SURVEILLANCE FOR AFRICAN SWINE FEVER AND CLASSICAL SWINE FEVER IN BENUE STATE, NIGERIA

BY

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A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATESTUDIES, AHMADU BELLO UNIVERSITY, ZARIA IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE DEGREE IN FOOD ANIMAL MEDICINE

DEPARTMENT OF VETERINARY MEDICINE,
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DECLARATION

I declare that the work in this dissertation entitled **"surveillance for African swine fever and classical swine fever in Benue State, Nigeria"**has been carried out by me in the Department of Veterinary Medicine, Ahmadu Bello University Zaria. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or any other institution.

AondoakaaASAMBE ____________________ ________________

Name of StudentSignature Date

CERTIFICATION

This dissertation entitled **"SURVEILLANCE FOR AFRICAN SWINE FEVER AND CLASSICAL SWINE FEVER IN BENUE STATE, NIGERIA"**by Aondoakaa ASAMBE meets the regulations governing the award of the degree of Master of Science in Food Animal Medicine of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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ABSTRACT

A serosurveillance study was conducted to detect the presence of antibodies to African swine fever virus (ASFV) and classical swine fever virus (CSFV) in pigs sampled frompiggeries and Makurdi central slaughter slab in Benue State, Nigeria. Four hundred and sixteen (416) pigs from 74 piggeries across 12 LGAs and 44 pigs at the Makurdi central slaughter slab were sampled for serum. The sera collected were analysed using Indirect Enzyme Linked Immunosorbent Assay (ELISA) test kit to test for antibodies to ASFV while competitive Enzyme Linked Immunosorbent Assay (ELISA) test kit was used to test for antibodies to CSFV. Questionnaire were distributed to 80 respondents consisting of farmers and slaughter slab workers to determine the risk factors associated with ASF and CSF infection. Of the 416 pigs from piggeries and 44 pigs sampled from the slaughter slab, 7 (1.7 %) and 6 (13.6 %) respectivelytested positive to ASFV antibodies and the detection rate was significantly associated with the pigs sampled in the different locations($p < 0.0001$),while none tested positive to CSFV antibodies in both locations. Out of the 12 LGAs sampled, Obi LGA had the highest ASFV antibody detection rate of (4.8%)and the detection rate was significantly associated with the pigs sampled in different LGAs ($p < 0.0001$).Piggeries located within 1km radius of a slaughter slab(OR=9.2, 95 % CI - 3.0 – 28.8), piggeries near refuse dump sites (OR=3.0, 95 % CI - 1.0 – 9.5)and piggeries where farm workers wear their work clothes outside of the piggery premises (OR=0.2, 95 % CI 0.1 - 0.7)were significantlyassociated ($p < 0.0001$), ($p = 0.05$)and ($p < 0.005$)with ASF infection of the sampled piggeries. Sanitary measures in piggeries were observed to be generally very poor,though respondents admitted being aware of ASF. The study concluded that antibodies to CSFV were absent in the sampled pigs in piggeries and at the Makurdi slaughter slab in Benue State. Also that antibodies to ASFV were detected both in sampled pigs in piggeries and at the Makurdi slaughter slab and may pose a great risk in the study area.It is therefore recommended that further studies be carried out to investigate possible sources of infection in ASFV positive pigs and also characterise the ASFV circulating in Benue State. Adherence to hygienic and proper sanitary measures in piggeries, routine surveillance and monitoring of ASFV and CSFV antibodies in pigs in Benue State to provide a comprehensive and readily accessible data base on these diseases to plan for the prevention of any fulminating outbreak were equally recommended.

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LIST OF ABBREVIATIONSAND SYMBOLS

%	Percentage
(p)	p-value
?	Question mark
<	Less than
=	Equality
>	Greater than
±	Plus or minus
≤	Less than or equal
≥	Greater than or equal
®	Trade mark
µl	Microlitres = 10^{-6}litres
Ab	Antibody
ASF	African Swine Fever
ASFV	African Swine Fever Virus
BD	Border Disease
BDV	Border Disease Virus
BNSG	Benue State Government
BVD	Bovine Viral Diarrhoea
BVDV	Bovine Viral Diarrhoea Virus
cDNA	Complementary Deoxyribonucleic Acid
CI	Confidence Interval
CSF	Classical Swine Fever

CSFV	Classical Swine Fever Virus
DIC	Disseminated Intravascular Coagulation
DIVA	Differentiate Infected from Vaccinated Animals
DNA	Deoxyribonucleic Acid
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-Linked Immunosorbent Assay
EU	European Union
FAT	Fluorescent Antibody Test
FITC	Fluorescent Isothiocyanate
FLD	Federal Livestock Department
HAD	Hemadsoption Test
HC	Hog Cholera
HRP	Horseradish Peroxidase
IFT	Immunofluorescence Test
IPT	Immunoperoxidase Test
LGA's	Local Government Areas
mAbs	Monoclonal Antibodies
min	Minutes
mls	Millilitres
nm	Nanometre
No	Number
^{0}C	Degree Centigrade
OD	Optical Density

OD_{NC}	Optical Density of Negative Control
OD_{PC}	Optical Density of Positive Control
OD_{Sample}	Optical Density of Samples
OIE	Office International des Epizootes
OR	Odd Ratio
PCR	Polymerase Chain Reaction
PRRS	Porcine Reproductive and Respiratory Syndrome
RNA	Ribonucleic Acid
rpm	Revolutions Per Minute
rRT-PCR	real-time Reverse Transcription Polymerase Chain reaction
RT-PCR	Reverse Transcription Polymerase Chain Reaction
S/N	Sample to Negative Ratio
S/P	Sample to Positive Ratio
SF	Swine Fever
Spp	Species
TCID	Tissue Culture Infectious Dose
USA	United States of America
VI	Virus Isolation
VNT	Virus Neutralization Test
X	Multiplication

CHAPTER ONE

INTRODUCTION

1.1 Background Information

African swine fever and classical swine fever are the two most feared and important contagious diseases of pigs worldwide (Penrith *et al*., 2011; Penrith *et al*., 2013) and are World Organization for Animal Health (OIE) notifiable diseases (Petrov and Salzungen, 2015). African Swine Fever (ASF) is also known as *Peste porcine Africaine, fiebre porcina Africana, maladie de Montgomery*(Penrith *et al*., 2013) while Classical Swine Fever (CSF) is known as *Hog Cholera, Peste du Porc, colera Porcina, Virusschweinepest* (Penrith *et al*., 2011; Petrov and Salzungen, 2015).

While outbreaks of ASF have a long and on-going history in Africa (Fasina *et al*.,2010), CSF up to now is considered an exotic disease within African member states with exception of Madagascar and Mauritius (Penrith *et al*., 2011;Aiki-Raji*etal*., 2014). However, very recently CSF was reported in domestic pigsslaughtered at an abattoir in Ibadan, South-western Nigeria (Aiki-Raji*etal*., 2014),thereby, making both diseases of high risks to the pig industry in Nigeria.

The two diseases exhibit similar clinical picturebut their causative agents differ greatly (Penrith, *et al*., 2013;Petrov and Salzungen, 2015). Afican swine feveris caused by African Swine Fever Virus (ASFV),a large, double-stranded DNA virus,belongingto its own genus;*Asfivirus*, the only member of the family *Asfarviridae*(Dixon *et al.* 2004), while CSFis caused by Classical Swine Fever Virus (CSFV),a small enveloped positive single-stranded RNA virus of the genus *Pestivirus,* family *Flaviviridae* (Petrov and Salzungen, 2015). Both viruses affect only members of the pig family *Suidae*

(Plowright *etal*., 1994; Moennig, 2000) and are highly contagious to pigs and their ancestor, the Eurasian wild boar (*Sus scrofa ferus*).African swine fevervirus is the only known DNA arbovirus that has a biological vector that can maintain and transmit the virus (Dixon *et al.* 2005).

The pig population in Nigeria is estimated at over 7.4 million and is concentrated mainly in the Middle Belt and Southern parts of the country (FAO, 1998b; NBS, 2010). Small holder farms raise most of these pigs(El-Hicheri, 1998; NBS, 2010 Cited by FLD, 2010).This important pig population contributes highly to the food security of the low income rural and periurban population (Ate and Oyedipe, 2011;El-Hicheri, 1998) thereby contributing significantly to the economic welfare of the rural population as well as playing an important role in the social and cultural life of most of the communities in the southern and middle-belt States of Nigeria, including providing cheap meat for traditional marriages and burial rites (Ate and Oyedipe, 2011). In addition, pigs provide a ready and regular source of cash to meet the rural families' needs, such as paying educational and health expenses; and procurement of farm inputs on a day to day basis (El-Hicheri, 1998; Ate and Oyedipe, 2011).

The pig industry in Nigeria can be classified into small holder farms(having fewer than 50 pigs in the herd at any point in time); medium holder farms (with 50 to 100 pigs in the herd at any point in time); and large holder farms (of over 100 pigs in the herd at any point in time) (Awosanya *etal.,* 2015). The pig farming industry in Nigeria has a significant presence in the north-centraland south-western Nigeria. The pig production system in Benue state Nigeria is predominantly semi-intensive (El-Hicheri, 1998; FAO, 1998a).

The ASF scourge has however adversely affected the bustling activities in the industry since the outbreak of African swine fever in Nigeria in 1997 (Babalobi*etal.*, 2007). The various state governments of the affected states have made efforts through farm extension services in educating the farmers on biosecurity measures following that outbreak (Awosanya *etal.*, 2015).

Globally, ASFV is present in Africa, Italy (Sardinia), Georgia, Latvia, Poland, Ukraine, Russia (Moscow) and some Caribbean countries, with an increasing risk of spreading to ASF-free countries in Europe and America (Sánchez-Vizcaíno*etal*., 2013; OIE, 2015). African swine fever is enzootic in Nigeria (Olugasa, 2007; Sánchez-Vizcaíno *etal*., 2009; Fasina, *et al.*,2012).

ASFV is often introduced into pig populations by infected pigs or the meat of infected pigs fed as swill, and this is the usual modes of introduction of CSFVthereby makingthe epidemiology of ASF and CSF similar (Haresnape *etal*., 1987; Penrith *et al.* 2013). Both ASFV and CSFVtolerate a wide range of temperatures and pH and can survive for long periods in meat (Mebus *et al.* 1997; Edwards, 2000).

Classical swine feverhas remained a problem in many parts of the world with both, an economic impact on swine production and a constraining effect on trade (Elbers *et al.*, 1999; Edward *et al.*, 2000; Awosanya *etal*., 2015).

Nigeria, other West and Central African countries endemic for ASF experience intermittent infections(Fasina *etal*., 2012). ASF first made an apparently unsustained incursion into South Western Nigeria in 1973 and thenbecame extinct (Owolodun *etal*., 2010a). However, there was a resurgence of outbreaksin West Africa from 1996 onwards, with the ASF virus entering Nigeria between August and September1997; and has continuedto spread acrossNigerian pig farms, causing sporadic outbreaks with attendant mortalities (Fasina *etal*., 2012).

1.2 Statement of Research Problems

African Swine Fever (ASF)is the main threat to the pig industry in Africa and has been sporadically occurring in different parts of Nigeria,causing deaths in pig populationsresulting in heavy economic losses (Babalobi *etal*., 2007; Costard *etal*., 2009a)and mortality approaching 100 % (Costard *etal*., 2009a).

Persistence infections with both ASFV and suspected CSFV appear to recur in the core pig-producing areas ofthe country having had significant economic and social impact inNigeria since 1997(Olugasa, 2007).

The status of CSF in Nigeria remains unknown thereby raising the suspicion of its existence or coexistence with ASF (Aiki-Raji*etal*., 2014).

However, there is, in particular, dearth of information on CSF even though Benue state is one of the largest pigs producing and pork consuming states in Nigeria.

Previous and recent outbreaks of ASF in Benue Statehave affected pig production in themidst of growing importance in pig farming and have affected livelihoods of pigkeepers in Nigeria as a result of direct loss of pigs and easy source of animal protein, due to mortality (Babalobi *et al*., 2007).

The reported presence of ASFV positive pigs in neighbouring state of Taraba (Abwage *et al*., 2015) appears to add to the existing threat on swine industry in Benue state as explained by the proposed theory of geographical contiguity and supported by the incidence of infections (Fasina *et al*., 2012).

Therefore, the continuous surveillance ond monitoring of both ASFand CSF in pigs in Benue State and indeed Nigeria needs not to be overemphasized since the presence and reintroduction ofthese viruses can be devastating to the pig population.

1.3 Justification of the Research

Worldwide, the goal of pig production operation is to make money or provide some advantage such as cheap source of animal protein for the family diet. So any disease threatening pig production threatens the food security of the human population and its social balance and thus the need of the surveillance for ASF and CSF diseases.

There are enormous potentials for pig production in Benue state, but the continuous maintenance of ASF in pigs in Nigeria is amajor constraints to the growth of the pig industry. Regular surveillance and monitoring of this disease will provide comprehensive and readily accessible data base that will help to contain any fulminating outbreak.

There is lack of regular surveillance or monitoring for ASF as well as lack of documented report on CSF; hence the need for this study.

Surveillance for ASF and CSF will help to ascertain the status andincrease awareness of the diseases to facilitate early detection in order to instituteappropriate control measures during outbreaks.

Also, the results from the study would contribute to the design of the disease control strategies and policy formulation.

1.4 Aim of the Study

The aim of the studywas to determine thepresence of antibodies to African swine fever virus and classical swine fever virus in pigs in piggeriesand in slaughtered pigs at Makurdi central slaughter slab in Benue State,Nigeria.

1.5 Objectives of the Study

Thespecificobjectivesofthe study were to determine the:

i. presence of antibodies to ASFV and CSFVinpigs in piggeries and in slaughtered pigs at the Makurdi central slaughter slab in Benue State, Nigeria.
ii. coexistence of antibodies to ASFV and CSFV in pigs in piggeries and in slaughtered pigs at Makurdi central slaughter slab in Benue State, Nigeria.
iii. risk factors associated with ASFV and CSFV infection in Benue State, Nigeria.

1.6 Research Question(s)

i. Does antibodies toASFV and CSFVexist inpigs in piggeries and in slaughtered pigs at Makurdi central slaughter slab in Benue State?
ii. What is the rate of antibodies to ASFV and CSFV coexistence in pigs in piggeries and in slaughtered pigs at Makurdi central slaughter slab in Benue State?
iii. What are the risk factors associated with ASFV and CSFV infection in pigs in Benue State?

CHAPTER TWO

LITERATURE REVIEW

2.1 African Swine Fever

2.1.1 Historical and current distribution of African swine fever

African swine feverwas first observed in settlers' pigs in Kenya in 1909 and was reported by Montgomery (1921) as an entity distinct from CSF. Reports of ASF in South Africa (Steyn, 1928, 1932) and Angola (Gago da Câmara, 1933) followed. While Gago da Câmara was able to determine that the disease observed in pigs was not *erysipelas*, as previously thought, but was caused by a virus, its identity as the disease described by Montgomery in East Africa was only confirmed in 1943 (Mendes, 1994).

In Kenya and South Africa, ASF affected pigs that belonged to settlers of European origin, but in Angola, pigs were also kept by the indigenous population and it was noted that those pigs showed increased resistance to ASF and that they appeared to serve as a source of infection for the pigs farmed by the Portuguese settlers (Mendes, 1994; Penrith *et al*., 2004a). Kenya confirmed an outbreak in 1994 (Penrith *et al*., 2004a) after more than 30 years of an outbreak from the disease (Magadla, 2015). ASF is endemic in domestic pigs in Malawi and Mozambique (Penrith *et al*., 2013). The disease has been persistent in the continent over the years, reaching the Indian Ocean Island of Madagascar in 1998 and Mauritius in 2007 (Rowlands *et al*., 2008; Costard *et al*., 2009b).

After World War II thepotential for producing pigs in the central highlands of Angola to provide meat for a burgeoning pork processing industry was recognised(Mendes, 1994).

Incursions of ASF into Europe focused attention on the disease. There were two separate outbreakes in Portugal in 1957 and again in 1960 (Wilkinson, 1989), with subsequent establishment of ASF in the Iberian Peninsula and spread to other countries in Europe as well as the Caribbean and Brazil. During the same period ASF was also reported from Malawi (Matson, 1960; Penrith *et al*., 2013) and Mozambique (Abreu *et al*., 1962; Mendes, 1971; Penrith *et al*., 2013). A link between warthogs and ASF was recognised early in East Africa (Montgomery, 1921), but the possible involvement of an arthropod vector was only confirmed much later (Plowright *et al*., 1969b), after Sánchez Botija (1963) reported the involvement of the argasid tick *Ornithodoros erraticus* in the epidemiology of ASF in Spain (Arias and Sanchez-Vizcaino, 2002).

Investigations in a number of countries in southern and eastern Africa revealed infection in *Ornithodoros moubata*ticks inhabiting both warthog burrows and pig shelters (Penrith *et al*., 2004a). By the mid-1970s, ASF had been reported either in warthogs or domestic pigs or both from most countries in southern and eastern Africa (Penrith *et al*., 2004a).

Other African countries where ASF was reported included: Malawi (Haresnape *etal*., 1985, 1987, 1988; Haresnape and Mamu, 1986;Haresnape and Wilkinson, 1989), Mali(Scott, 1965), Senegal in 1959 (Plowright *et al*., 1994; Bastos *et al*., 2003;Etter *et al*., 2011), Cameroon in 1982 and 1985 (Nana-Nukechap and Gibbs, 1985; Ekue and Wilkinson, 1990; Awa *et al*., 1999), Cote d'Ivoire which spread to Benin, Togo, Nigeria, Ghana and Burkina Faso between 1997 and 2003 (Penrith *et al*., 2013) and island of Madagascar in 1998 respectively (Roger *et al*., 2001; Costard *et al*., 2009a).

The only recentlyinfected African countries from which ASF has been eradicated, or apparently eradicated, are Ivory Coast (El Hicheri *et al*., 1998b) and the small island nations of São Tomé e Principe, which suffered an outbreak in 1979 (Lage and Oliveira da Silva, 1980, quoted by Wesley and Tuthill, 1984), and Mauritius, which suffered an outbreak in 2007 that was eradicated by 2008 (Lubisi *et al*., 2009).

Although Ghana succeeded in eradicating ASF within a year after its introduction in 1999, it has since 2002 suffered repeated outbreaks either resulting from incursions from its endemically infected neighbours or because the disease has become endemic. In the period 2009–2011, 26 countries have reported outbreaks or the endemic presence of ASF in domestic pigs(Penrith *et al*., 2004a).

Additionally, the sylvatic cycle in warthogs remains present in Botswana and Zimbabwe, but no outbreaks in domestic pigs have been reported. The number of countries reporting ASF outbreaks has grown markedly since 1990, mainly as a result of new countries becoming infected (Penrith *et al*., 2004b). ASF is thus widespread in sub-Saharan Africa and has become endemic in domestic pigs in countries where the sylvatic cycle occurs and in a number of countries that were newly infected in the last two decades (Penrith *et al.,* 2013).

2.1.2 African swine feverin Nigeria

The first unconfirmed outbreak of ASF in Nigeria was in 1973 at a commercial piggery in Ogun State Southwest Nigeria with 100% mortality whereas the first confirmed outbreak was reported by the Food and Agricultural Organization in September 1997 (El-Hicheri, 1998; FAO, 1998d; Babalobi *etal*., 2003, 2007).

The appearance of ASF in the neighbouring Republic of Benin was an important threat for Nigeria and is possible that the infection reported was brought in from Benin (El-Hicheri *et al.*,1998; FAO, 1998d) and the virus was subsequently isolated and the strain involved in the outbreak identified (Odemuyiwa *etal*., 2000).

A trend of high mortality levels were recorded throughout the country between September 1997 and October 1998. The mortality rates were between 85% and 100% with total mortality estimatedat 125, 000 across nine 9 states of the Federation valued at over $15 million (ProMED, 1998).

2.1.3 Aetiology of African swine fever

African swine fever virusis an icosahedral double stranded deoxyribonucleic acid*arbovirus* belonging to the genus *Asfivirus* of the family *Asfaviridae*.It is currently the only member of the family (Dixon *etal*., 2000 cited by Magadla, 2015).

The ASFV affects members of the *suidae* family (Penrith, 2013) and is the only known arthropod borne virus with a single molecule of linear double stranded DNA genome (Kleiboeker and Scoles, 2001; Penrith *etal*., 2004a). The DNA genome is between 170 and 190 kbp in size depending on the isolate (Dixon *etal*., 2004).Twenty-two different genotypes have been described, and virulence differs greatly from one isolate to another (Boshoff *et al*., 2007; OIE. 2009).

2.1.4 Survival of African swine fever Virus

African Swine Fever Virusis a resistant virus and can survive for long periods in a protein environment. ASFV remains infectious for months in blood when frozen, stored at 4°C and also highly stable at room and lower temperatures, where it can survive for 18 months (EFSA, 2010a). ASFV remains viable for long periods in faeces and tissues, including uncooked or undercooked pork products (McKercher *etal*. 1978; Mebus *etal*. 1997; Penrith and Vosloo, 2012).

The virus is destabilized by heat treatment at 60 degrees Celsius (^{o}C) for 20-30 minutes (Plowright and Parker, 1967; McKercher *etal*. 1978; Mebus *etal*. 1997). It is inactivated by many solvents that disrupt the viral envelope and by disinfectants (1% formaldehyde in 6 days, 2% NaOH in 1 day) and paraphenylphenolic disinfectants are very effective at inactivating the virus. The pH range in which the virus can survive is wide, with some infective virus remaining at pH4 or pH13 (EFSA, 2010b).The virus has been recovered in chilled and processed meat products up to six months after processing (Magadla, 2015).

2.1.5 Risk factors of African swine fever

The most serious constraints to pig production identified in many countries are lack of suitable feed at affordable prices, poor or no access to animal health services, cost of housing pigs, poor genetics of breeding stock and lack of knowledge and information about pig production and health (Adesehinwa *etal*., 2003; Mashatise *etal*., 2005; Halimani *etal*., 2007; Ironkwe and Amefule, 2008; Kagira *etal*., 2010; Karimuribo *etal*., 2011; Moreki and Mphinyane, 2011;Mutua *etal*., 2011; Nwanta *etal*., 2011; Petrus *etal*., 2011; ; Muhanguzi *etal*., 2012; FAO, 2012a,b).

High feed prices and low quality of available feed have been identified as a major constraint for smallhscale and commercial farmers in many African countries (Verhulst, 1993; Garcês *etal*., 1998; Chabo *etal*., 2000; Lekule and Kyvsgaard, 2003; Karimuribo *etal*., 2011; Moreki and Mphinyane, 2011).

Lack of resources to feed pigs is a major contributory factor to the maintenance of extensive pig-farming systems (Ngowi *etal*., 2007; Ndébi *etal*., 2009; Matos *etal*., 2011; Petrus *etal*., 2011; FAO, 2012b; Muhanguzi *etal*., 2012). Even in semi-intensive systems in Uganda where pigs are in theory permanently housed, they may be liberated for shorter to longer periods during the day to forage for themselves (Costard *etal*., 2009a; Mutua *etal*., 2011; Nissen *etal*., 2011; Muhanguzi *etal*., 2012).

Pig shelters with earth floors and with the type of walls that provide crevices in which *Ornithodoros*can hide occur throughout sub-Saharan Africa (Magadla, 2015). Because the ticks spend most of their time off the pigs and away from exposed surfaces in dark places, acaricides have proven to be of little value in controlling them, and it is necessary to burn the structures in order to get rid of the ticks (Penrith *etal*., 2004a).

Lack of resources to construct proper housing for pigs is a frequently stated constraint for producers, and therefore pig housing that offers shelter to *Ornithodoros* is likely to be a limiting factor for managing ASF in some areas, though no evidence of the presence of this ticks in Nigeria. When pigs are permanently confined, they are often at risk of ASF and other infectious diseases owing to poor or no biosecurity and poor hygiene (Penrith *et al*., 2013).

Lack of affordable feed results in housed pigs being fed household waste mostly kitchen wastefrom restaurants and other sources (Missohou *etal*., 2001; Costard *etal*., 2009a). This in turn poses a risk as ASF may be spread via food products containing contaminated meat (Penrith *etal*., 2004a; Costard *etal*., 2009a; Penrith and Vosloo, 2009).

The main risk factors for ASF associated with pig production systems are therefore increased exposure to infection in free-ranging husbandry systems and lack of basic biosecurity in semi-intensive and intensive husbandry systems, which may include feeding of kitchen waste that could include uncooked or undercooked pork (Penrith *et al*., 2013).

Housing pigs in structures that offer a suitable habitat in areas where a transmission cycle between pigs and tampans exists or where species of *Ornithodoros* are associated with pigs is an additional risk factor (Penrith *et al*., 2013).

2.1.6 African swine fever virushosts

Phacochoroerus africanus (common warthog), is the species of warthog mostly associated with the soft tick vector and ASF (Jori and Bastos, 2009). They are asymptomatic carriers of ASFV (Costard *et al.,* 2013) with minimal or no shedding of virus by seropositive warthogs (Kleiboeker and Scoles, 2001) and could as such act as reservoir of the disease (Magadla, 2015).

Common warthogs in the South African context, *Ornithodorosmoubata* (tampans)(Anderson *et al.,* 1998) are naturally adapted to open woodland savannah (Randi *et al.,* 2002; Muwanika *et al.,* 2007), where the virus is maintained and transmitted between wild and domestic suids *(Sus scrofa domestica)* by soft-shelled ticks of the *Ornithodoros* genus (Pini and Hurter, 1975). Common warthogs mainly inhabit burrows, which are excavated by aardvark (*Orycteropus afer*), for purposes of shelter, farrowing and rearing of their young (White and Cameron, 2009). However, in Nigeria only the domestic cycle which maintains the ASF virus within domestic pigs is most recognized and reported(Sánchez-Vizcaíno and Arias, 2012) despite reports on detection of ASFV in river hogs (Luther *etal*., 2007).

Bush pigs, although widely distributed are believed to be of less importance than warthog in the epidemiology of ASF (Costard *et al*., 2013; Jori *et al*., 2013). Infected bush pigs do not usually exhibit clinical signs of the disease and are able to transmit the disease directly by contact to domestic pigs (Costard *etal*., 2009a; Sánchez-Vizcaíno *etal*., 2012),while warthogs transmission into the domestic cycle occurs only via soft ticks (Bastos *et al.*, 2009).

The giant forest hogs, whose role in the epidemiology of ASF is regarded as negligible (Costard *et al*., 2013), are unlikely to play a significant role in the transmission or dissemination of ASFV (Jori and Bastos, 2009).

Infected African wild pigs, especially warthogs are normally in-apparent carriers of the virus and act as reservoir hosts. European wild boars and feral pigs, both of the species *Susscrofa*, are fully susceptible to ASF and show a similar clinical syndrome to domestic pigs (OIE, 2008; Arnot *etal*., 2009). Although wild boars are fully susceptible, Jori and Bastos (2009) suggested and it was confirmed in a study by Mur *etal*., (2012a) that wild boars cannot maintain the virus for long periods of time in the absence of other factors such as infected domestic pigs. Soft ticks are clearly involved in the maintenance of ASFV in Africa and have been regularly found in warthog burrows in eastern and southern Africa (Nesser *et al.,* 1994). African swine fever virus can persist and is amplified in *Onithodorosmoubata* ticks (Costard *et al.*, 2013); the virus can also cause mortality in ticks (Kleiboeker and Scoles, 2001).

2.1.6.1 Domesticpigs

When introduced into a region ofdomestic pig population, ASF typically is associated with high morbidity and mortality, and the rapid spread of outbreaks. However, in endemic regions, mortality rates have decreased and subclinical or chronic ASFV infections have become more frequent (Thomson, 1985; Allaway *etal*., 1995; Fasina *etal*., 2010; Owolodun *etal*., 2010b).

Infections with low virulence ASFV were shown to be responsible for the chronic forms observed in the Iberian Peninsula (1960–1995) and may have been the consequence of live attenuated vaccines used during the 1960s (Sanchez-Vizcaino *etal*., 2012).

Acquired immunity from previous exposure to lower doses of virus or to related viruses of reduced virulence have been proposed to explain the observed resistance to ASFV infection which may have emerged from circulation in domestic pig populations in Africa (Penrith *etal*., 2004a).

Subclinically and chronically infected or recovered pigs play an important role in the transmission of the disease by maintaining the virus. The disease persistence in endemic areas and sporadic outbreaks or introduction into disease-free zones (Wilkinson and Pensaert, 1989; Allaway *etal*., 1995; Leitao *etal*., 2001; Boinas *etal*., 2004; Sánchez-Vizcaíno and Arias, 2012) play major role in its epidemiology. Such Subclinically and chronically infected or recovered pigs were shown to remain infected for up to several weeks (Wilkinson, 1984) and transmit the disease to other susceptible pigs either via direct contacts or indirectly via tick bites or the ingestion of contaminated meat and products.

2.1.6.2 Wild suids

Warthogs are considered the original vertebrate hosts of ASF and are involve in sylvatic cycle with ticks of the *Ornithodorosmoubata* complex (Plowright *etal*., 1969b; Thomson, 1985). They are also considered the most important vertebrate reservoir for ASFV in Africa, due to their wide distribution and ecology which provides opportunities for contact with both *Ornithodoros* ticks living in burrows and domestic pigs (Plowright, 1981; Plowright *etal*., 1994; Jori and Bastos, 2009).

The role of bushpigs (*Potamochoeruslarvatus*) and red river hogs (*Potamochoerusporcus*) in the epidemiology of ASF has not yet been elucidated (Wilkinson, 1984; Haresnape *etal*., 1985; Thomson, 1985; Roger *etal*., 2001; Jori *etal*., 2007; Luther *etal*., 2007; Jori and Bastos, 2009).ASFV replication was demonstrated in bushpigs, and included transmission to pigs and soft ticks (Anderson *etal*., 1998; Oura *etal*., 1998).

Bushpigs are found in East, Central, Southern Africa and Madagascar (Roger *etal*., 2001; Jori *etal*., 2007; Jori and Bastos, 2009; Ravaomanana *etal*., 2011) but they are not considered important reservoirs for ASF, maybe because their nocturnal habits, low densities of population and non-use of burrows limit interactions with domestic pigs and soft ticks(Ravaomanana *etal*., 2011).

