African Swine Fever in Uganda: Epidemiology and Socio-economic Impact in the Smallholder Setting

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Doctoral Thesis
Swedish University of Agricultural Sciences
Uppsala 2017

Acta Universitatis agriculturae Sueciae
2017:26

Cover picture: Maria Nöremark

ISSN 1652-6880
ISBN (print version) 978-91-576-8825-5
ISBN (electronic version) 978-91-576-8826-2

Print: SLU Service/Repro, Uppsala 2017

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Abstract

In the last decade millions of people have been able to leave poverty, increasing the regional demand for meat and livestock products. In combination with reforms in market and agricultural policy, this has led to an increase in pig production in sub-Saharan Africa, most notably in Uganda. The growing pig sector could be an important contributor to poverty reduction among smallholder pig keepers. However, the growing pig population has been followed by an increase in African swine fever (ASF) incidence.

ASF is a contagious, typically very lethal, haemorrhagic, viral disease of domestic pigs. The overall goal of this doctoral project was to develop the understanding of ASF epidemiology in the smallholder setting in Uganda. Four studies were conducted in two districts in northern Uganda among smallholder farmers, other pig production value-chain actors, and a medium-sized farm. The studies included group- and individual interviews as well as biological and environmental sampling and testing for the virus. Data were analysed using semi-qualitative and quantitative methods. The thesis concluded that ASF was endemic in the study area, and that outbreaks could be detected using retrospective and real-time farmer reports. ASF outbreaks were associated with activities of humans, such as trade in pigs and pig products and free-range management systems. ASF outbreaks had long-term negative social and economic impact for pig production value-chain actors on all investigated levels in the value chain. For smallholder farmers, the impact was aggravated with increasing herd size. Trade and consumption of sick and dead pigs were commonly used as coping strategies. Farm-level biosecurity was insufficient for ASF protection and awareness of control methods did not guarantee their implementation. The continuous ASF transmission in the study area was not driven by lack of knowledge, but rather by cultural circumstances, taboos and poverty. Therefore, in order for control methods to be successfully and sustainably implemented, they need to be developed in participation with the communities, adapted to the local context, socially acceptable, flexible, and cost-effective.

Keywords: African swine fever, epidemiology, disease control, participatory, surveillance, smallholders, animal disease impact, low-income, Uganda

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"…the precautions now being exercised beneficially show that under the conditions at present existing the disease is one which can in large measure be avoided"

Eustace Montgomery, 1921. From: "On a form of swine fever occuring in British East Africa (Kenya Colony)"

Contents

List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Chenais E, Boqvist S, Sternberg-Lewerin S, Emanuelson U, Ouma E, Dione M, Aliro T, Crafoord F, Masembe C, Ståhl K (2017). Knowledge, Attitudes and Practices Related to African Swine Fever Within Smallholder Pig Production in Northern Uganda. *Transboundary Emerging Diseases* 64(1), 101-115.

II Chenais E, Sternberg-Lewerin S, Boqvist S, Liu L, LeBlanc N, Aliro T, Masembe C, Ståhl K (2016). African swine fever outbreak on a medium sized farm in Uganda: biosecurity breaches and within-farm virus contamination. *Tropical Animal Health and Production* dec13, doi:10.1007/s11250-016-1197-0.

III Chenais E, Sternberg-Lewerin S, Boqvist S, Emanuelson U, Aliro T, Tejler E, Cocca G, Masembe C, Ståhl K (2015). African swine fever in Uganda: qualitative evaluation of three surveillance methods with implications for other resource-poor settings. *Frontiers in Veterinary Science* 2(0051), doi: 10.3389/fvets.2015.00051.

IV Chenais E, Boqvist S, Emanuelson U, von Brömsen, C, Ouma, E, Aliro T, Masembe C, Ståhl K, Sternberg-Lewerin S. Quantitative assessment of social and economic impact of African swine fever outbreaks in northern Uganda. *Submitted manuscript.*

The contribution of Erika Chenais to the papers included in this thesis was as follows:

I Design and coordination of the study, part of the data collection, analysis and interpretation of the data, drafting the article, critical revision of the article.

II Design and coordination of the study, part of the data collection, interpretation of the data, drafting the article, critical revision of the article.

III Design and coordination of the study, part of the data collection, analysis and interpretation of the data, drafting the article, critical revision of the article.

IV Design and coordination of the study, part of the data collection, analysis and interpretation of the data, drafting the article, critical revision of the article.

Abbreviations

ASF	African swine fever
ASFV	African swine fever virus
C	Celsius
CKW	Community knowledge worker
DNA	Deoxyribonucleic acid
DVO	District veterinary officer
ELISA	Enzyme-linked immunosorbent assay
FAO	Food and Agriculture Organisation of the United Nations
FAOSTAT	FAO Statistic Division
GPS	Global Positioning System
HH	Household
NEPAD	New Partnership for Africa's Development
NGO	Non-governmental organisation
OIE	World Organisation for Animal Health
PCR	Polymerase chain reaction
PE	Participatory epidemiology
PRA	Participatory rural appraisal
spp	Species
UBOS	Uganda Bureau of Statistics
USD	US dollar
UPL	Universal probe library

1 Introduction

A domestic pig (*Sus scrofa domestica*) infected with African swine fever (ASF) will in most cases develop a severe haemorrhagic disease ending with death within a couple of days (Plowright *et al.*, 1994). If the diseased pig or its secretions come into contact with other pigs, most of these will become infected, and meet the same destiny (Taylor, 2006). This gloomy scenario was described already almost one hundred years ago, in the first scientific publication of the disease, by Eustace Montgomery in 1921. Since then we have learned a lot about ASF. We can now describe its epidemiology (Guinat *et al.*, 2016b; Korennoy *et al.*, 2016; Barongo *et al.*, 2015; Gulenkin *et al.*, 2011), the transmission cycles (Costard *et al.*, 2013; Penrith *et al.*, 2013) and the epidemiological implications of the wildlife and arthropod hosts (Jori *et al.*, 2013; Jori & Bastos, 2009; Bigalke, 1994; Thomson, 1985). We can model the spread (Guinat *et al.*, 2016b; Halasa *et al.*, 2016; Vergne *et al.*, 2016), culture, characterize (Malmquist & Hay, 1960), and sequence the virus (Boshoff *et al.*, 2007), determine the relationship between genotypes (Gallardo *et al.*, 2014; Gallardo *et al.*, 2011) and make precise diagnostic procedures (Gallardo *et al.*, 2015a).

During the last century, the way pigs are kept has changed in many parts of the world. All-in–all-out holdings with high biosecurity allow pig owners in high-income countries to take precautions to protect their pigs from infectious diseases, including ASF. Meanwhile, in many low-income countries, most notably in Africa where ASF is endemic, the traditional subsistence farming of poor smallholders has not changed (Costard *et al.*, 2009). In such systems pigs are kept without any protective biosecurity routines and are in daily contact with pigs from other farms. To protect the pigs from ASF infection in such conditions is challenging, but not impossible (Penrith *et al.*, 2013).

The ASF knowledge collected during more than a hundred years makes it possible to understand the disease, and thus to control it and avoid the devastating losses (Spencer & Penrith, 2014). However, disease epidemiology is never universal. The pathogen, the environment, the affected animals and the animal owners all interact and co-affect the epidemiology (Wilcox & Gubler, 2005). The disease impacts on people's livelihoods and conversely, people's decisions and behavior impact on the epidemiology. Because of this intricate web of interactions, disease epidemiology is context specific and needs to be studied for each situation where control is aspired. The paradox of the "worried well", emerging disease hot spots, and neglected disease cold spots describe how disease surveillance and research efforts are intensified in rich countries, despite both the impact of diseases on household level, and risks for disease emergence being higher in low-income countries (Perry *et al.*, 2013). Even if ASF has already reached the European Union (Olsevskis *et al.*, 2016) and now is approaching the Swedish border (Gavier-Widen *et al.*, 2015), the hot spot for both disease outbreaks and impact is still smallholder farms in sub-Saharan Africa.

This thesis is devoted to the epidemiology and socio-economic impact of ASF in smallholder settings in Uganda, a low-income country with a fast-growing domestic pig population and endemic ASF circulation.

1.1 Virus aetiology and properties

The causative agent of ASF is a large, enveloped DNA virus classified within the genus *Asfivirus*, *Asfarviridae* family (Takamatsu *et al.*, 2012). It is the only virus in both the genus and family, and the only arthropod-transmitted DNA-virus known to date (Bastos *et al.*, 2014; Plowright *et al.*, 1994). Currently 22 virus genotypes are described, based on the sequence of the p-72-protein coding gene (Muangkram *et al.*, 2015; Bastos *et al.*, 2003). All of these genotypes are present in Africa (Boshoff *et al.*, 2007). To reach higher molecular epidemiological resolution, with the purpose of studying the source and spread of outbreaks, further typological distinction can be done using genes coding for additional proteins (Gallardo *et al.*, 2011).

The ASF-virus (ASFV) is reputed for its stability and longevity when protected by organic material. The virus has reportedly remained infectious after more than a year at room temperature in serum; 18 months in serum with a preservative (Montgomery, 1921); after 12 months in ice-chests in whole blood with a preservative (Steyn, 1932); in fresh meat after freezing (Mebus *et al.*, 1997); and up to 399 days in traditionally dried and cured meat, prepared from experimentally infected pig slaughtered at peak virema (Mebus *et al.*, 1997; McKercher *et al.*, 1987). Virus survival has been theoretically modelled to several weeks in faeces and urine (Davies *et al.*, 2015). Likewise, the virus can persist in the arthropod vector, *Ornithodoros* spp. ticks, for long periods (months to several years) without a blood meal (Sanchez Botija, 1982; Parker *et al.*, 1969). Directly exposed, however, the virus is sensitive to most lipid solvents and detergents as well as heat-treatment (Takamatsu *et al.*, 2012). This sturdiness has obvious implications for the epidemiology and disease control.

