recent decades. More pigs of the same few breeds are kept on fewer, larger farms, with corresponding increased

FIGURE 1
Number of pigs (x 1 000 000) in the world by region (1961-2014)



Source: FAOSTAT, 2016

FIGURE 2
Global pig density per km²



Source: Robinson *et al.*, 2014

output of animal products. Large-scale production systems have achieved a high level of uniformity because they are based on the same genetic material and therefore use similar feed and housing infrastructure. But while these larger-scale operations are helping meet an increasing share of global pork demand, about 43 percent of pigs are still produced in backyard and other small-scale settings, particularly in the developing world (Robinson *et al.*, 2011).

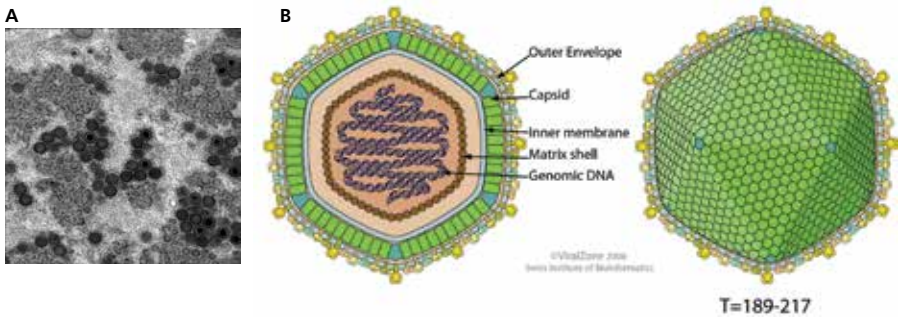
In the developing world, most pigs are still kept in traditional, small-scale, subsistence production systems in which they provide much more than meat. In such low-input systems, pigs produce added value for farmers by converting household waste into protein, while also providing manure to fertilize fields and fishponds. Hence, pork contributes to food security and nutrition, while live animals represent a financial safety net, play a significant role in cultural traditions, and supply additional cash for school fees, medical treatment, and small investments.

These two very different stakeholder groups have different priorities in adjusting production practices or investing in biosecurity to prevent and control pig diseases. Indeed, the backyard sector, characterized by low biosecurity, outdated husbandry practices and technologies, and poor awareness of, and compliance with, animal health regulations (outbreak reporting, movement control, certifications, vaccination, etc.) plays a major role in the introduction, spread, and maintenance of ASF and several other pig diseases.

THE ASF VIRUS

The causative agent of ASF is a unique, enveloped, cytoplasmic, double-stranded DNA arbovirus, which is the sole member of the family *Asfarviridae* (Figure 3). Although it

FIGURE 3
The ASF virus close-up



A. *Transmission electron micrograph of Vero cells infected with African swine fever virus. Mature virions, immature virions and membrane intermediates are visible. Mature virions approximately 200 nm in diameter.* (*Source:* The Pirbright Institute, UK).

B. *Diagram of the Asfarviridae virion.* (*Source:* Swiss Institute of Bioinformatics).

was generally considered that there is only one serotype of ASF virus, recent studies have reported the classification of 32 ASFV isolates in eight different serogroups based on a hemadsorption inhibition assay (HAI) (Malogolovkin *et al.*, 2015). However, genetic characterization of all the ASF virus isolates known so far has demonstrated 23 geographically related genotypes with numerous subgroups, illustrating the complexity of ASF epidemiology (Figure 4). The genotype is the reflection of the variability of a segment in a single gene and protein (VP-72) and is used for mainly phylogenetic and molecular epidemiological purposes (e.g. to identify the source of outbreaks). As far as is known, it does not determine the virulence, or other disease parameters.

ANIMALS AFFECTED

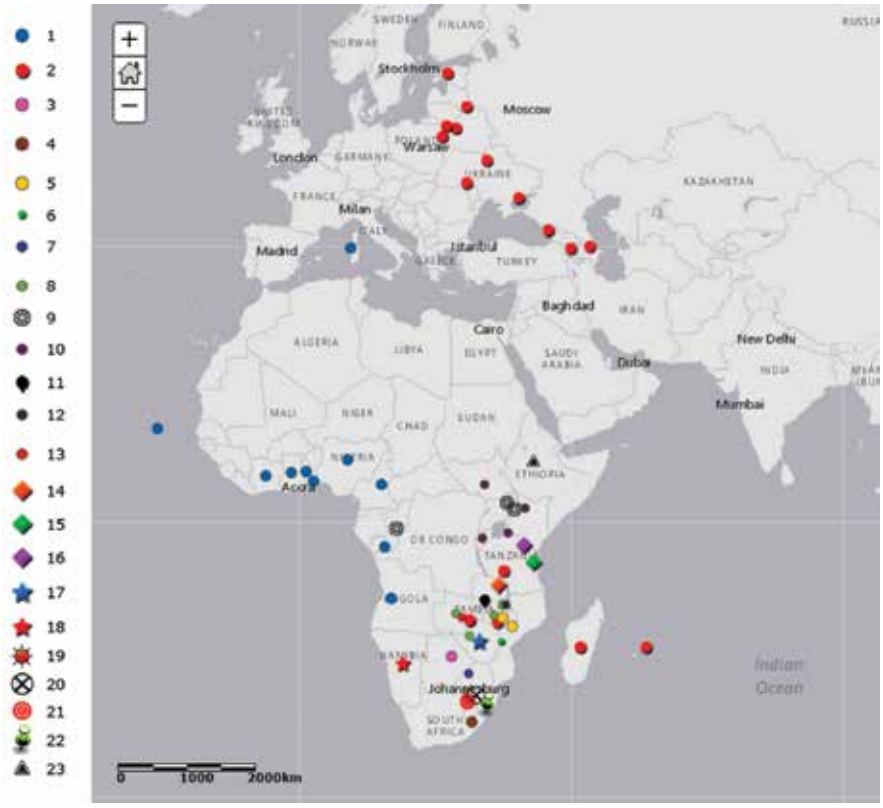
In the natural sylvatic cycle, the soft-bodied, eyeless *Ornithodoros* ticks (also known as tampans) are, together with African wild suids, the natural reservoir hosts of ASFV. They can transmit the virus through their bites.

All members of the pig family (*Suidae*) are susceptible to infection, but clinical disease is only seen in domestic and feral pigs, as well as in the closely related European wild boar. Wild African suids are asymptomatic carriers of ASF and act *as the* reservoir of the virus in parts of Africa (Figure 5). These include warthogs (*Phacochoerus africanus* and *P. aethiopicus*), bushpigs (*Potamochoerus porcus* and *Potamochoerus larvatus*) and giant forest hogs (*Hylochoerus meinertzhageni*).

GEOGRAPHICAL DISTRIBUTION OF ASF

African swine fever is currently widespread in sub-Saharan Africa, Eastern Europe and the Caucasus and the Italian island of Sardinia. With the increased circulation of ASF, there is growing global concern that the virus will spread further into other parts of the planet. Any country with a pig sector is at risk, and history has shown that the disease can jump thousands of kilometres into previously free countries, mostly via meat arriving aboard

FIGURE 4
The global genotypic diversity of ASFV



Source: INIA-CISA, 2016

aircraft and ships and then incorrectly disposed of, or meat carried by individual travelers. Of particular concern is the potential spread into East Asia. With China relying heavily on the pork industry and owning almost half of the world's domestic pigs, an ASF epidemic would have a catastrophic impact on trade and pig production, with serious implications for global food security.

Official information on the status and dates of ASF outbreaks can be obtained from the World Animal Health Information System (WAHIS) at the World Organisation for Animal Health (OIE).

Africa

African swine fever is considered endemic in most countries in sub-Saharan Africa (Figure 6) but is also very dynamic, with new areas often being affected. The upsurge is largely driven by the pig sector's tremendous growth in Africa, with some countries more than doubling their pig populations (e.g. Madagascar, Namibia, Uganda) in less than a decade (FAOSTAT – http://www.fao.org/faostat/). The other major contributing cause is the

FIGURE 5
African swine fever hosts

A. *Domestic pig/Sus scrofa domesticus (©FAO/Daniel Beltrán-Alcrudo).*

B. *European wild boar/Sus scrofa ferus (©Swedish University of Agricultural Science (SVA)/Torsten Mörner).*

C. *Bushpig/Potamochoerus porcus (©Swedish University of Agricultural Sciences (SLU) and Swedish Veterinary Institute (SVA)/Karl Stahl).*

D. *Warthog/Phacochoerus africanus (©University of Pretoria/Mary-Louise Penrith).*

E. *Giant forest hog/Hylochoerus meinertzhageni (©John Carthy).*

F. *Ornithodoros erraticus* (male & female) *(©Institute of Natural Resources and Agrobiology of Salamanca (IRNASA), of the Higher Council of Scientific Investigations (CSIC)/Ricardo Pérez-Sánchez).*

increased movement of people and products. The sector's growth is occurring despite disorganized and insecure marketing systems, which offer little incentive to producers to invest in improving pig production.

Most of this growth is taking place in smallholder or backyard systems with low biosecurity levels, posing clear disease challenges. Moreover, eradication of ASF in Africa is very difficult with currently available tools – there is no vaccine available nor are any compensation mechanisms in place. Prevention and control efforts should therefore focus on improved husbandry practices and biosecurity, and protection of areas not affected by the disease (through regulated trade and swine sector development programmes that stress awareness and prevention measures). At the same time, it should be recalled that ASF dynamics differ from one subregion to another.

East Africa

African swine fever was first detected in Kenya in 1909 following the introduction into the country of European domestic swine (Montgomery, 1921). In East Africa, the virus is maintained in a sylvatic cycle between warthogs and *Ornithodoros* ticks living in their burrows. The first outbreaks occurred in pigs belonging to European settlers, and it was found that by erecting fencing around farms to exclude warthogs and ticks, pigs could be farmed safely. However, pig farming has since increased in popularity in the region and large numbers of animals are kept

in insecure or free-range systems. This has resulted in repeated ASF outbreaks, largely as a consequence of pigs and pork, rather than wildlife, moving. Increased peri-urban pig production is reflected in outbreaks around bigger cities such as Kampala, Nairobi, Mombasa, and Dar es Salaam. The existence of a cycle of maintenance between domestic pigs and *Ornithodoros* in Kenya has also been identified (Gallardo *et al.*, 2011).

Southern Africa

The sylvatic cycle involving warthogs is present in the northern parts of the subregion (Botswana, Malawi, Mozambique, Namibia, Zambia, Zimbabwe and the northeastern parts of South Africa). In Malawi and Mozambique, a cycle involving domestic pigs and ticks has been identified or demonstrated to be highly likely. Angola and Mozambique report outbreaks regularly, while the other countries have sporadically experienced warthog-related ASF. Zimbabwe reported its first outbreak in free-range pigs in 2015 after more than 20 years of absence. The northeastern part of South Africa, where a high proportion of warthogs are infected with ASF virus, is demarcated as a control zone in which pig farming is only permitted under conditions of strict biosecurity. But sporadic outbreaks nevertheless result from illegal activity. The rest of South Africa, Lesotho, and Swaziland have remained historically free of ASF – although, in 2012 South Africa experienced its first outbreak outside the control zone in more than half a century due to the illegal movement of pigs to the free area. The Indian Ocean islands remained free of ASF until 1997, when the virus was introduced into Madagascar, where it has since become endemic. In 2007, Mauritius experienced an incursion that was eradicated the following year. The subregion shows a high level of genetic variation (Figure 2) linked to the presence of the sylvatic cycle.

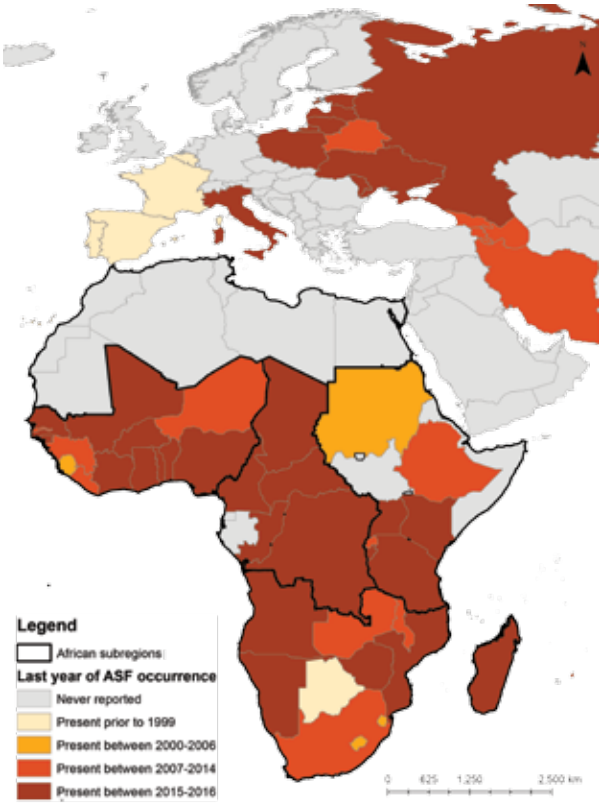
Central Africa

The Democratic Republic of Congo and the Congo Republic are historically endemically infected. It is likely that the sylvatic cycle is involved, at least in parts of those countries, as infected warthogs have been reported in Congo Republic (Plowright *et al.*, 1994; Saliki *et al.*, 1985). Other countries in the region have also reported outbreaks, notably Cameroon, which experienced its first incursion in 1982, not long after the pig population doubled. In 1973, the island country of Sao Tome and Principe experienced outbreaks that were rapidly eradicated. Chad reported its first outbreaks in 2010 in the south of the country, although there were anecdotal reports of ASF in Chad in the 1980s (Plowright *et al.*, 1994). Interestingly, ASF genotype IX, traditionally restricted to East Africa, has recently been recorded in the region, as has genotype I (Figure 2).

Western Africa

The first official report to the OIE of ASF in Western Africa was from Senegal in 1978, but a 1959 virus isolate from Dakar indicates that the virus was introduced at least two decades before. The disease in Western Africa appeared to remain restricted to southern Senegal and its neighbours (Guinea Bissau, Gambia and Cape Verde) until 1996, when Côte d'Ivoire experienced its first outbreak, and this was followed by an epidemic that involved most of the region's countries with any significant pig production (Benin, Nigeria, Togo, Ghana and Burkina Faso). The disease has since become endemic in most of these nations except

FIGURE 6
ASF status in domestic or wild hosts, as of April 2017



Iran only reported cases in wild boar.
Sources: AU-IBAR, EMPRES-i (FAO) and WAHIS (OIE), 2017

Côte d'Ivoire, which achieved a prolonged eradication within a year, until a new incursion in 2014. Niger and Mali reported their first outbreaks in 2009 and 2016 respectively. No sylvatic cycle involving wild suids and/or *Ornithodoros* ticks in maintaining the virus has been demonstrated. Only genotype I is circulating, suggesting introduction rather than evolution of the virus in the region (Figure 2).

Eastern Europe and the Caucasus

In 2007, ASF was introduced into Georgia. Of genotype II, the ASFV originated from south-east Africa and was most likely introduced via ship waste that was either turned into swill or was disposed of in an area accessible to scavenging pigs. The disease spread quickly throughout the Caucasus (Armenia in 2007 and Azerbaijan in 2008) and into the Russian Federation (2007). In the past few years, the disease has progressively spread westwards, entering Ukraine (2012), Belarus (2013), the European Union (Lithuania, Poland, Latvia and Estonia, 2014), and Moldova (2016) (Figure 6).

One of the main routes of infection in Eastern Europe is through the pork marketing chain, which brings in cheap, contaminated pork and pork products from infected areas. Swill feeding and improper disposal of carcasses then expose susceptible pig populations. The fact that ASFV remains infective from weeks to months in tissues and pork products enables it to persist in the environment (e.g. through carcasses), as well as in refrigerated and frozen meat and meat products.

In the affected EU Member States, wild boar are playing the main role in ASF infection, spread and maintenance. How they do so is not completely clear, but seems to depend largely on the population density of wild boar and their interaction with low-biosecurity pig production (free-ranging and scavenging pigs in particular). Carcasses of infected animals and food waste containing infected pork products are also thought to be involved.

To sum up, ASF is now firmly established (i.e. endemic) in some areas of the Caucasus and Eastern Europe, where it is not only causing considerable trade disruption but also inflicting significant damage on small-scale pig farmers.

Previous ASF incursions outside of Africa

In Europe, ASF was first introduced into Portugal from West Africa in 1957. After eradication of this incursion, an ASFV of genotype I reappeared in the country in 1960, and then spread across Europe (Italy, 1967; Spain, 1969; France, 1977; Malta, 1978; Belgium, 1985; and the Netherlands, 1986). It also hit the Caribbean (Cuba, 1971 and 1980; the Dominican Republic, 1978; and Haiti, 1979) and Brazil (1978). All countries successfully controlled the outbreaks after brief periods except for Spain and Portugal, where the struggle with the disease lasted several decades until the 1990s, and Italy's Mediterranean island of Sardinia, where ASF has been endemic since its introduction in 1978, circulating mainly in free-range settings and wild boar.

Transmission

The ASF virus persists in distinct cycles – traditionally, the sylvatic cycle, the tick-pig cycle and the domestic (pig-pig) cycle. More recently, a wild boar cycle has been described, which may sometimes be involved in the latter. The sylvatic cycle occurs only in parts of Africa and involves warthogs and ticks of the *Ornithodoros moubata* complex. The tick-pig cycle involves pigs and *Ornithodoros* spp. ticks, which have been described as infesting parts of Africa and the Iberian Peninsula.