Other wild suids (e.g. giant forest hog: *Hylochoerusmeinertzhageni*) have been reported to be infected with ASFV occasionally (Heuschele and Coggins, 1965a; Thomson, 1985), but their involvement in the epidemiology of the disease is considered negligible (Jori and Bastos, 2009).

In Europe, wild boar and feral pigs have been shown to have the same susceptibility to ASFV as domestic pigs (McVicar *etal*., 1981; Jori and Bastos, 2009). Evidence of infection was reported in the Iberian Peninsula (Wilkinson, 1984; Caiado *etal*., 1988; Perez *etal*., 1998), Sardinia (Laddomada *etal*., 1994), Cuba (Simeon-Negrin and Frias-Lepoureau, 2002), Mauritius (Lubisi *etal*., 2009) and Russia (Beltran Alcrudo *etal*., 2009).

2.1.6.3 Soft ticks

Onithodoros erraticus was first identified in Spain as a biological vector and reservoir for ASFV (Sanchez-Botija, 1963), which led to the discovery that ticks from*Onithodoros moubata* complex play a role in the epidemiology of the disease in Africa (Plowright *etal*., 1969a).

In Africa, *O. moubata* ticks are a source of infection with ASFV for both domestic and wild pigs. Transmission occurs during blood meals (Plowright *etal*., 1969b; Plowright, 1981) and infected ticks are able to retain the virus for long periods and transmit it to susceptible hosts. In addition, trans-stadial, trans-ovarial and sexual transmission that occurs in *O. moubata ticks* (Plowright *etal*., 1970, 1974; Wardley *etal*., 1983; Hess *et al*., 1989;Rennie *etal*., 2001) allows for the persistence of ASFV infection in the absence of viraemic hosts. However, ASFV infection can also cause mortality in ticks (Kleiboeker and Scoles, 2001), and the persistence of infection depends on the initial infection titre and thus the level of viraemia in infected pigs (Plowright, 1981; Haresnape *etal*., 1988).

Onithodorosmoubata ticks are widely distributed in Southern Africa and are also present in Madagascar (Uilenberg, 1963; Plowright *etal*., 1994; Roger *etal*., 2001), but there is limited evidence of their presence and distribution in Central Africa (Hoogstraal *etal*., 1954; Ekue and Wilkinson, 1990; De Glanville *etal*., 2010).

2.1.7 Transmission

African swine fever is transmitted by direct contact with infected animals, bite by the infected ticks (*Ornithodoros spp.*) and consumption of the infected pig meat or indirect by contact with fomite or substances such as clothing, furniture, bedding, footwear or feed contaminated by virus from urine, blood, faeces or saliva of infected pigs (Penrith and Vosloo, 2009).

There are some deviations concerning the role of the chronic carrier animals. Penrith and Vosloo (2009) argue that there is no evidence that recovered pigs can become long-term virus carriers, while Sánchez-Vizcaíno *et al*. (2012) signify that carriers demonstrated in both wild and domestic animals play a significant role for persistence and spread of the disease in endemic areas.

A review article done by Costard *et al.* (2009d) showed that pigs may remain persistently infected for 6 months and during this time act as a source of transmission to susceptible pigs. A study conducted by de Carvalho*et al.* (2012) showed that persistently infected animal can be viraemic and shed virus from oropharynx for at least 70 days post infection (Stegeman and Loeffen 2012).

2.1.8 Modes of infection

Four modes of infection with ASFV are recognized in suids: contact between sick and healthy animals, ingestion of infected meat, tick bites or bites from other vectorsand fomites (Costard*etal*., 2009; OIE, 2009)

Wild boars, domestic pigs and feral pigs can infect each other by direct contact, in particular when blood is present (Jori and Bastos, 2009). In contrast, field and experimental data indicate that direct contact is an unlikely means of transmission both amongst African wild suid species, and between them and pigs(Heuschele and Coggins, 1969; Anderson*etal*., 1998;Costard *et al.*, 2009; Costard*etal*., 2013;Jori *etal*., 2013). There is no reliable evidence of the transmission of virus from sows to foetuses during pregnancy (Penrith *et al*., 2004b). Whilst sexual transmission of this virus has not been documented in pigs, ASF virus is shed in genital secretions (Penrith and Vosloo, 2012).

All susceptible suids, regardless of their species or age, can be infected (Thomson*etal*., 1980; Jori and Bastos, 2009; Penrith and Vosloo, 2012; Costard *etal*., 2013). Under experimental conditions, tissues of African wild suids can contain sufficient virus particles to infect pigs by ingestion (Thomson*etal*., 1980), however under natural conditions the levels of virus in warthog tissues are likely to be too low to induce infections (Sánchez-Vizcaínoa*et al*., 2009; Penrith*et al*., 2004b).

Virus transmission by ticks of the genus *Ornithodoros* has been demonstrated in all suids (Anderson*et al*., 1998; Jori and Bastos, 2009; Costard *etal*., 2013). In contrast, there is no evidence at all for transmission of ASFV via hard ticks (family *Ixodidae*) (EFSA, 2010).

Some *Ornithodorosspecies* may have a life span of 15-20 years. At certain life stages they are able to survive 5-6 years without feeding, and to maintain and transmit the virus to pigs for at least two years (Oleaga-Pérez *etal*., 1990; Astigarraga *etal*., 1995;Boinas *etal*. 2011).

ASFV infection of naïve *Ornithodoros* ticks during blood meals depends on the host species involved. Naïve ticks can be readily infected when feeding on viraemic domestic or feral pigs, wild boars and bush pigs (Anderson*etal*., 1998;Oura *etal*., 1998); however, when feeding on warthogs, ticks only become infected after feeding on young warthogs during the viraemic phase.

Indirect contact through fomites may play a role in ASFV transmission. These modes of transmission seem only to be efficient when a high virus load is involved. Infectious blood is the main matrix by which the virus is indirectly transmitted (EFSA, 2010).

Airborne infections are unlikely. They may act only over short distances and, experimentally, the half-life of ASFV in the air was on average less than 20 minutes (Wilkinson, 1977; de Carvalho *et al.*, 2013a).

Other potential albeit to date unproven and therefore unlikely sources of ASFV include water (virus is rapidly diluted), and mechanical vectors such as rodents and birds (Penrith *etal*., 2004).

2.1.9 Incubation period

The incubation period varies from 5-15 days (OIE, 2009), with clinical signs ranging from peracute to subclinical or inapparent. It varies between 4-6 days [6-8 days in subacute cases] (Sánchez-Botija, 1982).

Experimentally, fever is a valid marker for onset and the duration of infectiousness was 1 to 7 weeks (de Carvalho*et al*., 2012; de Carvalho*et al.,* 2013b). Depending on the virulence of the viral strain and the response of the pig to the virus, some animals may survive infection (Perez *et al*., 1998).

Pigs that recover may shed the virus for up to a month after the disappearance of clinical signs (Penrith and Vosloo, 2012). Whilst some authors, claim that there is no evidence that recovered pigs can become long-term carriers of the virus (Vigário 1983, Wilkinson 1983; Penrith *et al*., 2004b), others suggest that these animals may be long-term carriers of the virus and represent a potential source of infection (Carrillo *etal*., 1994; Arias and Sanchez-Vizcaino, 2002; OIE. 2009, Sánchez-Vizcaínoa *etal*., 2009).

2.1.10 Transmission cycles

2.1.10.1 Sylvatic cycle

The sylvatic cycle have been well documented in southern and eastern Africa where it involves warthogs and ticks of the *O. moubata* complex (Plowright *etal*., 1969b, 1994; Thomson, 1985; Wilkinson and Pensaert, 1989). Young suckling warthogs are infected in burrows infested with soft ticks. A short period of viraemia follows and allows transmission of ASFV to naïve ticks during blood meals (Heuschele and Coggins,1965b, 1969; Thomson *etal*., 1980; Thomson, 1985).

Warthogs remain asymptomatically infected for life, but due to the absence of horizontal and vertical transmission between warthogs, the maintenance of infection is dependent on *O. moubata* ticks (Penrith *etal*., 2004b; Jori and Bastos, 2009). Tick colonies can maintain ASFV infection for up to 15 months in the absence of blood meals (Plowright *etal*., 1970, 1974; Hess *etal*., 1989), and allow subsequent transmission cycles with warthogs at the next farrowing season.

In areas with both warthogs and soft ticks, infection rates of the wild suids can be very high (Plowright *etal*., 1994), but theinfestation rates of warthog burrows and ASF infection rates of ticks can vary considerably (Plowright *etal*., 1969a,b;Bastos *etal*., 2009). Also, as indicated in the review by Jori and Bastos (2009), the presence of both warthogs and ticks in a region does not necessarily imply the existence of a sylvatic cycle. For example, sylvatic cycle have not been reported in West Africa, and warthogs or soft ticks have rarely been found infected (Taylor *etal*., 1977; Penrith *etal*., 2004b; Jori *etal*., 2007; Vial *etal*., 2007; Jori and Bastos, 2009).

2.1.10.2 Tick-pig cycle

Ornithodorosspecies ticks have frequently been found infesting pig pens in Africa and the Iberian Peninsula (Haresnape and Mamu, 1986;Haresnape *etal*., 1988; Wilkinson *etal*., 1988; Caiado *etal*., 1988; Haresnape and Wilkinson, 1989; Oleaga-Perez *etal*., 1990) and suspected to be responsible for the maintainance of the virus.

In some areas of Spain, the occurrence of ASF outbreaks was significantly associated with the presence of *O. erraticus* (Perez-Sanchez *etal*., 1994). In Portugal, ASF is thought to have re-emerged in 1999 on a previously infected farm due to the continued presence of *O. erraticus* ticks (Sanchez-Vizcaino *etal*., 2009; Boinas *etal*., 2011).

2.1.10.3 Domestic pig to domestic pig cycle

Once introduced into domestic pig populations, ASFV is transmitted through direct contact and infected swine meat (Plowright *etal*., 1994; Arias *etal*., 2002; Sánchez-Vizcaíno and Arias, 2012).

Pig trade and or movement and lack of biosecurity practices highly contribute to the local spread of ASF in endemic areas. All excretions and secretions of infected pigs can contain the virus, and ASFV may remain viable in blood and tissues for long periods. Transmission via direct contact can thus occur for several weeks (Wilkinson and Pensaert, 1989).

Due to the lengthy persistence of ASFV in tissues such as muscles, fat and bone marrow, pork products such as cured ham can remain infectious for several months (McKercher *etal*., 1987; Mebus *etal*., 1993, 1997; Farez and Morley, 1997). The access of pigs to poorly disposed infected carcasses, frozen, insufficiently cooked or cured pork products thus presents a risk of infection. ASFV can also persist in the environment for several days (Plowright *etal*., 1994; Sanchez-Vizcaino *etal*., 2009).

Factors found to increase the risk of outbreaks include: free-ranging (Allaway *etal*., 1995; Edelsten and Chinombo, 1995; Mannelli *etal*., 1997), the previous occurrence of the disease on the farm (Randriamparany *etal*., 2005), the presence of an infected pig farm in the neighbourhood or of an abattoir in the community, and visits by (para)veterinarians in Nigeria (Fasina *etal*., 2012).

Density of the road network, water bodies and of the domestic swine population are associated with outbreaks in Russia (Gulenkin *etal*., 2011), while Olugasa and Ijagbone (2007) found the movement of infected animals to be the most important factor for ASF spread.

The effect of emergency sale of pigs without detected signs in case of a suspected outbreak [a practice reported by small scale farmers in affected areas] (Randriamparany *etal*., 2005; Babalobi *etal*., 2007; Costard *etal*., 2009b; Fasina *etal*., 2010) contributes to the spread of the disease in domestic pig populations (Costard *etal*., 2012b).

2.1.10.4 Sylvatic to domestic cycles

The mechanisms of transmission from wildlife to pigs are not fully understood but different hypotheses have been discussed (Jori and Bastos, 2009). Transmission from African wild suids to pigs through direct contact seems unlikely (Heuschele and Coggins, 1969; Plowright *etal*., 1969b, 1994; Plowright, 1981; Thomson, 1985).It has only been reported once for bushpigs, under experimental conditions (Anderson *etal*., 1998). Interbreeding between bushpigs and domestic pigs has been suspected and could constitute an opportunity for ASF transmission (Roger *etal*., 2001; Jori and Bastos, 2009).

Opinions on the possibility of transmission through ingestion of infected meat differ. While some authors (Plowright *etal*., 1969b; Thomson *etal*., 1980; Thomson, 1985) argued that virus titres in wild suid carcasses are too low to induce infection, others stated that wild suid tissues may contain sufficient virus particles to infect pigs following ingestion (Wilkinson, 1984; Anderson *etal*., 1998). Furthermore, disposal of pig carcasses can potentially infect bush pigs or warthogs which may scavenge on these. However, soft tick vectors constitute the most likely mechanism of transmission from African wild suids to domestic pigs (Thomson, 1985; Wilkinson and Pensaert, 1989). This could happen when pigs and warthogs share spaces such as grazing or drinking areas, or when ticks are brought back to human settlements (Thomson, 1985; Jori and Bastos, 2009).

2.1.10.5 Wild boar cycle

The potential role of wild boars in the epidemiology of the disease is unclear. Evidence of infection in wild boars was reported in the Iberian Peninsula (Caiado*etal*., 1988; Perez *et al*., 1998), Sardinia (Laddomada *etal*., 1994), Russia (Beltrán-Alcrudo*etal*., 2009), Iran (Rahimi*etal*., 2010), Belarus (OIE, 2014), Lithuania and Poland (OIE, 2014).

Some authors suggested the unlikely persistenceof ASFV in wild boar population in the absence of cohabitation with infected domestic free-ranging pigs (Laddomada *etal*., 1994; Perez *et al.,* 1998; Ruiz-Fons *etal*., 2008; Mur *et al*., 2012b). Indeed the occurrence of the disease in wild boars is often associated with the disease in domestic pigs (Firinu and Scarano, 1988; Perez *et al*., 1998)

Similarly, it has been observed that in Sardinia and Spain, ASFV tends to disappear from wild boar populations when there is no contact with free-ranging infected pigs (Laddomada *etal*., 1994). Therefore, wild boars are not considered to play a major role as a virus reservoir in the absence of free-ranging, infected domestic pigs or other sources of infection (Perez *et al*., 1998; Rolesu, 2007; Sánchez-Vizcaínoa*etal*., 2009).

In their natural environment wild boars are unlikely to come into contact with infected soft ticks as they do not use permanent resting sites. However, contact with infected soft ticks may exceptionally occur where home ranges overlap with those of domestic pigs from infested pig pens (Jori and Bastos, 2009).

2.1.11 Prevalence of African swine fever in Nigeria

ASF outbreaks have been reported in pig farms across Nigeria (Odemuyiwa *et al.*, 2000; Olugasa, 2007;Kperegbeyi & Amotsuka, 2010; Owoludun *et al.*, 2010a,b,c; Saka *et al.*, 2010;Fasina *et al.*, 2012). Similarly, Abwage *et al.*, (2015) reported a seroprevalence of 13.2 % in Taraba State, Olugasa (2007) in his study reported a seroprevalence of 50% (Ondo), 52.5% (Oyo), 59.8% (Lagos) and 60.7% (Ogun) in south west Nigeria while Saka *et al.* (2010) reported 70% in Osun and 88% with 79.2% mortality in Lagos, Nigeria respectively.

2.1.12 Viral pathogenesis

The virus enters the host through oral, respiratory tract or through biting by ticks, then goes through the tonsil or dorsal pharyngeal mucosa and to the mandibular or retropharyngeal lymphnodesthen it spreads through viraemia (Plowright, 1994; Sánchez-Vizcaíno *et al.*, 2009)and in some cases the virus can go through bronchial, gastrohepatic or mesenteric lymph nodes. After its entrance into the host viraemia is observed after three days (Costard *et al*., 2009b).

Injuries to the endothelium lead to haemorrhges of the vessels and disseminated intravascular coagulation (DIC). Hemorrhages, effusions and oedema are preceded by thrombocytopenia, dysfibrinopaenia, coagulopathy and impaired vascular integrity (Hamdy and Dardiri, 1979; Anderson *et al*., 1998;Lubisi, 2005).

2.1.13 Diagnosis

2.1.13.1 Clinical signs

Peracute signs are characterized by sudden death with minimal clinical signs (Penrith *et al*., 2004a). Acute infection in domestic pigs is characterized by high morbidity, high fever of between 40^{o}C and 42^{o}C (FAO, 2000), congestion and cyanosis of the skin of the extremities and abdomen, abortion in pregnant sows and mortality rates of up to 100% (Penrith, *et al*., 2009; Penrith and Vosloo, 2009).

Highly virulent viruses can cause both per acute disease with sudden death and few clinical signs 3-4 days after infection (OIE. 2009) or acute disease, characterized by high fever (41-42°C), depression, loss of appetite, haemorrhages in the skin (tips of ears, tail, distal extremities, chest and abdomen), and death in 4-10 days (up to 20 days). Mortality rates may be as high as 100% (FAO, 2000;OIE, 2009, 2012).

Moderately virulent strains typically lead to sub-acute disease with mild clinical signs including mild fever, reduced appetite, depression and abortion in pregnant sows. Death may occur within 15–45 days and mortality rate varies around 30–70%. This form of the disease may be confused with many other conditions in pigs, not raising suspicion of ASF (OIE, 2009; 2012).

Low virulent strains produce subclinical infection; occasionally some animals may show weight loss, irregular peaks of temperature, respiratory signs, skin lesions, and arthritis. The disease develops over 2–15 months and the mortality rate is low

(Leifer*etal*., 2001; OIE, 2009, 2012). Sub-acute and chronic forms of the disease may result from insufficiently attenuated vaccine, as have been used in the 1960s in the Iberian Peninsula (Boinas *etal*., 2004).

2.1.13.2 Laboratory diagnosis

Different tests are available to detect the ASFV. The most sensitive and specific method is the Polymerase Chain Reaction, which is specially recommended for the identification of ASFV DNA in non-fresh or rotten tissue (OIE, 2009; Sánchez-Vizcaínoa *etal*., 2009).

Different versions of serological tests are available but the most commonly used is the Enzyme-linked Immunosorbent Assay (ELISA) (OIE, 2012;Cubillos *etal*., 2013). However, in acute disease, the death may occur before the animal may produce antibodies, and serological testing may fail to detect the disease in an early stage (Penrith *etal*., 2009; OIE, 2012).

Fluorescent antibody test (FAT): FAT assay is used as an additional method to haemadsoption test to detect antigen in tissues of suspect pigs in the field or those inoculated at the laboratory. This technique can be used to detect non adsorbing isolates of the ASFV that cannot be Fluorescent antibody test (FAT):detected by haemadsoption test. It can also be used to distinguish CPE of ASFV to other viruses like Aujeszky's disease virus (Sánchez-Vizcaíno, 2009; OIE Manual 2008; Sánchez-Vizcaíno, 2006).

Polymerase Chain Reaction (PCR): This technique is used to amplify a highly conserved region of the ASFV genome. Specific primer for *B646L* gene encoding the major capsid protein p72. Primers for diagnosis used are PPA1/PPA2 which targets the conserved region of *p72* gene (Aguero *et al*., 2003). The technique allows detection of both nonhaemadsorbing and low virulent ASFV and has a high specificity and sensitivity. It is also used in a wide range of circumstances and there is no need to isolate the virus during characterization of the virus (Steiger *et al*., 1992; Bastos *et al.*, 2003;OIE Manual, 2008).

Haemadsoption test (HAD): The haemadsorption test is based on the capacity of pig erythrocytes to adhere to surfaces of pig monocytes or macrophages cells infected with ASFV as described by Malmquist and Hay (1960). The technique is specific to viruses which have haemadsorbing capacity and it is very specific. This is a conclusive diagnostic test for ASF if the results are positive (OIE, 2012).

Enzyme linked immunosorbent assay (ELISA): The technique uses a combination of specific antibodies and specific conjugated photometric antibodies enzyme linked as described by Hamdy and Dardiri (1979). It is used to evaluate antibody in serum or fluid from tissues. ELISA is mostly used to detect the disease in endemic areas or where there is low virulent isolates. It is preferable to combine this technique with alternative serological test such as FAT or with antigen detection because the sensitivity of the technique becomes low when the samples used are poorly preserved (OIE, 2008, 2012).

Immunoblotting: This technique is based on the antibody-antigen binding principle and combines the resolution of the gel electrophoresis with the specificity of the immunochemical detection. It is sensitive and very specific that can detect even week positive samples (Sanchez-Vizcaino *et al*., 2009; Lubisi, 2005).

Counter immunoelectrophoresis: This technique is not sensitive although it can give results within 30 minutes thus, recommended to be used in screening pools of sera and not individual samples (OIE, 2008).

2.1.13.3 Pathology

Gross and microscopic findings may also vary with strain virulence (Greig and Plowright, 1970; Vigário,(1983). The gross pathological changes resemble those seen in CSF (Penrith *et al*., 2004a), with more severe lesion in acute cases and absence of lesions in some cases (OIE, 2009). The lesions include haemorrhages in the spleen, lymph nodes and kidneys, and sometimes cardiac haemorrhages, pulmonary congestion and insterstitial pneumonia (Arias and Sanchez-Vizcaino, 1992).

In cases of acute disease, carcasses are typically well-muscled with good fat reserves (FAO, 2000). There is widespread haemorrhages in organs, some abdominal lymph nodes which may resemble blood clots, small scattered haemorrhages in the kidneys, bladder and stomach lining, accumulation of blood in the vessels of multiple organs (spleen, lungs, intestines, and other abdominal structures), accumulation of blood-containing fluids in the chest and abdominal cavities (FAO, 2000; OIE, 2009).

In the subacute form there is fluids in body cavities (due to heart failure), enlarged and often haemorrhagic lymph nodes, signs of inflammation of the surfaces of the lungs and the heart, firm lungs with a mottled appearance, due to pneumonia, swollen and inflamed joints (FAO, 2000).

Chronic forms may present the following characteristics: Areas of severe lung damage, enlarged and firm lymph nodes, signs of inflammation of the surfaces of the lungs and the heart (FAO, 2000; OIE, 2009; TMVM, 2013).

2.1.14 Sample collection

For laboratory diagnosis of ASFV, blood samples and various tissue samples such as spleen, kidney, lung, liver, lymph nodes and tonsils may be submitted. The spleen and visibly affected lymph nodes are the predilection samples to collect (Aguero *etal*. 2004; OIE, 2009, 2012). Collaboration with hunting associations in Spainhas been an effective means of obtaining samples for surveillance of the disease in wild boar populations (Perez *etal*., 1998; Jori and Bastos, 2009).

2.1.15 Management and control of African swine fever

The control of ASF is complicated by the absence of both treatment and effective vaccine as well as the presence of the arthropod vectors (Penrith *et al*., 2004a). The sylvatic cycle can obscure and/or delay the successful eradication of the disease, whilst countries with sporadic outbreaks and no arthropod vectors have a higher likelihood of eradicating the disease (Magadla, 2015).

The control of ASF is based on rapid laboratory diagnosis, stamping out of affected pigs, strict movement control of both live pigs and pigs products and the enforcement of strict sanitary measures (Aguero *et al*., 2004; Lubisi *et al*., 2009). The rapid detection of infected animals reduces the potential transmission of the virus to uninfected herds and prevents the spread of the disease (Aguero *et al*., 2004). Prevention of contact between the warthog, their burrows and domestic pigs can limit the spread of the disease to domestic pigs and has proven to be successful (OIE, 2014). Sporadic outbreaks may occur in endemic areas when the virus spread from infected ticks or warthog to domestic pigs (Blood and Rodastis, 1989). These could be controlled by quarantine, culling of infected and in contact pigs and proper destruction of carcasses (Magadla, 2015).

To date, no vaccine or treatments are available (OIE, 2009; Sánchez-Vizcaínoa *etal*., 2009). In the future, vaccines may be added to the control options. Although, ASF vaccines are not yet available, a European Directive currently prohibits the use of ASF vaccines in the territory of the European Union (OJEC, 2002)

The measures taken vary according to the epidemiological situation. In Countries or areas free of ASF, there are national and international policies aim at guaranteeing that neither infected live pigs nor pig meat products are introduced to areas free of ASF.

At a national level, preventive measures include having a contingency plan in place, strict regulation of the import of animals and animal products, proper disposal of waste food from aircraft or ships, and efficient sterilization of domestic waste (OIE, 2009). Individuals also have the responsibility to apply measures to prevent import and spread of disease. Though some of the measures have a legal basis, such as not feeding suid offal (swill) to pigs, others are based on common sense such as not visiting a pig farm after hunting wild boar and other biosecurity measures (Pagani and Rijks, 2014).

In case of outbreaks, or suspicion of disease in previously ASF-free countries or areas, sanitary prophylaxis includes: rapid diagnosis (Gallardo *etal*., 2006); designation of the area as an infected zone, with zoning and control of pig movements; a survey of all pigs within the infected zone and the surrounding area to identify all infected animals/populations; the rapid slaughter of all animals on infected premises, proper disposal of cadavers and litter, and thorough cleaning, disinfection and acaricide treatment; detailed epidemiological investigation, with tracing of possible sources (up-stream) and possible spread (down-stream) of infection (OIE, 2009; Sánchez-Vizcaínoa *etal*., 2009; TMVM, 2013).

In countries or areas where ASF is endemic, disease control is primarily through the strict implementation of biosecurity measures. Consequently, proposed control methods include the separation of domestic pigs and wild suids, and proper disposal of carcasses and offal from domestic and hunted animals (Beltrán-Alcrudo *etal*., 2008).

At an individual level, a disease such as ASF that is primarily spread by direct transmission can be adequately controlled by preventing contact between domestic pigs and wild suids (Jori and Bastos, 2009; OIE, 2009). For instance, in endemic areas of South Africa, pig producers, whose premises are surrounded by a double fencing pig-proof barrier and implement biosecurity measures, have not experienced ASF since 1951 (Penrith and Vosloo, 2012).

Precautionary measures include: biosecurity when hunting wild boar; reporting of suspect cases such as cases with suspected lesion, unexplained deaths; compliance with regulations prescribed by national and local authorities and others as the authorities desired (Pagani and Rijks, 2014).

2.2 Classical Swine Fever

2.2.1 Historical and current distribution of classical swine fever

Classical swine fever is a serious, highly infectious and contagious viral and;OIE worldwide notifiable disease of domestic pigs and wild boar (Paton and Greiser-Wilke, 2003).

The disease is also known as Hog Cholera (HC) in the USA and Swine Fever (SF) or European swine fever in Europe, and needs to be differentiated from ASF which is caused by an icosahedral double stranded DNA virus, the sole member of the new genus *Asfivirus* in the family *Asfarviridae* (Dahle and Liess, 1992; Dixon *et al.*, 2008).

Classical Swine Fever was first reported in the 1830‘s in the Midwest of the USA (Moennig *et al.*, 1990; Moennig, 2000), although anecdotally it was seen in the state of Ohio as early as 1833 (Dahle and Liess, 1992; Moennig and Greiser-Wilke, 2008). The origin of the disease was reviewed by Hanson (1957) who stated that the disease originated in Europe and was introduced into the USA through the importation of new breeds of pigs (Edwards *et al.*, 2000). However, this was refuted by European authorities (Dahle and Liess, 1992), and consequently the real origin/source of the virus remains uncertain (Edwards *et al.*, 2000).

Transmission of the disease was demonstrated experimentally in 1903 through the use of bacteria-free filtrates indicating the viral nature of the disease (de Schweinitz and Dorset, 1903).

Milestones in the control of the disease involved the use of immunization by simultaneous inoculation of antiserum and virulent virus in 1907 (Niles 1910); the application of crystal violet inactivated vaccine in 1934 (McBryde and Cole, 1936); the use of attenuated live virus vaccines from 1951 (Cole *et al.*, 1962); and, most recently, the development of subunit marker vaccines (Rijn *et al.*, 1996). Meanwhile, significant advances in the laboratory diagnosis of infection have been made with the recognition of its relationship to bovine viral diarrhoea virus (Darbyshire, 1960), the application of fluorescent antibody techniques to detect antigen (Mengeling *et al.*, 1963), the use of ELISA for serology (Have, 1984), the development and application of monoclonal antibodies to the virus (Wensvoort *et al.*, 1986) and the use of molecular technology for epidemiological investigations (Lowings *et al.*, 1994) and diagnosis of infections (Edwards *et al.*, 2000).

The disease has, at some point of time, been distributed throughout the world including North and South America, Europe, Asia and Africa. However, several countries, including Australia, Canada, New Zealand, USA, and some European Union (EU) countries have succeeded in eradicating it (Moennig, 2000). Eradication was successfully implemented in Australia in 1963, Canada in 1964, USA in 1977, and France, Greece, Luxembourg, Netherlands, Portugal, Spain, and the UK at the end of 1989 (Dahle and Liess, 1992). Furthermore in 1990, Italy the last member state of EU vaccinating, stopped vaccination and since then it has been free from CSF (Saatkamp *et al.*, 2000).

2.2.2 Classical swine fever in Nigeria

At present as far as it is known, the only country in the African region that is confirm infected with CSF is Madagascar (Penrith *et al*., 2011). Recent findings suggest that

CSF is present in slaughtered pigs at Bodija abattoir in Ibadan, Nigeria (Aiki-Raji *et al.*, 2014**)**, although without an OIE official status (OIE, 2015).

2.2.3 Aetiology of classical swine fever

The CSF virus (CSFV) is a small (40-60 nm) enveloped ribonucleic acid (RNA) virus with a single stranded RNA genome with positive polarity (Collett *et al.*, 1989; Moennig and Plagemann, 1992; Paton and Greiser-Wilke, 2003; Moennig and Greiser-Wilke, 2008). The virus belongs to the *pestivirus* genus of the *Flaviviridae* family (Wengler, 1991) and it is related to the bovine viral diarrhoea (BVD) virus and the border disease (BD) virus of sheep (Moennig, 2000).

The genomic sequence of approximately 12,300 bases is known and infectious complementary deoxyribonucleic acids (cDNAs) have been produced in several laboratories (Meyers *et al.*, 1996).

Classical swine fever virusis relatively stable for an RNA virus (Vanderhallen *et al.*, 1999), but is antigenically and genetically diverse. Antigenic variability among CSFV isolates can be characterized using monoclonal antibodies (Edwards *et al.*, 1991) and the genetic variability evaluated by genomic sequencing (Le Poiter *et al.*, 2006b).