Other viral properties complicating control of ASF is the ability to evade the immunologic defense of the host, and the intrinsic immune response it triggers (Martins & Leitao, 1994). Natural infection will activate humoral and cellular immune responses, and generate production of antibodies (Gomez-Puertas *et al.*, 1996), if the pigs survive long enough for the immune response to be activated (Mur *et al.*, 2016; Gallardo *et al.*, 2015b). Such antibodies will neutralize virus in macrophages and cell lines (Takamatsu *et al.*, 2012), but the protection will seldom be enough to resist viral challenge *in vivo* (Neilan *et al.*, 2004; Martins & Leitao, 1994). Despite numerous research efforts, the virus-host interaction are not fully understood (Neilan *et al.*, 2004), and to date there is no safe and efficient vaccine (Sanchez-Vizcaino *et al.*, 2015).

1.2 Clinical signs and pathogenesis

ASF is a contagious, typically lethal, haemorrhagic infection of domestic pigs and European wild boars (*Sus scrofa*). The disease does not cause clinical disease in the indigenous African wild pigs (Jori & Bastos, 2009). The presentation of clinical signs can be hyper-acute, acute, chronic or even inapparent (Takamatsu *et al.*, 2012). The clinical presentation, including the incubation time, varies to some extent with the host, the virulence of the virus, dose and route of exposure (Sanchez-Vizcaino *et al.*, 2012). The incubation period ranges from a couple of days, up to a week (Plowright *et al.*, 1994). A common clinical presentation in outbreak situations is hyper-acute or acute hemorrhagic fever with very high (almost 100%) case fatality risk (Sanchez-Vizcaino *et al.*, 2015). This is valid (with some exceptions) for most sub-Saharan Africa (Plowright *et al.*, 1994), as well as for the current outbreaks in eastern Europe, Russia, and the Caucasus (Olsevskis *et al.*, 2016; FAO, 2013; Sanchez-Vizcaino *et al.*, 2013).

Typical signs, described in standard pig disease text books, include severe depression, pyrexia, anorexia, shivering and huddling together, ataxia, cyanotic discolouration of the skin, respiratory signs such as dyspnea, cough and sometimes nasal discharge, gastrointestinal signs such as vomiting, diarrhea or sometimes obstruction, abortion in pregnant sows, and ultimately death (Taylor, 2006). See Figures 1a and b.

Figure 1a. Pigs that have died from ASF. The sow in the foreground shows a classic clinical sign, a bluish discolouration of the skin (here in front of the right front leg). (Author's photograph)

Figure 1b. This pig that has died from ASF shows a common agonal sign in the form of foamy discharge from the snout. (Author's photograph)

The dramatic clinical signs most probably occur due to mass-destruction of macrophages and thrombocytes, causing serious impairments of the hemostasis (Blome *et al.*, 2013). Increased vassal permeability cause petechial bleeding and extravasation of blood components (Blome *et al.*, 2013). Macro-pathological signs vary according to the clinical character of the case and are dominated by the widespread haemorrhages and associated swelling of organs (notably extreme splenomegaly) (Taylor, 2006). Enlarged and haemorrhagic lymph nodes are commonly seen (Blome *et al.*, 2013).

1.3 Diagnostics

Identification of ASF-positive animals can be done based on virus isolation, detection of viral antigens, virus genome or antibodies against viral proteins (OIE, 2012). The recommended procedure for virus identification is virus isolation and the haemadsorption test (OIE, 2012). This procedure is time consuming, requires fresh tissue samples and high laboratory biosafety, and therefore it is seldom performed as a standard test today (Malmquist & Hay, 1960). For primary outbreaks, it is however necessary, in order to obtain the reference strain. Viral antigens can be detected by fluorescent antibody tests and by enzyme-linked immunosorbent assay (ELISA); the viral genome can be detected by both real-time and conventional polymerase chain reaction (PCR) assays (OIE, 2012). Detection of viral genome by PCR is the most sensitive technique of these; in addition it is rapid and can be done on putrefied samples. With PCR, the ASFV genome can be detected from tissues, serum and blood at a very early stage in the infection. It is therefore often the most suitable technique (OIE, 2012). Antibodies can be detected by indirect fluorescent antibody tests or ELISA. Detection of antibodies have a limited diagnostic value as their presence will not differentiate between ongoing or older infections. Furthermore, in the hyper-acute and acute forms of ASF, the pigs often die before antibodies have developed. ELISA-procedures are often fast, cheap and easily automated. For detection of antibodies, the OIE recommendations are to combine ELISA with other tests such as fluorescent antibody tests or the indirect immunoperoxidase test (Gallardo *et al.*, 2015a; OIE, 2012). In endemic areas, antibody detection can be of epidemiological value. In such cases it is recommended to combine antibody detection with detection of viral genome by PCR (OIE, 2012).

1.4 History

In 1903 and the following years, several outbreaks of an epizootic pig disease of previously unknown character were described in South Africa (for example

Robertson, 1905 and Theiler, 1905. Reviewed by De Kock, 1940). The clinical descriptions resemble ASF, but from the data at hand it is not possible to verify if these really were outbreaks of ASF, or some other epizootic pig disease. The authors attribute the outbreaks to "European swine fever", "hog cholera", "swine plague" or "pig typhoid", and as stemming from imported pigs. The first scientific publication and description of ASF, at the time of publication called "East African Swine Fever", was made by Montgomery in 1921. The publication describes outbreaks that occurred between 1909 and 1912 in what is today Kenya. Between 1910 and 1916, Montgomery further performed a large series of different experiments, and already in this first report, was able to describe the epidemiology in impressive detail. To honour this work, the disease was for some time called Montgomery's disease by certain authors (Hess, 1971). At the time of these first descriptions, the virus had probably already been present a long time on the African continent, circulating in a sylvatic transmission cycle between indigenous wild pigs and soft, argasid ticks. (Thomson, 1985). The disease successively spread throughout the domestic pig population and persisted in the southern and eastern parts of the African continent (Costard *et al.*, 2013). During the latter half of the 20th century, the disease gradually emerged from its south-east African foci and is now present in most parts of the African continent, wherever pigs are present (Penrith, 2009).

The first ASF occurrence outside Africa was reported from the Iberian peninsula in 1957 (Manso Ribeiro & Azevedo, 1961). Following this, several incursions were detected in Europe, South America and the Pacific. In all cases outside Africa, the epidemics were controlled and the disease eliminated, with the exception of the Italian island Sardinia where the disease has been present since 1978 (Mur *et al.*, 2014). In 2007 the disease escaped from Africa again (most probably from Madagascar), and emerged in the Black Sea port of Poti in Georgia (Rowlands *et al.*, 2008). This epidemic was not brought under control. The disease spread first to the surrounding countries Armenia, Azerbaijan and the Russian Federation (Oganesyan *et al.*, 2013), and then further to Ukraine and Belarus (EFSA, 2014). In 2014 it reached the EU member states Estonia, Lithuania (Gallardo *et al.*, 2014), Latvia (Olsevskis *et al.*, 2016) and Poland (Pejsak *et al.*, 2014). In 2016 the disease spread towards central Europe with cases confirmed in Moldova (*OIE Animal health data*).

1.5 Epidemiology

In viremic pigs, the virus is present in all body secretions and excretions (Greig & Plowright, 1970), although at higher concentration in blood (Guinat *et al.*, 2016a). Virus genomic material has been demonstrated in blood prior to the onset of clinical signs (Gallardo *et al.*, 2015b). The virus may remain viable for long periods in tissues, meat, processed products and faeces from infected pigs (Davies *et al.*, 2015; Turner *et al.*, 1999; Mebus *et al.*, 1997). However, under African conditions the virus is quickly inactivated by sunlight if not protected by organic material (Montgomery, 1921).

The case fatality risk in hyper-acute and acute cases often reaches 100%, whereas the morbidity is often much lower even with highly virulent genotypes (Guinat *et al.*, 2016a). The infectiousness can be described as the total number of new cases created by one infected individual or unit, throughout its infectious period. This parameter is called the basic reproduction ratio, R_0 (Dohoo *et al.*, 2009). Between-farm R_0 is commonly used for describing the transmission between farms or holdings, and within-farm R_0 for describing transmission between the individual animals within a farm or holding. Between-farm R_0 for ASF has recently been described for free roaming smallholder pig populations in northern Uganda, with results ranging from 1.77 to 3.24 (Barongo *et al.*, 2015). An R_0-value over one, and the continuous inflow of naïve pigs in the free-roaming smallholder management systems, explain the endemic disease status in many parts of sub-Saharan Africa (Penrith & Vosloo, 2009). Other publications have presented similar values for between-farm $R_{0,}$ for several settings and serotypes (between 1.65 and 3) (Korennoy *et al.*, 2016; Gulenkin *et al.*, 2011). For within-farm R_0 the reported values are slightly higher (1.4-7.46), with the exception of one very high value (18.0) from experimental infections with the Malta78-strain (Korennoy *et al.*, 2016; de Carvalho Ferreira *et al.*, 2013). The moderate R_0-values demonstrate that despite the dramatic consequences in infected individuals and herds, the disease is actually not that easily transmitted. Thus, it should be possible to control the infection in the domestic pig population.

1.5.1 Transmission

The epidemiology of ASF is complex with three distinct transmission cycles that include wild and domestic pigs, pig products, and an arthropod vector, see Figure 2.