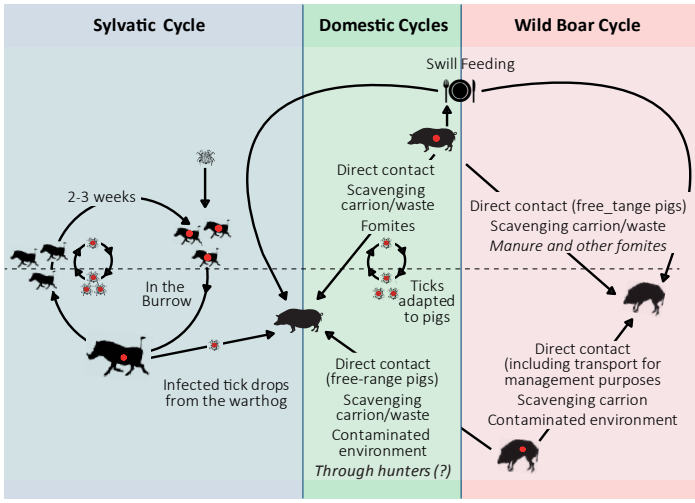
Transmission from the sylvatic cycle (African wild suids) to the domestic cycle (farmed pigs) occurs via indirect transmission by ticks. This can happen where pigs and warthogs share common grounds, particularly when warthogs establish burrows on farms, or when ticks are brought back to villages through the carcasses of warthogs killed for food.

SYLVATIC CYCLE

This cycle involves the natural hosts of the ASFV, i.e. warthogs and soft ticks of the *Ornithodoros moubata* complex, which act as biological vectors in Southern and Eastern Africa. However, information is scarce for other African regions. Also, the precise role of other African wild suids, e.g. bushpigs, still needs to be clarified.

The ASFV is maintained by tick-to-warthog transmission (Figure 7). Warthogs are infected by *Ornithodoros* bites in the first 6-8 weeks of life, while in the burrow (Figure 8).

FIGURE 7
Three ASF virus transmission cycles



Source: FAO, 2017

FIGURE 8
Warthog burrow

The natural habitat for Ornithodoros moubata ticks, Murchinson Falls National Park, Uganda.

They subsequently develop sufficient viraemia to infect other ticks. Following a short period when the virus is present in their bloodstream (2-3 weeks), the young warthogs recover, showing no clinical signs. In endemic areas, up to 100 percent of warthogs may have antibodies to ASFV. Virus can usually be recovered from the lymph nodes of warthogs of any age, although viraemia sufficient to infect ticks has only been found in neonates from burrows. It is likely that warthogs experience repeated infections when ticks feed on them, with low levels of virus remaining latent in the lymph nodes.

Tick populations can remain infected and infective for long periods due to transstadial, venereal and transovarial transmission of the virus in the tick population, allowing the virus to persist even in the absence of viraemic hosts. Infected ticks play an important role in the long-term maintenance of the disease, surviving for months in burrows and up to several years after feeding on an infected host.

TICK-PIG CYCLE

In the Iberian Peninsula, ASFV readily found a suitable host in *Ornithodoros erraticus*, local ticks that lived in pig shelters. The ticks then became involved in the maintenance of ASFV and its transmission to pigs, despite the absence of African wild pigs. The cycle has also been described in parts of Africa, where it is well documented in Malawi, Madagascar and Mozambique, although ticks probably do not play a prominent role in virus transmission within pig populations (Haresnape & Mamu, 1986; Quembo *et al.*, 2015; Ravaomanana *et al.*, 2010).

Several *Ornithodoros* tick species have been shown to be competent vectors of ASFV both in the field and experimentally (Table 1). However, what happens in the laboratory does not necessarily reflect what happens under field conditions. For *Ornithodoros* ticks to become competent vectors under field conditions, they need pigs as their preferred hosts, failing which natural transmission is likely to remain limited. Vector competence may also vary greatly inside species, or groups of closely related species, according to distinct population features. Although *Ornithodoros* ticks have been reported in currently infected areas in

TABLE 1
Ornithodoros ticks' geographic distribution and role in the transmission of ASF

Ornithodoros species	Geographical distribution	Trans-ovarial	Trans-stadial	To pigs	Comments
O. erraticus (O. marocanus)	Iberian Peninsula and Northern Africa	No	Yes	Yes	Inhabits pigsties and maintains a cycle in domestic pigs
O. moubata complex	Southern and Eastern Africa, Madagascar, one record from Sierra Leone (warthog burrow)	Yes	Yes	Yes	Depending on the subspecies, it may inhabit warthog burrows and maintain the sylvatic cycle in warthogs, but can also inhabit pigsties (maintaining a cycle in domestic pigs)
O. puertoricensis	Caribbean	Yes	Yes	Yes	Proved an efficient vector, but no virus detected despite large numbers collected in Haiti and Dominican Republic after ASF outbreaks
O.coriaceus	USA	No	Yes	Yes	Proved an efficient vector experimentally
O. turicata	USA	?	?	Yes	Proved able to transmit the virus to pigs experimentally
O. savignyi	Africa	?	?	Yes	Is a desert tick not associated with pigs or warthogs
O. sonrai	Sahel in North Africa (southward extension of range to south Senegal)				ASF viral genome detected by PCR in four out of 36 ticks on farms where outbreaks occurred in 2004 and 2005

Source: University of Pretoria

the Caucasus and southern parts of Eastern Europe, there is no indication of their involvement in the ASF epidemic cycle or of whether they could actually transmit the disease.

DOMESTIC CYCLE

In this cycle, the most commonly reported scenario in domestic pigs, the virus is maintained in pigs in the absence of wild suids and ticks (Figure 9). The virus may spread through direct contact via the oro-nasal route after contact with excretions from infected pigs, through ingestion of pork or other contaminated products, or indirectly through fomites. The virus is transmitted from one farm to the next almost exclusively due to human intervention, e.g. movement of animals or equipment, the feeding of infected materials, etc. This transmission route requires the existence of large, continuous populations of pigs for the virus to remain in circulation. However, even in the absence of infected pigs, sometimes the persistence of the virus in refrigerated or frozen meat allows it to persist for long periods of time, and reappear once those meat products are fed as swill.

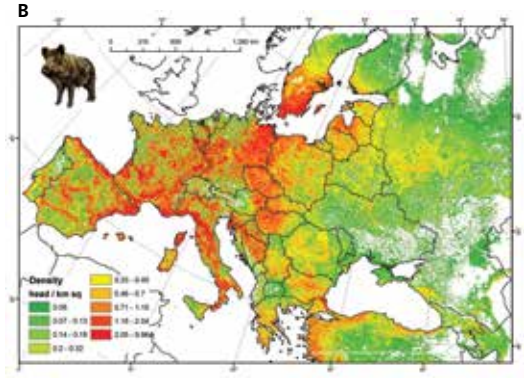
WILD BOAR CYCLE

In Eastern Europe, the Caucasus and Sardinia, wild boar populations play an important role in the maintenance of viral circulation and infection, particularly where there are free-ranging or scavenging populations of pigs in the area, or through some other biosecurity breaches, such as infected feed or leftovers being dumped, fences that allow nose-to-nose contact, etc. Some role may also be played by transportation of wild boar to hunting ranches and/or for management purposes, as well as by hunters (Figure 7).

FIGURE 9
Wild boar in Europe

A

B



A. *Half-eaten carcass of wild boar (©State Food and Veterinary Service, Lithuania/A. Marius Masiulis).*
B. *Wild boar density in Europe (Source: FAO, 2015).*

The exact role of wild boar is, however, still not completely understood. In the Caucasus and the Russian Federation, where wild boar densities are relatively low, their infection was not sustained for long periods, and mainly stemmed from spillover from domestic pigs. However, as ASF progressed westward into the dense wild boar populations of Poland and the Baltic States (Figure 9B), sustained transmission and continuous outbreaks were observed throughout the year. In these areas, wild boar are believed to be the true epidemiological reservoir of the virus, with most cases detected in the summer months.

In parts of Eastern Europe, where temperatures remain below 0 °C for much of the winter, a new, previously unseen epidemiological pattern is unfolding. The virus, present in infected carcasses in fields or forests, remains infective until the spring, when wild boar (and potentially free-ranging pigs, although uncommon) may scavenge on such remains and become infected (Figure 9A).

Human interventions, such as hunting, supplementary feeding, fencing, etc., have profound consequences on how epidemics evolve in wild boar populations. Hunting may lead to wild boar spreading ASF while escaping to other areas, but it can be also very useful in regulating the density of animals (and thus virus transmission). Different types of hunting also have different effects, e.g. driven hunts, targeting of females, etc. Similarly, supplementary feeding may increase transmission by encouraging high numbers of wild boar to congregate in feeding areas, while also allowing more wild boar to survive harsh winter conditions.

ASF TRANSMISSION AND RESILIENCE OF THE ASFV

The incubation period represents the time from infection (i.e. when the virus enters the animal) to disease (i.e. when the animal shows clinical signs). For ASF, it is between four and 19 days, depending on the virus, host and route. Virus excretion can begin up to two days prior to the appearance of clinical signs. The period when the pig is shedding virus

TABLE 2
Resilience of ASFV across a variety of environmental conditions

Item	ASFV survival time
Meat with and without bone and ground meat	105 days
Salted meat	182 days
Cooked meat (minimum of 30 minutes at 70 °C)	0
Dried meat	300 days
Smoked and deboned meat	30 days
Frozen meat	1 000 days
Chilled meat	110 days
Offal	105 days
Skin/Fat (even dried)	300 days
Blood stored at 4 °C	18 months
Faeces at room temperature	11 days
Putrefied blood	15 weeks
Contaminated pig pens	1 month

Source: adapted from Scientific Opinion on African swine fever, *EFSA Journal*, 2010; 8(3):1556.
The times given reflect the known or estimated maximum duration and will depend strongly on environmental temperature and humidity.

can vary depending on the virulence of the ASFV strain involved – pigs infected with less virulent ASFV strains could be persistently infectious for more than 70 days post-infection.

The virus is shed in saliva, tears, nasal secretions, urine, faeces, and secretions from the genital tract. Blood, in particular, contains large amounts of virus. Pigs can therefore become infected by contact with many different infected sources, mainly infected pigs, pork, and other pig-derived products (e.g. swill), and fomites (e.g. bedding). These infected animals and contaminated materials can be transported over long distances by vehicles and people.

Although ASF is associated with high lethality (most animals infected die), it is not as infectious as some other transboundary animal diseases such as foot-and-mouth disease. That means ASF usually spreads slowly within the herd, and some animals may not be affected.

In a suitable, protein-rich environment, the ASFV is stable over wide ranges of temperatures and pH levels for long periods, as well as resistant to autolysis and various disinfectants. Thus neither putrefaction, nor the maturing process, nor freezing of meat inactivates the agent. Consequently, the virus survives in excretions, carcasses, fresh meat, and certain meat products for varying periods of time. It may remain infective for at least 11 days in faeces, for 15 weeks in chilled meat (and probably longer in frozen meat), and for months in bone marrow or cured hams and sausages unless they have been cooked or smoked at high temperature (Table 2). This has very important implications for ASF spread. Undercooked, insufficiently smoked, dried, and salted pork, as well as blood, carcasses, and carcass meal can be infective if fed to pigs or discarded in communal waste sites where pigs or wild boar may feed. Cooking at 70 °C for 30 minutes inactivates the virus (Figure 10).

The introduction of new pigs into a herd or piggery often results in individuals fighting and biting each other. In the case of free-ranging or scavenging animals, infection can result from contact with infected roaming pigs, wild boar, their carcasses, or food leftovers. Additionally, using the same needle to vaccinate or treat several pigs can transmit the virus. Transmission via artificial insemination has not been proven, but may take place.

FIGURE 10
Inactivating the ASF virus in swill

Cooking swill (abattoir leftovers) prior to feeding to pigs in Kiambu, Kenya

Vector-borne transmission is also possible through bites from infected *Ornithodoros* species. Certain blood-sucking insects, namely *Stomoxys calcitrans*, have been shown to be able to retain and transmit ASFV for at least 24 hours after feeding on a sick pig (Mellor *et al.*, 1987), which is particularly relevant for transmission within herds.

Infection via large bodies of water such as lakes and rivers is unlikely as the virus rapidly becomes diluted and will not be present at infective levels.

Clinical presentation and postmortem findings

The disease is generally characterized by the sudden death of pigs. All ages and both genders may be affected. Animals segregated from the rest of the herd, for example sows with young suckling piglets, may be spared because of the rather low contagiousness of ASF. The spread of the disease within the herd (and numbers affected) may vary greatly from a few days to several weeks, depending on the type of pig production, management, and biosecurity measures. In fact, ASF, although highly lethal, is less infectious than some other transboundary animal diseases such as foot-and-mouth disease. Also, some indigenous pig breeds in Africa have developed some degree of tolerance to ASF. Wild boar, being the same species as domestic pigs, show the same clinical presentation.

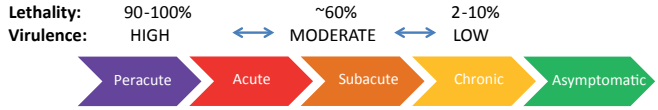
Clinical signs associated with ASFV infection are highly variable (see Table 3) depending on various factors: virus virulence, swine breed affected, route of exposure, infectious dose, and endemicity status in the area. According to their virulence, ASFVs are classified in three main groups: high virulence isolates, moderate virulence isolates, and low virulence isolates (Figure 11). The clinical forms of ASF range from peracute (very acute) to asymptomatic (unapparent). As shown in Figure 11, highly virulent ASFV isolates produce peracute and acute disease, moderately virulent isolates produce acute and subacute forms of disease. Low virulence isolates

TABLE 3
Main clinical signs and postmortem findings observed in the different forms of ASF

	Peracute ASF	Acute ASF	Subacute ASF	Chronic ASF
Fever	High	High	Moderate	Irregular or absent
Thrombocytopenia	Absent	Absent or slight (late)	Transient	Absent
Skin	Erythema	Erythema	Erythema	Necrotic areas
Lymph nodes	-	Gastrohepatic and renal with marbled aspect	The majority of lymph nodes resemble a blood clot	Swollen
Spleen	-	Hyperaemic splenomegaly	Partial hyperaemic splenomegaly or focal infarction	Enlarged with normal colour
Kidney	-	Petechial haemorrhages, mainly in cortex	Petechial haemorrhages in cortex, medulla and pelvis; peri-renal oedema	-
Lung	-	Severe alveolar oedema	-	Pleuritis and pneumonia
Gall bladder	-	Petechial haemorrhages	Wall oedema	-
Heart	-	Haemorrhages in epicardium and endocardium	Haemorrhages in epicardium and endocardium; hydropericardium	Fibrinous pericarditis
Tonsils	-	-	-	Necrotic foci
Reproductive alteration	-	-	Abortion	Abortion

Source: Extracted from Sánchez-Vizcaíno *et al.*, 2015

FIGURE 11
Clinical forms of African swine fever according to the virulence of the isolate involved



Source: FAO

have been described in endemic areas (in addition to the virulent viruses circulating) showing milder symptoms, and sometimes associated with subclinical or chronic ASF. Morbidity (i.e. the proportion of animals affected) will depend on the virus isolate and the route of exposure.

Although not precisely known, the incubation period in natural infections has been reported to vary from 4 to 19 days. Clinical courses of the disease range from less than seven days post-infection in acute forms, to several weeks, or even months, in chronic forms. The lethality rate depends on the virulence of the isolate, ranging from 100 percent characteristic of highly virulent strains, where pigs of all ages are affected, to less than 20 percent lethality in chronic forms. In the latter the disease may be fatal mostly in pregnant and young animals, and pigs suffering from a concurrent disease, or weakened for other reasons. The survival rate to highly virulent strains observed in some endemic areas may be higher owing to adaptation of the pigs to the virus.

PERACUTE

Characterized by high fever (41-42 °C), loss of appetite and inactivity. Sudden death may occur within 1-3 days before the development of any clinical sign. Often, neither clinical signs nor lesions in organs may be apparent.

ACUTE

Following an incubation period of 4-7 days (seldom, up to 14 days), animals with acute ASF display fever of 40-42 °C and lack of appetite; the animals look sleepy and weak, lie down and huddle (Figure 12), and show increased respiratory rate. Death often occurs within 6-9 days for highly virulent strains, or 11-15 days for moderately virulent isolates. Lethality often approaches 90-100 percent in domestic swine. The same signs are observed in wild boar and feral pigs. Acute forms are easily confused with other diseases, mainly classical swine fever, swine erysipelas, poisoning, salmonella, and other septicaemic conditions (see the next chapter for differential diagnosis). The infected pigs may show one or several of the following clinical signs in a variable percentage:

- bluish-purple areas and haemorrhages (spot-like or extended) on the ears, abdomen, and/or hind legs (Figure 12);
- ocular and nasal discharge;
- reddening of the skin of the chest, abdomen, perineum, tail, and legs (Figure 12);
- constipation or diarrhoea, which may progress from mucoid to bloody (melena);
- vomiting;
- abortion of pregnant sows at all stages of pregnancy;

FIGURE 12
Clinical signs of acute African swine fever

A. *Pigs are visibly weak with fever and huddle to stay warm.*
B-E. *Bloody diarrhoea and distinct hyperaemic (red) areas on skin of neck, chest and extremities.*
F. *Cyanosis (bluing) at the tips of ears.*
G-I. *Necrotic lesions on skin of the abdomen, neck and ears.*

- bloody froth from the nose/mouth and a discharge from the eyes (Figure 15);
- the area around the tail may be soiled with bloody faeces (Figure 12).

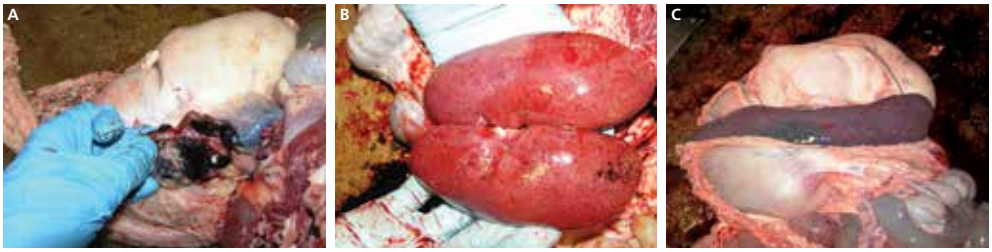
The colour changes and haemorrhages in the skin are easily missed in wild boar due to their darker skin and thick hair. The same applies to dark-skinned pig breeds.

Carcasses of pigs that die in the acute stage of the disease may be found in good body condition, although external clinical signs can be observed. The most recognizable postmortem findings (Figure 13) are: enlarged, edematous, and completely haemorrhagic lymph nodes similar to blood clots (particularly gastrohepatic, and renal); enlarged, friable, and dark-red to black spleen with rounded edges; and petechiae (spot-like haemorrhages) on the capsule of the kidneys.