2.2.4 Survival of classical swine fever virus

The CSFV, like many enveloped viruses, may be regarded as moderately fragile. It shows a short but variable survival time in the environment, depending on the physical conditions present, but importantly may remain viable for prolonged periods in favourable circumstances, for example, in stored meat (Edwards, 2000). The durability of the virus is affected by many physical and chemical variables, including temperature, humidity, pH, presence of organic matter, and exposure to various chemicals (Edwards, 2000). The stability of CSFV in the environment is of particular importance, since experience has shown that many outbreaks of the disease may be caused by vector-mediated spread of the virus (Moennig and Plagemann, 1992).

The virus may survive for long periods in manure, and experimental studies have suggested that the virus survives longer in solid manure than in liquid manure (Have, 1984). Survival time in various types of water has been reported to vary from 6 to 24 days at 20 ^{0}C (Pagnini *et al.*, 1984).

The virulence of the strains circulating in the field and the measures applied to control the disease determine, to a large extent, the course of an epidemic. Outbreaks of disease caused by highly virulent strains are easily recognised by the sudden onset of high mortality and morbidity. In contrast, epidemics caused by low virulent strains are characterised by indistinct signs of disease, slow spread of virus through the herd and the comparatively important role of the 'carrier sow syndrome'. The latter phenomenon may result in the birth of healthy looking, but persistently infected, immune-tolerant piglets. This, and the occurrence of chronic infections, is largely responsible for the perpetuation of the virus in the pig population (Terpstra, 1987).

The infectivity of CSFV can be inactivated by elevated temperatures e.g. 10 min at 60°C, or by ultraviolet radiation (Kubin, 1967). Due to the virus‘s lipid envelope, detergents and lipid solvents inactivate the virus with ease (McKissick and Gustafson, 1967). The inactivation rate of CSFV has been shown to be inversely related to the storage temperature. The average half-life for the virus has been shown to be between 2 and 4 days at 5°C, but only 1 to 3 hr at 30°C (Weesendorp *et al.*, 2008b).

Significant differences have been observed in the survival of virus in faeces kept at different temperatures, however not with virus in urine (Weesendorp *et al.*, 2008b). Survival time at temperatures above 100°C is less than 1 min (Downing *et al.*, 1977). In contrast inactivation occurred in 1 minute at 90°C, 2 min at 80°C, and 5 min at 70°C (Rehman, 1987). At lower temperatures, the virus is reasonably stable (depending on the suspending medium) which facilitates handling in the laboratory and shipment of diagnostic samples. In general, diagnostic samples should be kept cool (4°C wherever possible) although short periods at room temperature is considered not too deleterious (Edwards, 2000).

The CSFV is generally stable at neutral to slightly alkaline pH in the range 5–10, but is rapidly inactivated at pH 3 or below, and above pH 10 (Kubin, 1967). A sharp pH peak for virus survival in defibrinated blood has been demonstrated at pH 5.2 (Chapin *et al.*, 1939; Edwards, 2000).

Thermal inactivation curves may be derived for the virus at different temperatures but may vary with the virus strain (Kubin, 1967; Depner *et al.*, 1992). The half-life of virus is dependent upon both temperature and pH, with the effect of pH (below 4.0) being much more marked at 4°C than at 21°C (Depner *et al.*, 1992). In aerosols the virus remains infective for at least 30 min with a half-life ranging from 4.5 to 15 minutes (Weesendorp *et al.*, 2008a).

The virus can be inactivated by organic solvents, such as ether or chloroform, detergents, deoxycholate, or saponin (Moennig and Plagemann, 1992) and a wide range of chemicals, including chlorine-based disinfectants, detergents, phenolics, quaternary ammonium compounds, and aldehydes (formaldehyde, glutaraldehyde) (Liess and Schurian, 1973; Russell and Hugo, 1987). The virus can also be killed by pasteurisation or by thorough cooking. Treatment of virus-contaminated meat for 30 min at 65°C or 1 min at 71°C has been shown to render it non-infective (Keast and Helwig, 1966; McKercher *et al.*, 1978; Stewart *et al.*, 1979). Blood contaminated at 105 TCID50/ml can be inactivated at temperatures of 66°C for 60 min, 68°C for 45 min, or 69°C for 30 min (Edwards, 2000).

Infected pigs that are shedding large amounts of virus in their saliva, as well as lesser amounts in urine, faeces, ocular and nasal secretions, are a potent source of infection for other pigs. Importantly pigs start to shed virus several days prior to the development of clinical signs (Van Oirschot, 2004). Piglets born to carrier sows can shed large quantities of the virus in their faeces for months without showing clinical signs or developing an immune response (Terpstra, 1991). There is ample field evidence to indicate that the major mode of transmission of CSFV is directly from pig to pig (Terpstra and Wensvoort, 1988; Edwards, 2000).

Classical swine fever virus is relatively stable in moist excretions and fresh meat products, including ham and salami type sausages (Savi *et al.*, 1965). The virus has been reported to survive for more than 4 years in frozen pork (Edgar *et al.*, 1949), while in chilled fresh pork it can survive for up to 85 days (Birch, 1917; Doyle, 1933; Edwards, 2000). However, the virus is readily inactivated by heat, detergents, lipid solvents, proteases and common disinfectants (Stewart *et al.*, 1979; McKercher *et al.*, 1987).

Pig intestines used for the production of natural sausage casings may carry CSFV, therefore feeding pigs human food waste (swill) may result in the spread of virus to CSF-free animals (Wijnker *et al.*, 2008).

2.2.5 Risk factors of classical swine fever

Imported contaminated pig products have frequently resulted in the introduction of CSFV into previously disease-free regions (Paton and Greiser-Wilke, 2003). Feeding of untreated swill (kitchen waste) that contains infected pork is a major source of primary outbreaks in regions previously free from CSF (Sharpe *et al.*, 2001; Moennig and Greiser-Wilke, 2008).

Other important factors for the transmission of the virus from infected pigs include contact with wild pigs, and poor management and biosecurity practices, including a lack of suitable hygienic measures allowing exposure to contaminated fomites (Paton and Greiser-Wilke, 2003).

Epidemiological investigations and virus typing provided strong evidence that infected wild boars have been the source of numerous outbreaks in Europe (Fritzemeier *et al.*, 2000). The spread of the disease is facilitated by the movement of virus excreting pigs. The purchase of weaner pigs from different breeding farms or from markets carries a high risk of introducing the virus into susceptible populations (Beals *et al.*, 1970).

Semen from infected boars has also been shown to be significant in the spread of CSF (Elbers *et al.*, 1999; Floegel *et al.*, 2000; Stegeman *et al.*, 2000). If it occurs, airborne transmission of virus is probably only over short distances and mainly within a holding. However, there have been concerns that surrounding farms could be at risk from airborne spread during depopulation of affected premises (Laevens *et al.*, 2000; Elbers *et al.*, 2001b).

Indirect transmission may occur via people, wild animals and inanimate objects but the exact mechanisms whereby the virus spreads between neighbouring farms is poorly defined (Elbers *et al.*, 2001a, b; Laevens *et al.*, 2001).

Aside infection with CSFV, pigs can also be infected with the ruminant pestiviruses, Bovine Viral Diarrhoea Virus (BVDV) and Border Disease Virus (BDV). The presence of cattle on the same premises and a high density of sheep and/or goat herds within 3 km of the pigs have been identified as risk factors associated with a BVDV-seropositive breeding pigs. In addition, serological cross-reactions occur between the pestiviruses, providing potentially protective immunity, but also leading to confusion in the interpretation of the results from diagnostic tests (Loeffen *et al.*, 2009).

2.2.6 Classical swine fever virus hosts

Pestiviruses are not strictly host-species specific and can infect both domestic and wild animals (Vilcek and Nettleton, 2006). Wild pigs are found in many countries and are known reservoirs for a number of viruses, bacteria and parasites that are transmissible to both domestic animals and humans (Meng *et al.*, 2009). Infection with CSFV occurs under natural conditions in both domestic pigs and wild boars (*Sus scrofa*) (Kern *et al.*, 1999). This is important as infection of wild pigs with CSFV may complicate the success of an eradication program (Vilcek and Nettleton, 2006). The virus can be experimentally transmitted to probably all ruminants, but with certainty to goats, sheep, calves and deer (Dahle *et al.*, 1987).

Pestiviruses are able to cross the species barrier (Moennig, 1990). Many studies have shown that BVDV can naturally infect pigs, sheep, goats and a wide range of wild ruminants (Snowdon and French, 1968; Doyle and Heuschele, 1983; Dahle *et al.*, 1987). In a survey conducted in pigs in Northern Germany, 15-20% of all breeding pigs were found to be seropositive to BVDV (Liess *et al.*, 1974). Such cross-species transmission can be important when interpreting the results from a serosurveillance study.

2.2.7 Transmission

With respect to agent (viral) factors, virulence and mutation are important factors for disease transmission (Risatti *et al*. 2005). In addition, an association may exist between virulence and antigenicity, where strains that are antigenically related to BVDV appear to be less virulent.

Infection with highly virulent CSFV strains generally leads to death of infected animals, whereas isolates of moderate to low virulence induce a prolonged chronic disease (Van Oirschot, 1999).

In terms of host factors, the transmission of CSF is enhanced by many factors including: movement of virus excreting pigs within a population, population density, presence of susceptible and reservoir hosts, age structure of the population, and iatrogenic factors (Dahle and Liess, 1992).

Persistent infections are the most important mechanisms by which the disease perpetuates in the domestic pig population (Liess, 1984). Persistent infections are commonly established during gestation at a time when the immune response of the foetus is not capable of eliminating the virus. The optimal time for the establishment of persistent viraemia depends on the maturation of the foetal immune system (Moennig, 1990).

Transmission of the virus can occur via direct and indirect routes including contaminated fomites (Karsten *et al.*, 2005). With respect to spread via vehicles, trucks play a major role in the transmission of CSFV (Elbers *et al.*, 1999; Ribbens *et al.*, 2004). Transportation of weaners from different breeding farms to fattening farms has been identified as a significant risk factor for the spread of disease. Such transportation, often over long distances, may result in a large number of non-traceable contacts (Terpstra, 1991).

Acute, chronic or congenital infection can occur (Dahle and Liess, 1992). Congenital infections, in which the piglets are born `healthy', from an epidemiological point of view, are the most dangerous. These piglets may shed large quantities of virus for months without showing signs of disease or developing an antibody response (Van Oirschot and Terpstra, 1977).

The main route of infection in field cases is via the oronasal route (Moennig, 2000), by either direct or indirect contact with infected pigs or through contaminated feed, e.g. swill. In areas with a high density of pigs, virus spreads easily between neighbouring pig holdings (Terpstra and Wenvoort, 1988; Fritzemeier *et al.*, 2000).

The ability of the virus to persist in uncooked pork and processed pork that has not been heated to high temperatures for long periods [months when kept at cool temperatures and years if frozen] is of great importance for virus transmission over long distances and between continents (Mather *et al.*, 2011).

Farmers, veterinarians, inseminators and castrators potentially could also transmit CSF through the use of contaminated instruments. Use of hypodermic needles on more than one pig or more than one farm is also a very important method of spread. The disease can also spread when vaccinating teams use the same bottle of vaccine on different farms (AusVetPlan, 2009).

Disease transmission via the semen of infected boars may also occur (Elbers *et al.*, 1999; Risatti *et al.*, 2005). Tabanids are potential mechanical vectors of CSFV (Krinsky, 1976), however the virus is not transmitted biologically by any arthropod vectors, but it may be spread mechanically by arthropods as well as through scavengers such as dogs or wild birds (AusVetPlan, 2009).

Feral pigs (wild boar) can be infected by the virus, and it is therefore necessary to minimize contact between feral and domestic pigs by ensuring secure boundary fencing (Weesendorp *et al.*, 2008; AusVetPlan, 2009).

Transmission of CSFV is most commonly via the oronasal route, with primary virus replication in the tonsils. From the tonsils, it spreads to the regional lymph nodes, then via the peripheral blood to bone marrow, visceral lymph nodes, and lymphoid structures associated with the small intestine and spleen. The spread of virus within the pig is usually complete in less than 6 days (Le Poiter *et al.*, 2006b).

2.2.8 Route of infection

Pigs usually become infected by inhaling or ingesting the virus. Acutely infected pigs that are shedding large amounts of virus in their saliva, as well as lesser amounts in urine, faeces, ocular and nasal secretions are a potent source of infection for other pigs. Pigs start to shed virus for a few days before clinical signs develop (Terpstra, 1994; Van Oirschot, 2004), and continue to do so until antibodies develop, which usually happens about 11 days after infection (Penrith *et al*., 2011).

2.2.9 Incubation period

The incubation period for CSF is generally between 3 and 10 days (Moennig and Greiser-Wilke, 2008). Under field conditions, clinical signs may only become evident in a piggery 2 to 4 weeks after virus introduction (Laevens *et al.*, 1999). The severity of clinical signs mainly depends on the age of the animals and the virulence of the virus (Moennig *et al.*, 2003).

Pigs infected *in utero* are often persistently infected carriers, whether or not they are healthy at birth (Maclachlan and Scott, 2004). Pigs exposed postnatally are infective between 5 and 29 days post-infection, however with chronic infections this infective stage can last for over 30 days (Mengeling *et al.*, 1963; Dahle and Liess, 1992).

2.2.10 Transmission cycles

Maintenance of the virus in domestic pigs and wild boars is similar to that of ASF virus (Penrith *et al*., 2011). There is no long-term carrier state in recovered pigs, but large continuous populations of either free-ranging domestic pigs or wild boars can maintain indefinite circulation of the virus (Van Oirschot, 2004).

In North America and Western Europe CSF has been optimally controlled in domestic pigs, but it has not been possible to eradicate the virus from all the wild boar populations in Western Europe, and occasional outbreaks are registered when careless husbandry allows domestic pigs to come into contact with wild boars, or the entrails of wild boars infected with CSF are fed to domestic pigs. Such outbreaks are generally limited in extent and easily controlled. More serious outbreaks have occurred in recent decades in Belgium, the Netherlands and the United Kingdom, where molecular studies revealed the cause to be viruses of Asian origin that most probably came in with illegally imported pork that was subsequently fed as swill (Penrith *et al*., 2011).

Sexual transmission has been demonstrated to occur and the virus can persist in chilled or frozen semen, but its importance in the epidemiology of the disease has not been established (Penrith *etal*., 2011).

Mechanical transmission by biting flies may be possible. A study indicated that birds, rodents and domestic animals such as dogs and cats are not able to transmit the virus in infective quantities (Van Oirschot, 2004).

2.2.11 Prevalence of classical swine fever

Many studies have reported on the prevalence of CSF both in wild and domestic pigsin different countries. In the Netherlands, a survey on wild boar found that 11 of 116 (9%) wild boars were seropositive for CSFV (Stegeman *et al.*, 2000), while in France 80 of 12,025 (0.7%) wild boars tested from 1991 to 1998 were shown to be seropositive

(Albina *et al.*, 2000). Additionally, in France during two outbreaks of CSF from 2002 and 2003, of the 3337 samples from wild boar tested 188/2525 (7·45%) were positive on the ELISA, 65/152 (42·8%) were positive to the virus neutralization test (VNT), (4·1%) were positive to a PCR, and 15/84 (17·9%) had virus isolated from them (Pol *et al.*, 2008).

2.2.12 Viral pathogenesis

After an incubation time of 3 to 10 days, a clinical onset with highly varying stages of severity may occur (Moennig, 2000). Virus shedding already begins prior to occurrence of clinical signs (de Smit *etal*., 1999; van Oirschot, 1999; Floegel *etal*., 2000; Moennig, 2008) and sustains until development of antibodies or death. CSFV is excreted in large amounts via saliva and also, to a lesser extent, in urine, faeces, semen as well as ocular and nasal secretions (Ressang, 1973; van Oirschot, 1999; Pasick, 2008).

CSFV infection may either lead to peracute death or result in complete convalescence. Several factors from the virus' and the host's side, has shown to influence the clinical picture and outcome (Depner *etal*., 1995; Depner *etal*., 1997; Kaden *etal*., 2004).

Upon oronasal CSFV infection, primary replication takes place in tonsils and other local lymphoreticular tissues. Subsequently, virus progeny reaches regional lymph nodes via lymphatic vessels and enter the blood circulation (Dunne, 1973; Ressang, 1973; Liess, 1987). Thereafter, virus is disseminated into the spleen, bone marrow, visceral lymph nodes, intestinal lymphatic mesh, and other parenchymatos organs (Petrov, 2015).

Here, viral replication takes place within immune cells, particularly monocytes, macrophages and dendritic cells, which represent primary target cells (Moennig, 2000;

Gomez-Villamandos *etal*., 2001; Carrasco *etal*., 2004). Furthermore, endothelial and epithelial cells can get initially infected (Ressang, 1973; Trautwein, 1988) until later disease stages, almost every cell type is susceptible towards CSFV (Knoetig *etal*., 1999).

The virulence of the CSFV isolate seems to play the major role and, to a lesser extent, the dose and route of infection (Kaden *etal*., 2004). It was reported that moderately or low virulent strains of wild boar origin may lead to a more severe disease course or higher viral loads when introduced into domestic pigs (Kaden *etal*., 1999; Kaden *etal*., 2000).

In general, secondary or concomitant infections are characteristic for CSF. They occur in consequence of a severe immunosuppression promoted by the depletion of both, B- and T-lymphocytes (Summerfield *etal*., 2001). Immunosuppressive events were associated with the relatively late humoral and cellular immune response which is typical for CSF (Trautwein, 1988; Thiel, 1996).

In detail, a short-term leukocytosis occurs very early after infection followed by a leukopenia, particularly affecting lymphocytes (B- and T-cells) (Susa *etal*., 1992; Pauly *etal*., 1998; Summerfield *etal*., 1998; Markowska-Daniel *etal*., 1999; Gomez-Villamandos *etal*., 2000; Sato *etal*., 2000). Thereby, T-cell depletion develops rapidly after infection while B-lymphocytes are depleted in later disease stages (Susa *etal*., 1992).

2.2.13 Diagnosis

The clinical signs of CSF are extremely variable and may be confused with other diseases. Clinical signs can therefore only lead to a clinical suspicion of the disease and any suspicion of CSFV has to be confirmed by laboratory diagnosis. Laboratory diagnosis relies on either agent detection (detecting either viral proteins or genome) or antibody detection (Blome *etal*., 2006; Greiser-Wilke *etal*., 2007; EFSA, 2008).

2.2.13.1 Clinical diagnosis

It is difficult to make a clinical diagnosis of CSF, especially in older pigs (Paton and Greiser-Wilke, 2003), because of the presence of viral strains with only moderate virulence (Koenen *et al.*, 1996; Williams and Matthews, 1988). Although the diagnosis of CSF can be based on clinical and pathological findings (Edwards *et al.*, 2000), the clinical signs are often not pathognomonic for the disease (Le Poiter *et al.*, 2006b). The disease often has an incubation period of some weeks, requiring several cycles of amplification before it becomes clinically apparent (Paton and Greiser-Wilke, 2003). Furthermore, the recent emergence of porcine dermatitis and nephropathy syndrome also complicates the diagnosis, since it can have a similar clinical appearance to CSF. Therefore, confirmation of disease has to be supported by laboratory investigations (Edwards *et al.*, 2000), even for secondary cases during large outbreaks (Paton and Greiser-Wilke, 2003).

2.2.13.2 Laboratorydiagnosis

Laboratory tests are used to confirm the diagnosis of CSF and either detects viral antigen or, antibody to the virus (Le Poiter *et al.*, 2006b).

Virus isolation (VI): Virus isolation (VI) is based on the incubation of sample material on susceptible cells culture of porcine origin. If infectious CSF virus is present in the sample, it will replicate in the cells to an amount that can be detected, by immunostaining of the infected cells with conjugated antibodies. Classical swine fever specific antibodies are required to differentiate between CSFV and other pestiviruses (EFSA, 2008).

Suitable samples for isolation of CSF virus from live pigs are leukocytes, plasma or whole blood obtained from non-coagulated blood samples. Suitable tissue samples include tonsil, kidney, spleen ileum and different lymph nodes (EFSA, 2008).

Virus isolation is best suited for the investigation of samples from small numbers of animals rather than mass surveillance. The virus isolation procedure is labour intensive and requires at least three days before results are available. Two further cell culture passages may be necessary to detect lower amounts of virus in the sample. This may lead to an investigation time of more than 10 days before a final result is obtained. Samples that suffer from autolysis can be cytotoxic to the cell culture and consequently have limited value (EFSA, 2008).

Virus isolation is still considered the gold standard, even though by now the PCR is recognized as a more sensitive test (Depner *etal*., 2006a; Depner *etal*., 2007a). Virus isolationis still the most sensitive and specific method for virus detection (Le Poiter *et al.*, 2006a). Virus may be isolated from tissue homogenates, serum, plasma, buffy coat, and whole blood collected in heparin or EDTA (Terpstra, 2000).

It is critical that all cells, media, and reagents have been previously determined to be free of pestiviruses or antibodies against pestiviruses (Le Poiter *et al.*, 2006b). Although VI is the reference method in most CSFV eradication programs, it is labour intensive, time consuming, and incompatible with the rapid response required to prevent further spread of virus (Le Poiter *et al.*, 2006a).

The sensitivity of the VI is usually thought to be high, and in experimental infections, up to 95% sensitivity is reported (Dewulf *etal*., 2004). However, an evaluation of the VI during the 1997/98 outbreak in the Netherlands showed that the diagnostic sensitivity of the VI on tonsils in the field was only approximately 77%, which was comparable to the sensitivity of the FAT (Bouma *etal*., 2001). The sensitivity of the VI on blood samples may also be hampered by the presence of antibodies, although no quantitative data, especially from the field, is available on this (EFSA, 2008).

A positive VI is proof for the presence of infectious virus and any animal, tissue or blood sample being VI positive is assumed to be infectious to other pigs. A negative VI on the other hand does not mean that infectious virus is absent (McKercher *etal*., 1987; Panina *etal*., 1992; Mebus *etal*., 1993; Haegeman *etal*., 2006).

Polymerase chain reaction (PCR): Reverse transcriptase polymerase chain reaction (RT-PCR) is based on the amplification and subsequent detection of genome fragments. Small fragments of viral RNA are transcribed into DNA fragments during an RT-step, and are subsequently amplified by PCR to detectable quantities. Detection of amplicons is possible by gel electrophoresis, but nowadays mainly real-time RT-PCR’s are being used. These PCR’s use either SYBR green to detect amplicons, or, for enhanced specificity, hydrolysis of hybridization probes (Liu *etal*., 1991; Roehe and Woodward, 1991; Katz *etal*., 1993; Diaz *etal*., 1998; McGoldrick *etal*., 1998; Aguero *etal*., 2004; Belak, 2005).

A wide variety of samples are suitable for the PCR, but mainly whole blood samples and tissue samples will be used for the diagnosis of CSF. Beside whole blood, also serum, plasma or isolated leucocytes can be used. Tissue samples of preference are the same as for VI but kidney samples may be less suitable (EFSA, 2008).

Due to its high sensitivity, and the amplification of huge amounts of amplicons, the RT-PCR is also very sensitive to contamination or cross-contamination of samples, reagents or other materials. Separate rooms should be used for separate steps in the PCR diagnostics, for instance pretreatment of samples, preparing buffers and stock-reagents, RNA-isolation, and RT-PCR. Strict protocols should be in place with respect to movement of people, materials and samples between these rooms, or between these rooms and other rooms in the laboratory. Furthermore, retesting or independent confirmation of positive samples is always an option for doubtful results. For the same reasons, the real-time RT-PCR (rRT-PCR) requires appropriate laboratory equipment and skilled staff. For both RNA isolation and RT-PCR fully robotized solutions are available nowadays. An RT-PCR can be performed within several hours, but for high-throughput 24-48 hours between receiving samples and sending out results is more realistic. Using approved commercial kits can be useful as usually the reagents are ready for use, reducing the risk of contamination and saving time to perform the assay (EFSA, 2008).

It has been reported that pooling up to ten samples did not decrease the rRT-PCR sensitivity (Depner *etal*., 2006a; Le Dimna *etal*., 2008). In case of positive results for a pool, each of the ten samples has to be tested individually, limiting by the way the number of samples tested per day. However, the effect of the pooling on the diagnostic sensitivity of the PCR may be decreased when borderline positive samples are pooled (e.g. screening in vaccinated populations).

RT-PCR has been found to be the most sensitive method for detection of CSFV (Dewulf *etal*., 2004; Handel *etal*., 2004; Depner *etal*., 2006a; Depner *etal*., 2007a; Le Dimna *etal*., 2008). In carcasses from wild boar it is the method of choice, especially if the material is subjected to autolysis and virus is either inactivated or virus isolation is not possible any more due to cytotoxicity of the sample. With the RT-PCR, viral genome can be detected for a long time in certain tissue samples from animals that are fully recovered from an infection. In tonsils from pigs recovered after an infection, viral genome was detectable for at least 9 weeks (Loeffen *etal*., 2005). An RT-PCR positive result does not necessarily mean that infectious virus particles are present (Dewulf *etal*., 2005; Haegeman *etal*., 2006). rRT-PCR is also highly specific (Depner *etal*., 2006; Le Potier *etal*., 2006b, Le Dimna *etal*., 2008) especially if specific probes are being used.

Hybridization probes may be slightly more specific than hydrolysis probes, as the latter may be subject to non-specific degradation during high cycle numbers and therefore cause very weak-positive or doubtful results (Ciglenecki *etal*.,2008).

In general it can be said that from an RT-PCR negative result it can be concluded with a very high confidence that the tested animal or tissue sample is not infectious to other pigs, while on the other hand a sample that is RT-PCR positive, is not necessarily infectious (Dewulf *etal*., 2005; Haegeman *etal*., 2006, Le Potier *etal*., 2006b).

Depending on the vaccine, and the sample to be tested, rRT-PCR can also be used as a Differentiate Infected from Vaccinated Animaltest ‘genetic DIVA’(Beer *etal*., 2007). If the vaccine does not contain any genome (i.e. E2- subunit vaccines), or if the vaccine has deletions or substitutions on the primer sites (i.e. deletion mutants or chimaeric vaccines), an rRT-PCR positive result would be proof for an infection with field virus (Koenig *etal*., 2007). Newly developed C-strain specific real-time RT-PCRs can be used to test vaccinated animals for the presence of modified live vaccine, but in case of a positive result, infections with wild type virus can still not be ruled out. More importantly are therefore PCR’s that are specific for wild type virus (Li *etal*.,2007; Zhao *etal*., 2007) that can be used to detect or rule out wild type virus infections, independent of the vaccination status of the animal (EFSA, 2008).

Immunohistochemistry (IFT): The immunofluorescence test (IFT) or fluorescent antibody test (FAT) is based on detection of viral proteins with FITC-conjugated antibodies (Robertson *etal*., 1965). The immunoperoxidase test (IPT) is based on the detection of viral proteins with HRPO-conjugated antibodies. In the past both tests had often been used for the confirmation of secondary outbreaks. For the confirmation of primary cases IFT and IPT must be supported by other direct tests (Wensvoort *etal*., 1986; De Smit *etal*., 1999, 2000b).

The test can only be carried out post-mortem and the organs of preference are the tonsil, spleen, kidney, ileum, and several lymph nodes. From these organs, cryosections are cut for staining. A smear of bone marrow cells might also be used, for instance in case of feral pigs, if organs are not available or are subjected to autolysis (EFSA, 2008).

The test is relatively easy to perform, but requires experienced staff because interpretation of staining is not fully objective. Furthermore a cryostat is needed to cut the cryosections. The test can be performed within few hours. However, for testing larger amounts of samples (100-200 per day may be realistic), 24-48 hours between receiving samples and sending out results is more realistic (EFSA, 2008).

The IFT/FAT is often considered as less sensitive than VI, but an evaluation of the FAT and VI during the 1997/98 CSF outbreak in the Netherlands showed that in the field, the sensitivity of both tests on tonsils was almost equal (75%), (Bouma *etal*., 2001). The quality of the reagents should be controlled for the success of the test (EFSA, 2008).

The specificity of the test depends on the antiserum used. If polyclonal sera are used, positive samples need to be confirmed in a second test, especially to differentiate between CSFV and other pestiviruses. With monoclonal antibodies, the test is, however, highly specific (Bouma *etal*., 2001). Due to the introduction and implementation of the RT-PCR in many diagnostic laboratories, this test is not very commonly used anymore (EFSA, 2008).

Antigen enzyme linked immunosorbent assay (ELISA): The antigen ELISA is based on the detection of viral proteins, binding to antibodies in an ELISA plate (Shannon *etal*., 1993; Depner *etal*., 1995). The test is easy to perform and is relatively cheap and fast. However, a low sensitivity from 39% on wild boar samples, according to Depner *etal*., 2006, to 74.7% on experimental infected pigs according to Dewulf *etal*., 2004 has been described as this test needs a high virus charge to detect positivity. Its use has to be restricted to very recent infection when the viraemia is high. The specificity of this test was also considered as low as cross reaction to other pestiviruses were often recorded.

These intrinsic properties compared to most of the other diagnostic tests, especially RT-PCR, makes it usage less reliant as first choice for CSF detection (Dewulf *etal*., 2004; Depner *etal*., 2006; Depner *etal*., 2007).

Sequence analysis: Between 1970 and until the late 1990s, Germany was struck by several severe and less severe epidemics of CSF (Wachendörfer et *al*., 1978; Moennig and Plagemann, 1992; Fritzemeier *etal*., 2000). One aim was to find methods that would allow distinguishing isolates from individual outbreaks. This was a significant issue, because such information would be an invaluable tool for epidemiologists to trace primary and secondary outbreaks. First success was achieved using monoclonal antibodies against viral proteins for differentiating between Pestiviruses (Wensvoort *etal*., 1989; Greiser-Wilke *etal*., 1998; Paton *etal*., 1995). In addition, mabs were successfully used for typing CSF virus isolates and other Pestiviruses (Kosmidou *etal*., 1995; Paton *etal*., 1995). This method is work-intensive and was found to be closely correlated to the availability of the mabs. At that time, technological advances led to the implementation of the PCR in most laboratories, and automated DNA sequencing became practicable and affordable. It was then realized that isolates from individual outbreaks could be discriminated by genetic typing. For this, several different regions of the viral genome were used, and it was recognized that genetic typing had to be harmonised to ensure that results from different laboratories are comparable. Therefore, the three most widely used genomic fragments were evaluated, namely fragments of the 3' end of the polymerase gene (NS5B), (Lowings *etal*., 1994; Bjorklund *etal*., 1999), 150 nt of the 5'NTR (Stadejek *etal*., 1996; Greiser-Wilke *etal*., 1998) and a fragment (190 nt) of the gene coding for the E2 glycoprotein (Lowings *et al*., 1996). A standardised protocol was designed for typing new CSFV isolates, fixing the three genomic fragments to be used, the algorithms for calculation of the phylogenetic trees, and the nomenclature of the genetic groups (Lowings *etal*., 1996; Paton *et al*., 2000). The CSF viruses were divided into three groups with three or four subgroups each, namely 1.1-1.3, 2.1- 2.3, and 3.1-3.4 (Paton *etal*., 2000). Geographical distribution of the subgroups has been reviewed previously (Greiser-Wilke *etal*., 2000; Frias-Lepoureau and Greiser-Wilke 2002; Moennig *etal*., 2003).