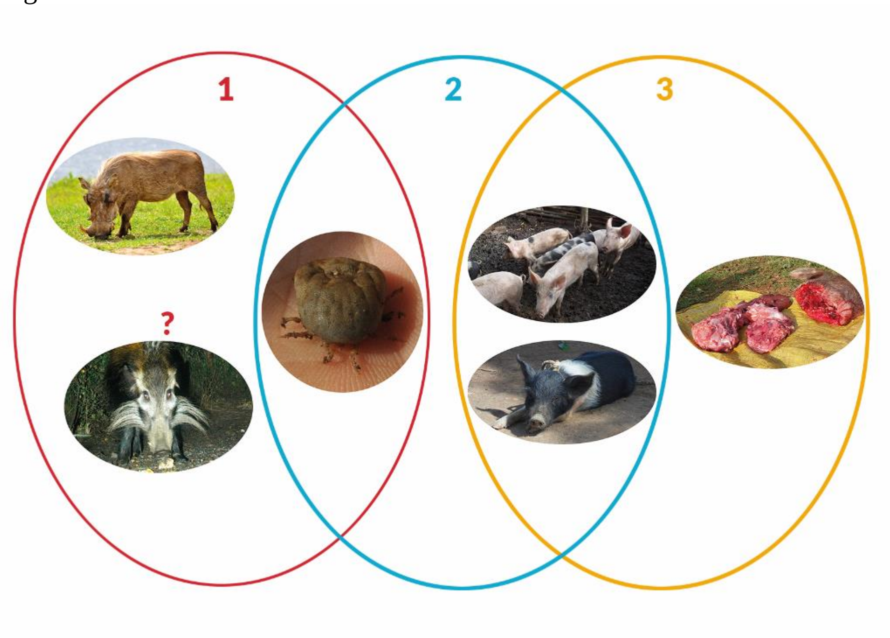

Figure 2. The three transmission cycles of ASF, with the main suspects of transmission depicted. The role of the bushpig remains unclear. Illustration: Linda Hallenberg, photographs by the author and Karl Ståhl.

In the <u>first transmission cycle</u>, the ancient, sylvatic cycle, the virus circulates between the natural reservoirs of the virus, i.e. African wild pigs and soft ticks of the *Ornithodoros* spp., without causing clinical disease in these pigs. In the ticks, virus can be transmitted transstadially, transovary and sexually (Takamatsu *et al.*, 2012). It is not known if the infection affects the ticks in any way (Bernard *et al.*, 2016).

The main suspect suid in this cycle is the common warthog (*Phacochoerus africanuus*) (Jori & Bastos, 2009). Whereas several of the African wild pig species are elusive, and data on their role in ASF epidemiology thus scarce, to date, much more is known about the warthog (Jori *et al.*, 2013). The warthogs are born, and spend their nights, in burrows (Wilkinson, 1984). These burrows are cohabited by *Ornithodoros* spp. ticks that normally live their entire lives in the same burrow (Jori & Bastos, 2009). Apart from newly infected, neonatal piglets, warthogs do not express viremia (Plowright *et al.*, 1969). The virus titers in the blood of the piglets seem to be high enough to transmit infection to ticks, but not to other wild or domestic pigs (Thomson, 1985). The ancient, sylvatic cycle is thus totally dependent on the tick for its continuation (Plowright *et al.*, 1969).

Other African wild pigs such as the bushpig (*Potamochoerus larvatus*), red river hog (*Potamochoerus porcus*) and giant forest hog (*Hylochoerus meinertzhageni*) do not live in burrows, and are thus assumed to be much less exposed to soft ticks (Jori & Bastos, 2009). To the author's knowledge there has only been a single report of ASFV from a giant forest hog (Heuschele & Coggins, 1965) and one from a red river hog (Luther *et al.*, 2007), therefore these species are not discussed further here. ASFV has been detected from free living bushpigs a few times (Okoth *et al.*, 2013; Detray, 1963), and in some areas their habitat coincide with endemic areas, but it is still unknown to what extent this pig species contribute to ASF transmission and persistence in endemic foci (Thomson, 1985). Experimentally infected bushpigs have been shown capable of infecting domestic pigs (Anderson *et al.*, 1998). However, the epidemiological value of this finding is unknown (Jori & Bastos, 2009). Warthogs and bushpigs are considered resistant to natural infection by direct transmission (i.e non-vector transmission). As a consequence, direct transmission between domestic pigs, warthogs and bushpigs probably has a limited role in the epidemiology. This applies to bushpigs in particular, as they are mostly nocturnal (Jori & Bastos, 2009). Anecdotal evidence of contact between domestic pigs and bushpigs in the form of inter-breeding exists, as does evidence that the species do not willingly mix (Kukielka *et al.*, 2016). Indirect transmission via watering points and common feeding areas has been suggested (Kukielka *et al.*, 2016; Payne *et al.*, 2016).

The *Ornithodoros* ticks normally feed quickly, and revert to hiding in crevices and irregularities in the burrows after a blood meal (Ravaomanana *et al.*, 2010). Sometimes, however, ticks can be incidentally carried by warthogs outside the burrow and thus fall off in the vicinity of domestic pigs (Plowright *et al.*, 1969). In order to propagate, the photophobic ticks require uneven surfaces to hide, making primitive pig sties in rough wood, straw or mud a suitable habitat (Ravaomanana *et al.*, 2010). Tick to pig transmission can occur when infected ticks feed on domestic pigs. Unlike their wild relatives, domestic pigs become viremic after infection, and subsequently spread the disease to other domestic pigs and back to the ticks (Plowright *et al.*, 1994). In this <u>second transmission cycle</u>, the tick-pig transmission cycle, the virus circulates between soft ticks and domestic pigs (Costard *et al.*, 2013; Jori *et al.*, 2013). Historically, such transmission happened sporadically in all areas where warthogs, ticks and domestic pigs cohabited (Thomson, 1985).

In the third transmission cycle, also non-sylvatic, the virus transmits from one domestic pig to another, or from pig products to domestic pigs, without involvement of sylvatic hosts or arthropod vectors. This transmission route has been denoted the domestic transmission cycle (Costard *et al.*, 2013; Jori *et al.*, 2013). This cycle has been identified as the main driver of disease in several areas with a high density of pigs, high occurrence of free-range management systems, and a generally low level of farm biosecurity (Penrith et al., 2013). Human behaviour, including legal and illegal activities in the pig production value chain, seem to be driving this transmission cycle in many settings.

1.5.2 Special aspects on ASF control in endemic areas in low-income countries

Contextual circumstances on the micro-, meso- and macro-level affect the epidemiology of ASF. To control spread, epidemiological knowledge is needed at all these levels. On the macro-level, the upsurge in pig keeping, as well as in national and international trade, following the positive global development (Delgado, 2003) can be mentioned as factors promoting ASF emergence (Penrith *et al.*, 2013; Costard *et al.*, 2009). Another factor hindering control on the macro-level is political unrest and the associated switch of focus from animal disease control to more urgent matters (Njabo *et al.*, 2016; ElMasry *et al.*, 2015). This was made evident after the introduction of ASF to Georgia in 2007. Cultural and local practices are increasingly recognised as important for understanding disease epidemiology and ecology on all levels (Janes *et al.*, 2012). On both meso- and micro-levels, poverty affects the possibilities to invest in, and implement, effective biosecurity routines as well as practices concerning pigs that are sick, that have been in contact with sick animals, died, or been slaughtered upon showing signs of disease (Leslie *et al.*, 2015).

Apart from the contextual specificities for low-income countries, ASF control is complicated by the lack of a vaccine and, in endemic areas in some African countries, by the presence of natural reservoirs (Penrith *et al.*, 2013; Jori & Bastos, 2009). However, with the example of South Africa, it has been shown that ASF can be controlled also in such areas. This can be done through strict separation of the domestic pig production from the wild vertebrate and arthropod ASF hosts, and by targeted biosecurity, including control of pig movements and improved management of slaughter and offal (Penrith & Vosloo, 2009).

1.5.2.1 Surveillance

Epidemiological understanding, which is the backbone of disease control, is normally gained through some form of disease surveillance system (Hasler *et al.*, 2011; Doherr & Audige, 2001). Resource allocation for the entire chain of surveillance is costly. Paradoxically, the need for surveillance is often greatest where it is most difficult to achieve, i.e. in resource-poor settings (Perry *et al.*, 2013; Perry & Sones, 2007). In low-income countries, current surveillance systems for endemic diseases are often inefficient and dysfunctional (de Balogh *et al.*, 2013; Forman *et al.*, 2012; Perry & Grace, 2009). Factors contributing to these failures are deteriorating administrative services, continuous budget reductions, and lack of veterinary personnel (Bendali, 2006). Lack of infrastructure as well as population and animal registers make surveillance difficult. Further, the surveillance systems in these settings are often not designed to meet the needs of the common smallholder farmer, but rather to meet trade requirements adapted to other circumstances and feasible for only a minority of commercial farmers (Thomson *et al.*, 2004).

Passive surveillance is generally considered the most appropriate form of surveillance for acute infectious diseases with high mortality such as ASF. To be effective, reporting compliance on all levels in the chain – from farmer to the concerned authorities – is paramount for passive surveillance. High levels of community participation and returned benefits of the surveillance improve reporting in the first level of the chain (Brookes *et al.*, 2017; Goutard *et al.*, 2015). In low-income countries, laws and regulations might provide detailed instructions on how to handle reported outbreaks, including compulsory culling, quarantine and trade regulations. However, they rarely or never include compensation to farmers for incurred losses from the outbreaks or the control efforts (Halliday *et al.*, 2012; Perry & Grace, 2009). Further, lack of capacity to enforce existing regulation is common and, as discussed by Halliday *et al.* (2012), a disincentive for reporting. Other frequent disincentives to reporting are stigmatization by peers, distrust of governmental authorities, and low disease awareness (de Balogh *et al.*, 2013; Halliday *et al.*, 2012). In these settings, true incentives to report disease outbreaks are rare. To overcome the challenges of ASF surveillance in low-income countries, there is a need for alternative surveillance methods (Doherr and Audige, 2001).