Postmortem examination usually reveals several of the following:

1. haemorrhages under the skin;
2. excess of fluids in the heart (hydropericardium with yellowish fluid) and body cavities (hydrothorax, ascites) (Figure 15);
3. petechiae on the heart's surface (epicardium), urinary bladder, and kidneys (on the cortical and renal pelvis) (Figure 14);

FIGURE 13
Some of the most recognizable postmortem lesions of acute African swine fever

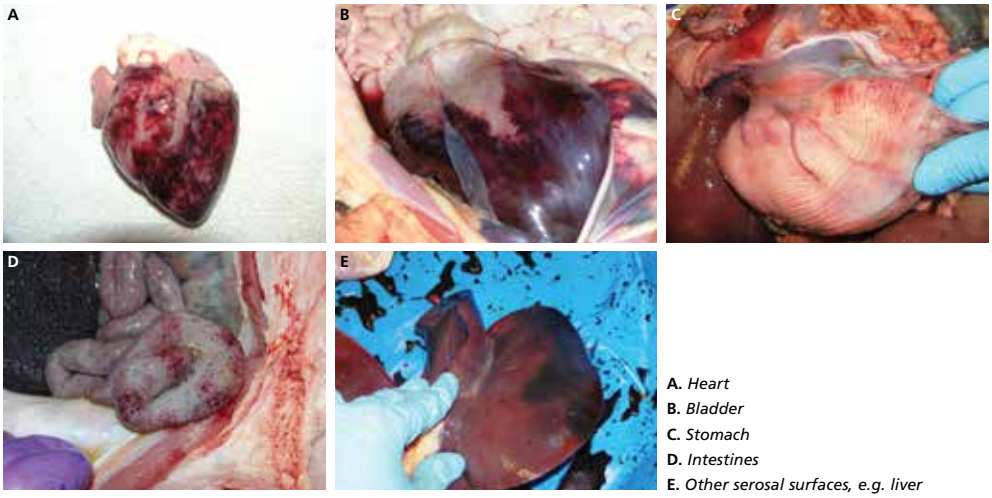


A. *The gastrohepatic and renal lymph nodes are recognizably haemorrhagic and enlarged when infected with ASFV. Non-diseased tissue is a healthy white/pink colour without inflammation.*

B. *Kidneys infected with ASFV have a notable petechiation (i.e. little pinpoint haemorrhages) on the cortex. Healthy renal tissue is a uniformly coloured light brown without any surface irregularities.*

C. *The spleen of pigs infected with AFSV is often enlarged, friable (fragile) and shows signs of infarction (dark area). Healthy spleens are uniformly coloured (red-brown) and textured.*

FIGURE 14
Haemorrhagic lesions of acute African swine fever



A. *Heart*
B. *Bladder*
C. *Stomach*
D. *Intestines*
E. *Other serosal surfaces, e.g. liver*

4. the lungs may present congestion and petechiae, with froth in the trachea and bronchus, and severe alveolar and interstitial pulmonary oedema (Figure 15);
5. petechiae, ecchymoses (larger haemorrhages), and excess clotted blood in the stomach and small and large intestines (Figure 14);
6. hepatic congestion and haemorrhages in the gall bladder.

Infected wild boar in Eastern Europe show the same clinical signs and necropsy findings, although due to their thick, dark fur, external clinical signs are less obvious (Figure 16).

FIGURE 15
Further lesions of acute African swine fever

A. *Pulmonary oedema and consolidation of lung tissue are evident.*
B. *Excess fluid around the heart and in body cavities.*
C. *Bloody froth may also be present in the trachea as well as the mouth and nose.*

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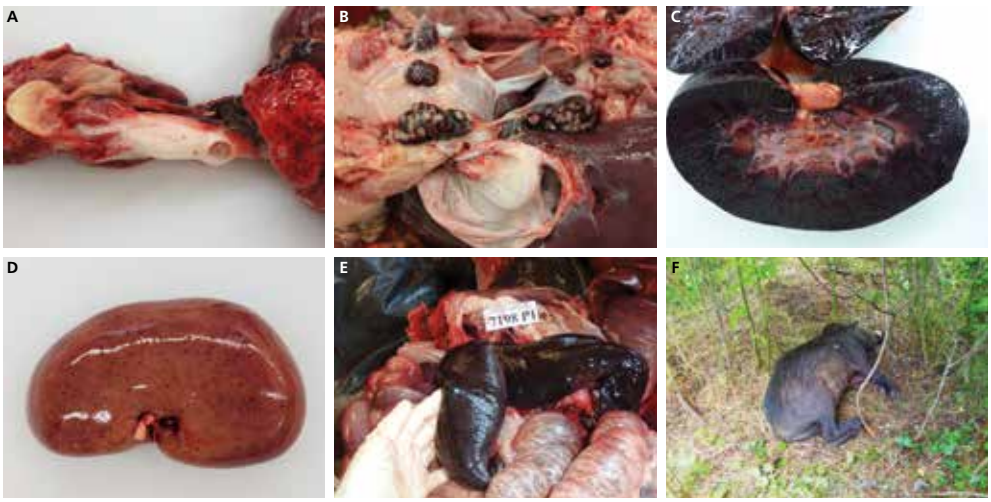
FIGURE 16
Characteristic necropsy findings and clinical signs in wild boar affected with acute African swine fever



A. *Froth in the trachea from severe lung oedema*
B. *Haemorrhagic gastrohepatic lymph node*
C. *Haemorrhagic kidney*
D. *Petechiation on the kidney's cortex*
E. *Spleen enlarged*
F. *Dead wild boar*

PHOTOS A-D: ©FLI. PHOTOS E-F: ©STATE FOOD AND VETERINARY SERVICE, LITHUANIA/MARIUS MASIULIS

SUBACUTE

Subacute forms of the disease are caused by moderately virulent isolates and may occur in endemic regions. Pigs usually die within 7-20 days, with lethality rate ranging from 30 to 70 percent. The survivors may recover after one month. Clinical signs are similar (although generally less intense) to those observed in the acute form, except for the more pronounced

FIGURE 17
Typical lesions observed in chronic forms of African swine fever

A-F. *Moderate to severe joint swelling, often combined with reddened areas of skin that become raised and necrotic.*
G. *Additional necropsy findings include edematous lymph nodes.*
H. *Pneumonia with caseous necrosis and mineralization of the lungs.*

vascular changes, mainly haemorrhages and oedemas. Fluctuating fever, accompanied by depression and loss of appetite, are also common. Walking may appear painful and the joints are often swollen with accumulated fluid and fibrin. There may be signs of laboured respiration and pneumonia. Pregnant sows may abort. Serous pericarditis (fluid around the heart) often evolves into a more advanced fibrinous pericarditis.

CHRONIC

Chronic forms often result in lethality rates that are typically less than 30 percent. They have been described in countries where ASFV has long been present, such as Spain, Portugal and Angola. Chronic forms stem either from naturally attenuated viruses, or from virus vaccine isolates released in field vaccination studies, as suspected in the Iberian Peninsula in the 1960s. Clinical signs begin 14 to 21 days post-infection with slight fever, followed by mild respiratory distress and moderate-to-severe joint swelling. This is often combined with reddened areas of skin that become raised and necrotic (Figure 17). Additional necropsy findings include pneumonia with caseous necrosis (sometimes with focal mineralization) in lungs, fibrinous pericarditis, and edematous lymph nodes, which can be partially haemorrhagic (mainly mediastinal lymph nodes) (Figure 17).

Differential diagnosis

African swine fever does not always manifest itself with the entire set of clinical signs described in the previous section. Clinical diagnosis can be difficult during the early stages of the disease, or when small numbers of animals are affected. Diagnosing ASF is often speculative, for symptoms may be confused with those of other diseases and/or conditions. Moreover, a number of pig (and wild boar) diseases can cause mortality at the rate observed in an acute ASF outbreak. **No diagnosis is conclusive until confirmed by the laboratory.**

In addition to the top differential diagnoses covered in this chapter (Table 4), additional conditions to consider may include other generalized septicaemia or haemorrhagic (bruising) conditions.

CLASSICAL SWINE FEVER (CSF)

The most important differential diagnosis of ASF is Classical swine fever, also known as hog cholera, which is caused by a *Pestivirus* in the Flaviviridae family. As with ASF, there are various clinical presentations or forms. Acute CSF presents almost identical clinical signs and postmortem lesions to acute ASF, and is also characterized by high fatality rates. Clinical signs may include high fever, lack of appetite, depression, haemorrhages (in the skin, kidneys, tonsils and gall bladder), conjunctivitis, respiratory signs, weakness, huddling, purple discolouration of skin, and death within 2-10 days. The only way to distinguish reliably between them is through laboratory confirmation. It is unwise to attempt vaccination against CSF until the diagnosis is confirmed, as ASF can easily be spread by untrained personnel during a vaccination campaign.

PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME (PRRS)

Also known as blue ear disease, PRRS is characterized by pneumonia in growing and finishing pigs and by abortions in pregnant sows. It is often accompanied by fever, skin flushing and in particular by bluish discolouration of the ears. Diarrhoea has also been described. Although mortality due to PRRS is generally not high, highly pathogenic PRRS viruses have decimated pig herds in China, Viet Nam and Eastern Europe over the last few years, associated with high mortality, high fever, lethargy, anorexia, cough, dyspnoea, lameness, and cyanosis/bluing (in ears, limbs and perineum). Necropsy findings include lesions in lungs (interstitial pneumonia) and lymphoid organs (atrophy of the thymus and swelling and haemorrhages in lymph nodes) and petechial haemorrhages in the kidneys.

PORCINE DERMATITIS AND NEPHROPATHY SYNDROME (PDNS)

One of the porcine circovirus-2 associated diseases (PCVAD), PDNS usually affects growers and finishers. Although the clinical signs are strongly suggestive, there is no specific diagnostic test. The syndrome is characterized by the presence of dark-red to purplish skin lesions that are often most prominent on the hindquarters and perineal

FIGURE 18
Haemorrhages in a pig with classical swine fever (CSF)

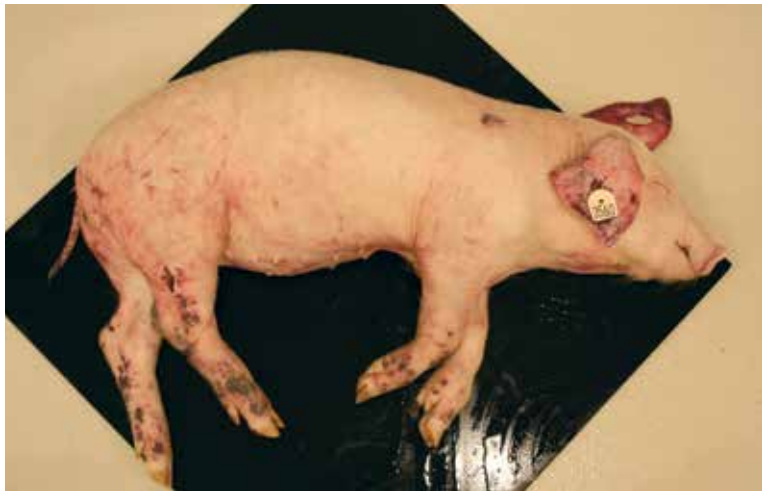


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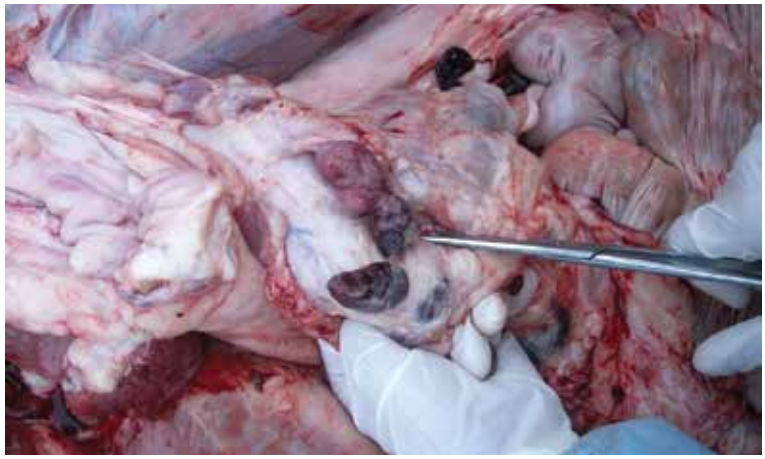
FIGURE 19
Enlarged haemorrhagic lymph node in a pig with highly pathogenic porcine reproductive and respiratory syndrome (PRRS)



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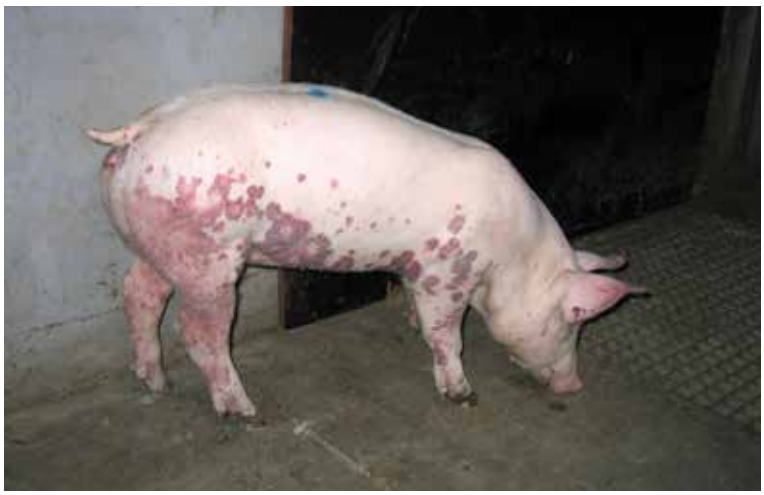
area, although in severe cases the flanks may also be affected. The lesions in the blood vessel walls are caused by necrotizing vasculitis (inflamed blood vessels), and are easily distinguished microscopically from those of ASF. The disease is also accompanied by anorexia, depression and severe nephrosis (inflamed kidney), which is usually the cause of death. Lymph nodes may also be enlarged. Morbidity is generally low but affected pigs very often die.

FIGURE 20
Pig suffering from porcine dermatitis and nephropathy syndrome (PDNS)



ERYSIPELAS

This bacterial disease caused by *Erysipelothrix rhusiopathiae* affects pigs of all ages and is as likely to affect pigs in small-scale and extensive farms as in commercial, intensive units. It can manifest itself in either acute or subacute forms. The acute form, usually seen in younger pigs, is characterized by sudden death, although mortality is usually much lower than in ASF. Two or three days after infection, affected pigs may show very characteristic diamond-shaped skin lesions associated with necrotizing vasculitis (inflamed blood vessels). In adult pigs this is usually the only clinical manifestation of the disease. As with acute ASF, the spleen may be congested and markedly enlarged. Other necropsy findings include congestion in lungs and peripheral lymph nodes, as well as haemorrhages in the cortex of the kidneys, heart and serosa of the stomach. Bacterial isolation can confirm the diagnosis and pigs respond well to treatment with penicillin. The microscopic changes differ from those typical of ASF.

AUJESZKY'S DISEASE

Aujeszky's disease, also known as pseudorabies, causes reproductive and severe neurological issues in affected animals, often leading to death. Although nearly all mammals can be infected, pigs are most frequently affected and are the reservoir host. Younger animals are the most severely affected, with mortality rates reaching 100 percent during the first two weeks of age. Piglets usually have a fever, stop eating, and show neurological signs (trembling, seizures, paralysis), and often die within 24-36 hours. Older pigs (over two months) may show similar symptoms, but usually have respiratory signs and vomiting, and are less likely to die. Sows and boars primarily develop respiratory signs, but pregnant sows can abort or give birth to weak, trembling piglets. Focal necrotic and encephalomyelitis lesions occur in the cerebrum, cerebellum, adrenals and other viscera such as lungs, liver or spleen. In fetuses or very young piglets, white spots on the liver are highly characteristic of infection by the virus.

FIGURE 21
Characteristic diamond-shaped skin lesions in a pig with erysipelas

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FIGURE 22
Piglet neurological issues due to Aujeszky's disease

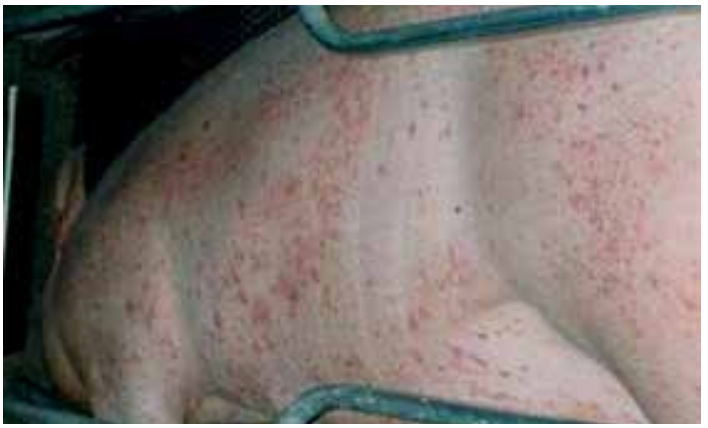
©BOEHRINGER INGELHEIM ANIMAL HEALTH GMBH/MARIKA GENZOW

SALMONELLOSIS (AND OTHER BACTERIAL SEPTICAEMIAS)

Younger pigs are usually affected. Animals treated in time may respond to antimicrobial therapy. Confirmation of the diagnosis is by bacterial culture. Features in common with ASF include fever, loss of appetite, respiratory or gastrointestinal disorders, and a congested, fevered carcass at slaughter. Animals may die 3-4 days post-infection. Pigs dying from septicaemic salmonellosis show cyanosis of the ears, feet, tail and abdomen. Necropsy findings may include petechial haemorrhages in the kidneys and on the heart's surface, enlarged spleen (but with normal colour), swelling of mesenteric lymph nodes, enlargement of the liver, and congestion of the lungs.