Phylogenetic analyses performed in different parts of the world confirmed that CSFV isolates that differ by genetic typing seem to be characteristic for certain geographic regions (Stadejek *etal*., 1996; Vilcek and Belak, 1997; Bartak and Greiser-Wilke, 2000; de Arce *et al*., 2005; Blacksell *et al*., 2005; Pereda *et al*., 2005; Kamakawa *et al*., 2006; Li *et al*., 2006; Sabogal *etal*., 2006; Chen *et al*., 2008).

Extensive use of the database and an increasing number of records, including isolates with identical sequences from related outbreaks in different regions, made it difficult for the user to select a standard dataset for genotyping new isolates. As a consequence, the database was supplemented with a module for searching for identical sequences, performing the alignment with a standard set of sequences, and calculating and graphically displaying the Neighbour-Joining phylogenetic tree (Dreier *etal*., 2007).

Antibody detection: In classical swine fever virus infected pigs, antibodies are usually detectable in serum sample from one to three weeks after infection. In pigs that have recovered from the disease, protective neutralising antibodies can be detected for several years or even for their lifetime (EFSA, 2008). Antibodies are also sporadically detectable in the terminal stage of lethally diseased animals. In some pigs with chronic form of classical swine fever, antibodies may be detectable for a few days at the end of the first month post-infection (Liess *etal*., 1976).

Pigs infected in utero may be immunotolerant against the homologue classical swine fever virus and produce no specific antibodies (Terpstra, 1987). However, maternal antibodies can be detected during the first weeks of life. The half-life of maternal antibodies against several viruses in nonviraemic healthy piglets can vary from approximately 8 days, found for CSF (Vandeputte *etal*., 2001), 12 days for swine influenza (Loeffen *etal*., 2003), 3 weeks for porcine parvo and foot-and-mouth disease (Fenati *etal*., 2008; Francis and Black, 1984), or more than 8 weeks for Aujeszky's disease depending on the level of maternal antibodies in the colostrums (Bouma *etal*., 1997). According to Kaden and Lange (2004) and Müller *etal*. (2005), the maternal derived antibodies were not detectable after three months of experimental oral immunisation of young female wild boars suggesting a quite high half-life value. Half-life values of maternal antibodies seem to be determined mainly by the increase in blood volume anyway (Francis and Black, 1984). Because domestic pigs grow much faster than wild boar, this would explain why maternal antibodies in wild boar can be detected much longer than in domestic pigs (EFSA, 2008).

Antibody ELISA: Several ELISA techniques using specific monoclonal antibodies have been developed, mainly: competitive or blocking ELISA and non-competitive ELISA's (Moser *etal*., 1996; Colijn *etal*., 1997; Wensvoort *etal*., 1988; Clavijo *etal*., 2001). The competitive or blocking ELISA is usually based on monoclonal antibodies. If the serum sample contains antibodies to CSF virus, the binding of a selected peroxidase-conjugated monoclonal antibody to virus antigen will be inhibited leading to reduced signal.

In general only serum samples will be used in ELISA's. Although meat juice are also being used for several other infections, including Salmonella and Aujeszky's disease (Nielsen *etal*., 1998; De Lange *etal*., 2003).

E^2-ELISA: The sensitivity of the E2-ELISA is in general comparable to that of the VNT, although the latter is more sensitive in samples obtained within 3 weeks after infection. If no antibodies can be detected in infected pigs, it is usually because they are chronically infected, with a persistent viraemia. The specificity is usually also high, in the range of 98 to > 99.5%. Part of the specificity problems may be caused by infections with other pestiviruses. Some non specific reactions can occur when the quality of the serum is poor. These quality problems are more frequent for wild boars sera even if the quality of the blood sampled by hunters has really been improved for the five last years (EFSA, 2008). Detection of antibodies does not necessarily mean that there is active infection.On the contrary, in most cases where antibodies are present, infectious virus will no longer be detectable. The E2-ELISA can be used as a DIVA test for vaccines that do not contain the E2 of CSFV. Such vaccines can either have the E2 replaced by that of another pestivirus (Van Gennip *etal*., 2000; De Smit *etal*., 2001a) or have it deleted (Van Gennip *etal*., 2002).

E^{RNS}-ELISA: The E^{RNS}-ELISA is based on the same principle as the E2-ELISA's, but instead detects antibodies against the ERNS -protein. The E^{RNS}-ELISA's were developed as companion tests for the E2-subunit vaccine (Van Rijn *etal*., 1999). Two commercially available E^{RNS}-ELISA's, A and B, were evaluated in a large EU-trial in the late 1990's (Floegel-Niesmann, 2001). At that time one of the ELISA lacked sensitivity, while the other one was deemed not to be specific enough. (Blome *etal*., 2006).

The sensitivity of the E^{RNS}-ELISA is in general somewhat lower than that of E2-ELISA's. Furthermore, it is not CSF-specific, but detects also antibodies against other pestiviruses. For a population where non-CSF pestivirus infections occur, the test is therefore less useful. While this test is developed in combination with the E2-subunit vaccine, it can be used as a DIVA test with any vaccine that does not contain ERNS, including live deletion mutants (Widjojoatmodjo *etal*., 2000). For chimeric vaccines, that contain ERNS from a non-CSF pestivirus (Van Gennip *etal*., 2000; Reimann *etal*., 2004), the test cannot be used as a DIVA test. In these cases the E^{RNS}-test B could be used, as it is CSF-specific, but lacks sensitivity (Floegel-Niesmann, 2001).

Virus neutralisation test: The virus neutralisation test is carried out by incubating serum samples in serial twofold dilutions with a known amount of virus together with a susceptible cell culture. In the absence of neutralizing antibodies, these cells will get infected and virus become detectable, while in the presence of neutralizing antibodies, the virus will be neutralized and no virus will grow. Detection of virus is usually done with an immune cytochemical method (IFT/IPT). The VNT is a laborious and time-consuming test. Furthermore, because virus is replicated, hygiene and containment procedures should be in place. Requirements for facilities, but also personnel are therefore much higher than for an ELISA.

The VNT is considered to be the gold standard of antibody detection. It is regarded as the most sensitive antibody test, but cross-neutralizing antibodies against non-CSF pestiviruses will readily be detected as well. To solve this problem, the VNT for CSFV antibodies is usually carried out in parallel with a VNT for BVDV antibodies and sometimes also a VNT for BDV antibodies. The VNT for the detection of antibodies against BVDV and BDV follows the same principles mentioned above for CSFV. If the CSF-titre is equal to or higher than the BVDV/BDV-titre, the presence of CSF antibodies is confirmed. This procedure results in a highly specific test, but this will be at the expense of the sensitivity. CSF infections in the presence of BVD antibodies will result in false-negative test results (Wieringa-Jelsma *etal*., 2006).

Few published papers estimates the specificity or sensitivity of the conventional test that were used for years. More recently, in studies of the different RT-PCR or rRT-PCR, a comparison was done with the well-established Virus isolation (gold standard) or with other antigen detection methods (FAT, Ag ELISA). Dewulf *etal*., (2004), shows the usual period of detection after an infection depending on the diagnostic method used, in comparison to the VI in whole blood.

The first test used for herd screening is a method known to be sensitive as rRT-PCR for viral genome detection or E2-ELISA for antibodies detection. Any positive sample is consequently again analysed with a different method as Virus isolation or Virus neutralisation test to check the specificity of the result. Therefore, the combination of the two tests gives a very high specificity, probably close to 100% (EFSA, 2008).

2.2.13.3 Pathology

Once the disease develops, pathological changes visible on post mortem examination are most often observed in the lymph nodes, spleen and kidneys of acute cases. The lymph nodes become swollen, oedematous and haemorrhagic. Haemorrhages in the kidney may vary in size from hardly visible petechiae to ecchymotic haemorrhages, and frequently occur on the surface of the cortex resulting in the characteristic turkey kidney pathological lesion, but are less common in the medullary pyramids and hilus. Kidney parenchyma may display a yellowish brown colour. Petechiae can also be observed in the urinary bladder, larynx, epiglottis and heart, and may be widespread over the serosa of the abdomen and chest (Van Oirschot, 1999).

Nonpurulent encephalitis is often also present (Gruber *et al.*, 1995). Infarctions of the spleen are considered to be pathognomonic for CSF, however they are rarely observed. These infarcts are a result of a disrupted blood flow to certain areas resulting from the occlusion of blood capillaries by thrombi (Sato *et al.*, 2000). In the spleen severe atrophy of the splenic corpuscles, swollen reticular cells in the mantle zone and follicular necrosis (which is a typical lesion of CSF) are observed on histology. In pigs with persistent CSF, the most common lesions are severe atrophy of the thymus and depletion of the lymphocytes and germinal follicles in the peripheral lymphoid organs (Sato *et al.*, 2000).

2.2.14 Sample collection

For virus isolation and antigen detection, the tonsils are considered essential. In addition, submandibular and mesenteric lymph nodes, spleen, kidneys, and the distal part of the ileum should be collected. In live pigs, tonsil biopsies and whole blood collected with anticoagulants are useful to diagnose HC. Sample collection should be targeted to pigs having fever or showing other signs of the disease. Each sample of tissue should be placed in a separate plastic bag and identified. The samples should not be frozen (interference with fluorescent antibody tissue section test) but kept at refrigeration temperature. The material should be transported and stored in leak-proof containers in accordance with national regulations for transportation of diagnostic biologic samples (Carbery *et al*., 1984).

Serum samples for antibody detection should be collected from animals that have recovered from suspected infection or from sows known to have been in contact with infected or suspected cases. A sufficient number of samples should be collected to ensure a high probability of detecting infection (Terpstra, 1990).

A complete set of tissues, including the whole brain, should be submitted in 10 per cent buffered formalin (OIE, 2010).

2.2.15 Management and control of classical swine fever

Unlike ASF, CSF can be well controlled by vaccination. The most widely used vaccines are live vaccines, most of which are based on the Chinese 'C' strain. They are highly effective and safe and have been successfully used in eradication programmes in many countries (Nielen *et al*., 1999).

In countries previously free from CSF, a non-vaccination policy combined with a total stamping-out, in the case of disease outbreaks, and eventual preventive slaughter of pigs in suspect and in-contact farms, is applied as necessary. Serological surveillance is undertaken in the domestic pig population. The surveillance system and number of samples collected depends on the prevalence of CSF, the wild boar population and the epidemiological situation of neighbouring countries(Edwards *et al.*, 2000).

In countries where the disease is endemic, control is generally based on vaccination. In some countries, the decision to use vaccination depends on the ownership or on the size of the farms. Programs of vaccination can vary as can the type of vaccines used in different countries. In some countries (e.g. Russia), it is recommended to vaccinate 3-week-old piglets, whereas in others (e.g. Bulgaria, Romania) pigs are not vaccinated until 10-12 weeks of age (Edwards *et al.*, 2000).

Recommendations are placed to prohibit the importation of pigs from infected countries. Quarantine measures and restrictions on the movements of pigs need to be employed within infected countries to control the spread of the disease (Edwards *et al.*, 2000).

Other precautions include slaughter of infected herds (although this may not be possible due to financial restrictions), and establishment of protection (approximately 3 km radius) and surveillance zones (approximately 10 km radius) around infected farms to control the spread of the disease. Swill feeding needs to be regulated(Edwards *et al.*, 2000).

Effective communication between veterinary authorities, veterinary practitioners and pig farmers are recommended. The disease reporting system must be effective and the policy for the importation of live pigs, and fresh and cured pig meat should be strictly implemented. Pigs should be quarantined before admission into a herd. Waste food or swill should be banned from being fed to pigs or if it is fed it must be properly sterilised. Efficient control of rendering plants should be established. Structured serological surveillance should be undertaken that is targeted at breeding sows and boars. An appropriate pig identification and recording system should be implemented (OIE, 2010).

In areas where CSF is endemic, vaccination with modified live virus strains is recommended (Van Oirschot, 2003; Suradhat *et al.*, 2007).

To eradicate CSF from a pig population, the transmission needs to be reduced to such an extent that the virus cannot maintain itself in the population. This might be obtained by control measures including slaughtering infected herds, culling of herds at risk, vaccination, improved hygiene measures and movement restrictions (Moennig, 2000). The most important control measures are the culling of infected herds, prohibition of transport, the tracing and testing of infectious contacts, and the implementation of hygienic measures and surveillance in the affected area (Klinkenberg *et al.*, 2003). The control policy depends upon the incidence and prevalence of the infection in both the domestic and wild pig populations. In countries where CSF is endemic in domestic pigs it is common practice to adopt systematic vaccination campaigns (Moennig, 2000; Van Oirschot, 2003), accompanied by routine diagnostic procedures and control measures (Van Oirschot, 2003) to minimise serious losses of pigs from the disease (Moennig, 2000). Vaccination overcomes some of the ethical dilemmas arising from large-scale culling of pigs during an outbreak (Klinkenberg *et al.*, 2003).

Control of animal-to-animal transmission of disease agents is a key concept in infectious disease epidemiology. To reduce disease transmission, movement controls are needed to be strictly implemented and subject to legislation (Fevre *et al.*, 2006).

Vaccination against CSF has a long history, leading to the development in the 1960s of a number of highly effective live attenuated vaccines. Prophylactic vaccination is still carried out in many parts of the world (Paton and Greiser-Wilke, 2003). The disease can be effectively controlled by vaccination with the live C-strain vaccine (Kortekaas *et al.*, 2011), and pigs can be protected against infection for at least 10 months following oral vaccination with C-strain‘live virus vaccine (Kaden and Lange, 2001).

Oral vaccination of wild boar may contribute to lowering the incidence of CSF, and consequently diminishing the threat of the introduction of virus to domestic herds. Disease-free countries should not vaccinate pigs but they should be aware of the disease and have a rapid response plan to counter any incursions. Once CSF is introduced into areas with a high pig density, an emergency vaccination program should be immediately instituted, to be of maximal benefit (Van Oirschot, 2003). Recently, marker vaccines have been developed to enable the differentiation of immunity induced from natural infection and that induced by vaccination (Vannier *et al.*, 2007).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

The study was carried out in 12 out of the 23 LGAs in Benue State, namely: Apa, Gboko, Gwer-west, Katsina Ala, Kwande, Makurdi, Obi, Ogbadibo, Oturkpo, Tarka, Ukum and Vandeikya LGA's and aslaughter slab located within Makurdi metropolis (Figure 3.1). Theselected LGA'shave high pig farming activities and also had previously suffered from ASF outbreaks (El Hitcheri *et al*., 1998).

Benue State lies in the North Central zone (middle belt area) of Nigeria and derives its name from the River Benue, the second largest and longest river in Nigeria (BNSG, 2011),with a land mass of 32, 518 sq km within the Lower River Benue at Longitude 7^0 47' and 10^0 0' East, Latitude 6^0 25' and 8^0 8' North of the Equator. It is bordered byNasarawa to the north, Taraba to the east, Cross River to the south, Enugu and Ebonyi to the south-west and Kogi to the westand the Republic of Cameroun on the south-east (BNSG, 2011) (Figure 3.1).

The State has a tropical sub-humid climate, with two distinct seasons namely the wet and dry seasons, with the wet season lasting between April to October with annual rainfall wthin the range of 100-200 mm and dry season from November to March. Temperatures are generally very high during the day, particularly in March and April (BNSG, 2011).The State has an estimated human population of 4,780,389 (NPC,2006);three senatorial districtsand 1,885 villages, andmost of the people in the

State are peasant farmers while the inhabitants of the riverine areas engage in fishing as their primary occupation (BNSG, 2011).

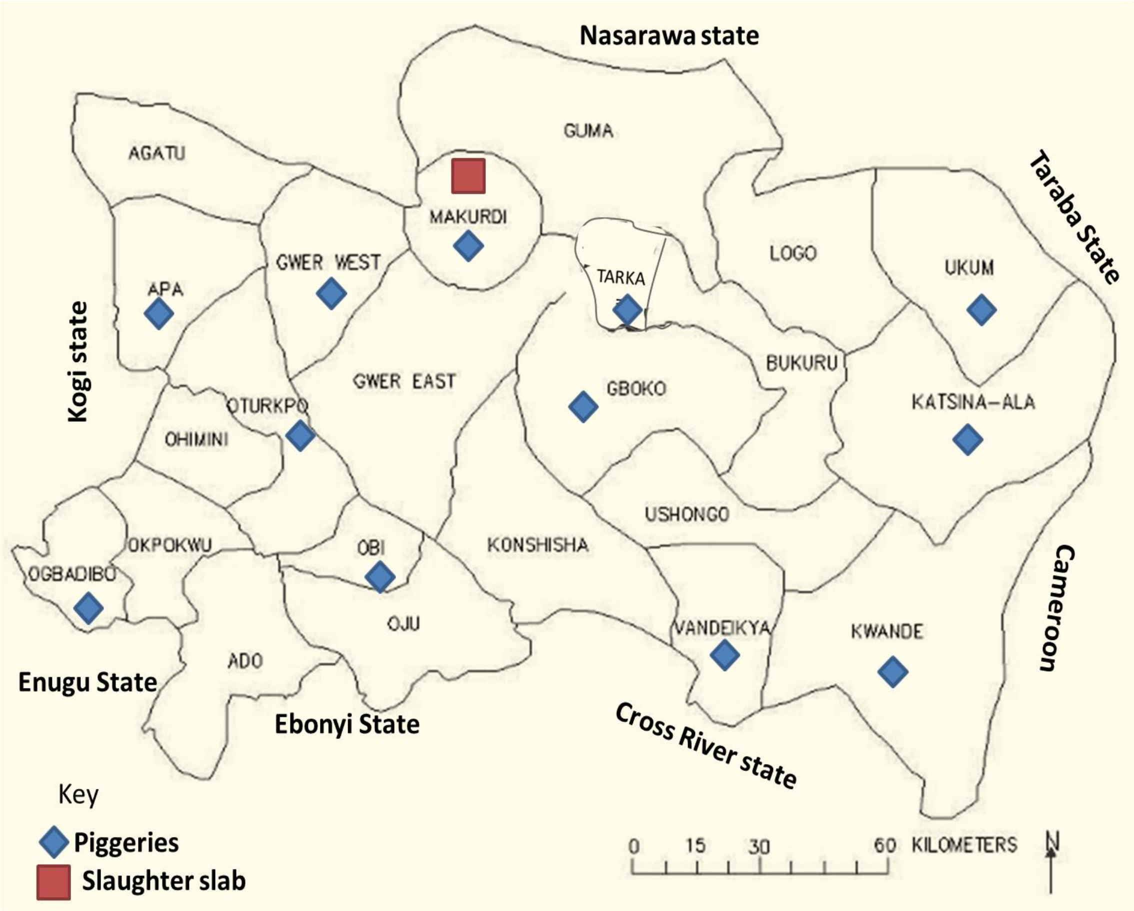

Figure 3:1: Map of Benue State showing the LGA's and locations of sampling sites
***Source**: Adapted from administrative map of Benue State 2014*

3.2 Study Design

A cross sectional study was conductedon a slaughter slab in Makurdi and 74 piggeries in 12 local governments areas (LGAs) across the 3senatorial zones;Benue North East (Katsina Ala, Kwande, Ukum and Vandeikya LGAs), Benue North West (Gboko, Gwer west, Makurdiand Tarka LGAs) and Benue South (Apa, Obi, Ogbadibo and Oturkpo LGAs) of Benue Statefrom July 2015 to December 2015.

The sampling LGAs were selected based on the availability of piggeries in the areas and willingness of the farmers to allow for their piggeries to be sampled. The number of pigssampledper piggery were also based onthe availability of pigs in the piggery and willingness of the farmers to allow for their pigs to be sampled.

3.3 Sample Size

A total of 460 sera (capacity of ELISA kits used) consisting of 44 serafrom pigs slaughtered at the Makurdi slaughter slab and 416 sera from pigs in piggeries in Benue State were used for the study.

3.4 Blood Collection

Venous blood (3 ml) was obtained from the cranial vena-cava of each pig sampled, using sterile 18G needle and 5 ml syringe into plain sample tubes and transported in ice packs to the microbiology laboratory at University of Agriculture Makurdi Veterinary Teaching Hospital. The blood samples were kept at room temperaturefor 60 minutes to clot and resultant sera were decanted into centrifuge tubes and centrifuged at 704 x g(rpm) for 20 minutes. The resultant serawere harvested and placed in sterile serum bottles and kept at -20^{0}C until used.

3.5 Questionnaire Design and Administration

A pre-testedstructured**,** interviewer-administered questionnaire was used to obtain data on demography, environmental and management factors; bio-security measures and African swine fever (ASF) and Classical swine fever (CSF) related signs. The same questionnairewas administered to respondents at piggeries and slaughter slabwhere samples were obtained (Appendix i). A respondent was someone who was actively involved in the daily activities of the piggerynot necessarily the piggery owner and also butchers solely engaged in the slaughtering of pigs.

3.6 Enzyme Linked Immunosorbent Assay Test for African Swine Fever Virus Antibodies

The serological assay was performed using the ELISA kit (ID Screen® African Swine Fever Indirectfrom IDvet, 310 rue Louis Pasteur, Grabels, France), according to the manufacturer's instructions. All reagents were brought to room temperature (21 ^{0}C) and homogenized (thoroughly mixed by inversion) before use.

One hundred and ninety (190) µl of the dilution buffer 14 was added to each well of the ELISA plate. Then 10 µl of negative control sera were added to two wells (A1 and B1) followed by the addition of 10 µl of positive control sera to wells C1 and D1,and finally 10 µl of each sample to be tested were then added to the remaining 92 wells on each of the 5 plates. The 5 plates were sealed and incubated at 21^{o}C for 45 minutes. The wells were then emptied of the reagents and washed 3 times each time with approximately 300 µl of wash solution. Then, 100 µl of the specific conjugate were added to each well on the 5 plates;the plates were sealed and incubated at 21^{o}C for 30 minutes. The wells on the 5 plates were then emptied and washed 3 times with approximately 300 µl of the wash solution with caution to avoid drying in between the washings. 100 µl of the substrate solution were added to each well and the plate incubatedin the dark (absence of light) at 21^{o}C for 15 minutes. Finally, 100 µl of the stop solution was added to each well to stop the reaction and the optical density (OD) of each well was read at 450nm using microplate reader.

In the presence of antibodies, a blue colouration appeared which became yellow after addition of stop solution whereas there was no colouration in the absence of antibodies.The resulting colouration is proportional to the quantity of specific antibodies present in the tested sample (Plate 3.1).

3.7 Enzyme Linked Immunosorbent Assay Test for Classical Swine Fever Virus Antibodies

The serological assay was performed using the ELISA kit (ID Screen®Classical Swine Fever E2 Competition from IDvet, 310 rue Louis Pasteur, Grabels, France) according to the manufacturer's instructions. All reagents were brought to room temperature (21^{o}C) and homogenized by inversion before use.

Then, 50 µl of dilution buffer 8 was added to each well followed by addition of50 µl of positive control sera to two wells (A1 and B1),50 µl of negative control sera to wells (C1 and D1) and 50 µl of each of the samples to be testedto the remaining 92 wells. The plates were sealed and incubated at 37^{o}C for 45 minutes. The wells were then emptied and washed 3 times with approximately 300 µl of wash solution. Precautions were taken to avoid drying of the wells in between washings. 100 µl of the specific conjugate were added, sealed and incubated at 21°C for 30 minutes. The wells were then emptied and washed 3 times with approximately 300 µl of the wash solution cautiously to avoid drying in between the washings. 100 µl of the substrate solution were added to each well and the plate incubatedin the dark at 21°C for 15 minutes. Finally, 100 µl of the stop solution was added to each well in order to stop the reaction and the optical density (OD) of each well was read at 450nm using microplate reader.

The resulting colouration was dependent on the quantity of specific antibodies present in the sample tested. In the absence of CSF antibodies, a blue colouration appears which becomes yellow after addition of the stop solution whereas there is no colouration in the presence of CSF antibodies in the tested sera samples (Plate 3.1).

3.8 Interpretation of Results

African swine fever: For each of the samples tested, sample-to-positive percentage (S/P%) was calculated thus:

$$\frac{OD_{sample} - OD_{NC}}{OD_{PC} - OD_{NC}} \times 100\%$$

Where OD_{sample}	optical density of the samples
OD_{NC}	optical density of negative control
OD_{PC}	optical density of positive control

And results interpreted as:

Sample-to-Positive (S/P) % ≤ 30 %	Negative
30% < S/P % < 40 %	Doubtful
S/P % ≥ 40 %	Positive

Classical swine fever: For each of the samples tested, sample-to-negative percentage (S/N %) was calculated thus:

$$\frac{OD_{samples}}{OD_{NC}}$$

Where OD_{sample} optical density of the samples

OD_{NC} optical density of negative control

And results interpreted as

Sample-to-Negative (S/N) % ≤ 50 %	Negative
50% < S/N % ≤ 60 %	Doubtful
S/N % > 60 %	Positive

3.9 Test Validation

African swine fever: The test was validated where the mean value of the Positive Control OD (OD_{PC}) was greater than 0.350 (OD_{PC}> 0.35) and the ratio of the mean values of the Positive and Negative Control ODs (OD_{PC} and OD_{NC}) were greater than 3 (OD_{pc}/OD_{Nc}> 3).

Classical Swine Fever: The test was validated where the mean value of the Negative Control (OD_{NC}) was greater than 0.7 (OD_{NC}> 0.7) and the mean value of the positive control (OD_{PC}) was less than 30% of the OD_{NC} (OD_{PC}/OD_{NC}< 0.3)

3.10 Data Analysis

Data obtained from serological tests and questionnaire were analysed usingthe Statistical Package for Social Sciences (SPSS) version 20.0. Descriptive statistics and univariate analysis(Chi square and Fisher's Exact Test) were used to test for association between categorical variables. P values ≤ 0.05 were considered significant.

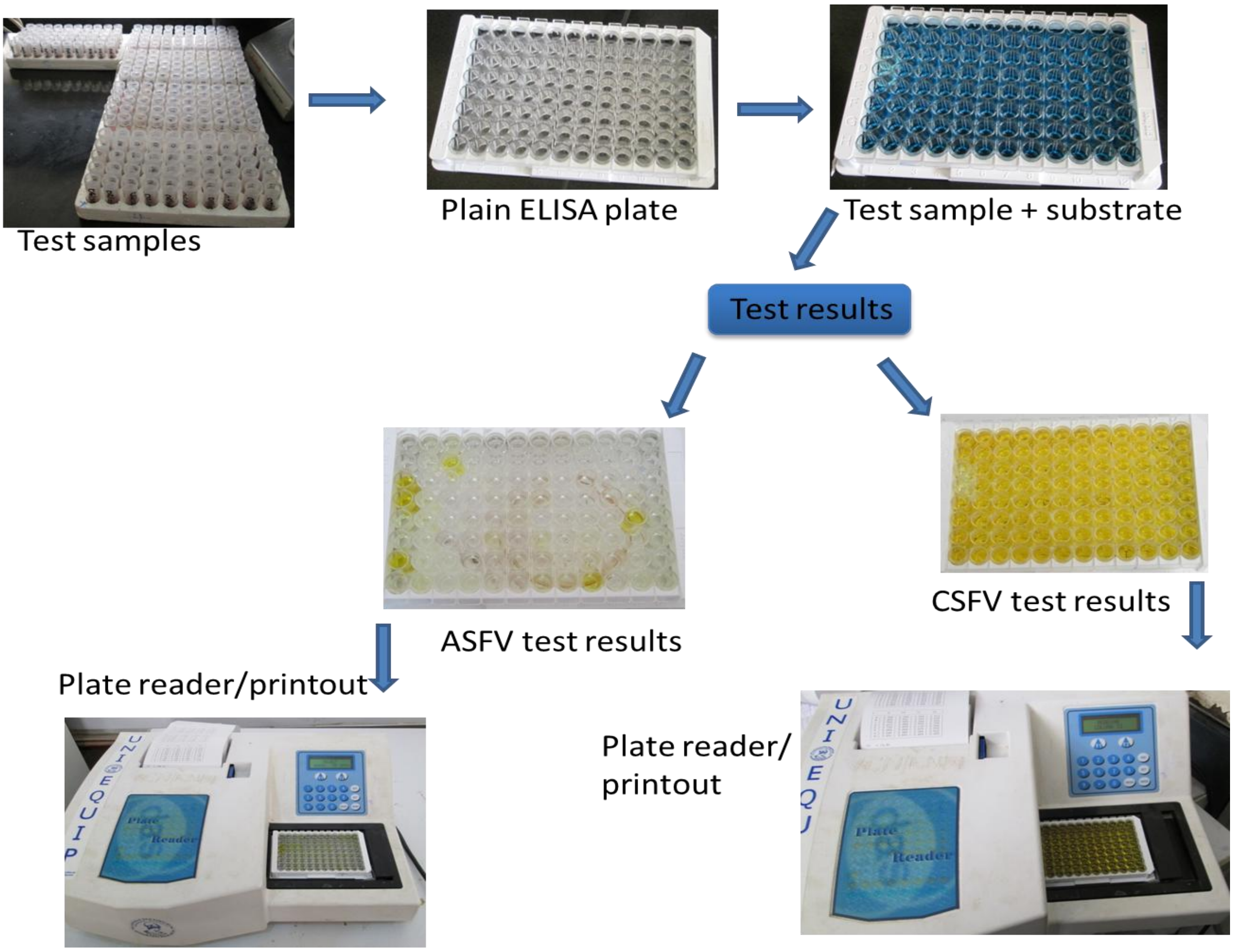

Plate 3:1: ELISA Test Kits for African Swine Fever and Classical Swine Fever

CHAPTER FOUR

RESULTS

4.1 African Swine Fever and Classical Swine FeverAntibody Detection Rates in Piggeries and Makurdi Slaughter Slab

A total of 416 and 44 pigs were respectively sampled in 74 piggeries and Makurdi slaughter slab. The ASFVantibody detection rate was higher in pigs sampled in the slaughter slab (13.6%) than the pigs sampled in the piggeries (1.7 %) and the detection rate was significantly associated with the pigs sampled in differentlocations($p < 0.0001$) (Table 4.1).