One such alternative is participatory disease surveillance, using participatory epidemiology (PE) tools (Mariner *et al.*, 2014). PE stems from the development of rapid rural appraisals in the 1970s (Catley *et al.*, 2012; Mariner & Paskin, 2000; Kirsopp-Reed, 1994; McCracken *et al.*, 1988; Chambers, 1983). PE comprises a set of methods widely used for various disease situations including emerging diseases (Jost *et al.*, 2010; Hussain *et al.*, 2008), endemic diseases

(Robyn *et al.*, 2012; Grace *et al.*, 2009), and the eradication of Rinderpest (Mariner *et al.*, 2012; Mariner & Roeder, 2003). These methods, are grounded in the perspective that local people possess important knowledge of their own situation and their environment (Pretty, 1995; Chambers, 1983). For example, farmers are generally able to identify animal diseases that are of importance to them (among others: Fischer *et al.*, 2016; Byaruhanga *et al.*, 2015; Catley *et al.*, 2012; Mariner & Roeder, 2003). Participatory methods effectively capture epidemiological knowledge, in particular qualitative information on interacting sociological, economic and ecological factors (Catley *et al.*, 2012; Grace, 2003; Mariner & Paskin, 2000). The flexibility offered by the methodology includes instant triangulation that can serve to avoid mis-classifications and can offer the possibility to probe for qualitative aspects and causality (Catley *et al.*, 2012; Mariner & Paskin, 2000). By involving the participants and letting them guide the discussions, a positive attitude and engagement of the respondents are preserved, ensuring adequate quality of the answers and thus the results (Catley & Mohammed, 1996).

Another category of surveillance alternatives that also encourages broad representation in participation, data contribution and information sharing, are systems gathering intelligence from the population by internet or mobile phone applications (Freifeld *et al.*, 2010). Surveillance applications for smartphones have been developed for different purposes and stakeholder categories (Larfaoui *et al.*, 2012; Lin & Heffernan, 2011; Robertson *et al.*, 2010). Other systems have used regular mobile phone calls (Jean-Richard *et al.*, 2014) or text messages (Syibli *et al.*, 2014; Thinyane *et al.*, 2010). Such technical solutions generally work well, especially for early detection of disease. As for any other surveillance system, sustainability, ownership and inclusion in national surveillance plans beyond project activities are crucial for the continued success (Goutard *et al.*, 2015). To achieve a sustainable surveillance system after the conclusion of research or development projects, the local institutions must be able to monitor, maintain and support the setup (Zaidi *et al.*, 2013; Asiimwe *et al.*, 2011). Several mobile phone surveillance systems have been shown to be highly sustainable with minimal initial investment. A key part in these success stories is the availability of mobile phones, and the opportunity to create two-way information sharing between the reporting farmer or professional and the information receiver (Syibli *et al.*, 2014; Asiimwe *et al.*, 2011). Feedback on diagnostic test results and advice on management of the disease problems can be achieved via a mobile phone system, for farmers as well as professionals, at different levels in the systems. Such feedback acts as a strong incentive to report (Syibli *et al.*, 2014).

1.5.2.2 Biosecurity

Farm biosecurity is largely non-existent in the smallholder subsistence farming systems dominating in low-income countries such as Uganda. Most pigs roam freely at least part of the year, and even if an enclosure for the pigs exists, some animals are frequently found outside the pens (often the piglets) (Dione *et al.*, 2014; Ikwap *et al.*, 2014). See Figure 3.

Figure 3. Free-roaming pig management systems have been identified as a risk factor for ASF transmission. (Author's photograph)

In similar settings in Kenya free-roaming domestic pigs move over relatively large areas, covering up to 10,000 m^2 during 24 hours, spending a lot of time outside the homestead (Thomas *et al.*, 2013). In settings where even the simplest biosecurity attributes such as buildings (or even outdoor pens limiting at least nose to nose contact between pigs and between pigs and discarded, infectious material) are absent, it is obvious that most other farm biosecurity routines will also be lacking. If the pigs are not confined, then restriction of visitor's access to pigs, change of clothing and boots at the entry to the pig sty, insect and rodent control, quarantine of new animals, environmental, feed and water hygiene and any other biosecurity measures become utopia.

The smallholder pig production value chain includes many critical points regarding biosecurity besides the immediate on-farm biosecurity. Some of these are middlemen (traders buying pigs from farmers to sell either immediately or later on for slaughter) entering onto farms, pigs bought for slaughter being resold as live pigs, middlemen and butchers keeping their own pig herd, unregulated and uncontrolled transport, trade, slaughter and risk material disposal, lack of appropriate slaughter places and waste disposal as well as lack of veterinary control of live pigs, slaughter and meat (Barongo *et al.*, 2015; Dione *et al.*, 2014).

1.6 Animal disease impact

In low-income settings animals serve multiple purposes: income generation, protein source, traction, waste management, manure source, and as social status symbols (Perry *et al.*, 2002). Because of this, the impact of animal disease is multidimensional (Zinsstag *et al.*, 2007), and includes social and economic effects along the value chain (Rich & Wanyoike, 2010). Perry *et al.* (2002) further note that the poorer the household is, the more diverse roles the livestock represent in the daily livelihood. Consequently, the impact diversifies with increasing level of poverty. In low-income settings, the impact keeps people poor and pushes those that have managed to escape poverty back again (Krishna *et al.*, 2004; Morens *et al.*, 2004). As a consequence, outbreaks of infectious animal diseases might disrupt the livelihoods of poor people in the same ways as civil unrest or other catastrophes (Wagstaff, 2006). In contrast to natural catastrophes, however, these diseases can be controlled, and the negative consequences are thus possible to mitigate.

ASF has severe economic impact, both in industrialised and low-income countries (Mur *et al.*, 2014; Swai & Lyimo, 2014; Sanchez-Vizcaino *et al.*, 2013; Fasina *et al.*, 2012). In the areas traditionally plagued by ASF, i.e. sub-Saharan Africa, most of the population is poor and a rural–urban poverty gap generally exists, with the rural population on the poorer side of that gap (*World Bank*, 2016). Outbreaks of ASF in low-income countries consequently affect poor people with far-reaching social and economic impact on the household level.

1.7 Uganda

Uganda is a low-income country situated in East Africa. The country lies north of Lake Victoria, mostly between latitudes 4°North and 2°South and at over 1000 meters altitude (Briggs & Roberts, 2010), see Figure 4. Rainfall varies greatly between regions, with a national average between 1000 and 2000 millimeters/year, creating agro-ecological zones ranging from very dry semi-desert to savannah, wood-lands and swamps. Administratively Uganda is divided, in falling order, into districts, counties, sub-counties and villages (UBOS, 2014). At the latest census the human population stood at about 35 million people, with an annual growth rate of more than three percent, and a strong concentration towards the bottom (younger) segments of the demographic pyramid (UBOS, 2014).

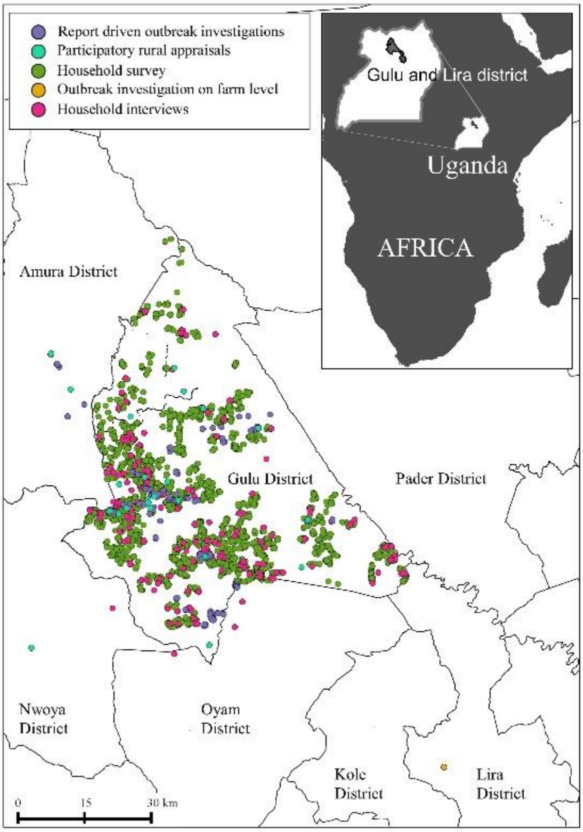

Figure 4. Geographical distribution of households, participatory rural appraisals and the farm included in the thesis.

1.7.1 Poverty in Uganda

During the years following the Millennium Development Goal agreement in year 2000, Uganda strived towards reducing poverty, and managed to fully achieve six out of the 17 millennium goals up to year 2015 (Ministry of Finance Planning and Development, 2015). The achieved goals include goal 1A: “Halve, between 1990 and 2015, the proportion of people whose income is less than 1.25 USD a day”. According to the same report, however, almost 7 million Ugandans are still living in absolute poverty, and another 15 million remain vulnerable. Here absolute poverty is defined as living on less than 1.25 USD a day, and vulnerability as living on only 10% more than that, with a risk of falling back into poverty. Poverty is complicated to measure, especially when it comes to creating figures that are comparable over time and between countries. To facilitate comparisons, since the issue of the World Development Report in 1990, the World Bank has aimed to apply a common standard for measuring extreme poverty (World Bank, 1990). The selected poverty line, initially set at 1 USD per day, has subsequently been adjusted to meet purchasing power, and now stands at 1.9 USD per day (*World Bank*, 2016). The "one dollar a day" was selected in 1985 based on poverty lines in the world's poorest countries at that time. In addition to this international poverty line, countries use national poverty lines, reflecting the specific poverty situation in each country (Levine, 2012). In Uganda, the national poverty line is based on household consumption and the cost of basic needs, and set to 1 USD/day (Ministry of Finance Planning and Development, 2014). For Uganda, both the World Bank and the national poverty line measurements show improving, but slightly diverting, trends. According to the World Bank 35% of the Ugandan population is still living in poverty, whereas, by using the national poverty line, the percentage has been reduced from 56.4% in 1992 to 19.5% in 2012 (*World Bank*, 2016). Regardless of the poverty line applied, a rural–urban poverty gap still exists in Uganda.

1.7.2 Pig keeping in Uganda

The growing middle class has increased the regional demand for meat and livestock products, creating market opportunities for livestock producers in many low-income countries (Delgado, 2003). In combination with market reforms and changes in agricultural policy, this has led to a remarkable increase in pig production in sub-Saharan Africa. The increase has so far been largest in Uganda with a 100-fold increase in the number of pigs in the last 50 years, from only 16,000 in 1961 to more than three million in 2011 (FAOSTAT, 2013; UBOS, 2008). Uganda now has the largest pig population in East Africa. Most are found in smallholder family farms in the rural areas, see Figure 5 (NEPAD & FAO, 2004).