FIGURE 23
Pig suffering from salmonellosis with cyanotic ears

FIGURE 24
Pig suffering from mycotoxin poisoning



POISONING

When a large number of pigs die suddenly, the possibility of poisoning should be considered. Few poisons result in the severe bleeding seen in ASF. Although coumarin-based rat poisons such as warfarin can cause widespread bleeding, they are unlikely to affect more than a few pigs in the herd. Certain fungal toxins found in mouldy feed such as aflatoxin and Stachybotrys toxin may cause haemorrhage and severe mortality. Accidental or malicious poisoning with pesticides can result in the death of pigs of all ages, but the death of all pigs in the space of 24-48 hours, usually with few if any clinical signs or postmortem lesions, should serve to distinguish such events from ASF. Poisoning is unlikely to be accompanied by fever.

TABLE 4
Summary of ASF differential diagnoses: clinical signs and postmortem differentials

CLINICAL SIGNS	Reportable disease	Vaccine available	Treatment options	Fever	Loss of appetite	Dull or depressed	Red to purple skin lesions	Respiratory distress	Vomiting	Diarrhea	Bloody diarrhea	High mortality	Sudden death	Abortion	CLINICAL SIGN DIFFERENTIALS	Enlarged dark-red to black & friable spleen	Hemorrhages on kidney	Hemorrhagic lymph nodes	Enlarged lymph nodes	Hemorrhages on mucous membranes	Excess fluid in body cavity & around heart	Pneumonia	POSTMORTEM DIFFERENTIALS
African swine fever (ASF)	X			X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	
Classical swine fever (CSF)	X	X		X	X	X	X	X	X	X		X	X	X	Conjunctivitis. Ataxia. Central nervous system signs in piglets, hunched posture. Constipation may progress to a yellow-grey diarrhea. Longer clinical course.		X	X	X	X			Necrotic or 'button' ulcers in the mucosa of the gastrointestinal tract, epiglottis and larynx. Encephalitis. CSF pigs lose weight quickly. Pale areas on edge of spleen.
Highly pathogenic PRRS	X	X		X	X	X	X	X	X			X		X	Intensity of respiratory distress.		X	X	X				Interstitial pneumonia. Absence of enlarged spleen. Atrophy of the thyme.
Erysipelas		X		X		X	X							X	Most often seen in animals reaching market weight. Characteristic diamond-shaped skin lesions.		X			X			Arthritis and vegetative endocarditis. Hemorrhages in pleura and peritoneum. Perypheral lymph nodes affected (rather than gastrohepatic and renal).
Salmonellosis (S. cholerasuis)			X	X	X	X	X	X		X	X				Yellowish diarrhea. Central nervous system signs including tremor, weakness, paralysis and convulsions.		X					X	Enteritis and occasional encephalitis. Necrotic endocarditis. Miliary foci of necrosis in the liver. Absence of vascular lesions in the spleen and nymph nodes.
Pasteurellosis			X	X		X		X							Signs vary in severity.							X	Adhesions between lungs and ribcage.
Aujeszky's disease or pseudorabies		X		X	X	X		X						X	Signs vary, depending largely on the immune status of the dam and the age of the pigs affected. Hypothermia, trembling and ataxia, seizures. Rhinitis and sneezing.							X	Focal necrotic and encephalomyelitis lesions occur in the cerebrum, cerebellum, adrenals and other viscera such as lungs, liver or spleen. In fetuses or very young piglets, white spots on liver are pathognomonic of their infection by the virus. Necrotic enteritis.
Porcine dermatitis and nephropathy syndrome (PDNS)				X			X						X		Most often seen in grower/finisher pigs.		X		X			X	Enlarged pale kidneys. Fluid in the body cavity, subcutaneous edema, gastric ulceration, and increased synovial fluid.

Immediate actions at farm level in the event of a suspected outbreak

Sections of this chapter have been extracted from the FAO manual, *Good Emergency Management Practices (GEMP): The Essentials* (FAO, 2011), which can be consulted for more in-depth information.

It is best to keep an investigation kit maintained in each local veterinary office so that the attending veterinarian can leave with minimal delay to undertake the investigation. Equipment should ideally include a digital camera, a GPS unit and some means of rapid communication (often a mobile phone, but could be a radio), as well as all the equipment needed to take, safely package and transport samples (GEMP, 2011).

Suspected ASF will usually be reported by the farmers themselves or by a private veterinarian. On encountering a suspected ASF outbreak, the following steps should be taken without delay at the farm/premises level based on the presumptive field diagnosis of ASF, even before laboratory confirmation:

- **Collect data** about the farm and animals affected (see Box 1).
- Infected and suspected farms must be placed under **immediate quarantine**, i.e. no people, vehicles, animals or pig products should enter or exit the farm until the diagnosis is confirmed.
- Establish **disinfection points** for people and vehicles at entrances and exits of the building housing pigs. Personnel and visitors leaving the farm should ensure that shoes, clothing and equipment are disinfected. If the veterinary officer or others need to come into contact with the sick animals or potentially infected materials, personal protective equipment should be used.
- Undertake **clinical inspection** of each farm subunit, clinical examination of selected animals and necropsy of dead (or euthanized) animals. When conducting a clinical examination of suspect animals, it is important to be systematic. It is also important to write down your findings as you perform the examination. A prepared form may help you do this efficiently. If large numbers of animals are present, you may need to prioritize which animals you examine. Initially, you may want to target those showing obvious clinical signs.
- **Appropriate samples** should be collected and sent as soon as possible to the laboratory for diagnosis (see the Section on Sampling, p. 39). In the case of many animals showing clinical signs, samples from approximately five of them should be sufficient to ensure a diagnosis.
- Conduct an **outbreak investigation** (also known as epidemiological enquiry – see p. 30).
- Neighbouring farmers or those who have bought from, or sold animals to, the farm recently, i.e. **dangerous contacts**, should be notified of the event so that they can

BOX 1

Basic information to be collected in the case of an emergency report on a disease outbreak (GEMP, 2011)

- disease or diseases suspected;
- exact geographical locations of the disease outbreak(s), including global positioning system (GPS) coordinates when available;
- names and addresses of affected farmers, farms or villages;
- livestock species affected;
- approximate numbers of sick and dead animals;
- approximate numbers of susceptible animals in the area;
- brief descriptions of clinical signs and lesions observed;
- date(s) when the disease was first noticed at the initial outbreak site and any subsequent sites;
- details of recent movements of susceptible animals to or from the outbreak farm or village;
- details of any recent movement of trucks and/or people from or towards other farms;
- any other key epidemiological information, such as presence of disease in wild or feral animals and abnormal insect activity;
- initial disease-control actions taken, including where and when.

check their animals (and report any symptoms detected to veterinary authorities), enclose them and stop the movements of pigs and products in and out of their premises. Service providers who have visited the farm recently should also be notified.

- Even with adequate cleaning and disinfection, personnel participating in outbreak investigations on a potentially infected farm should **not visit another farm** for at least 24 hours to prevent possible inadvertent spread of the disease.
- When facing an outbreak affecting free-range, scavenging pigs, the first step is to **bring back all non-confined animals** and keep them enclosed, or at least tethered/tied.

HOW TO CONDUCT AN OUTBREAK INVESTIGATION

This section is adapted from the EuFMD **online training course**.

An outbreak investigation, also known as an epidemiological enquiry, should determine: a) how long the disease has been present; b) the possible sources of introduction of the disease; c) what movements of animals, people, vehicles or other fomites could have spread the disease; and d) the magnitude of problem, by counting the number of cases, defining epidemiological units and estimating population at risk. This information is crucial in guiding decision-making on effective control strategy and also in monitoring control strategies once they are in place.

FIGURE 25
Sampling of pigs in Serbia

©FAO/KLAAS DIETZE

One of the first steps should be to define the epidemiological unit, which should include all pigs at a similar level of risk of exposure. This would be all susceptible animals under one management system or biosecurity compartment, i.e. usually the farm. However, a unit could extend to village level if there are no effective boundaries between farms. It is important to remember that geographically distant farms may be under one management system and form part of the same epidemiological unit.

Constructing a timeline is a useful way of representing the times during which infection and transmission of disease might have taken place, and therefore guiding an outbreak investigation. Timelines are used to determine time windows for introduction of the virus (based on the incubation period) and for spread to other premises (using the period of virus excretion).

Once a timeline has been established, the next step is to use it for source and spread tracing in order to establish contacts that could have led to virus transmission during the calculated timeframe. Risk factors for disease spread include:

- movements of animals or animal products (e.g. pork);
- personnel visiting the premises who were in direct contact with livestock on other farms, e.g. the veterinary surgeon or other pig farmers;
- farm workers visiting other livestock holdings;
- movements of vehicles or equipment between livestock holdings;
- direct contact with livestock at the farm boundaries;
- wild suids or their products.

Once possible sources of infection have been identified, it is important to prioritize them in order to carry out further epidemiological enquiries. This allows for rapid investigation and control of any contact liable to spread disease further. Contact occurring during the time period most likely for infection should be prioritized. Such prioritization is especially important where personnel and resources are limited, as it is often the case. The types of contact are also important. Priority should be given to:

BOX 2

Tips when interviewing a farmer during an outbreak investigation

Establish trust

- Explain the purpose of the interview.
- Avoid blaming or frightening the interviewee.
- Ask if the interviewee has any questions, and answer them fully.
- Take time to explain what you have found.

Keep calm

- An ASF outbreak is stressful for veterinarians as well as farmers. Try and project a tranquil image, talking calmly and quietly.
- Look after yourself – stay hydrated and remember to eat.

Keep an open mind

- Include "open" questions inviting full responses rather than yes/no answers.
- Remember to listen – an interviewee should be talking a lot more than you.
- Ask the same question in two or three different ways if you are not sure about the first answer.
- Draw on all personnel – farm workers often have more day-to-day contact with animals than the owner.

- larger premises where more animals are present;
- "hubs" where animals from multiple premises meet, including livestock markets and abattoirs;
- premises where regular animal movements take place, e.g. livestock dealers;
- direct animal contacts, e.g. animal purchases;
- neighbouring premises with pigs.

There are various ways of investigating possible contact:

Interviews

Carrying out an effective interview is a skilled job, especially when the farmer is likely to be under considerable stress. Farmers are often wary of outsiders, and particularly of government officials. It is vital to use time and patience to build a relationship. Also, do not plan on visiting more than one farm per day. Some tips are included in Box 2.

Other sources of information

Examine livestock and personnel movement records. Medicine records, diaries, delivery notes and invoices or receipts from deliveries may also hold valuable information. Remember that the farmer will be under considerable pressure and will find it hard to be precise, which makes records even more valuable.

BOX 3

Equipment needed to ensure good biosecurity when entering a farm

- one pair of good-quality gumboots that are easy to clean and disinfect;
- disposable biosecurity suit;
- waterproof suit if required (in cold and wet countries);
- overshoes or boot covers;
- examination gloves (make sure they are the right size);
- plastic mat;
- buckets (three ideally);
- detergent;
- disinfectant (approved for ASFV);
- scrubbing brushes (two);
- refuse bags (including biohazard bags);
- ziplock bags (for transporting phones or other equipment);
- disinfectant wipes for face;
- water (5 litres minimum);
- sealing tape;
- scissors;
- sampling and recording equipment (detailed lists in chapter VI);
- GPS device to record geocoordinates.

Besides interviewing the farmer, you should make a careful survey of the premises. The outer perimeter should be walked in order to establish any contact with neighbouring pigs or wild suids. It is often helpful to make a sketch map of the area, showing the location of animal housing, animal groups, entry and exit points and boundaries.

It may be appropriate, for epidemiological investigation and tracing purposes, to contact other visitors to the premises, for instance veterinarians, milk collectors, or artificial inseminators.

BIOSECURITY WHEN VISITING A FARM

This section has been adapted from the EuFMD **online training course**. A **detailed video** showing the main steps described below is also available at **https://www.youtube.com/watch?v=ljS-53r0FJk&feature=youtu.be**

Before departing:

- Remove all unnecessary equipment from the car.
- Arrange clean and dirty areas on the back seats and in the boot of the car lined with plastic sheeting.
- Make sure you bring all necessary equipment with you. It is helpful to have a checklist (see Box 3). It is helpful to have a standard list of the equipment required for setting up a disinfection point. There may be such a list in your contingency plans or manuals.

Figure 26
Disinfection procedures at the farm

©EuFMD

On arrival

- The car should not be driven onto the premises (leave it near the farm entrance).
- Choose a suitable location for your disinfection site on a clean and dry surface (preferably concrete), using a clear demarcation between the clean and dirty sides (the gate usually).
- Remove all unnecessary clothes and items (e.g. jacket, tie, watch) and empty your pockets.
- Electronic equipment (e.g. mobile phones) needed on the farm should be placed in sealed plastic bags to facilitate subsequent cleaning and disinfection. Phone should never be removed from bags while on the farm and should only be used through the plastic bag.
- Remove from the car all the items needed for disinfection that are to be taken onto the farm.
- You may need to bring your own water for making up detergents and disinfectants.

Preparation

- Lay down a plastic sheet on the clean side of the disinfection site.
- Place the items you will be taking with you to the farm on the dirty side of the disinfection site (e.g. black plastic bags and sample container).
- Make up one bucket of detergent and two buckets of disinfectant with the water you brought. The detergent and one disinfectant bucket remain on the dirty side, and will be used to clean off dirt picked up on the farm. The other disinfectant bucket will be on the clean side with its own brush.
- The disinfectant used will often be disease-specific. The concentration and contact time required should be carefully monitored.

Dressing (on the clean side)

- Take off shoes and leave them on plastic sheet.
- Disposable suit goes on first and fits inside boots. A set of gloves should be taped on.
- Waterproof suit (if required by weather conditions) goes over the boots. It has its own layer of disposable gloves, which can be changed when soiled.
- Overshoes should be worn to cover at least the soles and lower part of the gumboots.
- Don hood and double-check list before stepping off sheet and heading to farm.

Undressing (on dirty side)

- Before leaving the premises, use the farm's own facilities to clean very dirty areas.
- Clean sample container with detergent and brush before soaking in disinfectant for appropriate time, then place in sample bag on clean side.
- Wash off and disinfect the bag containing the phone any similar items taken to farm.
- Remove boot covers and dispose of in dirty-side plastic bags. Roll waterproof suit up (if worn) to top of boots before scrubbing boots with detergent and brush, especially bottoms (perhaps using screwdriver to clean between treads). Then use detergent to wash entire suit, including hood.
- Outer gloves come off and go into the dirty-side bags before the now-washed waterproof suit is removed and soaked in the disinfectant. After appropriate time the suit goes into a bag on the clean side.
- Boots can be rewashed quickly if necessary and properly disinfected.
- Inner gloves are untaped and placed in a dirty-side bag before the inner suit comes off (foot must come out of boot as suit is removed and then can go back into boot). The suit goes into a dirty-side bag for disposal.

On clean side

- Step out of boots and onto clean-side sheet before grabbing boots and disinfecting them on clean side (other disinfection bucket). Lastly, place them in a clean-side bag. Hands and glasses are also disinfected here, as well as your face with disinfectant wipes.
- Non-disposable equipment and samples are double-bagged and taped shut.

Regular shoes can be put back on.

- If the dirty-side buckets are personal, they should be disinfected and double-bagged before being taken away. Any buckets from the farm must stay on the dirty side.
- Bags can then go into the vehicle's dirty area.
- The farmer should be asked to take garbage for processing if necessary.
- Leave the farm and immediately take samples/equipment for processing.
- If there are no pigs on your premises you may return home, shower, and thoroughly wash hair. All clothes worn that day should be soaked in disinfectant for 30 minutes and washed with water over 60 °C. If there are pigs on your premises, complete this step elsewhere.
- Do not visit any premises with pigs for at least three days.

Alongside the procedures for cleaning and disinfecting yourself, you may also need to clean and disinfect the car. Ensure that there are no unnecessary items in the car and that it is clean before you begin your visit. Line the areas of the car used to store equipment with plastic, and establish clean and dirty areas inside. Also, ensure you follow local rules for disinfection of vehicles.

You should, if possible, clean and disinfect the exterior of the car before leaving an area that may have been contaminated, and repeat disinfection of the inside and outside of the car once you return to your base.

- Remove all plastic used to line the car and dispose of appropriately.
- Clean the exterior, using a power-washer or hose and a disposable sponge, removing all visible dirt. Do not forget to clean hidden areas such as wheel arches, tyre treads and the underneath of the car.

- Once all dirt has been removed, spray the exterior with disinfectant.
- Dispose of all rubbish inside, clean all dirt (taking care to dispose of this waste appropriately).
- Wipe steering wheel, gearstick, pedals, handbrake, etc. with a cloth dipped in disinfectant.

WHEN ENCOUNTERING SUSPECTED ASF IN WILD BOAR

First of all, it is key to have a clear suspect case definition for ASF in wild boar. Such definitions will likely change according to the epidemiological situation in the region/country, becoming more stringent as the risk increases. It usually includes any wild boar showing clinical signs or abnormal behaviour, or any hunted animal with lesions (postmortem), or any wild boar found dead, or killed in road incidents (especially in areas at risk).

The suspicion will usually be reported by hunters, although forest managers, hikers, mushroom pickers, etc. may do so too. Depending on the country, hunters may have a very prominent role in disease detection. Motivation of some sort, e.g. money, will be usually necessary to ensure their collaboration. It is important that each hunter in the area at risk is trained to recognize the clinical signs of ASF, to know what type of samples to take and how to take them, to notify the right authorities in good time, and to know how to dispose of carcasses. Hunters should also ensure that any hunted wild boar is dressed in a designated place, with offal or by-products disposed of appropriately, e.g. in special containers or pits.

In case of suspicion arising over an animal, hunters may be requested to store the entire carcass in a fridge (usually at the hunting station) until the laboratory results come in.

Suspect carcasses found in the forest should, if logistically feasible, be collected and transported (by car, sledge, etc.) to a safe disposal site for burning or rendering. Alternatively, they can be disposed of on-site by burning or burial.