All the pigs sampled in piggeries and at the Makurdi slaughter slab were negative for CSFV antibodies (Table 4.1).

Table 4:1African swine fever and classical swine fever antibody detection rates in piggeries and in makurdi slaughter slab

Category	**Number of pigs sampled**	**African swine fever +ve (%)**	**Classical swine fever + (%)**
Piggeries	416	7 (1.7)	0 (0.0)
Makurdi slaughter slab	44	6 (13.6)	0 (0.0)

χ^2 = 20.074, df = 1, p = 0.000

4.2 African Swine Fever and Classical Swine FeverAntibody Detection Rates in Pigs byLocal Government Areas

A total of 416 pigs were sampled from piggeries in the selected twelve LGAs and7 (1.7 %) were positive for ASF (Table 4.2).

The detection rates indicates that(4.8 %) were positive for ASF in Obiwith the highest among the LGAs; Kwande had (4.0 %); Vandeikya (3.0 %); Ukum (2.5 %); Makurdi (2.3 %)and Gboko (2.0 %).The LGAs of Apa, Gwer West, Katsina Ala, Ogbadibo, Oturkpo and Tarka all had (0.0 %) detection ratesand the detection rate was significantly associated with pigs sampled in the different LGAs ($p < 0.0001$)(Table 4.2).

All the pigs in the LGA's sampledwere negative for CSFVantibodies (Table 4.2).

Table 4:2: African swine feverand classical swine feverantibody detection rates in pigs by local government areas

Category	Number of pigs sampled	African swine fever+ve (%)	Classical swine fever+ve (%)
Apa	28	0 (0.0)	0 (0.0)
Gboko	50	1 (2.0)	0 (0.0)
Gwer west	27	0 (0.0)	0 (0.0)
Katsina Ala	35	0 (0.0)	0 (0.0)
Kwande	50	2 (4.0)	0 (0.0)
Makurdi	44	1 (2.3)	0 (0.0)
Obi	21	1 (4.8)	0 (0.0)
Ogbadibo	29	0 (0.0)	0 (0.0)
Oturkpo	36	0 (0.0)	0 (0.0)
Tarka	23	0 (0.0)	0 (0.0)
Ukum	40	1 (2.5)	0 (0.0)
Vandeikya	33	1 (3.0)	0 (0.0)
Total	**416**	**7 (1.7)**	**0 (0.0)**

Fisher's Exact Test = 210.272, df = 11, p = 0.000

4.3 African Swine Fever and Classical Swine FeverAntibody Detection Rates in Pigs bySex, Age, Breed and Management Systems

A total of 160 male and 256 female pigs were sampled in piggeries in which (1.3%) males and (2.0 %) females were positive for ASFV antibodies and the association was not significant ($p > 0.05$)(Table 4.3).

Considering age groups, pigs of age group > 6 months had the highest ASFV antibody detection rate (2.2%) than pig from age group 1 – 6 months (0.0 %)and the association was not significant ($p > 0.05$)(Table 4.3).

Of the breeds sampled, exotic breeds (2.4%) had higher ASFV antibody detection rate than cross breed(1.9%) and local breeds 0 (0.0%) respectively and the association was not significant ($p > 0.05$) (Table 4.3).

A total of 54 and 362 pigs were sampled from pigs raised under intensive and semi-intensive system of managements respectively. ASFV antibody detection rate was higher under intensive management system (3.7%) than semi-intensivesystem(1.4 %) and the association was not significant ($p > 0.05$)(Table 4.3).

All the pigs in these categories sampled were negative for CSFV antibodies(Table 4.3).

Table 4:3African swine fever and classical swine fever antibody detection rate in pigs bysex, age, breed and management systems

Category	Total sampled	ASF +ve (%)	Fisher's Exact Test for ASF/p-value	CSF +ve (%)
Sexofpigs				
Male	160	2 (1.3)	FET = 0.294,	0 (0.0)
Female	256	5 (2.0)	df = 1	0 (0.0)
Total	**416**	**7 (1.7)**	p = 0.712	**0 (0.0)**
Age of pigs				
□ 6 months	100	0 (0.0)	FET = 2.253,	0 (0.0)
> 6 months	316	7 (2.2)	df = 1,	0 (0.0)
Total	**416**	**7 (1.7)**	p = 0.204	**0 (0.0)**
Breeds of pigs				
Exotic	85	2 (2.4)	FET = 1.255,	0 (0.0)
Cross	260	5 (1.9)	df = 2,	0 (0.0)
Local	71	0 (0.0)	p = 0.561	0 (0.0)
Total	**416**	**7 (1.7)**		**0 (0.0)**
Management systems				
Intensive	54	2 (3.7)	FET = 1.528,	0 (0.0)
Semi-intensive	362	5 (1.4)	df = 1,	0 (0.0)
Total	**416**	**7 (1.7)**	p = 0.227	**0 (0.0)**

FET = Fisher's Exact Test

4.4 Risk Factors Associated with African Swine Fever and Classical Swine Fever

In the univariate analyses, location of piggerieswithin 1 km radius of a slaughter slaband ASF infection were significantly associated ($p < 0.0001$)and had 9.2(95 % CI 3.0 – 28.8)chances of ASF infection (Table 4.4).

Statistically significant association ($p < 0.05$)was observedin the location of piggeriesnear refusedumpsitesand had 3.0 (95 % CI 1.0 – 9.5) chances of ASF infection(Table 4.4).

Farms where farm workers wear their work clothes outside of the piggerypremises was also significantly associatedsignificantly associated ($p < 0.0001$)with ASF infection 0.2 (95 % CI 0.1 - 0.7) (Table 4.4).

The rest of the other estimated risk factors wereeither constant or not significantly associated($p > 0.05$)with ASF infection(Table 4.4). There was no statistical computation for CSF because all the factors were constant, (Table 4.4).

Table 4:4: Risk factors associated with African swine fever and classical swine fever

Category	OR(95% CI)	χ^2/p-values	Remarks
Slaughterslab within 1 km radius of piggery	9.2(3.0 - 28.8)	χ^2 = 20.704/p = 0.000	Significant
Nearby refuse dumpsitesto piggery	3.1(1.0 - 9.5)	χ^2 = 4.458/p = 0.035	Significant
Wearing of work clothes outside of the piggery premises	0.2(0.1 - 0.7)	χ^2 = 7.179/p = 0.007	Significant
Sharing of farm workers with other piggeries	Constant	Constant	Constant
Sharing of working utensils with other piggeries	Constant	Constant	Constant
Source of replacement stock	1.3(0.3 – 6.1)	χ^2 = 0.121/p = 0.728	Insignificant
Feeding of swill to pigs	0.5(0.1 - 2.2)	χ^2= 0.910/p = 0.340	Insignificant
Nearby piggery within 1 km radius of each other	1.3(0.3 – 6.1)	χ^2= 0.135/p = 0.714	Insignificant
Presence of functional foot dip on the piggery	1.0 (0.1 - 7.5)	χ^2 = 0.002/p = 0.962	Insignificant
Presence of ticks on pigs	0.7(0.2-3.2)	χ^2= 0.210/p = 0.647	Insignificant
Piggery perimeter fencing	0.7(0.1 - 5.6)	χ^2= 0.108/p = 0.743	Insignificant

4.5 Level of Compliance with Sanitary Measures Observed in Piggeries

All the 74 piggeries sampledhad quarantine or isolation unit within 100 m radius of the regular pig pen, 17 (23%) of the sampled piggeries had designated working clothes, 27 (36.5%) of the piggery workers in the sampled piggeries had bath at work. 62 (83.8%) of the sampled piggeries did receive or lent out service boars; 12 (16.2%)piggeries disinfected their pen floors daily, 12 (16.2 %) had routine pest control; 74 (100%)all of the sampled farms cleaned their working utensils daily and only 12 (16.2%) of them had designated footwear for their workers (Table 4.5)

It was observed that respondents generally took very poor sanitary measuressuch as lack of functional foot dip and routine cleaning of the pen and; had no measures in place such as proper hygienic practices to prevent their pigs from getting infected with ASF and CSF.

Table 4:5: Level of Compliance with Sanitary Measures Observed in Piggeries

Measures	Numberof piggeries observing measures (Yes %)
Quarantine section within 100m of the main piggery	74 (100)
Designated clothes for the piggery	17 (23.0)
No of piggeries where workers bath in the piggery after work?	27 (36.5)
Lend out service boars	62 (83.8)
Clean (wash/sweep) pen floor daily	12 (16.2)
Disinfect pen floor daily	12 (16.2)
Clean (wash) work utensils daily	74 (100)
Carcass burial within 1km	74 (100)
Piggery designated footwear	12 (16.2)
Routine pest control	12 (16.2)
Access by stray animals	12 (16.2)
Presence of rodents on the piggery	74 (100)

4.6 Respondents Level of Awarenessof African Swine Fever and Classical Swine Feverin Benue State

A total of 80 respondents were interviewed, 6 (7.5%) were from a slaughter slab while 74 (92.5 %) were from the piggeries. All 80 (100%) respondents admitted to being aware of ASF but only 23 (28.8%) were aware of CSF. No statistics were computed because awareness of ASF was a constant.The most common ASF related signs mentioned by the respondents were hyperaemia (reddening of skin mostly ears and snout) (67.5%)) followed by weakness or unwillingness of the pigs to stand (17.5 %) and abortion (15 %)in that order (Table 4.6).

Table 4:6: Respondents Level of Awarenessof African swine feverand classical swine feverin Benue State

Category	No of respondents	Yes(%)
Awarenessof ASF/CSF		
Have you ever heard of ASF?	80	80 (100)
Have you ever heard of CSF?	80	23 (31.1)
ASF signs mentioned	**No of respondents**	**Nothat mentioned signs**
Abortion	80	12 (15.0)
Hypaeremia	80	54 (67.5)
Weakness	80	14 (17.5)

4.7 Distribution ofrespondents qualification, sex, occupation and years of practice

A total of 460 pigs were sampled and also 80 respondentsinterviewed in selected LGA's and Makurdi central slaughter slab in Benue State to ascertain the risk factors which attributed to the occurrence of ASF. Of the 460 pigs sampled, 416 (90.4%) were from pig farms while 44 (9.6%) were sampled in Makurdi slaughter slab (Table 4.7).

Of the 80 respondents interviewed,74 (92.5%) were from piggeries while 6 (7.5%) were from Makurdi slaughter slab; 64 (80%)were males and 16 (20%) females;6 (7.5%) had no formal education; while 24 (13.8%); 39 (48.8 %)and 11 (30.0 %) had primary education, secondary education and tertiary education respectively (Table 4.6).Also, of the 80 respondents interviewed, 46 (57.5 %)were peasant farmers, 13 (16.2%) were farm attendants, 6 (7.5 %) were butchers while 15 (18.8 %) were civil servants (Table 4.7).

Similarly, of the 80 respondents interviewed, 20 (25.0%) had practiced for less than 5years, 34 (42.5 %) had practiced within the range of 5 to 10 years while 26 (32.5%) had practiced for more than 10 years as either pig farmers or butchers respectively (Table 4.7).

Table 4:7:Distributionof respondentsqualification,sex, occupation and years of practice.

Category	Frequency(%)
Qualification of respondents	
No formal education	6 (7.5)
Primary education	24 (13.8)
Secondary education	39 (48.8)
Tertiary education	11 (30)
Total	**80 (100)**
Sex of respondents	
Male	64 (80)
Female	16 (20)
Total	**80 (100)**
Occupation of respondents	
Piggery attendants	13 (16.2)
Butchers	6 (7.5)
Civil servants	15 (18.8)
Peasant farmers	46 (57.5)
Total	**80 (100)**
Yearsofpracticebyrespondents	
< 5years	20(25.0)
5–10years	34 (42.5)
>10years	26 (32.5)
Total	**80 (100)**

4.8 Distribution of pigs by System of management of piggeries, Breeds of pigs raised by piggeries, Herd size of piggeries, Duration of piggery existence, Previous ASF or CSFoutbreaksand Accessibility of piggeriesto potable water.

Of the 74 piggeries sampled, 12 (16.2 %) raised pigs under intensive system, 57 (77.0%) raised pigs under semi-intensive system and 5 (6.8%) raised pigs under extensive system (Table 4.8).

Of the 74 sampled piggeries, 11 (14.8%) raised exotic (pure) breed of pigs, 46 (62.2%) raisedcross breeds and 17 (23.0%) raisedlocal pigs (Table 4.8).

Fifty nineof the piggeries (79.7%) were small holder(< 50 pigs) herds, 9 (12.2%) were of medium holder (50 – 100 pigs) herds while 6 (8.1%) were large holders (> 100 pigs) herds (Table 4.8).

Of the 74 sampled piggeries, 17 (23.0%) had existed for < 5 years, 56 (75.6%) for between 5-10 years while 1 (1.4%) hadbeen in existence for more than 10 years (Table 4.8).

Regarding ASF and CSF outbreaks, only 12 (15.0 %) of the piggeries had previously encountered ASF outbreakwhile the remaining 62 (85.0%) had no previous ASF outbreak. None was reported for CSF(Table 4.8).

Most of the sampled piggeries 43 (58.1%) had no access to potable water while 31 (41.9%) had access to potable water (Table 4.8).

Table 4:8: Distribution of pigs by System of management of piggeries, Breeds of pigs raised by piggeries, Herd size of piggeries, Duration of piggery existence, previous African swine fever and classical swine fever outbreaksand Accessibility of piggeries to potable water.

Category	Frequency (%)
System of management of the piggeries	
Intensive	12 (16.2)
Semi-intensive	57 (77.0)
Extensive	5 (6.8)
Total	**74 (100)**
Breeds of pigs raised by piggeries	
Exotic	11 (14.8)
Crosses	46 (62.2)
Local	17 (23.0)
Total	**74 (100)**
Herd size of piggeries	
Smallholding (>50)	59 (79.7)
Medium (50-100)	9 (12.2)
Large (>100)	6 (8.1)
Total	**74 (100)**
Duration ofexistence of the piggeries	
< 5years	17 (23.0)
5–10years	56 (75.6)
>10years	1 (1.4)
Total	**74 (100)**
Previous ASF outbreak in the piggeries	
Yes	12 (15.0)
No	62 (85.0)
Total	**74 (100)**
Access to potable water by piggeries	
Yes	31 (41.9)
No	43 (58.1)
Total	**74 (100)**

CHAPTER FIVE

DISCUSSION

African swine fever virus antibodies were detected in screened sera from pigs in Benue State. ASF has been reported in pig farms in Nigeria and considered endemic (Odemuyiwa *et al.*, 2000;Otesile *et al*., 2005; Olugasa, 2007;Owolodun *et al*., 2007; Kperegbeyi and Amotsuka, 2010; Owolodun *et al.*, 2010 a, b, c;Sake *et al.*, 2010;Fasina *et al.*, 2012).

The detection rate of 1.7 % in piggeries was lower compared to the reported prevalence of 13.2% by Abwage *et al*. (2015) in Taraba State in which serum samples and ELISA were similarly used and this may be an indicationof some improvement in the control measures by individuals to eradicate the disease, as well as improved sanitary measure or hygiene and feeding methods that may differ in other studied areas that observed higher prevalence. Movement of pigs and pig products across borders from those infected areas had been reported to positively correlate with ASF seropositivity and outbreaks (Costard*et al*., 2009; Penrith and Vosloo, 2009) and thus was supported by the occurrence of ASFV seropositive pigs in Taraba State (Abwage *et al*.,2015) as well as inBenue State. This is also in line with the theory of geographical contiguity as had been proposed and thus supported by the incidence of infections in Kaduna/Plateau, Benue/Enugu and Osun/Ogun/Lagos during the 1997-1998 ASF outbreak and; Kwara/Oyo and Akwa-Ibiom/Rivers/Delta states during the 1999-2000 ASF outbreak (Fasina *et al*., 2012).

Similarly, the ASF detection rate in piggeries was far lower when compared to the reported prevalence of 65.2% by Olugasa (2007) and 28% by Awosanya *et al*. (2015) in commercial pig herds in south western Nigeria and 88% by Saka*et al.* (2010) in Lagos State respectively. The differences could be explained by the fact that previous ASF outbreaks in Nigeria, mostly, had originated from the south-western part of Nigeria and spread especially to the north central states strictly following the trade routes of pigs inthe country (El-Hicheri, 1998; Fasina *etal*., 2009) hence south-western states are suspected to be more endemic.

The higherASF detection rate of 13.6% observed in slaughtered pigs compared to 1.7%in piggeries could be due to the fact that most of the slaughtered pigs at Makurdi central slaughter slab were not only raised within Benue State but also came from other states like Nasarawa, Plateau, Kaduna, Adamawa and Taraba, most of which might have been pushed to the market following suspected ASF outbreak and consequently might have harboured the ASF virus (El-Hicheri, 1998; Fasina *et al*., 2012; Abwage *et al*., 2015). Also, this higher detection rate observed in slaughtered pigs could be attributed to pig farmers practices where during active ASF outbreaks, they often will not report to authorities but will quickly sell off pigsbefore they die and similarly cull off first for slaughter the unthrifty and sick pigs(Randriamparany *etal*., 2005; Babalobi *etal*., 2007; Costard *etal*., 2009b; Fasina *etal*., 2010).Fasina *etal*. (2009) had previously reported of higher ASF seroprevalence around the abattoir compared to pig farmsin Nigeria.

The detection of antibodies to ASFV in pigs in all the three senatorial districts and in slaughtered pigs at Makurdi central slaughter slab confirmed the presence of ASF and may suggest its endemicity (Costard *et al*. 2013)in Benue State. ASF have been considered endemic in Taraba state (Abwage *et al*., 2015); Delta State (Kperegbeyi and Amotsuka, 2010); Plateau State (Owoludun *et al.*, 2010); Lagos State (Saka *et al.*, 2010);south-western Nigeria (Olugasa, 2007); all major pig producing areas in Nigeria (Fasina *et al.,* 2012) and indeed across Nigeria (Odemuyiwa*et al.,* 2000).

The higher ASFVantibody detection rate observed in female than male pigs was not significant and contrasted the findings of Abwage *et al*. (2015) who reported higher pevalence in male pigs in Taraba state. The higher ASFVantibody detection rate in female could be explained by the fact that more female pigs are kept for breeding purposes and stay for longer period in the herds, while the male pigs are fattened and sold off except for a few that are kept as breeding boars and for other reasons (Lammers *et al*., 2007).

In this study, pigs of age group >6 months showed a higher rate of antibodies to ASFV than pigs of age group < 6 months. This could possibly be due to long persistence of ASFV antibodies for a period of time after exposure (Carrillo *etal*., 1994). There could also be differences in ASFV transmission rates among the various age groupsas reported by Olugasa (2007) and Awosanya *et al*. (2015) who also observed higher rates in older stock.

Highest ASFVantibody detection rate was observed in exotic breeds of pigs thancross and local breeds.This could possibly be due to the fact that local and cross breed pigs may be hardy with the ability to withstand or resist common diseases of swine like ASF more than the exotic breeds that may be naïve and succumb easily.Though, attempted breedingfor resistance to ASF by crossbreeding domestic pig resistant species has been with limited success(FAO, 2005).

The high ASF detection rate observed under intensively managed system could be explained by the poor hygienic practices observed in the visited piggeries. Fasina *et al*. (2012) suggested that purchasesof pigs routinely without screening during stocking or replacement and more regular individual contacts could be responsible for high rates of ASF seropositivity in confined farms in Nigeria.

The statistically significant association between ASF seropositivity and location of piggerieswithin 1 km radius of pig slaughter slabmay be as a result of pig farmers presentingsick and unthrifty pigs for slaughter at abattoirs firstwithout determining the cause of sickness, to which some may be ASF(Randriamparany *etal*., 2005; Fasina *etal*., 2010)thus contributing to ASF spread in nearby piggeries (Costard *etal*., 2012b). Since ASF virus is presentin tissues and body fluids of slaughtered sick pigs, massive environmentalcontamination and possiblenearby piggery infection may result.Similarly,rodents and wild birds are usually observed around open slaughter slabsenvironmentand they carry away intestinal content and viscera, which someare infectious and are disposed of indiscriminately to nearby piggeriesthusfacilitating the infection of naïve pigs.Also, farmers often participate in various processes on slaughter slab floors with the consequent risk of carrying the virus to their piggerieswith resultant infection as reported by Fasina *et al*., (2012)in major pigproducing areas in Nigeria.

The statistically significant association observed in the ASF seropositivity of piggeries located near refusedumpsites or carcass disposal siteagrees with the findings of Awosanya *et al*., (2015) andmay be relateddirectly to a local spread between and within piggeries and may occur throughdirect pig-to-pig contact, orby fomites especially in scavenging populations and possibly by stray animals such as dogs and pigs gaining access to the nearby piggeries (Fasina *et al*., 2012).

The high positive association between wearing of farm clothes outside and ASF occurrence is in agreement with similar observation by Awosanya *et al*., (2015) and couldnot be explained by the fact that ASF is reported to be transmitted by indirect contact through fomites though this mode of transmission is said to be efficient in a very high viral load(Mur *etal*., 2012d; de Carvalho *et al*., 2013).The movement of work clothes and contacts in and out of the farm at regular interval is suspected to serve as a vehicle for transmission.

The difference observed in the level of compliance with the assessed sanitary measures between seropositive and seronegative piggeries in this study is in agreement with previous report by Awosanya *et al*., (2015).

Respodents also admitted to high level of awareness ofASF but didnot adopt the basic hygienic and sanitary practices.However, Adesehinwe *et al*., (2003); Mashatise *et al*., (2005);Halimani *etal*., (2007); Ironkwe and Amefule, (2008); Kagira *etal*., (2010); Karimuribo *etal*., (2011); Moreki and Mphinyane, (2011);Nwanta *etal*., (2011); Petrus *etal*., (2011) and Muhanguzi *etal*., (2012)suggested lack of adequate knowledge and information about pig production and health as constraints to improved managementpractices in piggeries.

The signs of hyperaemia, weakness or unwillingness of the pigs to stand followed by abortionmentioned by the respondents though, may or may not be due to ASFshowsthe ability of the farmers to recognize such associated signs and therefore assist in early detection of an ASF infection. However, this maybe impeded by the farmers' unwillingness to report outbreaks which possibly could be explained by the adjudged low compensation by the government, if any, for the culling of affectedpigsbut it should be noted that early detection and reporting is critical to ASF control and eradication(Sanchez-Vizcaino, 2010) and hence the farmers should be made aware of this.

There is no evidence that CSF is present in Benue state at this time which is in contrast to the findings of Aiki-Raji *et al* (2014). This could be due to an altered trade routes of pigs (Bagidi, I. 2016. per personal communication) from the usual south-western Nigeria up north especially to the north central states (El-Hicheri, 1998; Fasina *etal*., 2010) particularly Benue State. Thus, this may even take longer period for the CSF and indeed any other disease of pigs reported in south-west, Nigeria to spread up north faster hence the absence of CSF in Benue State.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

This study concluded that:

i. Antibodies to ASFVexist in pigs in piggeries in Benue Stateat the rate of 1.7%
ii. Antibodies to ASFV existin pigs slaughtered at Makurdi slaughter slab at the rate of 13.6 %
iii. Antibodies to CSFV were absent in pigs in piggeries and in pigs slaughtered at Makurdi slaughter slab in Benue State
iv. Location of slaughter slab within 1 km radius of piggery; presence of refuse dumpsites and wearing of designated work clothes outside the piggery premises were risk factors identified in this study.
v. Sanitary measures in piggeries were generally poorin Benue State.
vi. Respondents were aware ofASF(100%) but less aware of CSF(31.1%) in Benue State

6.2 Recommendations

This studyrecommended that:

i. Further studiesbe carried out to investigate possible sources of infection in ASFV positive pigs and also characterise the ASF-virus circulating in Benue State.

ii. Adherence to hygienic and proper sanitary measures in piggeries, routinesurveillance and monitoring of ASF and CSF antibodiesin pigs in Benue State isrecommendedto provide a comprehensive and readily accessible data base on these diseases to plan for the prevention of any fulminating outbreak.

REFERENCES

Abreu E. F. deValadão F. G, Limpo Serra J. J. B, Ornelas Mário R., and Sousa Montenegro. (1962). Peste suína africana em Moçambique. *Anaisdos Serviços Veterinárias de Moçambique,*8:105-123.

Abwage S.A, Umaru G.A., Musa Y.B., Adamu Z., Akensire U.A., Njobdi A.B and Bello O.A, (2015). Detection of African swine fever virus (ASFV) antibodies in pigs in Taraba state north east Nigeria. *SokotoJournalofVeterinarySciences,* 13:20-25.

Adesehinwa, A.O.K., Aribido, S.O., Oyediji, G. O and Obiniyi, A. A. (2003). Production strategies for coping with the supply and demand of pork in some peri-urban areas of South-western Nigeria. *Livestock Research for Rural Development*,15(10):126-129.

Aguero, M., Fernandez, J., Romero, L J., Sanchez-Mascaraque, C., Arias, M. and Sanchez- Vizcaino, J. M. (2003). Highly sensitive PCR assay for routine diagnosis of African swine fever virus in clinical samples. *Journal of Clinical Microbiology*,41:431–434.

Agüero M., Fernández J., Romero L. J., Zamora M. J., Sánchez C., Belák S., Arias M and Sánchez-Vizcaíno J.M. (2004). A highly sensitive and specific gel-based multiplex RT-PCR assay for the simultaneous and differential diagnosis of African swine fever and Classical swine fever in clinical samples. *VeterinaryResearch*, 35:551-563.

Aiki-Raji C.O., Adebiyi A.I., Adeyemo I.A., Fagbohun O.A and Oluwayelu D.O. (2014). Seroprevalence of Classical Swine Fever Antibodies in Slaughtered Pigs at Bodija Municipal Abattoir, Ibadan, South West Nigeria. *European Journal of Scientific Research*, 126(4): 402-407.

Albina, E., Mesplède, A., Chenut, G., Le Potier, M.F., Bourbao, G., Le Gal, S., and Leforban, Y. (2000). A serological survey on classical swine fever (CSF), Aujeszky's disease (AD) and porcine reproductive and respiratory syndrome (PRRS) virus infections in French wild boars from 1991 to 1998. *Veterinary Microbiology*,77(1-2): 43-57.

Allaway, E., Chinombo, D., Edelsten, R., Hutchings, G. and Sumption, K. (1995). Serological study of pigs for antibody against African swine fever virus in two areas of southern Malawi. *Revue Scientifique et Technique Office*,14(3): 667–676.

Anderson, E. C., Hutchings, G. H., Mukarati, N and Wilkinson, P. J. (1998). African swine fever virus infection of the bushpig (*Potamochoerusporcus*) and its significance in the epidemiology of the disease. *Veterinary Microbiology*,62(1): 1–15.

Arias Mand Sánchez-Vizcaíno J.M. (1992). Manual de diagnóstico serológico de la peste porcina africana. *Monografías INIA*, 83:5-44.

Arias M. and Sanchez-Vizcaino J.M (2002). African swine fever eradication: The Spanish model. In: Morilla A. *etal*. (eds), Trends in Emerging Viral Infections of Swine, 1st edition. Iowa State University Press, Iowa, USA. pp. 133-139.

Arias, M., Sanchez-Vizcaino, J.M., Morilla, A., Yoon, K. J and Zimmerman, J. J. (2002). African swine fever. *TrendsinEmergingViralInfectionsofSwine*, Iowa State Press, Ames, pp. 119-124.

Arnot, L., du Toit, J. T and Bastos, A.D.S. (2009). Molecular monitoring of African swine fever virus using surveys targeted at adult Ornithodoros ticks: a re-evaluation of Mkuze Game Reserve, South Africa. *OnderstepoortJournalofVeterinaryResearch*, 76(4): 385-392.

Astigarraga A., Oleaga-Pérez, A., Pérez-Sánchez, R and Encinas-Grandes, A. (1995). A study of the vaccinal value of various extracts of concealed antigens and salivary gland extracts against Ornithodoros erraticus and Ornithodoros moubata. *VeterinaryParasitology*, 60: 133-147.

Ate, I. U and Oyedipe, E.O. (2011).Sow Reproductive Performance in Institutional Herds in Benue State, Nigeria. *JournalofReproductionandInfertility*, 2(2): 24-31.

Australian Veterinary Plan (2009). "Disease Strategy for Classical swine fever." http://www.animalhealthaustralia.com.au/fms/Animal%20Health%20Australia/ AUSVETPLAN/CSF3_0-11PROOF%2827Aug09%29.pdf. (Accessed on March 20, 2016).

Awa, D.N., Njoya, A., Ngo Tama, A. C and Ekue, F.N., (1999). The health status of pigs in Northern Cameroon. *Revue d Elevage et de Medecine Veterinaire des Pays Tropicaux*, 52: 93-98.

Awosanya E.J., Olugasa B., Ogundipe G and Grohn Y. P. (2015): Seroprevalence and risk factors associated with African swine fever on pig farms in southwest Nigeria. *BMCVeterinaryResearch*,11:133.

Babalobi O.O, Ayoade G.O., Olugasa B. O., Oluwayelu D. O and Oyedela O. (2003). Differential diagnosis of a swine epizootic of unknown aetiology in Ibadan, Oyo State, Nigeria. *Isreal Journal of Veterinary Medicine*, 58(2/3):86-89.

Babalobi O.O, Olugasa B.O, Oluwayelu D.O, Ijagbone I. F., Ayoade G.O and Agbede S.A (2007). Analysis and evaluation of mortality losses of the 2001 African swine fever outbreak, Ibadan, Nigeria. *Tropical Animal Health Production*, 39(7):533-542.

Bartak, P and Greiser-Wilke, I. (2000). Genetic typing of classical swine fever virus isolates from the territory of the Czech Republic. *Veterinary Microbiology*,77(1/2): 59-70.

Bastos, A. D. S., Penrith, M. L., Crucière, C., Edrich, J. L., Hutchings, G., Roger, F., Couacy-Hyman, E. and Thomson, G. R. (2003). Genotyping field strains of

African swine fever virus by partial p72 gene characterisation. *Archives of Virology*,148: 693-706.