Figure 5. A typical smallholder pig farm in northern Uganda. (Author's photograph)

In general pigs are found close to the bottom of the "livestock ladder", meaning that they are predominately kept by poor people, especially if compared to larger species such as cattle (Perry *et al.*, 2002). The growing pig sector in Uganda has been identified as a sector with high potential for contributing to poverty reduction (Doble, 2007; Ssewaya, 2003). Pig keeping represents a good opportunity for the predominantly rural population to raise money quickly by marketing pigs and pig products. Pig keeping further offers an attractive alternative to keeping ruminants, as they come with smaller investment costs, do not compete for grazing land, can be used for transforming kitchen waste into food, and have a short reproduction cycle presenting a quick financial turnover (Phiri *et al.*, 2003). However, pig production in Uganda is hampered by several factors preventing it to reach its full potential. Some constraining factors are: poor pig husbandry skills, poverty among the pig owners that prevent even minor investment and endemic as well as epidemic diseases (Nissen *et al.*, 2011; Perry & Grace, 2009; Waiswa *et al.*, 2009; Phiri *et al.*, 2003). In Uganda, as in many other African countries, an increased pig population has been associated with increased reporting of ASF (Penrith *et al.*, 2013). The increase in ASF reports probably mirrors both an increase in incidence as well as an increase in surveillance interests and capacities. ASF has been recognized as one of the biggest hurdles for development of the sector (Doble, 2007; Ssewaya, 2003). Several studies describe repeated outbreaks and endemic disease in the domestic pig population (Barongo *et al.*, 2015; Muhangi *et al.*, 2015; Atuhaire *et al.*, 2013; Muwonge *et al.*, 2012).

1.7.3 Rural pig value chains and economy

The pig sector in Uganda is dominated by smallholder farming systems (Dione et al., 2014; Ouma et al., 2014). Many farmers suffer from limited access to markets, technology, information and services because the sector is largely informal and poorly organized (Ouma et al., 2014). Lack of feed, or financial capital to buy feed, is another major constraint frequently mentioned by farmers (Nantima et al., 2015b). The value chain is short, with low levels of accumulated added value. Most of the pigs are sold in the neighbourhood: directly to the village butcher or to travelling middlemen (Dione et al., 2016; Lichoti et al., 2016; Ouma et al., 2014). The farmers can rarely choose between sales points, and it is commonly reported that ASF outbreaks cause farm gate prices of pigs and products to drop (Dione et al., 2014). The pork is sold directly from the butchers to consumers, to retailers (pork retail points are in Uganda called pork kiosks) or to restaurants (restaurants serving pork are in Uganda called pork joints). Apart from the initial purchase of the pigs, monetary inputs into the pig business are limited (Ouma et al., 2014; Waiswa et al., 2009).

2 Aims

The overall aim of the doctoral project was to develop the understanding of ASF epidemiology in Uganda. The specific objectives were to:

- Increase the knowledge of basic epidemiological parameters of ASF in the smallholder setting.
- Identify factors that affect the epidemiology in this setting.
- Assess factors influencing smallholders’ decisions regarding ASF control.
- Compare surveillance methods for ASF for their suitability in smallholder settings in endemic areas.
- Estimate the economic and social impact of ASF outbreaks at the household level in smallholder settings.

3 Comments on Materials and Methods

Details on the materials and methods in the four studies are described in each publication. Below is a general description of how data were collected and analysed.

3.1 Summary of study design

All four studies were observational, where studies I, III and IV were cross-sectional, with some retrospective elements in study III. Study IV was longitudinal. In study I, group interviews in the form of participatory rural appraisal (PRA) using PE tools were implemented. Study II constituted a case report from a confirmed ASF outbreak on a middle-sized farm. Study III compared the case-detecting capacity of three surveillance methods, and in addition includes biological sampling for ASFV of suspected outbreaks at household level. In study IV, structured individual interviews on household level were undertaken three times with a six-month interval.

Outbreaks of ASF were described at the household level (report-driven outbreak investigation and household survey in studies III and IV), farm level (study II), and the group level (study I and the PRA-part in study III); thus they were defined differently according to the respective study design. In studies I and IV as well as in the household survey and PRA in study III, case definition of ASF outbreaks was entirely based on the respondents' accounts of historical events in the pig herd. The ability of smallholder farmers in northern Uganda to correctly identify historical outbreaks of ASF was investigated during study I. Biological sampling for ASFV was performed in studies II and III.

3.2 Study area

The studies were carried out in and around two districts in northern Uganda, Gulu and Lira, see Figure 4. These areas were chosen based on previous studies indicating a high incidence of ASF (Barongo *et al.*, 2015; Ståhl *et al.*, 2014; Aliro *et al.*, 2012; Masembe, 2012), existing local contacts facilitating the research, as well as the post-conflict status of the area, which had created special challenges for pig farming and disease control. Both districts were severely affected by a civil war between the government and the Lord's Resistance Army between 1986 and 2006 (Finnström, 2008, Branch, 2013). In Gulu a large part of the population had to leave their rural homes and were internally displaced, either to rural refugee camps or Gulu town (Branch, 2013). Likewise, in the later years of the conflict, large parts of the population in Lira district were also internally displaced and relocated to the local towns (Jacobsen *et al.*, 2006). Even if no official peace agreement has yet been signed, northern Uganda is now slowly recovering from the political, social, economic and military unrest and people are starting to re-engage in agriculture (Bongyereirwe, 2010). As in the rest of Uganda the rural–urban poverty gap is prominent with a high concentration of poverty in the rural areas (UBOS, 2012). Pig farming is among the fastest growing livestock activities as communities resettle in their villages. Most parts of Gulu and Lira belong to the Northern-Teso agro-ecological zone, which is characterised by annual crops grown in two distinct rainy seasons (Kamanyire, 2000). Some parts of Lira district also belong to the cattle corridor, i.e. the parts of Uganda where cattle traditionally are kept.

Studies I, III and IV were carried out in Gulu district. At the time of the studies, Gulu covered 3,449 km^2 and was divided into two counties, 12 subcounties (plus Gulu municipality, divided into four divisions) and 294 villages (*Gulu district local government statistical abstract 2012/13*, 2013). According to preliminary results from the 2014 census the population in Gulu comprises more than 500,000 persons (UBOS, 2014). The most recent livestock census dates from 2008 and at that time Gulu district held approximately 25,000 pigs (UBOS, 2008). Study II was carried out on a farm in Lira district. Lira district covers 3,482 km^2, and consists of one county with nine subcounties plus Lira municipality (*Higher local government statistical abstract Lira district* 2009). According to preliminary results from the 2014 census the population in Lira comprises more than 400,000 persons (UBOS, 2014). The human population is heavily concentrated in Lira town. According to the livestock census, Lira district held almost 30,000 pigs (UBOS, 2008).

3.3 Sample selection and data collection

Randomised sample selection in observational studies in low-income countries is often a challenge as household and animal registers rarely exist. In the studies in this thesis, data were collected in five successive samplings, described below. One of these included the creation of a sampling frame, permitting random selection of households in the consecutive sampling. Some villages and/or households were included in more than one of the studies. Interview data, biological and environmental samples were collected.

The District Veterinary Office, under the Ministry of Agriculture, Animal Industry and Fisheries (MAAIF) has the official mandate to carry out investigations of animal disease in Uganda. Such investigations can include various methods of information collection, such as sampling of animals and interviews with animal owners. All handling of animals including sampling was carried out, or overseen, by District Veterinary Office staff in accordance with their national mandate. Oral or written informed consent was assured by all participants in the studies.

3.3.1 Report-driven outbreak investigations

During 18 months, all reports of ASF outbreaks in and around the Gulu district were investigated by the district veterinary officer (DVO) in Gulu. In brief, all reports of disease in pigs characterized by fever and mortality that reached the DVO in Gulu were included (n= 119). The affected households were subsequently visited by the DVO. At the visit blood and serum samples were collected from at least one clinically diseased pig where possible; otherwise, the samples were taken from apparently healthy pigs in those households that still had any surviving pigs at the time of visit.

After laboratory analysis of the samples, all villages with households with positive results were re-visited by the DVO. During this visit, key informants provided information pertaining to all households that had been affected by the recent ASF outbreak in each village. All these affected households (n=211) were included in the subsequent part, which comprised collection of additional data by household interviews using semi-structured questionnaires. The data collected included household location (GPS coordinates), starting month of the confirmed outbreak, number of pigs that had died or survived the outbreak, and number of pigs at the time of the visit.

The data collected in this way was used in study III, and for the purposive selection of participants in study I.

3.3.2 Participatory rural appraisals (PRAs)

Three separate series of PRAs were performed. Two series targeted farmers and one targeted other actors in the pig production value chain. Participants were selected on the basis of purposive sampling strategies (Mariner & Paskin, 2000; McCracken *et al.*, 1988) and the selection criteria varied for each PRA series. In the first farmer PRA series (36 PRAs, 419 participants), participants were selected from villages with confirmed ASF outbreaks in the report-driven outbreak investigations. In contrast, for the second farmer PRA series (8 PRAs, 105 participants), neither participants nor any other farmers in the same village had ever reported any ASF outbreaks to the DVO in Gulu. The selection of participants for the value chain actor PRA series (5 PRAs, 49 participants) was based on convenience sampling of known actors in the pig production value chain. Participants were included from both rural and semi-urban areas, and representing different activities in the value chain such as middlemen, butchers, pork kiosks and pork joints. All participants were invited by the DVO in Gulu.

Participants in the two farmer PRA series were invited via key informants and participants in the value chain actor PRA series via a personal letter. Information was triangulated within each PRA by cross-checking answers from several questions, and, in addition, for both farmer PRA series via key-informant interviews performed at the same time as the PRAs.

The PRAs covered topics related to the participants' knowledge, attitude and practices concerning ASF. The data collected in this way was used in studies I and III.