When clinical suspicion arises, the following immediate measures apply:

- Collect data about the animals affected (number, age, gender, postmortem lesions, location, etc.).
- Ensure that all those in contact with the carcass have their shoes, clothing and equipment disinfected. In the case of the veterinary officer and others coming into contact with sick/dead animals or potentially infected materials, personal protective equipment should be used.
- Conduct clinical inspections and postmortems on dead animals.
- Collect appropriate samples and ship them as soon as possible to the laboratory for diagnosis (see the "Laboratory diagnosis of ASF" section, p. 49). In some cases, particularly if carcasses are found in remote locations, hunters are expected to collect the samples themselves.
- Conduct an outbreak investigation (also known as epidemiological enquiry).
- Notify neighbouring farmers about the event so that they can check their animals for clinical signs and enclose them.
- Even after adequate cleaning and disinfection, personnel participating in an outbreak investigation on a potentially infected wild boar should not visit farms for at least 48 hours to avoid inadvertently spreading the disease.

When conducting an epidemiological investigation involving wild animals, the protocols will be different from those used on farms, given the different characteristics of wild populations. Interviewees will not be the animals' owners, but people regularly entering the forest, such as the head, or members, of the local hunting club, local forest rangers, etc. Questions to be asked include:

- Who hunted in the area – both local and visiting hunters?
- Any driven hunting (with beaters) during the last month or two?
- Geographical boundaries of the reserve?
- Management practices in the reserve?
- Biosecurity measures in place?
- Hunting hygiene?
- Any domestic pig populations in the area?

STANDARD OPERATING PROCEDURES (SOP) (GEMP, 2011)

SOP are crucial to ensure that suspect cases are investigated properly. They should include:

- notes for the safety of the investigators and animal owners;
- a list of equipment to be taken, including sample-handling equipment;
- criteria for establishing the extent of the infected area and, from this, the biosecurity entry point;
- biosecurity precautions to be taken when entering and leaving the location;
- restrictions to be imposed on arrival on movements of livestock, products, staff, vehicles and equipment;
- the examinations to be undertaken (numbers and types of animals);
- samples to be taken from animals with compatible signs;
- sample handling;
- procedure for submitting samples for testing; and
- procedure for communicating interim findings to the appropriate authorities.

SPECIALIST DIAGNOSTIC TEAM (GEMP, 2011)

It is recommended that a specialist diagnostic team (or teams) that can immediately be mobilized, be nominated within the country. Team members should be available and equipped to travel at short notice. Deployment should include all the equipment needed for outbreak investigation, for collection and transport of diagnostic specimens, and for rapid communications. The team should travel to the outbreak site accompanied by local veterinary staff, including the local veterinary practitioner. It should undertake clinical examinations, collect histories, make preliminary epidemiological investigations, trace the movements of suspect animals and collect a range of diagnostic specimens, both specifically for the suspected disease and for any endemic or exotic diseases that could be included in a differential diagnosis. The team should transport these samples back to the laboratory. It should also take any immediate disease-control measures needed at the outbreak site and should have the powers and legal authority to do so. In addition, it should be empowered to provide immediate instructions to local animal health officials. The team must report back immediately to the state/provincial/regional veterinary officer and the CVO on its assessment of the situation, including steps taken to secure a confirmatory diagnosis, and

advise on further disease-control strategies, including declaration of infected and surveillance zones. The composition of a diagnostic team varies with circumstances, but may include:

- a veterinary pathologist from the central or regional veterinary diagnostic laboratory;
- a specialist epidemiologist, preferably with first-hand experience or training in transboundary and emerging diseases, particularly the disease suspected;
- a veterinarian with extensive experience of endemic diseases;
- any specialist required for particular examinations.

Sampling, packaging and transport of samples

These practical guidelines are designed for field and laboratory teams.

SAMPLING

The starting point for any laboratory investigation of ASF is sample collection. An important consideration is the purpose of the investigation, for example disease diagnosis, disease surveillance, or health certification. Which animals to sample will depend on the objective of the sampling. For example, when investigating an outbreak (passive surveillance), sick and dead animals should be targeted, while the oldest animals should be sampled when checking if animals have been exposed to the disease (active surveillance).

Those in charge of sampling (and conducting clinical inspections) should have received previous training in the techniques available to restrain a pig (both for clinical inspection and for sampling).

A sampling team should bring sufficient quantities of sampling equipment (see Box 4) for the number of animals to be sampled, plus a margin for materials that may be dropped or become unusable for other reasons (e.g. vacutainers that lose vacuum etc.). Additionally, items for data collection, personal protection/biosecurity, and transport of samples must be packed (refer to "Materials for sample transport" in Box 4).

It is recommended to go with a field sampling form so that all samples and related information needed can be collected on-site. If submission of samples to a regional/international reference laboratory is foreseen, it is recommended to take samples in duplicate so that one set can be submitted while the other is safely stored, thus avoiding having to thaw and aliquot/divide samples before submission.

Samples should be taken with care and in accordance with the proper technique to avoid undue stress or injury to the animal, or harm to the sampler. They should be collected aseptically, taking care to avoid cross-contamination, and always using new needles for different individuals to avoid disease transmission. All samples awaiting testing should be considered infected and handled accordingly. All sampling material used on farms should be disposed of safely and according to local regulations, e.g. bagged and transported back to the laboratory for autoclaving/appropriate disposal.

Diagnostic laboratories require the submission of appropriate samples that are **clearly and permanently** labelled and that arrive at the laboratory in good condition.

Types of sample

a. Whole blood

Draw whole blood from the jugular vein, the inferior vena cava, or the auricular vein using sterile tubes (vacutainers) with anticoagulant (EDTA – purple stopper). If the animal

BOX 4

Sampling materials required

General materials

- labels and permanent markers;
- data collection forms, pens, clipboards;
- sharps bin for needle and scalpel disposal;
- autoclavable disposal bags.

Personal Protective Equipment (PPE requirements will vary, e.g. surveillance vs outbreak investigation)

- dedicated clothing (coveralls);
- rubber boots;
- boot covers;
- gloves;
- face mask;
- safety glasses for eye protection;
- disinfectant for hands;
- disinfectant for boots.

Materials for sample transport

- primary containers/tubes/vials (leakproof – should be clearly labelled);
- absorbent;
- containers or bags capable of withstanding 95 kPa as secondary packaging, hermetically sealable (i.e. leakproof), preferably plastic, for storage of sample containers and blood tubes from each animal;
- cool box (+4 °C), either electric to plug into car (preferable) or other, e.g. Styrofoam box filled with cooling materials (e.g. ice, frozen water bottles or cool pack as appropriate – some eutectic cold packs with special gel are commercially available and allow the desired temperature to be kept for up to a couple of days); portable -80 °C freezer/dry shipper/liquid nitrogen tank (only required if sampling takes place far from an appropriately equipped laboratory).

It is important always to maintain the above 'triple' containment structure when transporting samples.

Sampling materials for live animals

- materials for restraint of animals (e.g. snares, boards);
- cotton wool and disinfectant to clean sampling site;
- sterile vacutainers (10 ml) without anticoagulant (red stoppers) for serum collection;
- sterile vacutainers (10 ml) with EDTA (purple stoppers) for whole-blood collection;
- either vacutainer holders and vacutainer needles or 10-20 ml syringes; different sizes of needles appropriate for the size of the pigs and the sampling site (e.g. jugular vs. auricular vein);
- filter paper/dried blood spot (DBS) cards.

Materials for postmortem sampling

- sample racks or cryoboxes for cryovials;
- sterile 2 ml cryovials for organ collection (can be pre-filled with medium such as RNA later for sample preservation if the cold chain is not optimal);
- knives, knife sharpeners, shears, scalpel with blades, forceps and scissors;
- containers with disinfectant to sterilize knives, scissors etc. between organs and between animals, to avoid cross-contamination;
- securely sealable plastic pots filled with 10% neutral buffered formalin (1:10 organ volume: formalin volume ratio);
- materials for appropriate carcass disposal.

is already dead, blood can be taken from the heart, but it has to be done immediately. Avoid the use of heparin (green stopper) because it can cause inhibition of the PCR and/or false-positive reactions in the identification by the haemadsorption reaction (HAD) test. Blood is a target sample for virus detection using PCR and virus isolation. The plasma separated by centrifugation can be used for antibody detection with the indirect immunoperoxidase test (IPT) or indirect fluorescent antibody (IFA) test.

Dried blood spot (DBS) micro volume sampling on filter paper cards can be a convenient way to sample and store blood for further DNA and/or antibody detection. These cards are very useful in remote locations or when a cold chain is not available, such as in hunting conditions and rural areas in the tropics. However, genome and/or antibody detection tests have a lower sensitivity when using DBS ASF than with whole blood or serum. DBS samples are collected by applying a few drops of blood drawn by lancet, or using a sterile syringe needle, from the vein or skin, onto specially manufactured absorbent filter paper. The blood is allowed to thoroughly saturate the paper and is air-dried for several hours. Samples are stored in low-gas-permeability plastic bags with desiccant added to reduce humidity, and may be kept at ambient temperature, even in tropical climates.

b. Sera

Draw whole blood from the jugular vein, the inferior vena cava, or the auricular vein, or during the necropsy using sterile vacutainers without anticoagulant (red stopper). After returning to the laboratory, the blood should, to obtain the serum, be incubated for 14-18 hours at 4±3 °C for the separation of the coagulum. The coagulum is discarded and, after centrifugation for 10-15 minutes, the clear supernatant (serum) is recovered. If the serum is red, this indicates the sample is haemolyzed, which can produce false-positive reactions in ELISA tests. Haemolysis usually occurs when the animal is already dead, e.g. with wild boar. Serum can be tested immediately using antibody and virus detection techniques or stored at < -70 °C until further use. For future antibody detection, storage at -20 °C is also adequate, but for virus detection this is suboptimal.

c. Organs and tissue samples

Although all porcine organs and tissues can be used to check for the presence of ASFV (mainly in the acute and subacute forms of the disease), the target organs are spleen, lymph nodes, liver, tonsil, heart, lung, and kidney. Of these, spleen and lymph nodes are the most important as they usually contain the highest amounts of virus. Bone marrow is also useful in incidents involving dead wild animals, as it might be the only tissue that is comparatively well preserved if an animal has been dead for some time. Intra-articular tissues of joints can be examined to check for the presence of low virulent isolates. It is recommended to keep the samples at 4 °C and submit them to the laboratory as soon as possible (within 48 hours). If that is not possible for logistical reasons, samples can either be stored in a freezer or liquid nitrogen. For histopathological studies, samples in 10% buffered formalin can also be submitted in parallel. Although such samples cannot be used for further virus isolation studies, they can serve for PCR and immunohistochemistry.

BOX 5

Minimum amounts recommended for target samples

For antibody detection using ELISA, plus confirmatory techniques, the minimum amounts recommended are:

- Sera: 500 µl.

For ASF virus detection using PCR and virus isolation:

- Sera: 1 ml.
- Blood (EDTA-blood): 1 ml.
- Organs without formalin (minimum amount recommended): 5 g.

For virus detection by PCR, virus isolation and/or antigen ELISA, a 10% (w/v) clarified homogenized tissue suspension should be prepared in phosphate-buffered saline. After centrifuging, it is recommended to filter the supernatant and treat with 0.1% of antibiotic for 1 hour at 4±3 °C. The treated homogenate tissue can be used immediately for ASFV and genome detection, or stored at <-70 °C until further use. For PCR, it is recommended to process at 1/10 dilution of the supernatant in parallel with the undiluted material. Exudate tissue samples, mainly obtained from the spleen, liver, and lungs, are very useful to check for the presence of antibodies using IPT and IFA (Gallardo, 2015).

d. Soft tick samples

Ornithodoros soft ticks can be tested for ASFV and genome detection. The ticks can be collected from warthog burrows, crevices/holes in pigsties, and sometimes from rodent burrows inside pigsties. Different species will have different preferred locations and habitats. There are three techniques for collection: manual collection, carbon dioxide trapping and vacuum aspiration. After collection, ticks should be kept alive or directly stored in liquid nitrogen to ensure optimal conservation of the virus inside the ticks and to avoid DNA degradation.

PACKAGING AND TRANSPORT OF SAMPLES

To obtain the right diagnosis, it is essential that the right samples are selected, carefully packaged, labelled, and transmitted to the laboratory in the fastest possible way, with appropriate temperature control. ASF diagnosis is urgent and samples must be sent to the nearest appropriate laboratory by the most direct route. Samples must be accompanied by a submission form specifying the number and type of samples, the species, the sampling location (address, county, district, province, country of origin, as appropriate). Also to be listed are the tests required, name of the person submitting the sample, and the observed clinical signs, gross lesions, morbidity, mortality, number of affected animals, history and kinds of animals involved. In the case of domestic animals, the owner, name of the farm and type of farming system should be specified, plus a list of differential diagnoses. One must be able to cross-reference each sample to the source animal. The minimum required

information may vary depending on the laboratory, however. It helps to phone the laboratory before sampling to ensure that submission procedures are followed correctly and that the envisioned number of samples can be analysed or stored in an appropriate time frame.

Samples should arrive in the testing laboratory as soon as possible to avoid deterioration and ensure best results. They should be sent safely to avoid infecting other animals or persons during the trip, and also to avoid contaminating the samples themselves. Shipped samples must be delivered with adequate amounts of cooling materials, e.g. ice packs, to prevent deterioration. It is not possible to make an accurate diagnosis if the samples are not in good condition.

Land transport

National regulations must be followed when transporting samples to the nearest laboratory, even if samples are carried by veterinary services staff. For Europe, the base regulation is the *European Agreement concerning the International Carriage of Dangerous Goods by Road* (ADR).[1] For other areas, national regulations must be followed. If none are available, the UN Model Regulations,[2] explained in the *OIE Manual for Diagnostic Tests and Vaccines for Terrestrial Animals* (2016; Chapters 1.1.2 and 1.1.3), should be followed.

Triple packaging should be used even in the case of road transport. A detailed example of the characteristics of triple packaging is given in Figure 27.

Transport by air

These samples should be shipped in accordance with regulations,[3] and use of the "Triple packaging system" is required. Especially if the samples are transported by air, the sender must follow the *Dangerous Goods Regulation* (DGR) of the International Air Transport Association (IATA), and packaging should be in conformity with Packing Instruction 650[4] in the DGR.

African swine fever diagnostic samples are considered hazardous – they must be packaged and labelled correctly to prevent virus release. Therefore, products should be used that fulfil specifications (i.e. conform to the IATA requirements for the transport of diagnostic samples, such as 95kPa pressure test, drop test). To find suppliers for such receptacles and packaging, internet search keywords such as "95 kPa" together with "UN3373", and "vial", "tube" or "bag" usually return appropriate information.

- **Primary receptacles.** Samples should be stored in leakproof, water-resistant, sterile containers (these should be the primary receptacles) as shown in Figure 27). Each primary receptacle must not contain more than 1 litre. The lid of each container should be sealed with adhesive tape or Parafilm. These sealed primary containers should then

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1 *European Agreement concerning the International Carriage of Dangerous Goods by Road* (ADR) applicable as from 1 January 2015 (see p.110 of Volume 1) Available at: http://www.unece.org/trans/danger/publi/adr/adr2015/15contentse.html

2 *UN Recommendations on the Transport of Dangerous Goods – Model Regulations – Nineteenth revised edition* (see p. 80 of Volume II). Available at: http://www.unece.org/trans/danger/publi/unrec/rev19/19files_e.html

3 Basic regulations are set by the United Nations. Based on this, regulations are set for air, road and sea transport respectively by national and international authorities.

4 http://www.iata.org/whatwedo/cargo/dgr/Documents/packing-instruction-650-DGR56-en.pdf

BOX 6

Things to get ready/organize in advance

- Specific packing materials are required to transport diagnostic samples by air. Since such materials are often not produced domestically and have to be imported, it is advisable to keep some in stock.
- Dry ice is often required to transport diagnostic samples by air. Identify and confirm a supplier.
- Not all courier companies transport diagnostic samples. Find out which courier company in your country can do so. This is becoming more and more of a problem in many countries, delaying diagnosis and response.
- Not all airlines transport diagnostic samples. If it is planned to use airfreight, find out which airline flying to your country will accept them.
- Some airlines may not permit dry ice to be used. Find out in advance what the airline's policy is.
- Contact possible destination laboratories, ask for information regarding official documentation (e.g. import permits, export permits, etc.) required for importing diagnostic samples, and obtain a sample submission form, if available.

be packed individually in shock/absorbent material to soak up any possible leakage from containers or tubes and protect against shocks. It is essential to mark each container with waterproof ink to clearly identify the animal from which the sample was taken.

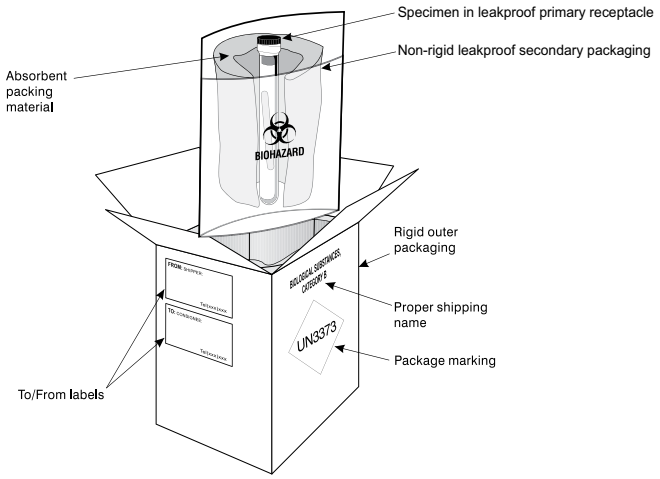
- **Secondary packaging.** All of these primary receptacles should be placed in a secondary leakproof, hermetically sealed, water-resistant container, e.g. plastic, metal. The secondary packaging must be capable of withstanding, without leakage, an internal pressure of 95 kPa (0.95 bar) in the range of -40 °C to 55 °C. Absorbent material should also be placed inside the second container. If multiple, fragile, primary receptacles are placed in a single, secondary container, they must be either individually wrapped or separated to prevent contact between them.