Bastos, A.D., Penrith, M. L., Macome, F., Pinto, Fand Thomson, G. R. (2004). Co-circulation of two genetically distinct viruses in an outbreak of African swine fever in Mozambique: no evidence for individual co-infection. *VeterinaryMicrobiology*, 103(3/4): 169-182.

Bastos, A. D. S., Arnot, L. F., Jacquier, M. D. and Maree, S. (2009). A host species-informative 1220 internal control for molecular assessment of African swine fever virus infection rates in the African sylvatic cycle Ornithodoros vector. *Medical and Veterinary Entomology*,23(4): 399-409.

Beals, T., Downey, W., Cowart, W., and Young, S.H. (1970). "A report on the involvement of markets in the spread of hog cholera." Paper presented at the 74th Annual meeting of the United States Animal Health Association, pp. 67-84.

Beer, M., Reimann, I., Hoffmann, B and Depner, K. (2007). Novel marker vaccines against classical swine fever. *Vaccine,* 25(30): 665-670.

Belak, S. (2005). The molecular diagnosis of porcine viral diseases: a review. *Acta Veterinaria Hungarica*,53:113-124

Beltran Alcrudo, D., Guberti, V., de Simone, L., DeCastro, J., Rozstalnyy, A., Dietze, K., Wainwright, S and Slingenbergh, J. (2009). African swine fever spread in the Russian Federation and the risk for the region. EMPRES FAO, Rome, pp. 9

Benue State Government (BNSG) (2011) History, tourist attraction, hotels and travel information. http//www.cometonigeria.com/search-by region/north-central/benue-state.

Birch, R.R. (1917). Hog Cholera transmission through infected pork. *American Veterinary Journal*,51: 303.

Bjorklund, H., Lowings, P., Stadejek, T., Vilcek, S., Greiser-Wilke, I., Paton, D. and Belak, S. (1999). Phylogenetic comparison and molecular epidemiology of classical swine fever virus. *VirusGenes*, 19:189-195.

Blacksell, S. D., Khounsy, S., Boyle, D. B., Gleeson, L. J., Westbury, H. A. and Mackenzie, S. (2005). Genetic typing of classical swine fever viruses from Lao PDR by analysis of the 5' non-coding region. *VirusGenes*, 31:349-355.

Blome, S., Meindl-Böhmer, A., Loeffen, W., Thuer, B and Moennig, V. (2006). Assessment of classical swine fever diagnostics and vaccine performance. *Revue scientifique et technique*,25(3): 25-38.

Blome S.,Gabriel, C and Beer, M. (2013). Pathogenesis of African swine fever in domestic pigs and European wild boar. *VirusResearch*, 173(1): 122-130.

Blood D.C and Radostits O.M. (1989). African swine fever. In: Veterinary medicine. 7th Edition, pp. 799-803

Boinas, F.S., Hutchings, G.H., Dixon, L. K and Wilkinson, P. J. (2004). Characterization of pathogenic and non-pathogenic African swine fever virus isolates from Ornithodoros erraticus inhabiting pig premises in Portugal. *Journal of General Virology,* 85(8): 2177-2187.

Boinas FS, Wilson AJ, Hutchings GH, Martins C and Dixon L.J (2011): The persistence of African swine fever virus in field-infected Ornithodoros erraticus during the ASF endemic period in Portugal. *PLoS ONE*, 6: 203-283

Boshoff, C. I., Bastos, A.D., Gerber, L. J and Vosloo, W. (2007), Genetic characterisation of African swine fever viruses from outbreaks in southern Africa (1973–1999). *VeterinaryMicrobiology,* 121(1–2): 45-55.

Bouma, A., De Jong, M. C. and Kimman, T. G. (1997). The influence of maternal immunity on the transmission of pseudorabies virus and on the effectiveness of vaccination. *Vaccine,*15: 287-294.

Bouma J., Stegeman A., Engel A., Kluijver E. P., Elbers A. R. W and De Jong M. C. M. (2001).*Evaluation of diagnostic tests for the detection of classical swine fever in the field without a gold standard. Journal ofVeterinaryDiagnostics,* 13:383-388.

Caiado, J.M., Boinas, F. S and Louza, A.C., (1988). Epidemiological research of African swine fever (ASF) in Portugal: the role of vectors and virus reservoirs. *Acta Veterinaria Scandinavica Supplementum,* 84: 136-138.

Carbrey, E. A., Erickson, G. A., and Metz, C. A. (1984). Diagnosis of Hog Cholera. *Preventive Veterinary Medicine,* 2(1/4): 103-108.

Carrasco, C. P., Rigden, R.C., Vincent, I.E., Balmelli, C., Ceppi, M., Bauhofer, O., Tache, V., Hjertner, B., McNeilly, F., van Gennip, H.G., McCullough, K. C and Summerfield, A. (2004). Interaction of classical swine fever virus with dendritic cells. *Journal of General Virology* 85: 1633-1641.

Carrillo C., Borca M.V., Afonso C.L., Onisk D.V and Rock D.L. (1994). Long-term persistent infection of swine monocytes/macrophages with African swine fever virus. *Journal of Virology,*68:580-583.

Chabo, R.G., Malope, P and Babusi, B. (2000). Pig productivity: a case study for south-eastern Botswana. *Livestock Research for Rural Development,* 12(3): 87-95.

Chapin, R.M., Powick, W.C., McBryde, C. N and Cole, C.G. (1939). The Influence of Hydrogen-Ion Concentration on the Survival of Hog-Cholera Virus in Defibrinated Blood. *Journal of the American Veterinary Medical Association,*95: 494-496.

Chen, N., Hu, H., Zhang, Z., Shuai, J., Jiang, L and Fang, W. (2008). Genetic diversity of the envelope glycoprotein E2 of classical swine fever virus: Recent isolates branched away from historical and vaccine strains. *Veterinary Microbiology*,127(3-4): 286-299.

Ciglenecki J.U., Grom J and Toplak I. (2008). Real-time RTPCR assay for rapid and specific detection of classical swine fever virus: comparison of SYBR Green and TaqMan MGB detection methods using novel MGB probes. *JournalofVirologicalMethods* 147: 257-264.

Clavijo, A., Lin, M., Riva, J., and Zhou, E.M. (2001). Application of competitive enzyme-linked immunosorbent assay for the serologic diagnosis of classical swine fever virus infection. *Journal of Veterinary Diagnostic Investigation*,13(4): 357-360.

Cole, C.G., Henley, R.R., Dale, C.N., Mott, L.O., Torrey, I. P and Zinober, M.R. (1962).*History of hog cholera research in the U.S. Department of Agriculture 1884-1960*, U.S. Departmentof Agriculture. Information, pp. 238-247.

Colijn, E.O., Bloemraad, M., and Wensvoort, G. (1997). An improved ELISA for the detection of serum antibodies directed against classical swine fever virus. *Veterinary Microbiology*,59(1): 15-25.

Collett, M.S., Moennig, V., and Horzinek, M.C. (1989). Recent advances in pestivirus research. *Journal of General Virology*,70(2): 253-266.

Costard, S., Porphyre, V., Messad, S., Rakotondrahanta, S., Vidon, H., Roger, F and Pfeiffer, D. U. (2009a). Multivariate analysis of management and biosecurity practices in smallholder pig farms in Madagascar. *Preventive Veterinary Medicine* 92(3): 199-209.

Costard, S., Wieland, B., de Glanville, W., Jori, F., Rowlands, R., Vosloo, W., Roger, F., Pfeiffer, D. U and Dixon, L. K. (2009b). African swine fever: how can global spread be prevented? *Philosophical Transactions of the Royal Society of London. SeriesB, Biological Sciences,* 364(1530): 2683-2696.

Costard, S., Randriamaparany, T., Humbert, C., Franco, S., Rasamoelina, H., Rakotoharinome, M., Raskotondrahanta, S., Albina, E., Roger, F and Pfeiffer, D.U. (2009c). Estimating the prevalence of African swine fever in Madagascar. In: 12th Symposium of the International Society for Veterinary Epidemiology and Economics, ISVEE 12, pp. 207-209.

Costard, S., Jones, B.A., Martinez-Lopez, B., Mur, L., De La Torre, A., Martinez, M., Sanchez-Viscaino, J.M., Pfeiffer, D.U and Wieland, B. (2012a). Risk of African swine fever introduction into the European Union through illegal importation of pork and products. In: 13th Symposium of the International Society for Veterinary Epidemiology and Economics, ISVEE 13.

Costard, S., Zagmutt, F. J., Porphyre, T., Roger, F and Pfeiffer, D.U., (2012b). Small-scale pig farmers’ behaviour, silent release of African swine fever and consequences for persistence. In: 13th Symposium of the International Society for Veterinary Epidemiology and Economics, ISVEE 13.

Costard S., Mur L., Lubroth J., Sanchez-Vzcaino J. M and Pfeiffer DU (2013). Epidemiology of African swine fever virus. *Virus Research,* 173(1): 191-197.

Cubillos C.,Gómez-Sebastian S., Moreno N., Nu˜nez M.C., Mulumba-Mfumu L.K., Quembo C.J., Heathe L., Etter E.M.C., Jori F., Escribano J.M and Blanco E, (2013): African swine fever virus serodiagnosis: A general review with a focus on the analyses of African serum samples. *VirologyResearch*, 173, 1: 159-167.

Dahle, J., Liess, B., and Frey, H.R. (1987): Interspecies transmission of pestivirus: experimental infection of swine with bovine diarrhoea virus and of cattle with swine fever virus. *Deutsche Tierarztliche Wochenschrift (German Veterinarians weekly magazine),*94(10): 590-594.

Dahle, J., and Liess, B. (1992). A review on classical swine fever infections in pigs: Epizootiology, clinical disease and pathology. *Comparative Immunology, Microbiology and Infectious Diseases,*15(3): 203-211.

Darbyshire, J.H. (1960). A serological relationship between swine fever and mucosal disease of cattle. *Veterinary Record,*72: 331.

de Arce, H.D., Ganges, L., Barrera, M., Naranjo, D., Sobrino, F., Frías, M.T, and Núñez, J.I. (2005). Origin and evolution of viruses causing classical swine fever in Cuba. *Virus Research,*112(1-2): 123-131.

de Carvalho, F. H. C., Weesendorp, E, Elbers, A. R., Bouma, A., Quak, S., Stegeman, J. A., and Loeffen, W. L. (2012). African swine fever virus excretion patterns in persistently infected animals: A quantitative approach. *Veterinary Microbiology,*160(4): 327-359.

de Carvalho, F. H. C., Weesendorp, E., Elbers, A. R. W., Bouma, A and Quak, S. (2013a). Transmission rate of African swine fever virus under experimental conditions. *VeterinaryMicrobiology*, 165(3-4): 296-304.

de Carvalho, F. H. C., Weesendorp, E., Elbers, A. R. W., Bouma, A and Quak, S. (2013b). Quantification of airborne African swine fever virus after experimental infection. *VeterinaryMicrobiology*, 165(3-4): 243-251.

De Glanville, W., Costard, S., Wieland, B., Pfeiffer, D. U. (2010). Risk mapping African Swine Fever in Africa: an exercise in uncertainty. In: *Geovet* 1–3 December 2010, Sydney, Australia.

De Lange, K., Haddad, N., Le Potier, M.F., Agier, C., Le Vee, M., Amar, P. and Toma, B. (2003). Specificity of three ELISA-gE kits for screening pig meat for antibodies to Aujeszky's disease. *Veterinary Records,*153: 621-624.

de Schweinitz, E.A., and Dorset, M. (1903). A form of hog cholera not caused by the hog-cholera bacillus. Washington D.C. Circular No. 41. U.S. Bureau of Animal Industry. Washington D.C. Cited by Edwards, S., Fukusho, A., Lefèvre, P.C., Lipowski, A., Pejsak, Z., Roehe, P., and Westergaard, J. (2000): Classical swine fever: the global situation. *Veterinary Microbiology,*73(2-3): 103-119.

de Smit, A. J., Bouma, A., Terpstra, C and van Oirschot, J. T.(1999). Transmission of classical swine fever virus by artificial insemination. *VeterinaryMicrobiology,* 67:239-249.

de Smit, A.J., Bouma, A., de Kluijver, E.P., Terpstra, C and Moormann, R. J. (2001a). Duration of the protection of an E2 subunit marker vaccine against classical swine fever after a single vaccination. *Veterinary Microbiology*,78(4): 307-317.

de Smit, A.J., Eble, P.L., de Kluijver, E. P., Bloemraad, M and Bouma, A. (2000b). Laboratory experience during the classical swine fever virus epizootic in the Netherlands in 1997-1998. *Veterinary Microbiology*,73(2-3): 197-208.

Depner, K.R., Bauer, T., and Liess, B. (1992). Thermal and pH stability of pestiviruses. *OIE Revue Scientifique et Technique*,11(3): 885-893.

Depner, K.R., Müller, A., Gruber, A., Rodriguez, A., Bickhardt, K., Liess, B., (1995). Classical swine fever in wild boar (Sus scrofa)--experimental infections and viral persistence. *Dutsch Tierarztl Wochenschr*, 102(10): 381-384.

Depner, K.R., Hinrichs, U., Bickhardt, K., Greiser-Wilke, I., Pohlenz, J., Moennig, V., and Liess, B. (1997). Influence of breed-related factors on the course of classical swine fever virus infection. *Veterinary Record*,140(19): 506-507.

Depner, K.R., Strebelow, G., Staubach, C., Kramer, M., Teuffert, J., Botcher, L., Hoffmann, B., Beer, M., Greiser-Wilke, I and Mettenleiter, T. (2006a). Case report: the significance of genotyping for the epidemiological tracing of classical swine fever (CSF). *Dutsch Tierarztl Wochenschr* 113(4): 159-162.

Depner, K., Bunzenthal, C., Heun-Munch, B., Strebelow, G., Hoffmann, B. and Beer, M. (2006b).Diagnostic evaluation of a real-time RT-PCR assay for routine diagnosis of classical swine fever in wild boar. *Journal of Veterinary Medicine Series B-Infectious Diseases and Veterinary Public Health* 53

Depner, K., Hoffmann, B and Beer, M. (2007).Evaluation of real-time RT-PCR assay for the routine intra vitam diagnosis of classical swine fever. *Veterinary Microbiology* 121, 338-343

Dewulf, J., Laevens, H., Koenen, F., Mintiens, K. and de Kruif, A.(2004). Efficacy of E2-sub-unit marker and C-strain vaccines in reducing horizontal transmission of classical swine fever virus in weaner pigs. *PreventiveVeterinaryMedicine*, 65:121-133.

Dewulf, J., Koenen, F., Ribbens, S., Haegeman, A., Laevens, H and De Kruif, A. (2005). Evaluation of the epidemiological importance of classical swine fever infected, E2 sub-unit marker vaccinated animals with RT-nPCR positive blood samples. *Journalof Veterinary Medicine. B Infectious Disease Veterinary Public Health*,52: 367-371.

Diaz de Arce, Nunez, J.I., Ganges, L., Barreras, M., Frias, M. T and Sobrino, F. (1998). An RT-PCR assay for the specific detection of classical swine fever virus in clinical samples. *Veterinary Research Journal*,(29): 431-440.

Dixon, L. K., Costa J. V., Escribano, J. M., Rock, D. L., Venuela, E. and Wilkinsons, P. J. (2000).*Family Asfaviridae. In: Virus Taxonomy, Seventh Report of the International Committee on Taxonomy of Viruses*. Academic Press, London,pp.165.

Dixon L.K., Abrams C.C., Bowicl G., Goatley L.C., Kay-Jackson P.C., Chapman D., Liverani E., Nix R., Silk R and Zhang F. (2004). African swine fever virus protein involved in evading host defence system.*Veterinary immunology and immunopathology*, 100:117-134.

Dixon, L.K., Escribano, J.M., Martins, C., Rock, D.L., Salas, M. L and Wilkinson, P. J. (2005).*Virus Taxonomy. VIII. Report of the ICTV.* (Edited by Fauquet, C. M., Mayo, M. A., Maniloff, J., Desselberger, U. and Ball, L. A.), Elsevier Academic Press, London, pp. 135-143.

Dixon, L.K., Abrams, C.C., Chapman, D. G and Zhang, F. (2008).*Animal Viruses: Molecular Biology*. Edited by Mettenleiter, Thomas C and Sobrino, Francisco, African swine fever Virus. Madrid

Downing, D.R., Carbrey, E. A and Stewart, W.C. (1977). Preliminary findings on a thermal inactivation curve for hog cholera virus. *Agricultural research seminar on hog cholera/classical swine fever and African swine fever, held at the Tierarztliche Hochschule, Hannover,* September 6-11, 1976, pp. 94-98.

Doyle, T.M. (1933). The viability of the virus of swine fever in bone marrow muscle and skin of preserved carcases. *Journal of Comparative Pathology*,46: 25.

Doyle, L.G and Heuschele, W.P. (1983). Bovine viral diarrhea virus infection in captive exotic ruminants. *Journal of American Veterinary Medical Association*,183(11): 1257-1259.

Dreier, S., Zimmermann, B., Moennig, V and Greiser-Wilke, I. (2007). A sequence database allowing automated genotyping of Classical swine fever virus isolates. *Journal of Virological Methods*,140: 95-99.

Dunne, H.W., (1973): Hog cholera (European swine fever). *Advance Veterinary Science Comparative Medicine*, 17: 315-359.

Edelsten, R. M and Chinombo, D. O. (1995). An outbreak of African swine fever in the southern region of Malawi. *Revue Scientifique et Technique de Office International des Epizooties*, 14(3): 655–666.

Edgar, G., Hart, L and Hayston, J.T. (1949). Studies on the viability of the virus of swine fever. *International Veterinary Congress*, pp. 387-391.

Edwards, S., Moennig, V., and Wensvoort, G. (1991). The development of an international reference panel of monoclonal antibodies for the differentiation of hog cholera virus from other pestiviruses. *Veterinary Microbiology*,29(2): 101-08.

Edwards, E., Fukusho, A., Lefevre, P. C., Lipowski, A., Pejsak, Z., Roeche, P. and Westergaard, J. (2000), Classical swine fever: the global situation. *Elsevier*. *VeterinaryMicrobiologyJournal,* 73(2): 103-119

Edwards, S. (2000): Survival and inactivation of classical swine fever virus. *Veterinary Microbiology*,73(2-3): 175-181.

Ekue, N. F and Wilkinson, P. J. (1990). Absence of Ornithodoros moubata, the vector of 588 African swine fever virus, from the main pig producing area of Cameroon. *TropicalAnimalHealthandProduction,* 22(2): 127–131.

Elber, A. R., Stegeman, A., Moser, H., Ekker, H. M., Smak, J. A. and Pluimers, F. H. (1999). The classical swine fever epidemic 1997-1998 in The Netherlands, descriptive epidemiology. *PreventiveVeterinaryMedicine*, 42:157-184.

Elbers, A.R.W., Moser, H., Ekker, H.M., Crauwels, P.A.A., Stegeman, J.A., Smak, J.A., and Pluimers, F.H. (2001a). Tracing systems used during the epidemic of classical swine fever in the Netherlands, 1997-1998. *Revue Scientifique et Technique De Office International Des Epizooties,* 20(2): 614-629.

Elbers, A.R.W., Stegeman, J.A., and de Jong, M.C.M. (2001b). Factors associated with the introduction of classical swine fever virus into pig herds in the central area of the 1997/98 epidemic in the Netherlands. *Veterinary Record,*149(13): 377-382.

El-Hicheri K, Gómez-Tejedor C, Penrith M-L, Davies G, Douati A, Edoukou G D, Wojciechowski K. (1998). L'epizootie de peste porcine africaine de 1996 en Côte d'Ivoire. *Revue Scientifique et Technique, Office International des Epizooties* 17: 660–673

El-Hicheri, K. (1998). Emergency assistance on control and eradication of an outbreak of African swine fever in Western Nigeria. *Report of the FAO Consultancy Mission to Nigeria.* TCP/NIR/7822(E). FAO, Rome.

Etters, E. M. C., Seck, I., Grosbois, V., Jori, F., Blanco, E., Vial, L., Akakpo, A. J., Bada-Alhambedji, R., Kone, P. and Roger, F. L. (2011). Seroprevallance of African swine fever in Nigeria. *Emerging Infectious Disease Journal*,17(1): 49-54.

European Food Safety Authority. (2008).*AnnextoTheEFSAJournal,*932:1-18;933:1-16

European Food Safety Authority. (2010). Scientific opinion on African swine fever. *EFSAJournal,* 8(3): 1556.

European Food Safety Authority. (2010a). Scientific opinion on African Swine Fever. *EFSA Journal*, 8(3): 149.

European Food Safety Authority. (2010b). Scientific opinion on the role of tick vectors in the epidemiology of Crimean-Congo hemorrhagic fever and African Swine Fever in Eurasia. *EFSAJournal,* 8(8): 12-16.

Farez, Sand Morley, R. S. (1997): Potential animal health hazards of pork and pork products. *Revue Scientifique et Technique, Office International des Epizooties*16(1): 65-78.

Fasina, F.O., Shamaki, D., Makinde, A. A., Pitt, A.S.F.(2009). Field surveillance andlaboratory diagnoses of African swine fever in Nigeria (IAEA-CN-174-151).Book of Synopses, FAO/IAEA International Symposium on SustainableImprovement of Animal Production and Health, 8-11 June 2009, Vienna, Austria,pp. 301-302.

Fasina, F. O., Shamaki D., Makinde, A. A., Lombin, L. H., Lazarus, D. D., Rua'i S. A., Adamu, S. S., Agom D., Pelayo, V., Soler, A., Simon A., Adedeji, A. J., Yakubu M. B., Mantip S., Benshak, A. J., Okeke, I., Anagor, P., Mandeng, D. C., Akanbi, B. O., Ajibade, A. A., Faramade, I., Kazeem, M. M., Enurah, L. U., Bishop, R., Anvhuelo, R., Martin, J. H and Gallardo, C. (2010).Surveillance of African swine fever in Nigeria, 2006-2009. *Transboundary Emerging Diseases,* 57(4): 244-253.

Fasina F. O., Agbaje, M., Ajani, F. L., Talabi, O. A., Lazarus, D. D., Gallardo, C., Thompson, P. N and Bastos, A. D. S. (2012). Risk factors for farm-level African swine fever infection in major pig-producing areas in Nigeria, 1997-2011. *Preventive Veterinary Medicine,* 107(1-2): 65-75.

Federal Department of Livestock (FDL) Nigeria (2010): Report on study and mapping of poultry farming systems and pig production areas by support for the Integrated National Action Plan on Animal and Human Influenza (SPINAP) Project in Nigeria. Official document.

Fenati, M., Armaroli, E., Corrain, R. and Guberti, V. (2008). Indirect estimation of porcine parvovirus maternal immunity decay in free-living wild boar (Sus scrofa) piglets by capture-recapture data. *Veterinary Journal*,27: 65-77.

Fevre, E.M., Bronsvoort, B.M.C., Hamilton, K.A and Cleaveland, S. (2006). Animal movements and the spread of infectious diseases. *Trends in microbiology (Regular edition)*,14(3): 125-131.

Firinu, A and Scarano C. (1988). African swine fever and classical swine fever (hog cholera) among wild boar in Sardinia. *Revue Scientifique et Technique - Office International des Epizooties,* 7(4): 909-915.

Floegel, G., Wehrend, A., Depner, K. R., Fritzemeier, J., Waberski, D and Moennig, V. (2000): Detection of Classical Swine Fever virus in semen of infected boars. *Veterinary Microbiology*,77(1-2): 109-116.

Floegel-Niesmann, G., (2001): Classical swine fever (CSF) marker vaccine. Trial III. Evaluation of discriminatory ELISAs. *Veterinary Microbiology,*83: 121-136.

Food Agricultural Organization of the United Nations. (1998b). African swine fever in West Africa: Togo, Senegal, Gambia and Guinea-Bissau. [French] Mission report.pp.1-16. Retrieved March 25, 2016, from http://www.fao.org/docrep/field/382969.htm.

Food Agricultural Organization of the United Nations. (1998d). African swine fever in Nigeria hits rural poor. News and Highlights. Retrieved March 25, 2016 from http://www.fao.org/NEWS/1998/981201-e.htm.

Food Agricultural Organization of the United Nations. (2000). Recognizing African swine fever: A field manual. FAO Animal Health Manual. Retrieved December 15, 2015 from http://www.fao.org/docrep/004/x8060e/x8060e00.htm.

Food Agricultural Organization of the United Nations.(2005). Animal genetic resources and resistance to disease. In: The state of agricultural biodiversityin the livestock sector. pp. 109. Retrieved June 10, 2017, from ftp://ftp.fao.org/docrep/fao/010/a1250e/a1250e05.pdf

Food Agricultural Organization. (2012). Secteur porcin Republique Democratique du Congo. Revues nationales de l'élevage de la division de la production *et de la santé animales de la* FAO 2. Rome.

Francis, M.J and Black, L. (1984). Effect of the sow vaccination regimen on the decay rate of maternally derived foot-and-mouth disease antibodies in piglets. *Research in Veterinary Science,*37: 72-76.

Frias-Lepoureau, M. T and Greiser-Wilke, I. (2002).*An update on classical swine fever virus molecular epidemiology*. Edited by Morilla, A., Yoon, K. J. and Zimmerman, J. J., Trends in emerging viral infections of swine. Ames, pp.134-154.

Fritzemeier, J., Teuffert, J., Greiser-Wilke, I., Staubach, C., Schluter, H and Moennig, V. (2000). Epidemiology of classical swine fever in Germany in the 1990s. *Veterinary Microbiology,*77: 29-41.

Gago da Câmara N. J. (1932). História da peste suína em Angola. *Pecuária,*1: 25-40

Gallardo, C., Blanco E, Javier, M. R., Carrascosa, A.L., Sanchez-Vizcaino, J.M.(2006). Antigenic properties and diagnostic potential of African swine fever virus protein pp62 expressed in insect cells. *Journal of clinical microbiology.*, 44: 950-956.

Garcês, A., Casey, N. H., Otto, F. (1998). Factors limiting pig breeding in Maputo, Mozambique. *IsraelJournalofVeterinaryMedicine*, 53(1): 21-24.

Gomez-Villamandos, J.C., Ruiz-Villamor, E., Bautista, M.J., Quezada, M., Sanchez, C.P., Salguero, F.J., Sierra, M.A. (2000). Pathogenesis of classical swine fever:

renal haemorrhages and erythrodiapedesis. *Journalof ComparativePathology,* 123(1): 47-54.

Gomez-Villamandos, J.C., Ruiz-Villamor, E., Bautista, M.J., Sanchez, C.P., Sanchez-Cordon, P.J., Salguero, F. J., Jover, A. (2001). Morphological and immunohistochemical changes in splenic macrophages of pigs infected with classical swine fever. *Journal of Comparative Pathology,* 125(2-3): 98-109.

Greig, A and Plowright W. (1970): The excretion of two virulent strains of African swine fever virus by domestic pigs. *JournalofHygiene*, 68(4): 673–682.

Greiser-Wilke, I., Depner, K., Fritzemeier, J., Haas, L. and Moennig, V. (1998). Application of a computer program for genetic typing of classical swine fever virus isolates from Germany. *JournalofVirologicalMethods*, 75:141-150.

Greiser-Wilke, I., Fritzemeier, J., Koenen, F., Vanderhallen, H., Rutili, D., De Mia, G. M., Romero, L., Rosell, R., Sanchez-Vizcaino, J. M and San Gabriel, A. (2000). Molecular epidemiology of a large classical swine fever epidemic in the European Union in 1997-1998. *VeterinaryMicrobiology*, 77:17-27.

Greiser-Wilke, I., Blome, S and Moennig, V. (2007): Diagnostic methods for detection of Classical swine fever virus--Status quo and new developments. *Vaccine*,25(30): 5524-5530.

Gruber, A., Depner, K., and Liess, B. (1995): Experimental-infection of weaner pigs with a field isolate of hog-cholera classical swine fever virus derived from a recent outbreak in lower saxony pathological findings. *Wiener Tierarztliche Monatsschrift*,82(6): 179-184.

Gulenkin, V.M., Korennoy, F.I., Karaulov, A.K., Dudnikov, S. A. (2011). Cartographical analysis of African swine fever outbreaks in the territory of the Russian Federation and computer modeling of the basic reproduction ratio. *PreventiveVeterinaryMedicine,* 102(3): 167–174.

Haegeman, A., Dewulf, J., Vrancken, R., Tignon, M., Ribbens, S. and Koenen, F. (2006). Characterisation of the discrepancy between PCR and virus isolation in relation to classical swine fever detection. *Journal of Virological Methods* 136, 44-50.

Halimani, T.E., Ndlovu, L. R., Dzama, K., Chimonyo, M and Miller, B.G. (2007). Growth performance of pigs fed on diets containing Acacia karroo, Acacia nilotica and Colophospermum mopane leaf meals. *LivestockResearchforRuralDevelopment* 19: 12.

Hamdy, F. M and Dardiri, A. H. (1979). Enzyme linked immunosorbent assay for diagnosis of African swine fever. *Veterinary Record,*105: 445-446.

Handel, K., Kehler, H., Hills, K and Pasick, J. (2004): Comparison of reverse transcriptase-polymerase chain reaction, virus isolation, and immunoperoxidase assays for detecting pigs infected with low, moderate, and high virulent strains of classical swine fever virus. *Journal of Veterinary Diagnostic Investigation*16: 132-138.

Hanson, R.P., (1957). Origin of hog cholera. *Journal of the American Veteinary Medical Association,*131: 211-218

Haresnape, J.M., Lungu, S. A and Mamu, F.D. (1985). A four-year survey of African swine fever in Malawi. *Journal ofHygiene*, 95: 309-323.

Haresnape, J. M and Mamu, F.D. (1986). The distribution of ticks of the Ornithodoros moubata complex (Ixodoidea: Argasidae) in Malawi, and its relation to African swine fever epizootiology. *TheJournalofHygiene*, 96(3): 535-544.

Haresnape, J. M., Lungu, S. A and Mamu, F. D (1987). An updated survey of African swine fever in Malawi. *Epidemiology and Infection,* 99: 723-732

Haresnape J.M., Wilkinson, P. J and Mellor, P. S. (1988). Isolation of African swine fever virus from ticks of the Ornithodoros moubata complex (Ixodoidea: Argasidae) collected within African swine fever enzootic area of Malawi. *EpidemiologicalInformation*, 101(1): 173-185.