3.3.3 Household survey

A household survey was delivered by 41 community knowledge workers (CKWs). The CKWs were peer-selected local residents affiliated with the Uganda branch of the Grameen Foundation (http://www.grameenfoundation.org). The CKWs are trained in delivering surveys and extension services using mobile phones provided by the organisation. The survey consisted of two parts: the first comprised questions related to the respondents' pig keeping and the second a poverty score developed by the Grameen Foundation (*Progress out of poverty*, 2014). Each CKW was assigned one parish in Gulu, in most cases the home parish where they also performed their regular extension services. Parishes not covered by any CKWs were excluded from the study after having controlled that all 12 subcounties in Gulu were covered. The CKW system was not in place in Gulu municipality, but five parishes from two divisions in the municipality were still included in the study for a broader representation of households. These parishes were covered by CKWs from other parts of Gulu district and chosen by convenience selection. All villages in the included parishes were surveyed. In each village the selection of households was done by convenience selection of the first pig-keeping household, followed by a snowball sample selection technique (the interviewed household indicated the closest neighboring household keeping pigs) for the subsequent households, i.e., not strictly random (Goel & Salganik, 2009; Atkinson & Flint, 2001; Kaplan *et al.*, 1987). Households were thus not identified or selected based on participation in the other samplings. To focus the sampling on the population of interest, pig keeping was set as an absolute inclusion criterion. In each village, all pig-keeping households were included in the study, if less than 20 in total. If the village had more than 20 pig-keeping households, the snowball selection process ended with the twentieth household. The respondent in each household was an adult household member that was at home and available at the time of visit, and who had sufficient knowledge of the family's pig keeping to adequately answer the questions.

The data collected in this way was used in study III (questions related to pig keeping) and IV (poverty score). All sampled households (in total 4000) were included in a list used as sampling frame for study IV.

3.3.4 Outbreak investigation on farm level

An outbreak investigation was performed on a middle-sized farm in Lira district.

The study farm was run by a non-governmental organisation (NGO) and established to financially support the organisation's main, humanitarian, activities. The objective of the farm was to produce piglets and pork and offer training in pig husbandry for local pig farmers. As such the farm represented a larger-scale farming operation located in an area with typical smallholder family farms. Thus, it was vulnerable to the various challenges, including the high need for biosecurity which a pig farm in an ASF endemic setting faces.

The farm had 35 adult pigs and 103 piglets and growers, all of exotic (typical European white landrace) breed. The pigs were kept in a purpose-built, fenced compound with two stables. Some of the authors of study II (EC, KS, TA) were contacted about disease and mortalities in the pigs. Already at the first contact, ASF was suspected based on the clinical signs and the location in northern Uganda, and some preliminary advice to contain the disease and prevent further spread was given.

The investigation included five farm visits, with interviews with farm representatives, site assessment, and collection of biological and environmental samples. Interviews were informal, with simultaneous note-taking. Photographs were taken as part of the assessment, and feedback in the form of investigation reports was provided to farm representatives following each visit. Information regarding biosecurity was extracted from the interview data and from observations made during the farm visits.

Biological samples (blood, serum and organ samples) were collected on two occasions. Environmental samples were taken on one occasion. Places to be sampled were chosen on the basis of visible blood contamination and interviews indicating biosecurity high-risk spots. The material collected was in most cases soil, but also samples of feed, water, manure and pig hair.

The data collected this way was used in study II.

3.3.5 Household interviews

Data were collected using structured face-to-face individual interviews. In a longitudinal design, structured interviews with 200 randomly selected pig-keeping households were undertaken three times with a six-month interval. Participants were randomly selected from the list of pig-keeping households obtained from the household survey. The random number function in Microsoft

Excel (Microsoft, Redmond, WA) was used for the sample selection. The number of selected households was based on time and resources available for fieldwork. If a selected household could not be reached at the time of the first interview it was replaced with the nearest pig-keeping household in the same village. For the second and third interviews, no households were replaced; those that could not be reached were left out of the study. The respondent was an adult household member that was at home and available at the time of the visit, and who had sufficient knowledge of the family's pig keeping to adequately answer the questions. Whenever possible, the same person in each selected household was interviewed on all three occasions.

Interviews were conducted at the respondents' homes, or other places in the close vicinity (such as fields, working places and markets). Data collected included family and pig herd demographics, social and economic characteristics of the household, pig trade and pig business.

The data collected this way was used in study IV.

3.4 Data handling and editing

3.4.1 Interview data

Data from the report-driven outbreak investigations and the PRAs were collected on paper questionnaires and entered into Microsoft Excel spreadsheets (Microsoft, Redmond, WA) as soon as possible after each interview. For the household survey, data were collected using a smartphone-based application. Answers were continuously registered on the phones during the interview. Immediately after each interview, questionnaire data were transferred to a cloud-based server of the mobile network or, if no mobile network was available, saved on the mobile phones and transferred automatically as soon as the mobile network was reached. Data were transferred to the author of this thesis in Microsoft Excel spreadsheets (Microsoft, Redmond, WA). Data from the outbreak investigation were summarized in the form of an investigation report after each visit. Data from the household interviews were collected on paper questionnaires and entered by single entry into a web-based database shortly after each round of interviews (EasyResearch, QuestBack International HQ, Oslo, Norway).

3.4.1.1 Translation

All interview protocols used were constructed in English and delivered in the local language (Luo) with answers noted in English. The questionnaires used in the report-driven outbreak investigations were constructed in English and delivered by the DVO in Luo with notes taken in English. For the PRAs and the household survey, common agreement on the translation from English to Luo was reached by the interviewers. All interviewers had been trained in research ethics and the implementation of the respective questionnaires prior to the start of the studies. Four facilitators/note-takers, constituting two teams, performed all the PRAs. The household surveys were delivered by 41 CKWs. Surveys were displayed on mobile phones in English and the questions asked in Luo. The household interviews were implemented by two of the facilitators that had performed the PRAs. To provide consistency and supervision, the author of this thesis was present at most of the PRAs, some household surveys, and half of the household interviews.

3.4.2 Biological and environmental samples

Biological samples from the report-driven outbreak investigations as well as biological and environmental samples from the outbreak investigation on farm level were transported to the laboratory at Makerere University in Kampala. Samples were kept cool with ice in a cooler bag during transport. On arrival to the laboratory, serum samples were centrifuged to separate serum and blood, which were then stored separately at -20°C until analysis.

3.5 Analyses

Data were managed, analysed and visualised using basic packages in R software version 3.1.0 (R Core Team 2015).

3.5.1 Statistical analysis

Descriptive and summary statistics, including measures of central tendency and dispersion, as well as measures of associations were calculated in studies I, III and IV. Depending on the type of data, parametric or non-parametric tests were used for statistical hypothesis testing. In study III a spatial cluster analysis was performed; in study IV, variables associated with ASF occurrence as per a conceptual model were evaluated by linear regression. The outcome variables in the models were gross margin in pig business, pig value change, and (the natural logarithm of) the number of times that meat was served in the household since the last interview. A logarithmic scale was used as it improved model fit. The gross margin in pig production was calculated as all recorded incomes in the pig production minus all recoded costs in the pig production. The pig value change was calculated as the change in the value of the pig herd during the applicable time period for each interview occasion, using the product of the change in number of pigs in each category (i.e. the difference in numbers of pig entering and pigs leaving the herd), and the measured average sale prices for each pig category.

3.5.2 Laboratory analysis

Laboratory analyses in study II were done at the Molecular Biology Laboratory at Makerere University, Kampala and in the national reference laboratory, National Animal Disease Diagnostics and Epidemiology Centre in Entebbe. Laboratory analyses in study III were done at the Molecular Biology Laboratory at Makerere University, Kampala. Blood samples were analysed for the presence of ASFV nucleic acids using a commercially available real-time PCR and a Universal Probe Library (UPL) probe PCR. Environmental samples were analysed for the presence of ASFV nucleic acids using a real-time PCR assay with internal control (IC). Blood samples were analysed for the presence of ASF antibodies using a lateral flow device test. For all laboratory analyses, standard protocols were used.

4 Results

The main results of the four studies are summarized below. More detailed results are given in each publication.

4.1 Demographic characteristics of the included populations including ASF occourence (Studies I-IV)

The participants in studies I, III and IV were mostly smallholder pig farmers. Study I also included other pig value-chain actors (middlemen, butchers, owners of pork kiosks, owners of pork joints and dealers in pig skins). Most of these value-chain actors were engaged in several activities, and, more than half of them kept pigs. The number of pigs in the households included in studies III and IV were on average less than four, including piglets, see Table 1.

Table 1. Herd demographic data from studies III and IV. ASF=African swine fever.

	Study						
	III		IV				
	Method		Interview occasion				
Variable	**Report-driven outbreak investigations**	**House-hold survey**	**First[1]**	**Second**	**p-value[2]**	**Third**	**p-value[2]**
Pigs (No.)							
Range	0-70	1-40	0-29	0-20		0-39	
Average (all)	1.9	3.3	3.7	3.4		3.4	
Average ASF Yes	*	*	*	1.1		1.2	
No	*	*	*	3.6	<0.001	3.7	<0.001
Cattle (No.)							
Range	*	*	0-46	*	*	*	*
Average (all)			2.8				
Sheep (No.)							
Range	*	*	0-12	*	*	*	*
Average (all)			0.3				
Goats (No.)							
Range	*	*	0-25	*	*	*	*
Average (all)			4.9				
Poultry (No.)							
Range	*	*	0-100	*	*	*	*
Average (all)			11.0				

[1] In Study IV the variables refer to a specific time period. For the first interview occasion this was the 12 months preceding the interview and for the second and third interview occasions, since last interview (approximately 6 months).
[2] Difference in averages between household that described ASF outbreaks (ASF yes) compared to those that did not (ASF no) for each specific interview occasion.
*=Data not recorded or analysis not applicable.

Likewise, the farmers included in study IV kept just a few animals of other animal species on average. Despite the smallholder character of the farming, pig trade was frequent and in study IV most households had sold or acquired at least one pig during the 12 months preceding the first interview, see Table 2.