CAUTION 1) Dry ice must not be placed inside the primary or secondary receptacles because of the risk of explosion. 2) The primary receptacle must be capable of withstanding, without leakage, an internal pressure of 95 kPa (0.95 bar) in the range of -40 °C to 55 °C.[5]

- **Rigid outer packaging.** The secondary container must be secured in outer packaging with suitable cushioning material. It should have successfully passed the drop test at a height of 1.2 m and be labelled with the UN3373 mark. The outer packaging

[5] *WHO Guidance on regulations for the Transport of Infectious Substances 2015-2016* (pp. 28-31 of the English version for diagnostic specimen packaging). Available in Arabic, English, French and Russian at http://www.who.int/ihr/publications/who_hse_ihr_2015.2/en/

FIGURE 27
Example of the triple packaging system for the packing and labelling of Category B infectious substances.



must not contain more than 4 litres in the case of liquid or more than 4 kg in the case of solid substances. These quantities exclude ice, dry ice or liquid nitrogen when used to keep samples cold.

Samples that must be shipped at 4 °C, usually for short shipments (1-2 days)

Packaged as indicated above, these samples should be shipped with refrigerants (in sufficient quantity to maintain the desired temperature) within thermally insulated, robust boxes meeting the IAEA Packing Instruction 650 if transported by air.

Samples that must be shipped frozen (-20 °C or -70 °C)

For shipments that take more than three days: these materials should be also packaged as specified, adding enough dry ice to the thermally insulated box to maintain the temperature. It is important to ensure that the secondary packaging is secured at the centre of the box because as the dry ice decomposes, the secondary container can become loose. The carbon dioxide gas (CO_2) resulting from the decomposition of the dry ice lowers the pH and deactivates the virus; therefore all primary and secondary containers must be hermetically sealed. When dry ice is used to keep specimens cold during the transport, the outer packaging must permit the release of gas (i.e. must not be hermetically sealed) to prevent a build-up of pressure that could rupture the packaging. Never freeze whole blood, or serum that contains coagulant.

1. Labelling and marking

The outside of the box (rigid outer packaging)[6] should be labelled with the following identification:

1. label for "biological substance Category B" (Figure 28), with the proper shipping name reading, "Biological substance, Category B", next to it;
2. full name, address and telephone number of sender;
3. full name, address and telephone number of addressee;
4. full name and telephone number of a responsible person, knowledgeable about the shipment, e.g. RESPONSIBLE PERSON: First name LAST NAME, +123 4567 890;
5. label reading "conserve at 4 degrees Celsius" or "conserve at -70 degrees Celsius".

If dry ice is used:

6. label for "dry ice" (Figure 29);
7. UN number and the proper shipping name of dry ice followed by the words "AS COOLANT". The net quantity of dry ice in kilograms, must be clearly written near the Figure 29, e.g. UN 1845, DRY ICE, AS COOLANT, NET. ## KG.

2. Documentation

Samples shipped to a laboratory must be accompanied by a submission form supplied by that lab or, if this is not available, by a covering letter. This letter should include relevant information concerning the owner of the animal, name and district of the farm, type of farming system, animal(s) involved, history, clinical signs and postmortem lesions. Test(s) required must be indicated.

Documentation for the shipment: if the shipment crosses a national border, an import or export permit, plus a copy of the permit for the recipient laboratory to accept infectious substance for diagnosis, etc. will sometimes be needed. Such requirements vary from one country to another. Always ask the recipient laboratory if such documents are needed to import diagnostic samples.

3. Transport

Before dispatch of samples, contact the recipient laboratory as early as possible and inform them about the intended shipment, including detailed information and approximate date and time of arrival. It is better to arrange the shipment with a courier offering door-to-door service, with delivery directly to the laboratory. As soon as the samples are dispatched, the courier should give the destination laboratory their company name and, if available, the shipment's tracking number and/or air waybill number. If airfreight is used, a prior arrangement with the recipient laboratory to pick up the shipment on arrival at the airport is necessary (some international laboratories have such a system, but not all of them). The recipient laboratory must be informed of the name of the airline, the flight number and the air waybill number as soon as available. It is prohibited for people to transport infectious substances as checked or carry-on baggage, or to carry them on their person.

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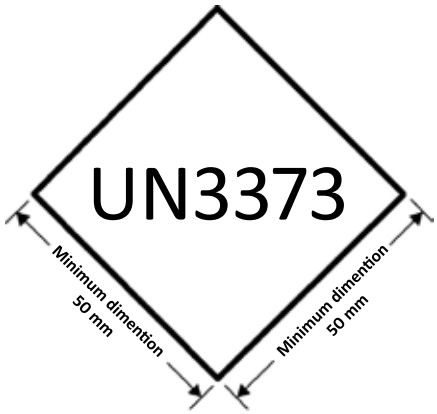
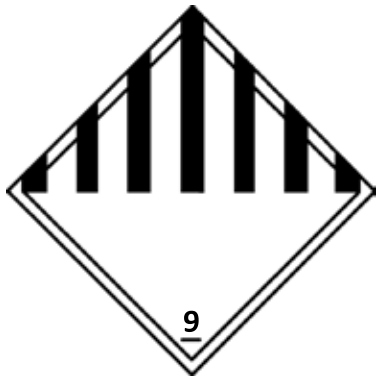
[6] Refer to *WHO Guidance on regulations for the Transport of Infectious Substances* if OVERPACK is used.

FIGURE 28
Marking for infectious substances of Category B



Minimum dimensions: 100 × 100 mm (for small packages: 50 × 50 mm), 1 label per package.
Colour: black and white.

FIGURE 29
Marking for Miscellaneous dangerous substances



Minimum dimensions: 100 × 100 mm (for small packages: 50 × 50 mm), 1 label per package.
Colour: black and white.

Transport of isolated/cultured ASF virus

Isolated/cultured ASF virus must be transported as a Category A infectious substance. The UN number is UN2900, the proper shipping name is "Infectious substances affecting animals (African swine fever virus)", and packaging in conformity with the Packing Instruction 620 must be used. The labelling and marking on the outside of the box are also different.

While dangerous goods regulations require all personnel involved in transport to undergo appropriate training, especially for the transport of Category A infectious substances, personnel must undergo training in accordance with the apposite requirements, including attending approved courses, passing examinations and receiving certification (valid for two years). For more information, refer to the "WHO Guidance on regulations for the Transport of Infectious Substances".

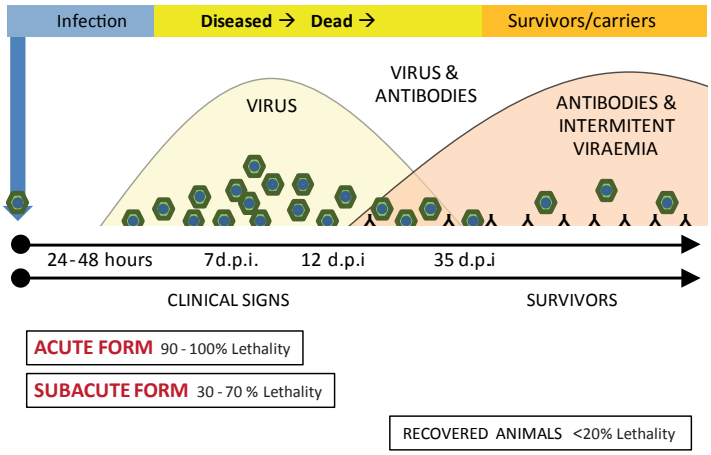
Laboratory diagnosis of ASF

Since there is no vaccine available, rapid and reliable early detection of the disease is essential for the implementation of strict sanitary and biosecurity control measures to prevent the spread of the disease. Diagnosis of ASF means the identification of animals that are, or have previously been, infected with ASFV. An appropriate diagnosis therefore involves the detection and identification of ASFV-specific antigens, or DNA and antibodies, to obtain relevant information to support control and eradication programmes. It is important to consider the course of the disease when choosing the diagnostic test (Figure 30). Since each animal could be at a different stage of the disease, **both virus and antibody detection tests should be carried out** in outbreaks and control/eradication programmes.

The incubation period in natural infections has been reported as varying from 4 to 19 days. About two days before clinical signs develop, ASF-infected animals begin to shed large amounts of the virus. Virus shedding can vary depending on the virulence of the ASFV strain involved. Seroconversion occurs at about 7-9 days post-infection and antibodies can be detected for the rest of the animal's life (Figure 30).

A positive test for the presence of the virus (i.e. antigen) indicates that the tested animal was undergoing infection at the time of sampling. On the other hand, a positive ASFV antibody test indicates an ongoing or past infection, where the animals have recovered (and may remain seropositive for life).

FIGURE 30
Virus and antibody circulation in blood over time and in relation to the stage of ASF virus infection, as observed in European domestic pigs in Iberian Peninsula and Western Hemisphere (1960-1995)



Since late 2015, epidemiological serological data in Eastern Europe has shown a significant increase in the incidence of seropositive animals, particularly evident in wild boar populations in the affected EU countries. These results suggest that some animals are surviving for over a month, may be able to recover from ASF infection, and in certain cases, even remain subclinically infected, as previously described in the Iberian Peninsula, Americas, and in Africa. Antibody detection techniques are therefore essential to obtain complete information in support of control and eradication programmes.

DETECTION OF ASF VIRUS

ASFV genome detection by polymerase chain reaction (PCR)

Polymerase chain reaction (PCR) is used to detect the ASFV genome in porcine samples (blood, organs, etc.) and ticks. Small fragments of viral DNA are amplified by PCR to detectable quantities. All validated PCR tests allow viral DNA detection even before the appearance of clinical signs. PCR enables the diagnosis of ASF to be made within hours of sample arrival to the laboratory. PCR provides a sensitive, specific, and rapid alternative to virus isolation for the detection of ASFV. PCR provides higher sensitivity and specificity than alternative methods for antigen detection, such as the antigen enzyme-linked immunosorbent assay (ELISA) and the direct fluorescent antibody test (FAT). However, the extreme sensitivity of the PCR makes it susceptible to cross-contamination, and proper precautionary measures should be taken to minimize and control this risk.

Conventional and real-time PCRs recommended by the OIE in the *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* (2016) have been fully validated over time and are useful tools for routine diagnosis of the disease. Other real-time PCR procedures have proved to provide higher sensitivity than OIE-prescribed, real-time PCR methods for ASFV genome detection in recovered animals. Primer sets and probes used in these molecular techniques are repeatedly designed within the VP72 coding region, a well-characterized and highly conserved region of the ASFV genome. A wide range of isolates belonging to all the 22 known p72 virus genotypes can be detected with these PCR assays, even in inactivated or degraded samples.

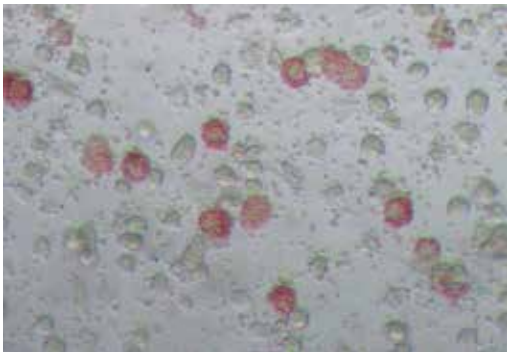
PCR is the tool of choice in the case of peracute, acute, or subacute ASF infections. Furthermore, since PCR detects the viral genome, it may be positive even when no infectious virus is detected by virus isolation, making it a very useful tool for the detection of ASFV DNA in pigs infected with low- or moderately virulent strains. Although PCR is not informative about the infectivity of the virus, it can provide quantitative information.

ASF virus isolation

Virus isolation is based on the inoculation of sample material onto susceptible primary cell cultures of porcine origin, monocytes, and macrophages. If the ASFV is present in the sample, it will replicate in the susceptible cells, producing cytopathic effect (CPE) in the infected cells. Cell lysis and CPE usually occur after 48-72 hours of haemadsorption. The importance of this finding relies on its specificity because none of the other pig viruses are capable of haemadsorbing in leukocyte cultures. When the virus replicates in these cultures, most of the ASFV strains produce the haemadsorption reaction (HAD) due to adsorption of pig red blood cells on ASFV-infected leukocytes forming "rosettes" (Figure 31).

However, it is important to point out that the CPE, in absence of haemadsorption, could be due to the cytotoxicity of the inoculum, the presence of other viruses such as Aujeszky's disease

FIGURE 31
Haemadsorption reaction (HAD)



virus, or to a non-haemadsorbing ASFV isolate. In these cases, the presence of ASFV on the cell sediment must be confirmed by other virological assays such as FAT or by the use of PCR. If no change is observed, or if the results of the FAT and PCR are negative, the supernatant must be sub-inoculated into fresh cultures for up to 3-5 passages before discounting the presence of ASFV.

Virus isolation and identification by HAD are recommended as a reference test for the confirmation of positive results of a prior antigen-positive test (ELISA, PCR or FAT). They are also recommended when ASF has already been confirmed by other methods, particularly in the case of a first outbreak of ASF in an area. In addition, virus isolation is essential if the objective is to obtain virus stocks for future molecular and biological characterization studies.

ASF antigen detection by direct fluorescent antibody test (FAT)

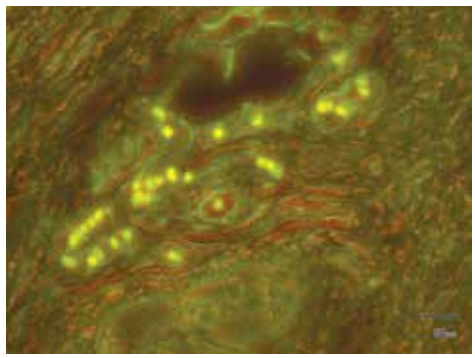
The FAT can be used to detect ASFV antigen in pig tissues. The principle of the test is the microscopic detection of viral antigens on impression smears or thin cryosections of organ material. Intracellular antigens are detected using fluorescein isothiocyanate (FITC)-conjugated specific antibodies. FAT can also be used to detect ASFV antigen in leucocyte cultures in which no HAD is observed, and can thus identify non-haemadsorbing strains of ASFV. It also distinguishes between the CPE produced by ASFV and that produced by other viruses, or due to the cytotoxicity of the inoculum.

Positive and negative controls are used to ensure that the slides are interpreted correctly. This is a highly sensitive test for cases of peracute and acute ASF and can be carried out fairly rapidly. It is a robust test, but has been largely replaced by PCR and reagents are no longer widely available. However, it is important to note that in subacute and chronic disease, the FAT has a significantly decreased sensitivity (40%).

ASF antigen detection by antigen ELISA test

Viral antigens can also be detected using ELISA, which is cheaper to set up than PCR methods and allows large-scale testing of samples in a short time without special laboratory equipment. However, as in the case of the FAT, in subacute and chronic disease the antigen ELISA has a significantly decreased sensitivity. In addition, field samples are often in poor

FIGURE 32
Localisation of ASFV by immunofluorescence antibody test (FAT) in ASFV-infected tonsil



Fluorescent inclusion bodies or granules appear in the viral factories in cells in the cortex.

©INIA-CISA

condition and therefore also decrease the sensitivity of the test. It is thus recommended to use the antigen ELISA (or any other ELISA) only as a "herd" test and in conjunction with other virological and serological tests.

DETECTION OF ASF ANTIBODIES

Serological assays are the most commonly used diagnostic tests due to their simplicity, comparatively low cost, and their necessitating few specialized pieces of apparatus or facilities. Since there is no vaccine against ASF, the presence of ASFV antibodies always indicates current or historic infection. Furthermore, ASFV antibodies appear early after infection and persist up to several years. However, in peracute and acute infections the pig often dies before antibodies become detectable. It is therefore recommended that in the early stages of an outbreak, samples are taken for detection of viral DNA as well.

For the detection of ASF antibodies, the recommended tests include the ELISA test for antibody screening followed by the immunoblotting test (IB) or indirect fluorescent antibody (IFA) test as confirmation. The antibody detection by indirect immunoperoxidase test can be used as an alternative confirmatory test for the detection of ASF antibodies in porcine sera and in tissue exudate. It can be easily applied to a large number of samples, does not require expensive fluorescence microscope equipment, and provides appropriate sensitivity.

ASF antibody detection by ELISA test

The ELISA test is a very useful technique, widely used for large-scale serological studies of many animal diseases. Some of the most notable characteristics of this method are high sensitivity and specificity indexes, high speed, low cost and easy interpretation of results. Large populations can be rapidly screened thanks to the automatic equipment available.

The ELISA uses tagging to identify ASF antibodies in serum samples. In this technique, the antibodies are tagged with certain enzymes. When an antigen and antibody bind to each other, the enzyme causes a reaction that produces a colour change, thereby identifying the presence of ASF. A variety of commercial and "in-house" methods such as indirect or blocking ELISA tests are currently available for ASF antibody detection.

FIGURE 33
ASF antibody detection by immunoblotting (IB)

Sera incorrectly handled or badly preserved (due to inadequate storage or transportation) and haemolyzed samples may yield up to 20% false-positive results. Therefore, all positive and doubtful samples by ELISA must be confirmed by alternative serological confirmatory tests.

The IB technique is a rapid and sensitive assay for the detection and characterization of proteins. It works by exploiting the specificity inherent in antigen-antibody recognition. This test involves the production of antigenic strips bearing the virus antigens. It involves solubilization, electrophoretic separation, and transferring of proteins onto membranes (usually nitrocellulose). The membrane is overlaid with a primary antibody for a specific target and then with a secondary antibody labelled to visualize the positive reaction.

The first viral proteins that induce ASF-specific antibodies in pigs invariably react by IB in all the infected animals. Positive reactions begin with sera obtained from animals 7-9 days post-infection, and up to several months post-infection in surviving animals. Sera from animals vaccinated against other viruses can induce false-positive reactions. In those cases, alternative confirmatory tests such as IPT or FAT should be used.

ASF antibody detection by indirect fluorescent antibody (IFA) test

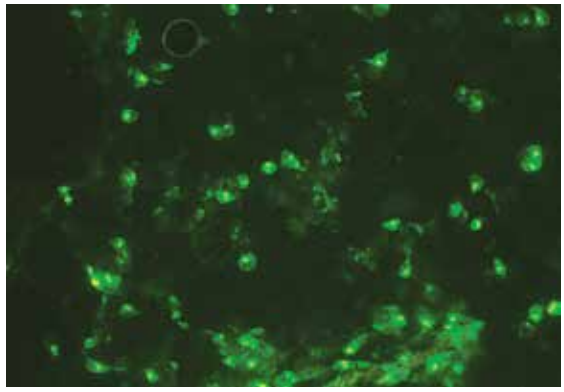
The test is based on the detection of ASF antibodies that bind to a monolayer of green monkey kidney cells infected with an adapted ASFV. The antibody-antigen reaction is detected by a labelled fluorescein conjugate. Positive samples show specific fluorescence in the cytoplasm of the infected cells. The IFA is a rapid technique with high sensitivity and specificity for the detection of ASF antibodies from sera, plasma or tissue exudates.