Haresnape, J. M and Wilkinson, P. J. (1989). A study of African swine fever virus infected ticks (*Ornithodoros moubata*) collected from three villages in the ASF enzootic area of Malawi following an outbreak of the disease in domestic pigs. *Epidemiology Infectious* 102(3): 507-522.

Have, P. (1984): Detection of antibodies against swine fever virus by enzyme-linked immunosorbent assay (ELISA). *Acta Veterinaria Scandinavica,*25(3): 463-465.

Hess, W.R., Endris, R. G., Lousa, A and Caiado, J.M. (1989): Clearance of African swine fever virus from infected tick (Acari) colonies. *JournalofMedicalEntomology,* 26(4): 314-317.

Heuschele, W. P., Coggins, L. (1965a). Isolation of African swine fever virus from a giant forest hog. *BulletinofEpizooticDiseasesofAfrica,* 13: 255-256.

Heuschele, W.P., Coggins, L. (1965b). Studies on the transmission of African swine fever by arthropods. Proceedings of the United States Livestock Sanitary Association. 69:(94-100).

Heuschele, W. P and Coggins, L. (1969). Epizootiology of African swine fever virus in warthogs. *BulletinofEpizooticDiseaseofAfrica,* 17(2): 179-183.

Hoogstraal, H., Salah, A.A., Kaiser, M.N., (1954): Summary of the known distribution and biology of Ornithodoros erraticus. *The Journal of the Egyptian Public Health Association,* 24(5): 127-138.

Ironkwe, M. O and Amefule, K. U. (2008). Appraisal of indigenous pig production and management practices in Rivers State, Nigeria. *Journal of Agriculture and Social Research,* 8:1-7.

Jori, F., Vial, L., Ravaomanana, J., Le Glaunec, G., Etter, E., Akakpo, J., Sarr, J., Costard, S., Perez, R and Roger, F. (2007). The role of wild hosts (wild pigs and ticks) in the epidemiology of African swine fever in West Africa and Madagascar. In: International Conference of the Association of Institutions for Tropical Veterinary Medicine, pp. 8-22.

Jori, F and Bastos, A. D. S. (2009). Role of wild suids in the epidemiology of African swine fever. *EcoHealth,*6: 296-310.

Jori, F., Vial, L., Penrith, M., Perez-Sanchez, R., Etter, E., Albina, E., Michaud, V and Rogers F. (2013). Review of the sylvatic cycle of African swine fever in sub-saharan Africa and the Indian Ocean. *Virus Research,* 173:212-227

Kaden, V., Steyer, H., Strebelow, G., Lange, E., Hubert, P., Steinhagen, P. (1999). Detection of low-virulent classical swine fever virus in blood of experimentally infected animals: comparison of different methods. *ActaVirologica,* 43(6): 373-380.

Kaden, V., Lange, E., Fischer, U and Strebelow, G. (2000). Oral immunisation of wild boar against classical swine fever: evaluation of the first field study in Germany. *VeterinaryMicrobiology,* 73(2-3): 239-252.

Kaden, V and Lange, B. (2001). Oral immunisation against classical swine fever (CSF): onset and duration of immunity. *Veterinary Microbiology,*82(4): 301-310.

Kaden, V and Lange, E. (2004): Development of maternal antibodies after oral vaccination of young female wild boar against classical swine fever. *Veterinary Microbiology,*103(1–2): 115-119.

Kagira, J.M., Maingi, N., Kanyari, P.W.N., Githigia, S.M., Ng'ang'a, J.C andKaruga, J.W. (2010). Characteristics of the smallholder free-range pig production system in western Kenya. *TropicalAnimalHealthandProduction,* 42(5): 865-873.

Kamakawa, A., Thu, H. T. V and Yamada, S. (2006). Epidemiological survey of viral diseases of pigs in the Mekong delta of Vietnam between 1999 and 2003. *Veterinary Microbiology,*118(1-2): 47-56.

Karimuribo, E.D., Chenyambuga, S.W., Makene, V.W., Mathias, S. (2011). Characteristics and production constraints of rural-based small-scale pig farming in Iringa region, Tanzania. *Livestock Research for Rural Development,* 2(8): 231-272.

Karsten, S., Rave, G and Krieter, J. (2005). Monte Carlo simulation of classical swine fever epidemics and control: II. Validation of the model. *Veterinary Microbiology,*108(3-4): 199-205.

Katz, J. B., Ridpath, J. F., Bolin, S. R. (1993). Presumptive diagnostic differentiation of hog cholera virus from bovine viral diarrhea and border disease viruses by using a cDNA nested-amplification approach. *Journal of Clinical Microbiology,* 31: 565-568.

Keast, J. C and Helwig, D.M. (1966). Viability of virulent swine fever virus in cooked and uncooked ham and sausage casings. *Australian Veterinary Journal*,42(4): 131-35.

Kern, B., Depner, K.R., Letz, W., Rott, M., Thalheim, S., Nitschke, B., Plagemann, R., and Liess, B. (1999). Incidence of Classical Swine Fever (CSF) in Wild Boar in a Densely Populated Area Indicating CSF. Virus Persistence as a Mechanism for Virus Perpetuation. *Journal of Veterinary Medicine, Series B*,46(1): 63-68.

Kleiboeker, S.B., Scoles, G.A. (2001). Pathogenesis of African swine fever virus in Ornithodoros ticks. *Animal Health Research Reviews*, 2(2): 121–128.

Klinkenberg, D., Everts-van der Wind, A., Graat, E.A. M and de Jong, M.C.M. (2003). Quantification of the effect of control strategies on classical swine fever epidemics. *Mathematical Biosciences,*186(2): 145-173.

Knoetig, S.M., Summerfield, A., Spagnuolo-Weaver, M and McCullough, K.C. (1999). Immunopathogenesis of classical swine fever: role of monocytic cells. *Immunology*, 97(2): 359-366.

Koenen, F., Caenegem, G. van, Vermeersch, J.P., Vandenheede, J and Deluyker, H. (1996). Epidemiological characteristics of an outbreak of classical swine fever in an area of high pig density. *Veterinary Record,*139(15): 367-371.

Koenig, P., Hoffmann B., Depner, K. R., Reimann, I., Teifke, J. P and Beer, M. (2007). Detection of classical swine fever vaccine virus in blood and tissue samples of pigs vaccinated either with a conventional C-strain vaccine or a modified live marker vaccine. *VeterinaryMicrobiology*, 120(3-4): 343-351.

Kortekaas, J., Ketelaar, J., Vloet, R.P. M and Loeffen, W.L. (2011). Protective efficacy of a Classical swine fever virus C-strain deletion mutant and ability to differentiate infected from vaccinated animals. *Veterinary Microbiology*,147(1-2): 11-18.

Kosmidou, A., Ahl, R., Thiel, H. J and Weiland, E. (1995). Differentiation of classical swine fever virus (CSFV) strains using monoclonal antibodies against structural glycoproteins. *Veterinary Microbiology*,47(1-2): 111-118.

Kperebeyi J. I and Amotsuka P. (2010). An outbreak of African Swine Fever (ASF) in Delta State Polytechnic Piggery Farm, Ozoro, Nigeria. *African Journal of General Agriculture*, 6(1): 25-29.

Krinsky, W.L. (1976): Animal disease agents transmitted by horse flies and deer flies (Diptera: Tabanidae). *Journal of Medical Entomology*,13(3): 224-75.

Kubin, G. (1967).*In vitro* characteristics of swine fever virus. *Zentralblatt fur Veterinarmedizin*,14B: 543-52.

Laddomada, A., Patta, C., Oggiano, A., Caccia, A., Ruiu, A., Cossu, P and Firinu, A. (1994). Epidemiology of classical swine fever in Sardinia: a serological survey of wild boar and comparison with African swine fever. *VeterinaryRecord*, 19: 183-187.

Laevens, H., De Kruif, A., Koenen, F., Dewulf, J and Mintiens, K. (2001). Evaluation of the potential of dogs, cats and rats to spread classical swine fever virus. *Veterinary Record* 149(7): 212-213.

Laevens, H., Koenen, F., de Kruif, A., Dewulf, J and Mintiens, K. (2000): Airborne transmission of classical swine fever virus under experimental conditions. *Veterinary Record*,147(26): 735-738.

Lage, M.C. D and Oliveira da Silva, R.D. C. (1980). Peste suína africana em S. Tomé e Principe. *Repositorio de Trabalhos do Instituto Nacional de Veterinaria*, 12: 23–28.

Lammers P. J., Stender D. R and Honeyman M. S. (2007).Replacement gilts and sow longevity. Reproduction and genetics. *IPIC NPP*, 420:1-3

Le Dimna, M., Vrancken, R., Koenen, F., Bougeard, S., Mesplede, A., Hutet, E., Kuntz-Simon, G. and Le Potier M.F. (2008). Validation of two commercial real-time RT-PCR kits for rapid and specific diagnosis of classical swine fever virus. *Journal of Virological Methods*,147(1): 136-42.

Le Potier, M.F., Mesplede, A and Vannier, P. (2006a). Classical swine fever and other pestiviruses, In: Straw, B.E., Zimmerman, J.E., D'Allaire, S., Taylor, D.J. (Eds.) *Diseases of Swine*. Blackwell Publishing, Ames, Iowa, pp. 309-322.

Le Potier, M., Le Dimna, M., Kuntz-Simon, G., Bougeard, S. and Mesplède, A. (2006b). Validation of a Real-Time RT-PCR Assay for Rapid and Specific Diagnosis of Classical Swine Fever Virus. In: Basel (Ed), New Diagnostic Technology: Applications in Animal Health and Biologics Controls. *Developmental Biology. Basel*126, Basel, Karger, pp. 179-186.

Leifer, I., Hoffmann, B., Hoper, D., Bruun Rasmussen, T., Blome, Leitão A., Cartaxeiro C., Coelho, R., Cruz, B., Parkhouse, R. M., Portugal, F., Vigario, J. D and Martins, C. L. (2001). The non-haemadsorbing African swine fever virus isolate ASF/NH/P68 provides a model for defining the protective anti-virus immune response. *Journal of General Virology*, 82(3): 513 – 523.

Lekule, F. P and Kyvsgaard, N. C. (2003). Improving pig husbandry in tropical resourcepoor communities and its potential to reduce risk of porcine cysticercosis. *ActaTropica*, 87(1):111–117.

Li, X., Xu, Z., He, Y., Yao, Q., Zhang, K., Jin, M., Chen, H. and Qian, P. (2006). Genome comparison of a novel classical swine fever virus isolated in China in 2004 with other CSFV strains. *VirusGenes*, 33:133-142.

Li, Y., Zhao, J. J., Li, N., Shi, Z., Cheng, D. and Zhu, Q. H. (2007). A multiplex nested RT-PCR for the detection and differentiation of wild-type viruses from C-strain vaccine of classical swine fever virus. *JournalVirologicalMethods* 143:16-22.

Liess, B and Schurian, E. (1973). In the suspension test with two types of virus, the toga-group: the European swine and bovine viral diarrhea-mucosal disease. *Archive of Food Hygiene* 24: 154-157.

Liess, B., Frey, H. R., Prager, D., Hafez, S. M and Roeder, B. (1974). "The course of the natural swine fever virus infection in individual swine and investigations on the development of inapparent SF infections." Paper presented at the Seminar on diagnosis and epizootiology of classical swine fever April 30th-May 2nd 1974, Amsterdam, Netherlands.

Liess, B., Frey, H. R and Bucksch, K. (1976). Hog cholera viral antigens (brief review) and some recent results on the relationship to envelope antigens of bovine viral diarrhea-mucosal disease virions. *In*: Studies on virus replication, edited by Becker Y. Seminar, Bussels EEC publication, 54 (51): 333-346.

Liess, B. (1984): Persistent infections of hog cholera: A review. *Preventive Veterinary Medicine*, 2(1-4): 109-113.

Liess, B., (1987): Pathogenesis and epidemiology of hog cholera. *Annals of veterinary research*,18(2): 139-145.

Liu, S.T., Li, S.N., Wang, D.C., Chang, S.F., Chiang, S.C., Ho, W.C., Chang, Y. S. and Lai, S.S. (1991). Rapid detection of hog cholera virus in tissues by the polymerase chain reaction. *Journal of Virological Methods,*35: 227-236.

Loeffen, W.L.A., Nodelijk, G., Heinen, P.P., Van Leengoed, L.A., Hunneman, W.A. and Verheijden, J. H. (2003). Estimating the incidence of influenza-virus infections in Dutch weaned piglets using blood samples from a cross-sectional study. *Veterinary Microbiology,*91: 295-308.

Loeffen, W.L. A., Smits-Mastebroek, L and Quak, S. (2005). Persistence of CSF-virus in E2-vaccinated and subsequently infected pigs. In: Proceedings of the 6th ESVV Pestivirus Symposium, Thun, Switzerland, pp. 42.

Loeffen, W.L.A., van Beuningen, A., Quak, S and Elbers, A.R.W. (2009). Seroprevalence and risk factors for the presence of ruminant pestiviruses in the Dutch swine population. *Veterinary Microbiology,*136(3-4): 240-45.

Lowings, P., Ibata, G., Needham, J and Paton, D. (1996). Classical swine fever virus diversity and evolution. *Journal of General Virology,*77(6): 1311-21.

Lowings, J.P., Paton, D.J., Sands, J.J., Mia, G.M and Rutili, D. (1994). Classical swine fever: genetic detection and analysis of differences between virus isolates. *Journal of General Virology,*75(12): 3461-68.

Lubisi, B. A., Bastos, A. D. S. Dwarka, R. M and Vosloo, W. (2005). Molecular epidemiology of African swine fever in East Africa. *Archive Virology* 150: 2439-2452.

Lubisi, B.A., Dwarka, R. M., Meenowa, D and Jaumally, R. (2009). An investigation into the first outbreak of African swine fever in the Republic of Mauritius. *TransboundaryandEmergingDiseases*, 56(5): 178-188.

Luther, N. J, Shamaki, D., Joannis, T. M and Fasina FO (2008): A five-year survey of African swine fever outbreaks in Plateau State, Nigeria. *Sokoto Journal of Veterinary Sciences*, **7**(2): 34-37.

Maclachlan, N.J and Scott, J.L. (2004).*Veterinary microbiology*. Edited by Hirsh, D. C, Maclachlan, N.J. and Walker, R.L. 2nd edition, pp.67-74.

Magadla N. R. (2015). The prevalence of African swine fever determinants along the control zone in South Africa. (Master of veterinary science dissertation, University of Pretoria, South Africa).

Malmquist, W. A.,and Hay, D. (1960). Haemadsorption and cytopathic effect produced by African swine fever virus in swine bone marrow and buffy coat cultures. *American Journal of Veterinary Research*,21: 104 – 108.

Markowska-Daniel, I., Pejsak, Z., Winnicka, A., Collins, R, A. (1999). Phenotypic analysis of peripheral leukocytes in piglets infected with classical swine fever virus. *Researchin VeterinaryScience,* 67(1): 53-57.

Mashatise, E., Hamudikuwanda, H., Dzama, K., Chimonyo, M and Kanengoni, A. (2005): Socio-economic roles, traditional management systems and reproductive patterns of mukota pigs in semi-arid north-eastern Zimbabwe. *Bunda Journal of Agriculture, Environmental Science and Technology*, 3 (1): 97–105.

Matos, C., Sitoe, C., Afónso, S., Banze, J., Baptista, J., Dias, G., Rodrigues, F., Ataná-sio, A., Nhamusso, A., Penrith, M. L and Willingham III, A. L. (2011). A pilot study of common health problems in smallholder pigs in Angónia and Boane dis-tricts, Mozambique. *Journal of the South African Veterinary Association*,82(3): 166–169.

Matson, B.A., (1960). An outbreak of African swine fever in Nyasaland. *Bulletin of Epizootic Diseases of Africa*, 8: 305–308.

McBryde, C. N and Cole, C.G. (1936). Crystal-Violet Vaccine for the Prevention of Hog-Cholera; Progress Report. *Journal of the American Veterinary Medical Association* 89: 652-663.

McGoldrick, A., Lowings, J.P., Ibata, G., Sands, J. J., Belak, S and Paton, D.J. (1998). A novel approach to the detection of classical swine fever virus by RT-PCR with a fluorogenic probe (TaqMan). *JournalofVirologicalMethods,* 72: 125-135.

McKercher, P.D, Hess, W. R and Hamdy, F. (1978). Residual viruses in pork products. *Applied and Environmental Microbiology*,35(1): 142-145.

McKercher, P. D., Yedloutschnig, R.J., Callis, J.J., Murphy, R., Panina, G.F., Civardi, A., Bugnetti, M., Foni, E., Laddomada, A and Scarano, C. (1987). Survival of viruses in «Prosciutto di Parma» (Parma Ham). *Canadian Institute of Food Science and Technology journal*,20(4): 267-272.

McKissick, G. E and Gustafson, D.P. (1967).*In vivo* demonstration of lability of hog cholera virus to lipolytic agents. *American Journal of Veterinary Research*,28: 909-914.

McVicar, J.W., Mebus, C.A., Becker, H.N., Belden, R.C and Gibbs, E.P. (1981). Induced African swine fever in feral pigs. *Journal of the American Veterinary Medical Association*, 179(5): 441–446.

Mebus, C.A., House, C., Ruiz Gonzalvo, F., Pineda, J.M., Tapiador, J., Pire, J.J., Bergada, J., Yedloutschnig, R. J., Sahu, S., Becerra, V and Sanchez-Vizcaino, J.M. (1993). Survival of foot-and-mouth disease, African swine fever, and hog cholera viruses in Spanish serrano cured hams and Iberian cured hams, shoulders and loins. *Food Microbiology*, 10(2): 133–143.

Mebus, C.A., Arias, M., Pineda, J. M., Tapiador, J., House, C and Sanchez-Vizcaino, J. M. (1997). Survival of several porcine viruses in different Spanish dry-cured meat products. *FoodChemistry*, 54(4): 555–559.

Mendes A. M. (1971). Algumas doenças dos animais em Angola e Moçambique e sua importância na higiene das carnes. *RevistaPortuguesa das Ciencias Veterinarias* 66:271–286

Mendes A. M. (1994). A história de peste suína africana em Angola. *Revista Portuguesa dasCiencias Veterinarias* 89: 110–120

Meng, X.J., Lindsay, D. S and Sriranganathan, N. (2009). Wild boars as sources for infectious diseases in livestock and humans. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*,364(1530): 2697-2707.

Mengeling, W.L., Pirtle, E. C and Torrey, J.P. (1963). Identification of hog cholera viral antigen by immunofluorescence. Application as a diagnostic and assay method. *Canadian Journal of Comparative Medicine* 27: 249-252.

Meyers, G., Thiel, H. G and Rumenapf, T. (1996). Classical swine fever virus: recovery of infectious viruses from cDNA constructs and generation of recombinant cytopathogenic defective interfering particles. *Journal of Virology*,70(3): 1588-1595.

Missohou, A., Niang, M., Foucher, H and Dieye, P.N., (2001). Pig production systems in Lower Casamance (Senegal). *Cahiers Agricultures*, 10: 405–408.

Moennig, V., Schagemann, G., Dahle, J., Greiser-Wilke, I and Leder, L. (1990). A new approach to the diagnosis of hog cholera. *Deutsche Tierarztliche Wochenschrift*,97(2): 91-93.

Moennig, V. (1990): Pestiviruses: a review. *Veterinary Microbiology*,23(1-4): 35-54.

Moennig, V and Plagemann, P.G.W. (1992). The Pestiviruses. *Advances in Virus Research*,41: 98.

Moennig, V. (2000). Introduction to classical swine fever: virus, disease and control policy. *VeterinaryMicrobiology,* 73(2-3): 93-102.

Moennig, V., Floegel-Niesmann, G and Greiser-Wilke, I. (2003): Clinical Signs and Epidemiology of Classical Swine Fever: A Review of New Knowledge. *The Veterinary Journal* 165, no. 1: 11-20.

Moennig, V and Greiser-Wilke, I. (2008). "Classical Swine Fever Virus." In *Encyclopedia of Virology*, edited by Mahy, B.W.J. and van Regenmortel, M.H.V 525-32. Oxford: *AcademicPress*.

Montgomery, R. E. (1921). On a form of swine fever occurring in British East Africa (Kenya colony). *Comparative Pathology* 34: 159 – 191.

Moreki, J.C and Mphinyane, H.G., (2011). Opportunities and challenges of pig production in Botswana. Livestock Research for Rural Development 23(4): 30-87.

Moser, C., Ruggli, N., Tratschin, J. D and Hofmann, M.A. (1996). Detection of antibodies against classical swine fever virus in swine sera by indirect ELISA using recombinant envelope glycoprotein E2. *Veterinary Microbiology,*51(1–2): 41-53.

Muhanguzi D, Lutwama V and Mwiine F.N (2012). Factors that influence pig production in Central Uganda: Case study of Nangabo Sub-County, Wakiso district. *Veterinary World*, 5(6):346-351.

Müller, T., Teuffert, J., Staubach, C., Selhorst, T and Depner, K. R. (2005). Long-Term Studies on Maternal Immunity for Aujeszky's Disease and Classical Swine Fever in Wild Boar Piglets. J*ournal of Veterinary Medicine Series B,*52(10): 432-436.

Mur, L., Martínez-López, B., Costard, S., de la Torre, A., Jones, B. A., Pfeiffer, D., Sánchez- Vizcaíno, J.M and Wieland, B. (2012a). General framework for the risk assessment of African swine fever introduction into the European Union. In: 9th International Congress of Veterinary Virology, 4–6 September, Madrid, Spain.

Mur, L., Martinez-Lopez, B., Sanchez-Vizcaino, J.M., (2012b). Risk of African swine fever introduction into the European Union through transport-associated routes: returning trucks and waste from international ships and planes. *BMC Veterinary Research* 8(1): 149.

Mutua, F.K., Dewey, C.E., Arimi, S.M., Ogara, W.O., Githigia, S.M., Levy, M and Schelling, E. (2011): Indigenous pig management practices in rural vil-lages of Western Kenya. *LivestockResearchforRuralDevelopment* 23(7): 144-147.

Muwanika, V.B, Nyakaana, S., Siegismund, H. R. and Arctander P. (2007). Population genetic structure of the common warthog (*Phacochoerus africanus*) in Uganda. Evidence for a strong philopatry among warthogs and social structure breakdown in a disturbed population. *African Journal of Ecology,* 45(1): 22-30.

Nana-Nukechap, M. F and Gibbs, E.P.J. (1985). Socioeconomic effects of African swine fever in Cameroon. *Tropical Animal Health and Production*, 17(3), 183–184.

National Bureau of Statistics (NBS), (2010). The review of the Nigerian economy 2010. http://www.nigerianstat.gov.ng

National Population Commission (NPC), Nigeria. (2006): Data for national development. 2006 census. www.population.gov.ng

Ndébi, G., Kamajou, J and Ongla, J. (2009). Analyse des contraintes au développement de la production porcine au Cameroun. Tropicultura 27(2): 70–76. Ndébi, G., Kamgnia, B.D and Tchoumboué, J., 2004. Etude des marges dans les circuits de commercialisation du porc au Cameroun. *Tropicultura,* 22(3): 104–109.

Ngowi, H.A., Mlangwa, J.E.D., Carabin, H., Mlozi, M.R.S., Kassuku, A.A., Kimera, S. I and Willingham III, A.L (2007): Financial efficiency of health and pig management education intervention in controlling porcine cysticercosis in Mbulu District, northern Tanzania. *Livestock Research for Rural Development,* 19(62): 19-36.

Nielen, M., A.W. Jalvingh, M.Meuwissen, P.M. Horst, S and Dijkhuizen, A. A. (1999). Spatial and stochastic simulation to evaluate the impact of events and control measures on the 1997–1998 classical swine fever epidemic in the Netherlands. II. Comparison of control strategies. *PreventiveVeterinaryMedicine*, 42: 297-317.

Nielsen, B., Ekeroth, L., Bager, F and Lind, P. (1998). Use of muscle fluid as a source of antibodies for serologic detection of Salmonella infection in slaughter pig herds. *Journal of Veterinary Diagnostic Investigations,*10: 158-163.

NILES, (1910): Cited by Cole *etal*. 1962.

Nissen, S., Poulsen, I.H., Nejsum, P., Olsen, A., Roepstorff, A., Rubaire-Akiiki, Cand Thamsborg, S.M., (2011): Prevalence of gastrointestinal nematodes in growing pigs in Kabale District in Uganda. *Tropical Animal Health and Production* 43(3): 567-572.

Nwanta, J.A., Shoyinka, S.V.O., Chah, K.F., Onunkwo, J.I., Onyenwe, I.W., Eze, J.I., Iheag-wam, C.N., Njoga, E.M., Onyema, I., Ogbu, K.I., Mbegbu, E.C., Nnadozie, P.N., Ibe, E.C and Oladimeji, K. T. (2011). Production characteristics, disease prevalence, and herd-health management of pigs in Southeast Nigeria. *Journal of Swine Health and Production,* 19(6): 331-339.

Odemuyiwa S. O, Adebayo I. A., Ammerlaan W., Alaka O. O., Oyedele O. I., Soyelu K. O, Olaleye, D. O., Otesile, E. B and Muller, C. P. (2000). An outbreak of African swine fever in Nigeria: virus isolation and molecular characterization of VP72 gene of a first isolate from West Africa. *Virus Gene,* 20(2): 139-142.

Office international des epizooties (OIE). (2008). African swine fever. In: Manual of diagnostic tests and vaccines for terrestrial animal mammals, birds and bees. [http://www.oie.int/e ng/normes/ mmanual/2008/pdf/2.08.01_ASF.pdf].

Office international des epizooties (OIE) (2009). Event Summary: African swine fever, Mauritius.[http://www.oie.int/wahis/public.php?page=eventsummary&thiscountrycode=MUS&reportid=63 69].

Office international des epizooties (OIE). (2010).*Terrestrial Animal Health Code*, 19(1-2): 2. Paris.

Office international des epizooties (OIE). (2012). WAHID interface animal health information African swine fever [athttp://web.oie.int/wahis/public.php] site visited on.

Office international des epizooties (OIE).(2014). Weekly Disease information:[http://www.oie.int/]wahis_2/public/wahid.php/Diseaseinformation/WI/index/newlang/en.

Office international des epizooties (OIE). (2015). Classical swine fever.List of CSF free Member Countries. http://www.oie.int/animal-health-in-the-world/official-disease-status/classical-swine-fever/

Oleaga-Perez, A., Perez-Sanchez, R and Encinas-Grandes, A. (1990). Distribution and biology of Ornithodoros erraticus in parts of Spain affected by African swine fever. *VeterinaryRecord*, 126(2): 32-37.

Oleaga-Perez, A., Perez-Sanchez, R., Astigarraga, A and Encinas-Grandes, A. (1994). Detection of pig farms with *Ornithodoroserraticus* by pig serology. Elimination of non-specific reactions by carbohydrate epitopes of salivary antigens. *VeterinaryParasitology* 52(1–2): 97–111.

Olugasa B.O. (2007): Serological evidence of African swine fever virus infection in commercial pig herds in South west Nigeria. *African Journal of Livestock Extension*, 5(1): 61-66.

Olugasa, B.O., Ijagbone, I.F., (2007): Pattern of spread of African swine fever in south-western Nigeria, 1997–2005. *VeterinariaItaliana,* 43(3): 621-628.

Otesile, E.B., Ajuwape, A.T., Odemuyiwa, S.O., Akpavie, S.O., Olaifa, A.K., Odaibo, G.N., Olaleye, D. O and Adetosoye, A. I. (2005): Field and experimental investigations of an outbreak of African swine fever in Nigeria. *Revue d Elevage et de Medecine Veterinaire des Pays Tropicaux*,58(1–2): 21-26.

Oura, C.A., Powell, P. P., Anderson, E and Parkhouse, R.M. (1998). The pathogenesis of African swine fever in the resistant bushpig. *Journal of General Virology,* 79(6): 1439–1443.

Owolodun, O. A, Yakubu, B., Antiabong, J. P., Adefalujo, O. K., Ogedengbe, M. E., Obisakin, E. T and Shamaki, D. (2007). Molecular assessment of African swine fever In North-central Nigeria. *Bulletin of Animal Health and Production in Africa,* 55(2): 96-103.

Owolodun, O.A., Bastos, A.D., Antiabong, J.F., Ogedengbe, M.E., Ekong, P. S and Yakubu, B. (2010a). Molecular characterisation of African swine fever viruses from Nigeria (2003–2006) recovers multiple virus variants and reaffirms CVR epidemiological utility. *VirusGenes,* 41(3): 361-368.

Owolodun, O.A., Obishakin E.T, Ekong P.S and Yakubu, B. (2010b). Investigation of African swine fever in slaughtered pigs, Plateau state, Nigeria, 2004-2006. *TropicalAnimalHealthProduction* 42:1605–1610

Owolodun, O. A., Yakubu, B., Antiabong, J. F., Ogedengbe, O. E., Luka, P. D and Audu,J. B. (2010c): .Spatio-temporal dynamics of African swine fever outbreaks in Nigeria, 2002–2007. *Transbound Emerging Diseases,* 57(5):330-339.

Pagani, P and Rijks, J. (2014). African swine fever in wild boar and African wild suids. In: AfricanSwineFever. Edited by Rachel Thomas. *Dutchwildlifehealthcentre*

Pagnini, P., Rossi, G., Tommasis, B., de Matteo, R and Martone, F. (1984). Resistance of bovine diarrhoea virus to physical agents and various types of water. *Acta Medica Veterinaria,*30(2): 277-284.

Panina, G.F., Civardi, A., Cordioli, P., Massirio, I., Scatozza, F., Baldini, P and Palmia, F. (1992). Survival of hog cholera virus (HCV) in sausage meat products (Italian salami). *InternationalJournal ofFoodMicrobiology*, 17: 19-25.

Pasick, J. (2008). Advances in the Molecular Based Techniques for the Diagnosis and Characterization of Avian Influenza Virus Infections.*TransboundaryandEmergingDiseases*, 55(8):329-338

Paton, D.J., Sands, J.J., Lowings, J.P., Smith, J. E., Ibata, G. and Edwards, S. (1995). A proposed division of the pestivirus genus using monoclonal-antibodies, supported by cross-neutralization assays and genetic sequencing. *Veterinary Research,*26(2): 92-109.