Pig farming was of low-input–low-output character, with little investments apart from the initial purchase of the pigs. This included feed and reproduction costs, meaning that most pigs were left to feed themselves by scavenging and were bred by a communal or free-roaming boar. Start-up investments (pigs and housing) were on average low and few households expanded their pig business during the duration of Study IV.

Yearly household incidence of ASF varied between the studies, but outbreaks were frequently reported in studies I, III and IV, see Table 3.

Table 2. *Data on pig business from study IV. HH=household, ASF=African swine fever.*

	Interview occasion				
	First	Second	p-value[2]	Third	p-value[2]
Variable[1]					
Pigs have left the herd (No. HH)	Yes No	Yes No		Yes No	
All	174 21	167 29		136 60	
ASF Yes		15 0		23 0	
No		152 29		113 60	
Pigs that left the herd (No. pigs)					
Range	0-52	0-27		0-23	
Average (all)	5.6	4.6		2.8	
Average ASF Yes		7.9		6.9	
No		4.3	<0.05	2.2	<0.01
Pigs have entered the herd (No. HH)	Yes No	Yes No		Yes No	
All	186 10	134 62		76 114	
ASF Yes		9 6		9 14	
No		125 56		67 100	
Pigs that entered the herd (No. pigs)					
Range	0-52	0-29		0-39	
Average (all)	7.1	4.7		2.6	
Average ASF Yes		3.5		2.9	
No		4.8	0.26	2.5	0.78
Purchased pigs (No. pigs)					
Range	0-11	0-8		0-8	
Average (all)	1.6	0.5		0.5	
Average ASF Yes		0.9		0.5	
No		0.5	0.46	0.5	0.50
HH have sold pigs (No. HH)	Yes No	Yes No		Yes No	
Average (all)	139 52	127 62		92 101	
Average ASF Yes		6 9		12 10	
No		121 53		80 91	
Sold pigs (No. pigs)					
Range	0-29	0-22		0-17	
Average (all)	3.5	2.7		1.5	
Average ASF Yes		0.5		2.0	
No		2.9	<0.001	1.4	0.38
Dead pigs (No. pigs)					
Range	0-38	0-24		0-15	
Average (all)	1.9	1.7		1.1	
Average ASF Yes		7.4		4.9	
No		1.2	<0.01	0.5	<0.001
Price at sale, growers (USD)[3]					
Range	4.4-88	1.2-82		8.8-78	
Average (all)	20	28		33	
Average ASF Yes		38		37	
No		28	0.53	33	0.62
Price at purchase, growers (USD)					
Range	0.7-132	0.9-117		12-63	
Average (all)	16	18		21	
Average ASF Yes		15		21	
No		17	0.40	21	0.14

[1] Variables refer to a specific time period. For the first interview occasion, this was the 12 months preceding the interview and for the second and third interview occasions, since last interview (approximately 6 months).
[2] Difference in averages between household that described ASF outbreaks (ASF yes) compared to those that did not (ASF no) for each specific interview occasion.
[3] Difference in average sell price between interview occasion one and two and between interview occasion one and three, $p<0.001$, between interview occasion two and three, $p=0.16$.

Table 3. *Disease estimates from studies III and IV. PRA=participatory rural appraisal, NA=not applicable.*

	Study					
		III			**IV**	
		Method			**Interview occasion**	
	Report-driven outbreak investigations	**Household survey**	**PRA**	**First**	**Second**	**Third**
No. of participants/ households	211	4000	524	200	198	196
No. of outbreaks	211[1]	1225[2]	94[3]	40[4]	15[4]	23[4]
Household yearly incidence (%)	NA	15	NA	20	15	23
Village yearly incidence (%)	8.8[5]	31	NA	NA	NA	NA

[1] ASF outbreak defined as a household in a village from which ASF had been laboratory confirmed, with the individual, affected households identified by a key informant.
[2] ASF outbreak defined as high mortality among pigs at the household level as reported by the respondent. Retrospective interview covering the last 12 months.
[3] ASF outbreak defined on village level as stated by the participants. Retrospective group interview covering the last 12 months.
[4] ASF outbreak defined based on the respondents' classification of cause of death or description of classical signs. Retrospective interview covering the last 12 months for the first interview and the time since last interview for the second and third interview (approximately 6 months).
[5] Calculated based on outbreaks starting during a period of 20 months and Gulu district having 294 villages.

Less than half of the households sampled in study IV had off-farm income and almost half of the households did not have any household members with education other than primary school. Some of the household demographic variables were included in a poverty score. The distribution of the poverty score was narrow with a low average, signifying a homogenously poor population (see Figure 6) where most of the sampled households had a high probability of falling under the national poverty line.

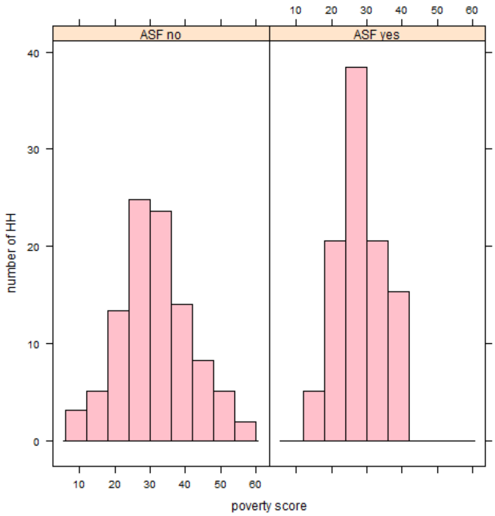

Figure 6. Distribution of the poverty score for households that did (ASF yes) or did not (ASF no) describe ASF outbreaks at the first interview occasion in study IV. The poverty score was created from a number of non-monetary indicators and represents the households' probability of falling under the national poverty line; a lower poverty score represents a higher probability.

4.2 Knowledge, attitudes and practices related to ASF (Study I)

Most participants were well aware of the routes for ASF spread and methods to control transmission. They could accurately describe the clinical signs of ASF. All PRAs correctly identified pork, pork products, offal and trade in live pigs as the main risk factors for spreading, or acquiring, ASF. Each value-chain actor PRA further mentioned four specific business-related routes of spread; infected pork was mentioned as an example in all of them. More than 40 different control methods were mentioned in the farmer PRAs. The most frequent was confinement of pigs, followed by moving the affected pig away from the house, village and other pigs, administration of drugs, and not buying pork. However, most participants said they were aware of more control methods than what they used. Factors preventing farmers from adoption of the known methods were: lack of knowledge on how to implement them; lack of capital for construction and investments; lack of income; and a cultural taboo against throwing away food. Factors mentioned by the value-chain actors were: absence of slaughter slabs in the area; no veterinarian present at slaughter; the need for maximizing profits; "the love for pork"; lack of capital; and the fact that some regarded pig business only as a supplementary income. Factors that farmers mentioned as possible incentives for adopting control methods were: training in biosecurity, pig health and management; provision of new or improved pigs; mass vaccination; and farm visits by veterinarians. Value-chain actors mentioned improvement of the existing, or construction of new, slaughter slabs as well as appointment of veterinary inspectors; training in biosecurity; increased legal enforcement and institutionalisation of local trade regulations.

Most participants considered consuming pork from pigs that had died from ASF a public health risk. All middlemen participating in study I had sold pigs with signs they recognised as ASF. Most of the butchers had also sold carcasses or meat with signs they recognised as ASF. Most participants in the value-chain actor PRAs confirmed that they considered themselves to have some responsibility for diseases control.

4.3 Temporal variations (Studies I and III)

The ASF outbreaks described in study I showed a seasonal pattern, coinciding with seasonal peaks in several potential risk factors. Slaughter and sales occurred almost exclusively around festivities such as Easter, Independence Day in October, Christmas and New Year, and in August (pigs being sold to obtain money for school fees). Furthermore, pigs were kept free-range between October and April and tethered or fenced-in during the cropping season (May-September). The seasonality of pigs kept on free-range as well as pig slaughter and pork sale coincided with the seasonality of ASF occurrence. The same temporal variation of outbreaks was not obvious in study III.

4.4 Biosecurity breaches and within-farm virus contamination during an ASF outbreak (Study II).

The outbreak started with an adult boar presenting with clinical signs of ASF. The following days a few pigs died each day, most of them adult pigs. Between days 10 and 16 of the outbreak, the cumulative death figures started to show signs of exponential growth. On the day 34 of the outbreak, 133 pigs had either died or been slaughtered upon showing clinical signs of ASF. Three months after the onset of the outbreak, all pigs at the farm had either died or been slaughtered and the farm was empty.

The first impression of the farm setup was that the farm biosecurity standards seemed to be rather high, and fit for pig farming in an ASF endemic area, see Figure 7.

Figure 7. The setup of the farm in study II. (Author's photograph)

However, interviews and in-depth assessment of the farm site revealed that biosecurity protocols were lacking, including routines, layout and location of specific activities. Specific biosecurity risks are described in detail in the publication. Most notably, the slaughter took place inside the compound, less than three meters from one of the stables that had partly open sides, without drainage, and on an unpaved surface. Further, there had been a high turnover of staff, causing biosecurity routines to be lost in the transition process. According to the stated routines, rubber boots and overalls were provided for the staff and overalls were washed daily. At the visits it became apparent that all procedures were not being followed. Pigs that fell sick during the outbreak were in general not euthanized or slaughtered immediately. When the outbreak started, disinfectant boot-baths were placed at both entrances to the stables and at the gate to the compound. However, the boot-baths were placed on an un-paved surface and used without prior cleaning of the boots, causing heavy contamination of the water by organic material. In addition, the boot baths were placed without any demarcation of the clean and dirty sides.

The cause of the outbreak, including severe environmental contamination, was confirmed by the presence of ASFV in biological and environmental samples. Organ samples from one dead pig, taken during the first week of the outbreak as well as three out of seven blood samples from clinically diseased pigs and one out of seven blood samples from apparently health pigs taken approximately one month into the outbreak were positive for the presence of ASFV. Out of 35 environmental samples, 33 were positive for the presence of ASFV.