ASF antibody detection by indirect immunoperoxidase test (IPT)

The IPT is an immune-cytochemistry technique on fixed cells to determine the antibody-antigen complex formation through the action of peroxidase. In this procedure, green monkey kidney cells are infected with ASFV isolates adapted to these cell cultures. The infected cells are fixed and used as antigens to determine the presence of the specific antibodies against ASF in the samples. As is the case with FAT, IPT is a rapid technique with high sensitivity and specificity for the detection of ASF antibodies from sera, plasma or tissue exudates. Interpretation of the results is easier than FAT, because of the enzymatic visualization system employed.

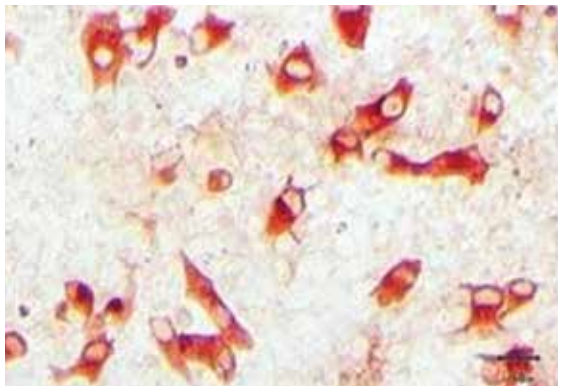
In conclusion, current available diagnostic tests allow one to confidently diagnose ASF by combining both virus and antibody detection. Real-time PCR is the most widely used for virological diagnosis, providing sensitive, specific, and swift detection of ASFV

FIGURE 34
ASF antibody detection by indirect fluorescent antibody (IFA) test



Positive samples show specific fluorescence in the cytoplasm of the infected cells.

FIGURE 35
ASF antibody detection by indirect immunoperoxidase test (IPT)



Positive samples show specific red staining in the cytoplasm of the infected cells.

DNA. Due to the possibility of a cross-contamination, a unique positive PCR result from a single animal in a free area (e.g. a wild boar), or a single positive PCR result within a group of animals, should be confirmed by additional virus detection tests and should be combined with serological, pathological and epidemiological findings. Since PCR detects viral DNA presence and not live virus, it is highly recommended to get virus isolation from infected samples prior to the confirmation of an outbreak if a new region is affected.

Keeping test limitations in mind, validated ELISA tests are the technique of choice for ASF antibody detection, particularly for screening serum samples. Confirmatory tests such as IB, IFA or IPT are crucial to identify false-positive ELISA results. In addition, IFA and IPT are the recommended techniques for the analysis of tissue exudates and plasma samples, providing a complete picture of the epidemiology, and allowing to determine the time of infection.

An accurate ASF diagnosis must include the virological and serological results together with the clinical, pathological, and epidemiological findings. Table 5 summarizes the characteristics of the main laboratory diagnostic techniques for ASF.

TABLE 5
African swine fever laboratory diagnostic techniques at a glance

ASSAY FOR VIRUS DETECTION	TIME	SENSITIVITY	SPECIFICITY	SAMPLE TYPE	COST	COMMENTS
Polymerase Chain Reaction (PCR)*	5-6 hours	XXX	XX	Tissues, blood, ticks or cell cultures	$$	Most common method Susceptible to contamination Detects live or dead virus
Haemadsorption Test (HA)	7-21 days	XX	XXX	Porcine macrophage cells	$$$$	GOLD STANDARD Only used in a few reference laboratories
Direct Fluorescence Antibody test (FAT)	75 min	XXX (for early detection)	XXX	Cryostat sections. Impression smears. Cell culture of macerates	$$$	Recommended when PCR is unavailable or lack of experience Needs a fluorescent microscope Lack of sensitivity after the first week post-infection
Enzyme-Linked Immunosorbent Assay (ELISA)	3 hours	X (for early detection)	XX	Serum, macerates	$	Not routinely used Lack of sensitivity after the 1st week post-infection

ASSAY FOR ANTIBODY DETECTION	TIME	SENSITIVITY	SPECIFICITY	SAMPLE	COST	COMMENTS
Enzyme-Linked Immunosorbent Assay (ELISA)*	3 hours	X	X	Serum	$	Screening test In-house and commercial kits available
Immunoblotting	3 hours	X	X	Serum	$$$$	Confirmatory test No commercial kits
Indirect Fluorescent Antibody (IFA) test	4 hours	XXX	XX	Tissue exudates, serum or plasma	$$$	Confirmatory test No commercially available reagents Needs a fluorescent microscope

(*): most commonly used

Prevention and control

African swine fever is different from most other transboundary animal diseases (TADs) in that no vaccines or drugs are available to prevent or treat it. Therefore it is particularly important that ASF-free areas are maintained as such. Preventing the entry of ASFV into both domestic and wild suid populations, and controlling and eradicating the disease as soon as it is detected, are the best ways of minimizing its impact. There are, however, also successful examples of ASF eradication, e.g. Brazil, Portugal, Spain or Côte d'Ivoire.

Prevention starts with stringent measures at the borders and raising awareness among all stakeholders involved. Early detection, early diagnosis, early response, and good communication are critical in minimizing the spread of the disease after incursion. In order to understand what measures will be most effective, it is important to bear in mind how ASF is transmitted: i.e. mainly via the movement of infected pork and animal products (followed by ingestion); from direct contact between live animals, including wild suids; and through bites by *Ornithodoros* ticks.

Action can be taken at the institutional or individual (e.g. farmer) level, with most measures relating to the improvement of biosecurity. Prevention and control activities/measures can be implemented either through private or public initiatives, but reaching an optimal level generally requires a combination of both. Farmers play a key role, but they may need technical and financial support.

Sections of this chapter are extracted from two FAO Manuals, which can be consulted for more detailed information: *Good Emergency Management Practices (GEMP): The Essentials* (FAO, 2011), and *Good practices for biosecurity in the pig sector* (FAO, 2010).

AWARENESS

Raising awareness, together with the provision of information/technical assistance and training of all relevant stakeholders, is a cross-cutting approach with a direct positive impact in the implementation of all disease-prevention, control and surveillance activities. Awareness is therefore considered the most cost-effective measure. Awareness helps pig producers to take prompt, efficient decisions when adopting prevention and control measures.

Everyone in contact with pigs should be made aware of how to prevent and respond to ASF, starting with official veterinarians and farmers, but also including operators along the market chain, i.e.: individuals involved in the transport, marketing and butchering of pigs; service providers (e.g. private veterinarians, feed distributors, etc.); and in some cases the general public. When wild boar are present, hunters, rangers and forestry services should also be targeted.

It is very important to establish regular contact between veterinary services (professional and/or paraprofessional) and livestock farmers/traders. This should not only take the form of routine visits but also feature "house calls" to investigate and provide assistance on disease problems. In this way, farmers will have the confidence to seek official veterinary help when confronted by an unusual and potentially disastrous disease like

FIGURE 36
Training veterinarians on how to conduct a pig postmortem in Signani, Georgia

©FAO/MIKHEIL SOKHADZE

ASF. This "bottom-up" approach will also make it possible to take farmers' views into consideration when developing prevention and control tools and strategies. For countries that rely heavily on the private sector for official veterinary services, an additional interface between them and the veterinary authority is needed (GEMP, 2011).

All stakeholders should be made aware of the potential severity of ASF, of how to prevent and recognize it (i.e. clinical presentation), and of the need to immediately report any suspicion of ASF to the veterinary services (i.e. passive surveillance). The latter is particularly important since farmers may accept significant pig losses as "normal". Information on measures to reduce the likelihood of infection should also be provided. The dangers of swill feeding and other biosecurity breaches should be stressed, particularly to smallholder pig producers. In the event of ASF entering a country, outbreaks should be well-publicized, emphasizing the need to enhance biosecurity at all levels, to inspect pigs regularly, and to promptly report suspicious lesions and deaths to the authorities. Even information on the control policy, e.g. culling, compensation and restocking, will help farmers to understand their role in the whole process and make them more willing to cooperate.

Livestock traders, dealers and marketers, despite being important target groups for public awareness campaigns, are often overlooked. The movement of animals through livestock traders is often a key factor in the spread of epidemic diseases like ASF. The need for building a climate of trust between animal health officials and livestock traders is as important as it is with farmers. The general themes should also be similar, although emphasis should be placed on the importance of sourcing animals from disease-free areas, not buying or selling sick pigs, or pigs from groups where some have been sick, following any rules about quarantine, vaccination, testing or identification of animals, and the keeping of records. The potential consequences of ASF for internal and international trade should be emphasized (GEMP, 2011).

The development and dissemination of awareness information and training is usually handled through extension and outreach services, mostly by public authorities (and sometimes NGOs), rather than the private sector. There are numerous approaches for

FIGURE 37
Training pig farmers in Burkina Faso

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delivering the information, e.g. leaflets, booklets, posters, TV and radio messages, meetings organized by religious leaders or village chiefs, etc. The format/s will depend on the target group/s. In some cases, however, more thorough training is needed. As for awareness materials, there are multiple formats available, from online courses to traditional, face-to-face training. When there is a need to deliver information to a large number of people, a training-of-trainers model might be the best approach. Also known as "cascade training", these programmes are designed to train people who in turn train others.

PREVENTION

The risk of introducing ASFV (or any other pathogen) is reduced by adopting good biosecurity practices, not just on the farm, but at every step of the supply chain, e.g. at live-animal markets, slaughter sites, while transporting the animals, etc. Special attention should be paid to small commercial and backyard operations, which are characterized by low biosecurity standards, and to live-animal markets, which bring together animals from many sources. Both are key in the spread of ASF, and although the same biosecurity concepts apply, specific measures and manuals have been specifically developed for them.

Biosecurity measures should be used to avoid the entry of pathogens into a herd or farm (external biosecurity), but also to prevent or slow down the spread of disease to uninfected animals within a herd or farm after the pathogen has arrived (internal biosecurity), and to stop infecting other premises or wild suids. As with regulations put in place by the government on farm biosecurity, needs and expectations will vary significantly depending on the pig production system and local geographic and socio-economic conditions (i.e. ranging from large-scale, closed farms through to smaller, scavenging, village systems). Global biosecurity issues are relevant to all environments and production systems, but are particularly challenging in the backyard sector of developing and transition countries. However, the wide range of options available to improve biosecurity, some of them as simple as improved record-keeping, means that all farms can improve their disease-prevention and control practices.

The ability of farmers to implement on-farm biosecurity measures depends on the characteristics of their production system, their technical knowledge, and their financial resources. Those in charge of biosecurity improvement programmes should have a thorough knowledge of the diversity of systems and an understanding of the people involved in pig production, e.g. their motivations for keeping animals and their available resources. Keeping these factors in mind will help to develop strategies for implementing sustainable biosecurity measures on farms and along the production and marketing chains.

There are differences between on-farm biosecurity measures put in place before an outbreak (bio-exclusion) and after one has occurred (bio-containment), though for proper disease prevention and management, these measures are closely linked. To help separate ASF prevention from general disease-prevention techniques, efforts should take into account its transmission routes. Listed below are some of the most relevant biosecurity measures. More information on biosecurity can be obtained from the FAO manual on *Good practices for biosecurity in the pig sector*.

Swill feeding

Feed is an important control point for both ASF and other diseases. Due to its nature, swill is inherently a convenient, affordable, but hazard-laden food. Swill feeding presents a very high risk of introducing several diseases into healthy populations. An effective ban on swill feeding would be ideal, but compliance at household level is unlikely since it would defeat one of the main motivations for keeping pigs, i.e. minimum feed inputs thanks to swill feeding or scavenging. In any case, pigs should not be fed swill that might contain pork, and swill should be boiled for 30 minutes, with periodic stirring, and cooled before feeding to pigs.

Containment of pigs

The construction of pigsties, which enable hygienic conditions to be maintained, should be encouraged. Also, perimeter fencing will prevent direct contact and subsequent disease spread from domestic pigs to wild boar (and feral pigs) and vice versa, and from wild African suids to domestic pigs. Perimeter fencing will also help limit access by both wild and domestic suids to garbage, leftovers or carcasses that may have been contaminated. Fencing designed to keep wild or domestic pigs in or out must extend to a depth of at least half a metre below ground, as they are accomplished at digging. Overall, authorities should discourage pig production systems based on scavenging since they give pigs access to potentially contaminated garbage or carcasses, and allow them contact with infected wild boar, or other scavenging or feral pigs.

However, as with swill feeding, traditional ways of keeping pigs cannot easily change, as many producers will not find it worthwhile to confine (and feed) their pigs. A significant part of the pig sector survives because its animals are allowed to range freely. Thus, any move to create a more closed system, with a consequent increase in feed costs, is likely to be resisted by many smallholder farmers.

It is difficult to introduce effective biosecurity if pigs can scavenge at will for most of the day. However, some simple precautions can be recommended at minimum cost in terms of outlay and time. Perimeter fencing around the whole village, although not

FIGURE 38
Examples of pig production systems with different levels of biosecurity

A. *Scavenging pigs in Kisumu, Kenya*
B. *Low-biosecurity premises in Gulu, Uganda*
C. *Medium-sized farm in Kiambu, Kenya*
D. *Highly biosecure farm in South Africa*

always practical, can be considered, because the pigs within a village are assumed to have the same health status. It is useful to point out the advantages of confinement to prevent theft, road accidents and predation. Generally speaking, biosecurity for outdoor systems needs greater focus on the control of feedstuffs, water and pasture, as well as wildlife and human visitors.

Cleaning and disinfection

As with farms, equipment and premises should be frequently cleaned and disinfected. Organic matter should be cleaned from sheds, equipment, vehicles, etc. before disinfection. Vehicles and personnel (shoes, equipment, etc.) should be disinfected on entering and leaving farms. Disinfectants proven to be effective include detergents, hypochlorites and glutaraldehyde. The ASFV is susceptible to ether and chloroform. The virus is inactivated by 8/1000 sodium hydroxide (30 minutes), hypochlorites – 2.3% chlorine (30 minutes), 3/1000 formalin (30 minutes), 3% ortho-phenylphenol (30 minutes) and iodine compounds (OIE, 2013). Effective commercial products are also available. The environmental impact of these agents should be taken into account. Equipment that cannot be easily disinfected should be exposed to sunlight.

FIGURE 39
Improperly discarded dead pig outside a farm in Kisumu, Kenya

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Other biosecurity measures

- Visitors must be kept to a minimum and only allowed in after cleaning and disinfection of footwear, or a change of clothing and footwear, particularly in the case of high-risk visitors such as livestock owners and animal health professionals. People working with pigs should avoid contact with other pig populations.
- Vehicles should not enter the farm, and pig loading and unloading in particular should take place outside the perimeter fences. Pig-carrying trucks should be cleaned and disinfected after unloading.
- Sharing of equipment among farms/villages should be discouraged without previous proper cleaning and disinfection.
- Dedicated work clothing and footwear should be provided.
- As far as practicable, farms should be run as closed herds, with limited new animal introductions.
- Newly purchased animals should come from trusted sources and be quarantined (i.e. kept in isolation for observation) for at least 14 days.
- Adequate distances should be maintained between farms.
- Rearing must be age-segregated (all-in-all-out management).
- Dead pigs, effluents and discarded parts from slaughtered pigs should be disposed of appropriately, out of the reach of wild or free-ranging pigs.
- Pigs should not be returned to the farm from live-animal markets. However, if they do return, they should be put in quarantine for 14 days before mingling with other pigs.
- Staff should be trained in good sanitary and hygiene practices, and in disease recognition.
- Wild birds, vermin and other animals should be kept away from animal sheds and from animal feed and water supplies.

Risk analysis and import/export procedures

Biosecurity is a concept that can be also applied at national level. As in a farm, preventing ASF from entering disease-free countries depends on stringent policies for the safe importation of swine and high-risk products, i.e. pork and pork products, semen, hides, etc. Such preventive measures will decrease the frequency and impact of disease incursions. The **OIE Terrestrial Animal Health Code** (2016) provides detailed guidelines. According to GEMP (2011), one should:

- Keep well informed for early warning of changes in distribution or epidemiology in affected countries and trading partners. Information should also be gathered on the country's ports of entry, the pig and pork supply chains, the distribution of farms by production system, wild suids, live-animal markets, slaughterhouses, etc. These data will help to conduct a risk analysis of all potential routes of entry and spread. This should be conducted at regular intervals, with frequency depending on the estimated risk. Subsequent measures should be dynamic, proportionate to the estimated risk.
- Prevent entry of the disease agent in legal imports through additional, targeted restrictions in accordance with accepted international standards. Import restrictions will allow low-risk trade to maximize effectiveness of the quarantine barrier.
- Customs, regulatory and quarantine services should be equipped to effectively intercept illegal/unregulated foodstuffs and other hazardous materials at international airports, seaports, and border crossings. Confiscated materials should be destroyed or disposed of safely, and not dumped within reach of scavengers (animals and humans). Past events suggest that particular attention should be paid to the proper disposal of waste food from aircraft, ships or vehicles from infected countries, preferably by incineration or, if available, by rendering.
- Consider establishing pre-embarkation and post-entry testing for diseases of concern, depending on the level of risk and provided that capacity exists for reliable testing.
- Establish and strengthen cross-border meetings and information exchanges with neighbouring administrations.

CONTROL

When encountering a suspected outbreak, it is important to take appropriate and immediate action. Veterinarians as well as farm owners, workers, and other industry stakeholders must all work to contain and prevent the further spread of the disease. Because ASF-infected animals begin to shed large amounts of the virus 48 hours before clinical signs develop, containment of bedding, feed, and animals (both live and slaughtered) on the infected premises is crucial.