Paton, D. J., McGoldrick, A., Belak, S., Mittelholzer, C., Koenen, F and Vanderhallen, H. (2000). Classical swine fever virus, a ring test to evaluate RT-PCR detection methods. *VeterinaryMicrobiology,*73:159-174.

Paton, D. J and Greiser-Wilke, I. (2003). Classical swine fever: an update. *Research in Veterinary Science* 75(3): 169-78.

Pauly, T., Konig, M., Thiel, H. J and Saalmuller, A. (1998). Infection with classical swine fever virus: effects on phenotype and immune responsiveness of porcine T lymphocytes. *Journal ofGeneralVirology,* 79 (1): 31-40.

Penrith, M.L., Thomson, G. R and Bastos, A.D.S. (2004a). African swine fever. In: Coetzer, J.A.W., Tustin, R.C. (Eds.), *Infectious Diseases of Livestock*, volume 2, 2nd edition. Oxford University Press, Oxford, UK, pp. 1088–1119.

Penrith, M.L., Thomson, G.R., Bastos, A.D., Phiri, O.C., Lubisi, B.A., Du Plessis, E.C., Macome, F., Pinto, F., Botha, B and Esterhuysen, J. (2004b). An investigation into natural resistance to African swine fever in domestic pigs from an endemic area in southern Africa. *Revue Scientifique et Technique de l Office International des Epizooties* 23(3): 965-977.

Penrith, M. L and Vosloo, W. (2009). Preparation of African swine fever contingency plan. *FAO Animal production and health manual* 8.

Penrith, M. L., Vosloo, W., Jori, F. and Bastos, A. D. S. (2009).Review of African swine fever: transmission, spread and control. *Journal of South African Veterinary Association*, 80(2): 58-62.

Penrith, M. L., Vosloo, W and Mather, C. (2011). Classical Swine Fever (hog cholera): review of aspects relevant to control. *TransboundryEmergingDisease*, 58(3): 187-196.

Penrith, M. L., Vosloo W and Jori F., (2013). African swine fever virus eradication in Africa. *Journal of Virus Research*,173(1):228-246.

Pereda, A. J. I., Greiser-Wilke, B. Schmitt, M.A. Rincon, J.D. Mogollon, Z.Y., Sabogal, A.M. Lora, H., Sanguinetti, and Piccone, M.E. (2005). Phylogenetic analysis of classical swine fever virus (CSFV) field isolates from outbreaks in South and Central America*VirusResearch*, 110: 111-118.

Perez, J., Fernandez, A.I., Sierra, M. A., Herraez, P., Fernandez, A and Martin de las, M. J. (1998). Serological and immunohistochemical study of African swine fever in wild boar in Spain. *VeterinaryRecord,* 143(5): 136–139.

Perez-Sanchez, R., Astigarraga, A., Oleaga-Perez, A and Encinas-Grandes, A. (1994). Relationship between the persistence of African swine fever and the distribution of Ornithodoros erraticus in the province of Salamanca, Spain. *VeterinaryRecord*,135(9): 207-209.

Petrov A and Salzungen B. (2015). Design and validation of optimized tools for Classical and African swine fever surveillance and pathogenesis research. (Inaugural- Dissertation zur Erlangung der Doktorwürde der Tierärztlichen Fakultät der Ludwig-Maximilians-Universität München, Germany).

Petrus, N.P., Mpofu, I., Schneider, M. B and Nepembe, M. (2011). The constraints and potentials of pig production among communal farmers in Etayi Constituency of Namibia. *Livestock Research for Rural Development,* 23(7):23-59.

Pini, A and Hurter, L. R. (1975). African swine fever: an epizootiological review with special reference to the South African situation. *Journal of the South African Veterinary Association*, 46(3): 227-232.

Plowright, W and Parker, J. (1967). Stability of ASFV with particular reference to heat and pH inactivation. *ArchivesofGesamteVirusforsch*, 21:382-402.

Plowright, W., Parker, J and Pierce, M.A., (1969a). African swine fever virus in ticks (*Ornithodorosmoubata*) collected from animal burrows in Tanzania. *Nature*, 221: 1071–1073.

Plowright, W., Parker, J., Pierce, M. A. (1969b). The epidemiology of African swine fever in Africa. *Veterinary Record*, 85: 668-674.

Plowright, W., Perry, C. T and Peirce, M.A. (1970). Transovarial infection with African swine fever virus in the argasid tick, *Ornithodoros moubata porcinus*, Walton. *Research in Veterinary Science*, 11(6): 582–584.

Plowright, W., Perry, C. T and Greig, A. (1974): Sexual transmission of African swine fever virus in the tick, Ornithodoros moubata porcinus, Walton. *Research in Veterinary Science*, 17(1): 106–113.

Plowright, W. (1981). African swine fever. In: Davis, J.W., Karstad, L.H., Trainer, D.O. (Eds.), *Infectious Diseases of Wild Mammals*. Iowa State University Press, Ames, IA, pp. 178–190.

Plowright, W., Thomson, G.R., Neser, J.A., Coetzer, J.A.W., Thomson, G. R and Tustin, R. C. (1994). African swine fever. In: Coetzer, J.A.W., Thomson, G.R., Tutsin, R.C. (Eds.), Infectious Diseases of Livestock with Special Reference to Southern Africa, volume 1, Oxford University Press, Cape Town, pp. 567–599.

Pol, F., Rossi, S., Mesplede, A., Kuntz-Simon, G and Potier, M. F. (2008). Two outbreaks of classical swine fever in wild boar in France. *Veterinary Record*, 162(25): 811-816.

ProMED, (1998): PRO/AH> African swine fever – Russia (10): control, food chain. Archive Number: 20121008.1329760. http://www.promedmail.org/

Rahimi, P., Sohrabi, A., Ashrafihelan, J., Edalat, R., Alamdari, M., Masoudi, M., Mostofi, S and Azadmanesh, K (2010). Emergence of African swine fever virus, northwestern Iran. *Emerging Infectious Diseases*, 16: 1946-1948.

Randriamparany, T., Grenier, A., Tourette, I., Maharavo Rahantamalala, C., Rousset, Dand Lancelot, R. (2005). Epidemiological situation of African swine fever in Lake Alaotra Region (Madagascar) and possible consequences on the organization of disease control and surveillance. *Revue d Elevage et de Medecine Veterinaire des Pays Tropicaux*, 58: 15-20.

Randy, E., D'huart J., Lucchini, V and Aman R. (2002). Evidence of two genetically divergent species of warthog *phacochoerus africanus* and *p. aethiopicus* (artiodactyla; suiformes) in East Africa. *MammalianBiology-zeitschriftfursaugetierkunde,* 67:91-96.

Ravaomanana, J., Jori, F., Vial, L., Perez-Sanchez, R., Blanco, E., Michaud, V and Roger, F. (2011). Assessment of interactions between African swine fever virus, bushpigs *(Potamochoerus larvatus)*, *Ornithodoros* ticks and domestic pigs in north-western Madagascar. *Transboundary and Emerging Diseases*, 1682–1865.

Rehman, S. (1987). Virucidal effect of the heat treatment of waste food for swine. *Tierarztliche Umschau,* 42(11): 892-896.

Reimann, I., Depner, K., Trapp, S and Beer, M. (2004): An avirulent chimeric Pestivirus with altered cell tropism protects pigs against lethal infection with classical swine fever virus. *Virology* 322:143-157.

Rennie, L., Wilkinson, P. J and Mellor, P.S. (2001). Transovarial transmission of African swine fever virus in the argasid tick Ornithodoros moubata. *Medical and Veterinary Entomology,* 15: 140-146.

Ressang, A. A. (1973). Studies on the pathogenesis of hog cholera. I. Demonstration of hog cholera virus subsequent to oral exposure. *ZentralblVeterinarmed* B, 20(4): 256-271.

Ribbens, S., Dewulf, J., Koenen, F., Laevens, H and de Kruif, A. (2004). Transmission of classical swine fever. Areview. *The Veterinary quarterly*,26(4): 146-155.

Rijn, P.A. van, Bosser, A., Wensvoort, G and Moormann, R.J.M. (1996). Classical swine fever virus (CSFV) envelope glycoprotein E2 containing one structural antigenic unit protects pigs from lethal CSFV challenge. *Journal of General Virology*,77(11): 2737-2745.

Risatti, G.R., Holinka, L.G., Lu, Z., Kutish, G.F., Tulman, E.R., French, R.A., Sur, J.H., Rock, D. L and Borca, M.V. (2005). Mutation of E1 glycoprotein of classical swine fever virus affects viral virulence in swine. *Virology*,343(1): 116-127.

Robertson, A., Bannister, G.L., Boulanger, P., Appel, M and Gray, D. P. (1965). Hog cholera virus. Demonstration of the antigen in swine tissues by the fluorescent antibody technique. *Canadian Journal of Comparative Medicine and Veterinary Science*,29: 299-305.

Roehe, P. M and Woodward, M. J. (1991): Polymerase chain reaction amplification of segments of pestivirus genomes. *Archive Virologica Supplementum*3: 231-238.

Roger, F., Ratovonjato, J., Vola, P and Uilenberg, G. (2001).*Ornithodoros porcinus* ticks, bushpigs, and African swine fever in Madagascar. *Experimental and Applied Acarology,* 25(3): 263-269.

Rolesu S. (2007). Geographical information systems: a useful tool to approach African swine fever surveillance management of wild pig populations. *VeterinariaItalia*, 43(3): 463-467.

Rowlands, R. J. V., Michaud, L., Heath, G., Hutchings, C., Oura, W., Vosloo, R., Dwarka, T., Onashvili, E., Albina, E and Dixon L. K. (2008): African swine fever virus isolate, Georgia, 2007. *Emerging Infectious Diseases,*14: 1870-1874.

Ruiz-Fons, F., Segalés, J and Gortázar, C. (2008). A review of viral diseases of the European wild boar: Effects of population dynamics and reservoir rôle. *The Veterinary Journal*,176(2): 158-169.

Russell, A. D and Hugo, W.B. (1987).*Disinfection in Veterinary and Farm Animal Practice.* Edited by Linton, A.H., Hugo, W.B. and Russell, A.D., Chemical disinfectants. Oxford, London.

Saatkamp, H.W., Berentsen, P.B.M and Horst H.S. (2000). Economic aspects of the control of classical swine fever outbreaks in the European Union. *Veterinary Microbiology,* 73(2-3):221-237

Sabogal, Z.Y., Mogollón, J.D., Rincón, M. A and Clavijo, A. (2006): Phylogenetic analysis of recent isolates of classical swine fever virus from Colombia. *Virus Research*,115(1): 99-103.

Saka J. O., Adesehinwa, A. O. K and Ajala, M. K. (2010). Incidence of African swine fever disease and its associated implications on pig production in Lagos State, Nigeria. *Bulgarian Journal of Agricultural Science*,16(1): 80-90.

Sanchez, B. C. (1982). African swine fever: New developments. *Revue Scientifique et Technique* 1(4): 1065-1094.

Sanchez-Botija, A. (1963). Reservorios del virus de la peste porcina Africana. Investigacion del virus de la P.P.A en los artropodos mediante la prueba de la hemoadsorcion. *Bulletin de l Office International des Epizooties,* 60: 895-899.

Sánchez-vizcaíno, J. M. (2006).*African Swine Fever Diseases of Swine*. (Editors by Straw, B., D'Allaire, S., Mengeling, W. and Taylor, D.), Iowa State University, USA, pp. 298.

Sanchez-Vizcaino, J.M., Martinez-Lopez, B., Martinez-Aviles, M., Martins, C., Boinas, F., Vial, L., Michaud, V., Jori, F., Etter, E., Albina, E and Roger, F. (2009).Scientific review on African swine fever, External Scientific Report submitted to EFSA,pp. 1-141.

Sánchez-Vizcaíno, J. M. (2010). Early detection and contingency plans for African swine fever. Compedium of technical items presented to the OIE World Assembly of Delegates and to OIE Regional Commissions, 129-168.

Sanchez-Vizcaino, J. M., Mur, L. and Marinez-Lpez, B. (2012). African swine fever. An epidemiological update. *Transboundary and Emerging Diseases,*59(1): 27-35.

Sánchez-Vizcaíno, J. M and Arias, M. (2012).Africanswinefever In: *Diseases of swine*. Zimmerman J, Kar-riker LA, Ramirez A, Schwartz KJ, Stevenson GW, editors. John Wiley & Sons, Iowa, USA, pp.396-404.

Sánchez-Vizcaíno J. M, Mur, L and Martínez-López, B. (2013). Africanswinefever(ASF): Five years aroundEurope. *Veterinary Microbiology*, 165(1–2):45-50

Sato, M., Mikami, O., Kobayashi, M., and Nakajima, Y. (2000). Apoptosis in the lymphatic organs of piglets inoculated with classical swine fever virus. *Veterinary Microbiology*, 75(1): 1-9.

Savi, P., Torlone, V and Titoli, F. (1965). Research on the survival of the virus of classical swine fever in certain meat products. *Bulletin Office International des Epizooties*,63: 87-96.

Scott G. R (1965): Prevention, control and eradication of African swine fever. *Bulletin de l'Office International des Epizooties*,63:751–764

Shannon, A.D., Morrissy, C., Mackintosh, S. G and Westbury, H.A. (1993). Detection of hog cholera virus antigens in experimentally-infected pigs using an antigen-capture ELISA. *Veterinary Microbiology*,34: 233-248.

Sharpe, K., Gibbens, J., Morris, H and Drew, T. (2001). Epidemiology of the 2000 CSF outbreak in East Anglia: preliminary findings. *Veterinary Record*,148(3): 91.

Siméon-Negrin, R. E and Frias-Lepoureau, M. T. (2002). Eradication of African swine fever in Cuba (1971 and 1980). In: In: Morilla A. *et al*. (eds), *Trends in Emerging Viral Infections of Swine*, 1st edn. Iowa State University Press, Iowa, USA. pp. 125–131.

Snowdon, W. A and French, E.L. (1968). The bovine mucosal disease-swine fever virus complex in pigs. *Australian Veterinary Journal*,44: 179-84.

Stadejek, T., Warg, J and Ridpath, J. F. (1996). Comparative sequence analysis of the 5' noncoding region of classical swine fever virus strains from Europe, Asia, and America. *Archive of Virology*,141: 771-777.

Stark, K.D. C. (1998): System for the prevention of African swine fever; the role of Free-Living Host in Africa. *OnderstpoortJournalofveterinaryResearch*, 52:201-209.

Stegeman, A., Elbers, A.R.W., Nes, A.V, Smak, J. A and Verheijden, J.H.M. (2000). Transmission of classical swine fever virus by artificial insemination during the 1997-1998 epidemic in the Netherlands: a descriptive epidemiological study. *The Veterinary quarterly*,22(4): 228-233.

Stegeman, J. A and Loeffen, W. L. A. (2012). African swine fever virus excretion patterns in persistently infected animals: A quantitative approach. *Veterinary Microbiology*,160: 327-340.

Steiger, Y., Ackermann, M., Mettraux, C and Kihm, U. (1992). Rapid and biologically safe diagnosis of African swine fever virus infection by using polymerase chain reaction. *Journal of Clinical Microbiology,*30(1): 1-8.

Stewart, W.C., Downing, D.R., Carbrey, E.A., Kresse, J.I and Snyder, M.L. (1979). Thermal inactivation of hog cholera virus in ham. *American Journal of Veterinary Research,*40(5): 739.

Summerfield, A., Knotig, S. M and McCullough, K.C. (1998): Lymphocyte apoptosis during classical swine fever: implication of activation-induced cell death. *Journal of Virology* 72(3), 1853-1861.

Summerfield, A., McNeilly, F., Walker, I., Allan, G., Knoetig, S. M and McCullough, K.C. (2001): Depletion of CD4(+) and CD8(high+) T-cells before the onset of viraemia during classical swine fever. *Veterinary Immunology and Immunopathology,* 78(1): 3-19.

Suradhat, S., Damrongwatanapokin, S and Thanawongnuwech, R. (2007). Factors critical for successful vaccination against classical swine fever in endemic areas. *Veterinary Microbiology,*119(1): 1-9.

Susa, M., Konig, M., Saalmuller, A., Reddehase, M.J and Thiel, H.J. (1992). Pathogenesis of classical swine fever: B-lymphocyte deficiency caused by hog cholera virus. *JournalofVirology* 66(2): 1171-1175.

Taylor, W.P., Best, J.R and Colquhoun, I.R. (1977). Absence of African swine fever from Nigerian warthogs. *Bulletin of Animal Health and Production in Africa,* 25: 196-203.

Terpstra, C and Wensvoort, G. (1988). The protective value of vaccine-induced neutralising antibody titres in swine fever. *VeterinaryMicrobiology*, 16(2): 123-128.

Terpstra, C. (1987): Epizootiology of swine fever. *Veterinary Quarterly,*9(l.1): 50S-60S.

Terpstra C., Woortmeyer, Rand Barteling S.J. (1990). Development and properties of a cell culture produced vaccine for hog cholera based on the Chinese strain. *Dutsch.Tierarztl. Wochenschr*, 97: 77-79.

Terpstra, C. (1991). Hog cholera: an update of present knowledge. *British Veterinary Journal,* 147: 397-406.

Terpstra, C. (1994). Hog cholera. In Coetzer, J.A.W., and R.C. Tustin (eds), Infectious Diseases of Livestock, 2nd edition. pp. 654-657. Oxford University Press, South Africa.

The merck veterinary manual. (2013). Overview of African Swine Fever. In: The Merck Veterinary Manual. Published by Merck Sharp & Dohme Corporation. a subsidiary of Merck & Company Incorporated, Whitehouse Station, N.J. USA

Thiel, H. J., Plagemann, P. G. W and Moennig, V. (1996). Pestiviruses. In: Knipe, D.M., Howley, P. M. (Ed.), *FieldsVirology*. *Lippincott-Raven*, *Philadelphia*, pp. 1059-1073.

Thomson, G. R., Gainaru, M. D and Van Dellen, A. F. (1980). Experimental infection of warthog (*Phacocherusaethiopicus*) with African swine fever virus. *Onderstepoort Journal of Veterinary Research*,47: 19-22.

Thomson, G.R. (1985). The epidemiology of African swine fever: the role of free-living hosts in Africa. *Onderstepoort Journal ofVeterinaryResearch*, 52(3): 201-209.

Trautwein, G. (1988). Classical swine fever and related infections, Pathology and pathogenesis of the disease. Martinus Nijhoff, Boston, pp. 27-54.

Uilenberg, G. (1963). Existence de Ornithodoros porcinus Walton, 1962 (Argasidae) a Madagascar. *Revue d Elevage et de Medecine Veterinaire des Pays Tropicaux*, 16(2): 147–149.

van Gennip, H. G., van Rijn, P. A., Widjojoatmodjo, M. N., de Smit, A. J and Moormann, R. J. (2000). Chimeric classical swine fever viruses containing envelope protein Erns or E2 of bovine viral diarrhoea virus protect pigs against challenge with CSFV and induce a distinguishable antibody response. *Vaccine*, 19:447-459.

van Gennip, H.G., Bouma, A., Van Rijn, P. A., Widjojoatmodjo, M. N and Moormann, R.J., (2002), Experimental non-transmissible marker vaccines for classical swine fever (CSF) by trans-complementation of E(rns) or E2 of CSFV. *Vaccine*, 20: 1544-1556.

Van Oirschot, J. T and Terpstra, C. (1977). A congenital persistent swine fever infection. Clinical and virological observations. *Veterinary Microbiology*,2(2): 121-132.

Van Oirschot, J.T. (1999).*Classical Swine Fever (Hog cholera)*. Edited by Straw, B.E., Sylvie, D' Allaire, Mangeling, W. L. and Tailor., D.J., *DiseasesofSwine*,. IowaState University Press, Iowa, USA.

Van Oirschot, J. T. (2003). Vaccinology of classical swine fever: from laboratory to field. *Veterinary Microbiology*,9(4): 367-384.

Van Oirschot, J.T. (2004). Hog cholera. In: Coetzer, J.A.W., Tustin, R. C. (Ed.), 2nd ed. Vol. Infectious Diseases of Livestock. Oxford University Press, South Africa, pp. 975-986.

van Rijn, P.A., van Gennip, H. G. P and Moormann, R.J.M. (1999). An experimental marker vaccine and accompanying serological diagnostic test both based on envelope glycoprotein E2 of classical swine fever virus (CSFV). *Vaccine*,17(5): 433-440.

Vandeputte, J., Too, H.L., Ng, F.K., Chen, C., Chai, K. K. and Liao, G. A. (2001). Adsorption of colostral antibodies against classical swine fever, persistence of maternal antibodies, and effect on response to vaccination in baby pigs. *American Journal of Veterinary Research,*62: 1805-1811.

Vanderhallen, H., Mittelhozer, C., Hofmann, M. A and Koenen, F. (1999). Classical swine fever virus is genetically stable *in vitro* and *in vivo*. *Archives of Virology,*144(9): 1669-1677.

Vannier, P., Capua, I., Le Potier, M.F., Mackay, D.K.J., Muylkens, B., Parida, S., Paton, D. J and Thiry, E. (2007). Marker vaccines and the impact of their use on diagnosis and prophylactic measures. *Revue Scientifique Et Technique-Office International Des Epizooties,*26(2): 351-372.

Verhulst, A. (1993). Lessons from field experiences in the development of monogastric animal production. In: Mack, S. (Ed.), Strategies for Sustainable Animal Agriculture in Developing Countries. Proceedings of the FAO Expert Consultation held in Rome, Italy, 10–14 December 1990. FAO. *AnimalHealthandProduction* 107: 261-271.

Vigário J.D. (1983). Experimental studies with African swinw fever virus carriers. In African swine fever. Wilkinson P. J. (ed). EUR. 8466 EN. Commission of the European Communities, Luxembourg, 63-66.

Vilcek, S and Belak, S. (1997). Organization and diversity of the 3'-noncoding region of classical swine fever virus genome. *Virus Genes,*15: 181-186.

Vilcek, S and Nettleton, P.F. (2006). Pestiviruses in wild animals.*Veterinary Microbiology*,116(1-3): 1-12.

Wachendorfer, G., Reinhold, G.E., Dingeldein W., Berger J., Lorenz, J and Frost J.W. (1978). Analyse der Schweinepest-Epizootie in Hessen in den Jahren 1971-1974. *Dtsch. Tierarztl. Wochenschr,* 85:113-120.

Wardley, R.C., Andrade, C.d.M., Black, D.N., de Castro Portugal, F.L., Enjuanes, L., Hess, W.R., Mebus, C.A., Ordas, A., Rutili, D., Sanchez-Vizcaino, J.M., Vigario, J.D., Wilkinson, P.J., Moura Nunes, J.F and Thomson, G. (1983). African swine fever virus. *Archives of Virology*, 76(2): 73–90.

Weesendorp, E., Landman, W.J. M., Stegeman, A and Loeffen, W.L.A. (2008). Detection and quantification of classical swine fever virus in air samples originating from infected pigs and experimentally produced aerosols. *Veterinary Microbiology*,127(1-2): 50-62.

Weesendorp, E., Stegeman, A., and Loeffen, W.L.A. (2008): Survival of classical swine fever virus at various temperatures in faeces and urine derived from experimentally infected pigs. *Veterinary Microbiology*, 132(3-4): 249-259.

Wengler, G. (1991). Family Flaviviridae. In: Fifth report of the International Committee on Taxonomy of Viruses, Berlin, Germany.

Wensvoort, G., Terpstra, C., Boonstra, J., Bloemraad, M and van Zaane, D. (1986). Production of monoclonal antibodies against swine fever virus and their use in laboratory diagnosis. *Veterinary Microbiology*, 12(2): 101-108.

Wensvoort, G and Terpstra, C. (1988). Bovine Viral Diarrhea Virus Infection in Piglets born to Sows Vaccinated against Swine Fever with Contaminated Vaccine. *Research in Veterinary Science,* 45:143-148.

Wensvoort, G., Terpstra, C., De Kluyver, E.P., Kragten, C and Warnaar, J. C. (1989). Characterization of pestivirus strains with monoclonal antibodies against hog cholera virus. Epitopes on structural proteins of hog cholera (swine fever) virus. Ph.D. Thesis. University of Utrecht, The Netherlands, pp. 41–51.

Wesley, R. D and Tuthill, A. E. (1984). Genome relatedness among African swine fever virus field isolates by restriction endonuclease analysis. *Preventive Veterinary Medicine*,2: 53-62.

White, A and Cameron, E. (2009). Communal nesting is unrelated to burrow availability to in the common warthog. *Animal behaviour*, 77:87-94.

Widjojoatmodjo, M. N., van Gennip, H. G., Bouma, A., van Rijn, P. A and Moormann, R. J. (2000). Classical swine fever virus Erns deletion mutants, trans-complementation and potential use as nontransmissible, modified, live-attenuated marker vaccines. *Journal of Virology*, 74:2973-2980.

Wieringa-Jelsma, T., Quak, S and Loeffen, W.L. (2006). Limited BVDV transmission and full protection against CSFV transmission in pigs experimentally infected with BVDV type 1b. *Veterinary Microbiology*,118: 26-36.

Wijnker, J.J., Depner, K. R and Berends, B.R. (2008). Inactivation of classical swine fever virus in porcine casing preserved in salt. *International Journal of Food Microbiology*, 128(2): 411-413.

Wilkinson, P. J. (1977). Transmission studies with African swine fever virus. Infection of pigs by airborne virus. *JournalofComparativePathology*, 87: 487-495.

Wilkinson, P.J. (1983). Studies in pigs infected with African swine fever virus (Malta/78). In African swine fever. Wilkinson, P. J. (ed). EUR. 8466 EN. Commission of the European Communities, Luxembourg, 74-84.

Wilkinson, P. J. (1984). The persistence of African swine fever in Africa and the Mediterranean. *PreventiveVeterinaryMedicine*, 2(1–4):71–82.

Wilkinson, P.J and Pensaert, M. (1989). African swine fever Virus, Virus Infections of Porcines. *ElsevierScience*, New York, pp. 17-35.

Williams, D. R and Matthews, D. (1988). Outbreaks of classical swine fever in Great Britain in 1986. *Veterinary Record*, 122(20): 479-483.

Zhao, J. J., Li, Y., Li, N., Shi, Z., Cheng, D., Zhu, Q. H., Tu, C., Tong, G.Z and Qiu, H.J. (2007). A multiplex nested RT-PCR for the detection and differentiation of wild-type viruses from C-strain vaccine of classical swine fever virus. *Journal of Virological Methods,*143(1): 16-22

APPENDICES

Appendix i

QuestionnaireforPigFarmers

Q. NO.............. **Date …………**

STRUCTURED QUESTIONNAIRE TO ASSESS THE RISK FACTORS OF AFRICAN SWINE FEVER AND CLASSICAL SWINE FEVER IN BENUE STATE

INTERVIEW SCHEDULE FOR INDIVIDUALS

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Instructions: Kindly complete the following details; you are required to tick one answer only.

L.G.A: ____

Ward: ____

Name of Owner/respondent (optional) ________________________________

Occupation of respondent

1. Peasant ( )
2. Civil servant ( )
3. Businessman ( )

Sex of respondent

1. Male ( )
2. Female ( )

Educational Status of Respondent

1. Tertiary education ( )
2. Secondary education ( )
3. Primary education ( )
4. No education {Illiterate} ( )

Years of farm's existence

1. < 5years ( )
2. 5 - 10 years ( )
3. >10 years ( )

Years of practice by respondents

1. < 5 years ( )
2. 5 - 10 years ( )

3. > 10 years ( )

What breeds of Pigs are on your farm?

1. Local ( )
2. Exotic ( )
3. Cross ( )

Type of holder farm and the number of pigs

1. Large holder farms(≥ 101) ( )
2. Medium holder farms(51-100)( )
3. Small holder farms (≤ 50) ( )

Is there a Perimeter fence around the farm?

1. Yes ( )
2. No ( )

Are there Rodents on the farm?

1. Yes ( )
2. No ( )

Do other animals have access to your farm?

1. Yes ( )
2. No ()

Do you observe presence of wild pigs around your farm?

1. Yes ( )
2. No ()

Do you dispose or bury carcass near your farm?

1. Yes ( )
2. No ( )

Do you have a nearby pig farm within 1 km radius?

1. Yes ( )
2. No ( )

Do you have slaughter slabs within 1 km radius of the farm?

1. Yes ()
2. No ()

Do you have refusedump sites within 1 km radius of the farm?

1. Yes ( )
2. No ( )

Do you have a quarantine/isolation unit within 100 m radius of the regular pens?

1. Yes ( )
2. No ( )

Do your farm workers have designated working clothes?

1. Yes ( )
2. No ( )

Do your farm workers take shower/bath at work?

1. Yes ( )
2. No ( )

Do your workers Snack or eat while working?

1. Yes ( )
2. No ( )

Do your workers wear work clothes outside of the farm?

1. Yes ( )
2. No ( )

Do you lend out or receive service boars?

1. Yes ( )
2. No ( )

Do you clean your pen floor daily?

1. Yes ( )
2. No ( )

Do you disinfect your pen floor daily?

1. Yes ( )
2. No ( )

Do you clean your working utensils daily?

1. Yes ( )
2. No ( )

Do you disinfect working utensils daily?

1. Yes ( )
2. No ( )

What is your Source of replacement stock?

1. External ( )
2. Internal ( )

Do you feed swill to your pigs?

1. Yes ( )
2. No ( )

Do you feed uncooked swill to your pigs?

1. Yes ( )
2. No ( )

Do you Share farm workers among fellow farmers?

1. Yes ( )
2. No ( )

Do you share working utensils with other farms?

1. Yes ( )
2. No ( )

Do you have designated work footwear for your farm workers?

1. Yes ( )
2. No ( )

Do you have a functional foot dip?

1. Yes ( )
2. No ( )

Are there reported presences of ticks on pigs in your farm?

1. Yes ( )
2. No ( )

Do you routinely control pests on your farm premises?

1. Yes ( )
2. No ( )

System of Management in the Farm

1. Intensive system ( )
2. Semi-intensive ( )
3. Extensive system ( )

Are you aware that CSF (hog cholera) can affect pigs?

1. Yes ( )
2. No ( )

Are you aware that ASF can affect pigs?

1. Yes ( )
2. No ( )

What is the most common swine fever related signs frequently heard of by you?

1. Abortion ( )
2. Hyperaemia ( )
3. weakness or unwillingness of the pigs to stand ( )

Do you routinely seek veterinary services for your pigs?

1. Yes ()
2. No ()

THANK YOU FOR YOUR TIME AND RESPONSES