Once a disease has been detected and confirmed, it is essential to: 1) activate contingency plans; 2) assess the initial outbreak (e.g. size, geographical spread, epidemiology) to judge what control measures may be required; 3) implement the control measures as quickly and completely as possible; 4) monitor progress and adjust policies accordingly; 5) continue to exchange information and data with neighbouring administrations; and 6) communicate with the public and all stakeholders, including the OIE (GEMP, 2011).

The policy used to control and eradicate a disease will be greatly influenced, at least initially, by how widespread and severe the initial incursion was before it was detected. The wider the spread of the disease and the more locations affected, the less likely it will be that culling

BOX 7

Plans and documents required in any comprehensive risk-mitigation and response system

- An **emergency preparedness** plan outlines what a government needs to do before an outbreak. This also includes things that all stakeholders need to do, and the preparation of a contingency plan.
- A **contingency plan** details what a government will do in the event of a disease incursion, beginning from the point when a suspect case is reported. This also includes things that all stakeholders need to do.
- An **operations manual** is a comprehensive set of instructions (also called standard operating procedures [SOPs]) that tells field staff and others how to undertake specific tasks required by the contingency plan.
- A **recovery plan** is the blueprint for the safe recovery or restoration of normal activities, although possibly with procedures and practices modified in the light of experience gained during the outbreak.

as a main eradication tool will be effective. Culling is most effective when it can be carried out within the first few days of a location being affected. This requires that the disease is spotted fast and that once detected, affected animals can be culled quickly with compensation. If this cannot be done, it is likely that movement controls and other actions will be needed. It is therefore vitally important to establish the geographical spread and number of affected locations early in the outbreak, i.e. surveillance. Almost invariably, the index case (the first case found) is not actually the primary, or first-occurring case (GEMP, 2011).

Just as important as the first actions is the end phase, when the clinical disease has apparently disappeared. If undetected pockets of infection remain, many of the benefits gained from the eradication campaign may eventually be lost. A common mistake is to divert resources or discontinue surveillance and control efforts, since the clinical disease has seemingly disappeared and the socio-economic losses are over. But if surveillance is abandoned prematurely, ASF is likely to flare up again.

Emergency planning (GEMP, 2011)

Emergency preparation is key in the effective control of disease emergencies. However, it should take place during the prevention phase, i.e. in "peacetime". It is essential to agree in advance and have a clear understanding of who will be responsible for what activities, and to establish a single chain of command and line of communication. These channels and responsibilities are often organized differently from peacetime. A key benefit of planning is that it prompts a wide range of people who are likely to become involved to think carefully about what challenges may arise. This enables some gaps or deficiencies to be addressed before an outbreak.

Emergency planning is greatly enhanced by farmer participation. Farming communities are more likely to cooperate in a disease emergency if they see that quick, decisive action

is being taken and that this will ultimately benefit them. They also need to know that their contributions and inputs are considered during planning and review.

These plans and instructions are living documents that should be reviewed at regular, planned intervals and updated to reflect any changes since the last revision (at least every five years).

Responders need to be regularly trained in disease recognition, reporting and response procedures, outbreak investigations and analyses, etc. Regular desktop and field simulation exercises involving all stakeholders help to practice the implementation of contingency plans and operations manuals. This type of regular training and practice is key in maintaining a real ability to implement control measures as well as in spotting gaps in the current system.

Legal framework (GEMP, 2011)

To take rapid disease-control actions, adequate legal powers must be in place. This includes powers to enter a farm (for disease surveillance, prevention and control purposes), to cull and destroy infected and in-contact animals, to establish quarantines and movement controls, to proclaim infected and disease-control zones, to provide compensation, etc.

Establishing legal powers takes time so they must be in place before any outbreak occurs. As it is not possible to devise a set of regulations for each disease, there should be a general set of legal powers and regulations linked to a list of notifiable or prescribed diseases.

Sometimes, it may also be necessary to enlist the assistance of the police and armed forces in law enforcement, e.g. in policing livestock movement restrictions, and quarantining and protecting personnel involved in response activities.

For countries operating under a federal system, there should be harmonization and consistency of legislation throughout the country. The same applies between countries in regions with unrestricted exchange (i.e. free-trade pacts) of livestock and animal products, e.g. the Economic Community of West African States (ECOWAS), the Southern Africa Development Community (SADC), the Common Market for Eastern and Southern Africa (COMESA), the East African Community (EAC), the Eurasian Economic Union (EEU) or the European Union (EU).

Financing (GEMP, 2011)

Experience has shown that delay in obtaining finance is one of the major constraints to rapid response in emergency disease outbreaks. The immediate application of even modest funds will very likely save major expenditure later. Forward financial planning is therefore an essential component of preparedness. The finance plan should cover both ongoing costs (e.g. surveillance, risk analysis) and costs that are likely to arise during an emergency (e.g. control). The latter costs will be reflected in the associated contingency plan.

The funds may cover the cost of the whole eradication campaign. More typically, they will cover the initial phases of the campaign, pending a review of the outbreak and the control programme and of the funds required to finalize eradication. In some countries, it may be desirable for funds to be provided from both the government and the private sector for emergency programmes against some diseases (i.e. cost-sharing arrangements).

BOX 8

Basic principles of emergency outbreak communication

Adapted from WHO's *Outbreak Communication* (2005) and the Centers for Disease Control and Prevention's *Crisis Emergency Risk Communication* (2014).

- TRUST is the goal – each communication builds or erodes trust.
- TRANSPARENCY is the tool – tell stakeholders everything you can, proactively and voluntarily.
- Announce EARLY – even with incomplete information, to control rumours and establish leadership; provide frequent updates.
- LISTEN to the public and respond – build messages to show you are listening to the public's concerns, even when those concerns seem unreasonable.
- PLAN your communication for the extreme demands of an outbreak.

Communication

An important aspect of disease control is communication with stakeholders at all levels, from producers to the general public. It is best to agree on who will give interviews and restrict media communications to those designated and trained.

Movement control

The spread of ASF mostly occurs as a result of human activity rather than through wild boar movements or other vectors. Disease spread due to the movement of live animals and animal products can be controlled by adequately enforced movement restrictions, which need to be well-supported by legislation. It is best if the owners of the animals or animal products understand the need for restricting movement, and that complying with such requirements is in their own interests.

It is, unfortunately, relatively common for pig farmers to sell animals for slaughter or to market their meat as soon as disease is suspected. The marketing of sick animals and infected meat is a serious risk. Incubating or excreting, sick pigs can disseminate ASF, particularly when sold at live-animal markets.

At the farm level, following an outbreak or suspected case strict quarantine should be imposed as soon as possible, i.e. no pigs, pork or potentially infected materials are allowed off the property. No one should leave the farm without changing (or disinfecting) their clothes and footwear. When free-roaming, pigs should be immediately rounded up and enclosed.

In the area around the outbreak (the control zone), authorities must prevent any illegal trade of dead or sick animals and their products. The exact borders of these control zones do not need to be circular, but should take into account and use natural barriers and administrative borders as well as any relevant information. The borders of these zones must be clearly marked by road signs.

Variable restricted-movement areas and periods can be established to prevent disease spread. Such limitations will be most effective when they have minimal impact on the

FIGURE 40
Roadblocks and signs limiting access to outbreak area and protection zone in Lithuania

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animal owners. It is recommended that: 1) all animal holdings be registered and a census of all animals conducted; 2) all susceptible animals on those holdings undergo veterinary inspection periodically; and 3) susceptible animals (or their products) not be moved from their holdings, unless to emergency slaughter under official supervision.

Inspecting animals and setting up checkpoints are important parts of the process of implementing movement controls. However, checkpoints on major roads may cause unacceptable disruption or be too expensive to maintain. Also, pigs can be smuggled outside restricted areas by hiding them in vehicles or by using unguarded minor roads (GEMP, 2011).

Stamping out and disposal

Actively infected and excreting animals are the greatest source of ASFV. Such animals may also lead to indirect infection by contaminating inanimate objects (i.e. fomites), including vehicles, clothing and, in particular, people's footwear. Replication of ASFV effectively ceases when the animal is killed. Still, the carcasses may remain contaminated for a long period after death, hence the need for prompt and effective disposal (GEMP, 2011).

Stamping out consists in the culling of infected animals, plus, usually, all other susceptible animals in the holding, and sometimes neighbouring premises or dangerous contacts, i.e. those connected through movements of animals, people or vehicles. There is rarely, if ever, a place for wide-range culling such as ring culls based purely on geographical location. The slaughter of animals must be conducted on-site and humanely, with animal welfare in mind. Slaughter capabilities may easily become overwhelmed, so careful planning of resources, equipment and personnel is key. This is particularly true when killing large, commercial pig herds.

After stamping out is completed, carcasses must be disposed of on-site if possible in a safe manner, i.e. they should be burnt, composted, rendered or buried, to prevent carcasses being consumed, and to avoid feral pigs, wild boar and other scavengers (including humans) accessing them. The disposal of large numbers of pigs in a short time presents major logistic, but also environmental problems.

FIGURE 41
Stamping out and disposal operations

A. *Culling in a CO^2 chamber in Lithuania.*
B. *Disposal operations in the Russian Federation.*
C. *Disposal in Lithuania.*

A. AND C. ©STATE FOOD AND VETERINARY SERVICE, LITHUANIA/MARIUS MASIULIS; B. ©VNIIVVIM

The single most important challenge arising from stamping out is that pig owners object to having their animals killed in the absence of timely and adequate forms of compensation. Without such mechanisms, it is likely that reporting will be reduced and that the disease will spread through the illegal movement of infected animals and products. Therefore, no stamping-out campaign should be applied in the absence of a sound compensation programme.

Cleaning and disinfection

The destruction of carcasses should be followed by the thorough cleaning and disinfection of all premises, vehicles and equipment. Though disinfection with an approved product can help to eliminate the virus, ASF can survive in protein-rich settings for long periods of time and across a wide variety of environments. Organic matter should be removed from sheds, equipment, vehicles and any other surface that was in contact with infected materials. Vehicles (particularly the underside, the bed if carrying live pigs and the cab) and personnel (shoes, equipment, etc.) should be disinfected after cleaning on entering and leaving farms.

Proven effective disinfectants include detergents, hypochlorites and glutaraldehyde. The ASFV is susceptible to ether and chloroform. The agent is inactivated by 8/1000 sodium hydroxide (30 minutes), hypochlorites – 2.3% chlorine (30 minutes), 3/1000 formalin (30 minutes), 3% ortho-phenylphenol (30 minutes) and iodine compounds (OIE, 2013). Effective commercial products are also available. The environmental impact of these agents should be taken into account. Equipment that cannot be easily disinfected should be exposed to sunlight.

Compensation (GEMP, 2011)

A compensation policy is the cornerstone of any control policy that requires the killing of animals or the destruction of property. Compensation is key in encouraging farmers to report outbreaks early. While compensation may be thought of as being expensive, the incentive it creates for rapid reporting has a strong effect on the overall size and cost of an outbreak. It is very likely to save money overall.

Compensation can take various forms, which have been extensively debated. The exact compensation strategy to be implemented needs to be carefully evaluated, taking into account the local context and involving those affected in the discussions. Compensation can be in cash or goods, e.g. replacement animals. But irrespective of whether cash or animals are offered, pig farmers should be consulted, if possible before any outbreak takes place. The advantage of cash is that it allows livestock keepers to choose the type and numbers of animals they wish to buy, and, just as important, to control the timing. However, the disbursement of cash is open to corruption and theft.

Compensation should be paid for any animals killed as part of a compulsory culling campaign, whether they are infected or killed as dangerous contacts, or for welfare purposes as sometimes happens. In effect, the government buys the animals and then kills them. Compensation should also be paid for products and property destroyed as part of a compulsory campaign. Since one of the major roles of compensation is to encourage the early reporting of disease, it should not be paid for animals that have already died or been killed by the producer before the disease was reported and confirmed.

For compensation to be effective, it needs to be paid soon after the losses are incurred. Planning should consider how funds for compensation can be easily and quickly disbursed to those eligible.

Compensation should be based on a fair market price for the animals at the time of culling, and where possible, their full market value. However, some recommend compensation just below market value, arguing that farmers should also contribute some of the funds, e.g. 10 percent. Compensation arrangements that are inadequate or too generous can encourage forms of behaviour that are damaging to the control efforts.

The lack of adequate and timely compensation for culled animals may lead to: 1) outbreaks not being reported; 2) emergency slaughter by farmers either for their own consumption or sale; 3) hiding of animals or their movement to other premises; or 4) inappropriate carcass disposal in areas accessible to domestic, feral or wild swine. Compensation that is too generous can encourage risky behaviour in the hope that animals will become infected so that compensation will be paid.

The greatest loss incurred by producers is often the loss of production during the outbreak rather than the value of the animals killed, or even the losses due to movement restrictions (e.g. not being able to sell animals). However, these losses are not predictable because they depend on the overall duration and severity of the outbreak. Therefore, other support mechanisms (e.g. financial and social, beyond compensation) should be considered as part of the plan to assist affected farmers to recover.

Restocking

Once the disease is deemed to have been contained, the rehabilitation of the farm or region to its pre-outbreak production is the final step in ASF control. Following a massive outbreak, some owners may not wish to restock or continue animal breeding. But the majority will wish to return to their traditional way of life and will have to restock.

Before any restocking, farms must be free of the pathogen. This can be achieved through cleansing and disinfection, often carried out twice. In addition, it is advisable to improve farm biosecurity before restocking. Following cleaning and disinfection, empty

premises should not be restocked for 40 days at least, but the period will depend on the prevailing situation and should be risk-based rather than arbitrary. If sentinel pigs are introduced, which is highly recommended, animals should be monitored (clinically and serologically) to detect possible reinfections. If there is no evidence of infection after 40 days, the sentinels may be used as part of the restocking programme.

Pigs for restocking should, if possible, be bought locally or in neighbouring areas. Such animals are adapted to local conditions and they are usually those animals that farmers know best. Buying from several sources means purchasing animals that have different health and immune status. Mixing them together under stress can lead to cross-infection.

Tick control

Elimination of *Ornithodoros* ticks from infected pigsties is a challenge, particularly when involving old buildings, because of tick longevity, endurance and ability to hide in cracks that cannot be reached by acaricides. The destruction of tick habitat (e.g. covering over cracks where ticks can hide and/or building new facilities with materials that leave no cracks) helps to lower their numbers and transmission potential. Infested buildings should not be used as pigsties. They should be isolated so that pigs cannot enter them, or destroyed and rebuilt elsewhere. Farmers able to rebuild previously contaminated housing should do so. This is also the best time in which to consider other possible biosecurity upgrades.

Acaricides and other pesticides may be used on bedding or, depending on the product, applied directly to the skin of pigs.

Since blood-sucking insects can mechanically spread ASF virus within herds, insect-control programmes are advisable on infected premises.

Wildlife control

No realistic measures can be taken in the wild suid or *Ornithodoros* populations to prevent the ASF transmission between them. The only option is to implement prevention measures to protect domestic pigs from being infected. In parts of southern and east Africa where the sylvatic cycle occurs, adequate fencing or permanent housing of domestic pigs has been demonstrated to provide complete protection – for almost a century. The fencing or wall must extend below the surface for at least 0.5 metres to prevent burrowing by warthogs and the recommended height is 1.8 metres. In addition, in South Africa the area where the sylvatic cycle occurs is known and monitored by surveillance of *Ornithodoros* in warthog burrows around the perimeters.

If ASF becomes established in wild boar (or feral pig) populations, effective control is much more challenging. The strategy is to minimize contact between wild boar and domestic pigs through fencing of piggeries, limiting the numbers of free-ranging or feral pigs, and ensuring the proper disposal of kitchen and slaughtering waste. There is much controversy about how to best control ASF in wild boar. The removal of wild boar carcasses during epidemics followed by the decontamination of the site, although very resource-consuming, has been widely and successfully used in Eastern Europe. Increasing hunting pressure may be counterproductive, since it may push wild boar to escape to other areas. Supplementary feeding will keep wild boar within a known, well-defined area, thus limiting dispersal of the animals and the virus. However, it will also foster close contact between animals, thus

FIGURE 42
Removal and decontamination of ASF-suspected wild boar in Ignalina, Lithuania

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promoting disease transmission. Fencing of open areas to avoid the movement of wild animals is difficult and costly to implement and maintain. It disturbs wildlife movements and migrations, and its efficacy is questionable since wild suids will find their way under or over fences. The use of repellents is also problematic. Hunters and hunting clubs, as well as forestry services, are important partners in surveillance and control of ASF in wild boar.

Zoning and compartmentalization

Where the disease is present in only one part of a country, then zoning becomes an important strategy towards progressive elimination or eradication efforts, while allowing trade from free zones or compartments. For zoning to be applied, it is key for national authorities to establish infected and disease-free zones and enforce tight controls on the movement of pigs and products between zones. Compartmentalization is a different approach based on the creation of sub-populations with their supply chains under a common biosecurity management system. These sub-populations are clearly defined and separated from all sub-populations of different or potentially different status. Compartmentalization is highly suitable for commercial pig farms and enables business to continue even in an infected area. The costs and responsibility for compartments are shouldered by the producer and his/her suppliers, but monitoring and approval remain the responsibility of the competent veterinary authority.

Sources of assistance

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African swine fever (ASF) is a contagious viral disease that causes a haemorrhagic fever in pigs and wild boar, and is often associated with lethality of up to 100 percent. As a result, ASF can severely impact on the productivity of pig farming. This not only threatens food security and challenges the livelihoods of pig producers and other actors along the supply chain, but can also have major repercussions on international trade.

With an extremely high potential for transboundary spread, the disease is today considered endemic in sub-Saharan Africa, Sardinia (Italy), and parts of the Caucasus and Eastern Europe. There exists a permanent risk of further spread of ASF from these areas due to the transboundary movements of individuals, pork products, fomites, and infected wild boar. Any country with a pig sector is at risk. The backyard sector, characterized by low biosecurity, is particularly vulnerable.

In the absence of any effective vaccine or treatment, the best strategy against ASF is to set up an early detection strategy, coupled with an early response mechanism for outbreaks. In that context, the awareness and training of veterinary professionals and others in the front line will be crucial.

The purpose of this manual is to provide veterinary professionals, para-professionals, and laboratory diagnosticians with the information they need to promptly diagnose and react to an outbreak or case of ASF. Pig farmers, hunters and forest managers will also benefit from reading it.

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