4.5 Surveillance methods for resource-poor settings (Study III)

The total number of described outbreaks is illustrated in Table 3. The geographical distribution of the included households is displayed in Figure 4.

4.5.1 Report-driven outbreak investigations

During a 15-month study period, outbreaks were reported from 119 households in 43 villages. ASFV was detected in at least one sample from every investigated village. On the subsequent visit, following the test results, key informants reported a total of 211 affected households from these 43 villages, all of which were interviewed. During the outbreaks that preceded the interviews, the median case fatality risk in the included households was 100 (10^{th} percentile 50, 90^{th} percentile 100).

4.5.2 Participatory Rural Appraisals

A total of 524 participants, representing 56 different villages, were included in 44 PRAs. A total of 94 outbreaks was described in the PRAs. Out of these outbreaks 74 had occurred in the two years preceding the PRAs. Based on all 94 described outbreaks, the median proportion of farmers in a village affected by each outbreak was 79% (10^{th} percentile 46, 90^{th} percentile 97). The median case fatality risk during these outbreaks was 80% (10^{th} percentile 50, 90^{th} percentile 96).

4.5.3 Household survey

In total, 4,000 households were included in the survey of which 1,225 households reported outbreaks with pig deaths or sickness. Out of these 1,125 households stated the month and year of the outbreaks and 1,101 of those dates were within the two years preceding the study. On average, 15% of the households and 31% of the villages had an outbreak of ASF each year. The median case fatality risk was 67% (10^{th} percentile 20, 90^{th} percentile 100).

4.6 Social and economic impact of ASF (studies I, II and IV)

In study IV, a total of 40 households described historic ASF outbreaks during the 12 months preceding the first interview. At the second interview, 15 households described outbreaks of ASF since the first interview, and at the third interview 23 households described outbreaks since the second interview, see Table 3. One of the households described outbreaks on both these interview occasions. At both the second and third interview occasions, households that had described ASF outbreaks since the last interview had fewer pigs than households that had not described outbreaks, see Table 2. Increasing herd size (measured either as number of pigs or as pig units, taking into account the different values of the pig categories) was positively associated both with a higher gross margin in the pig business and pig value change. The interaction between ASF outbreaks and the herd size variables showed that ASF outbreaks were negatively associated with economic output, measured both as gross margin in the pig business and pig value change at the second interview occasion. Likewise, at the third interview occasion, ASF outbreaks before the second interview occasion were negatively associated with economic output, measured both as gross margin in the pig business and pig value change. At the third interview occasion ASF outbreaks before the third interview were also negatively associated with the pig value change, whereas a positive association was seen between ASF outbreaks before the third interview and the gross margin in the pig business. In conclusion, ASF outbreaks were always negatively associated with economic output in the long-term, whereas at the third interview occasion, a short-term positive association could be seen between ASF outbreaks and the outcome variable gross margin in the pig business. Both models explained only a limited part of the variance in the data.

Approximately one-fifth of the participants in the first of the farmer PRA series in study I had discontinued pig farming after experiencing ASF. Reasons given were: lack of funds to buy new pigs, no pigs available on the market, and fear of new ASF outbreaks. Consequences of the outbreaks included failure to pay school fees, failure to pay for farm labour leading to less cultivated land, decreased meat content in the family diet, feelings of discouragement, lost hope, fear, failure to pay medical expenses, loss of important pig breeds, increase in pig prices, public health problems, postponed marriages, and disputes with neighbours. These semi-qualitative results informed on the selection of social impact variables for the quantitative analyses in study IV. Subsequently, school attendance, possibility to pay school fees and medical expenses, postponement of family gatherings and frequency of meat consumption in the household were selected as social impact variables in study IV. For the second interview occasion in study IV, an increasing number of pig units were associated with more frequent servings of meat. No other consistent significant associations could be established. The model with (the natural logarithm of) the number of times that meat was served in the household since the last interview as a social impact variable explained very little of the variance in the data.

Of the 50 participants in the value-chain actor PRAs in study I, only one had ceased his business after an ASF outbreak. This was attributed to lack of money to buy new pigs. The value-chain actor PRAs cited both negative and positive impacts of ASF outbreaks, with negative impacts dominating. All these PRAs mentioned loss of clients as a negative effect. A positive impact of the outbreaks was that farmers were eager to sell both their healthy and diseased pigs, leading to lower farm-gate prices and an opportunity for middlemen to make a profit.

Employees at the farm in study II expressed feelings of stress and depression from seeing the pigs sick and dying, and their inability to control the outbreak. The farm manager left his post voluntarily and two staff members lost their jobs due to the outbreak as no farm labour was needed once all the pigs had died. The employees emotional stress during the outbreak possibly aggravated the outbreak, as diseased pigs were not immediately euthanized or slaughtered “as they might recover”. In total, 35 adult pigs and more than a hundred piglets or growers died or were slaughtered with clinical symptoms of ASF during the outbreak. The farm estimated the market value of a pig at slaughter to 90 USD and the total loss (including costs for restocking, decontamination, repair and extra work, but excluding the gain made from slaughter during the outbreak) at approximately 20,000 USD. The study farm did not only suffer substantial economic loss, but aspirations and non-financial investments were shattered. As the study farm was meant to serve as a teaching farm, the loss of the stock and failure to control the disease could also have had a larger impact on society. This was further accentuated as the study farm was meant to finance the humanitarian activities of the NGO.

5 Discussion

The poverty and associated low-input–low-output character of the pig businesses in the sampled populations (study IV) have implications: for the interpretation of the results from the studies, for the formulation of disease control advice, and for achieving deeper understanding of the farming context and the disease epidemiology. Study IV showed that the monetary input in the pig business, apart from the initial purchase of the pigs, was close to zero in many cases. An extreme low-input system, including the use of free-range management systems, has an undisputable financial advantage in terms of the small monetary investments. In certain circumstances, free-range management might be part of a well-adapted strategy, apart from drawbacks such as reduction in growth due to increased energy needs connected to the pigs' physical exercise (Thomas *et al.*, 2013), and increased risk of contracting infectious diseases such as ASF (Nantima *et al.*, 2015c). If ASF is endemic and the households do not see that they can avoid infection, it might be wise to invest as little as possible in each pig. The challenges to keep even commercial farms in ASF endemic areas ASF-free were demonstrated in study II. These challenges are even greater for smallholder farmers. Despite the low inputs, outputs, and gross margins in the pig businesses, the pigs constitute an important social and economic asset for the households. In poor households, pigs are veritable piggy-banks used to barter for agricultural labour and to pay for school fees and other cash expenses (Mbuthia *et al.*, 2015). This was demonstrated in study IV with the use of the variable "pig value change", including a hypothetical value of all pigs that left or entered the herd. This variable had a better model fit than "gross margin in pig business" as a measure for economic impact of ASF at the third interview occasion.

"Lack of knowledge" is often mentioned as a reason for continuous disease spread in transmission cycles driven by human activities. As a logical sequel, various trainings are proposed as a quick fix for the problem. Study I showed that the level of knowledge was high concerning most aspects of ASF, although with some knowledge gaps. The results propose cultural circumstances, taboos and poverty as factors affecting implementation of known control methods, apart from factual knowledge. Studies from Russia, Georgia and Sardinia report similar importance of cultural and social factors in the understanding of ASF transmission and control failures (Mur *et al.*, 2014; Gogin *et al.*, 2013; Zaberezhnyĭ *et al.*, 2012). Several participants in study I mentioned a cultural taboo of throwing away food and the importance of pork in the family diets. Indeed, most participants in the value-chain actor PRAs stated that they had sold live pigs, carcasses or pork from pigs they thought were infected with ASF. In study II slaughter of pigs with signs of ASF was an economically informed decision.

To achieve ASF control globally, nationally and locally, many different aspects of the epidemiology need to be considered (Costard *et al.*, 2009). One important piece of the puzzle is successful implementation of biosecurity advice in the local context (Coffin *et al.*, 2015). Simplicity, adaptability, acceptance and cost-effectiveness are vital for success in this regard. If these are lacking, biosecurity advice, and ASF control, will probably fail. For example, in study I, most farmer participants were aware of, but did not practice, several control methods. In study II, boot-baths were rendered ineffective by organic contamination. A recommendation to implement biosecurity barriers and change of boots instead of disinfectant boot-baths are examples of simple, adaptable and cost-effective biosecurity advice. Another example of biosecurity advice that needs to be better adapted to the cultural and social surroundings concerns the practice of slaughter of sick pigs. As these practices are common, they need to be considered while formulating disease control information, despite possible violations of national animal health laws and regulations (Coffin *et al.*, 2015; Leach & Scoones, 2013). The case fatality risk after infection with highly virulent ASFV, such as the genotype IX circulating in Uganda (Gallardo *et al.*, 2011), is very high, as confirmed in all studies included in this thesis (studies I, II, III and IV). The highest viral loads are observed when pigs express clinical signs (Davies *et al*. 2015). Early euthanasia or safe slaughter must therefore be considered key for reducing disease transmission and the total amount of virus in circulation. Given the local situation with widespread poverty and related protein deficiency – and the fact that ASF is not a zoonotic disease – the author argues that legal, safe, emergency slaughter of selected animals would be a better alternative than the current common practice of fright sales and illegal slaughter (studies I and II). In this regard, safe slaughter includes both a biosafe slaughter procedure with disposal of the remains, and heat treatment of the meat. Such procedures were applied during the eradication of ASF on Cuba (Simeon-Negrin & Frias-Lepoureau, 2002), and similarly for brucellosis control in the former Soviet Union (Denisov *et al.*, 2013). This is in line with the commodity-based approach for food safety standards, suggesting that different commodities pose very different risks for disease transmission and that these specific risks ought to be reflected in the risk management of animal origin food production (Naziri *et al.*, 2015; Thomson *et al.*, 2013; Thomson *et al.*, 2004). Furthermore, the infectiousness of ASF is not extremely high, Guinat *et al*. (2014) describe low to moderate transmissibility between pigs. This means that transmission can be interrupted if strict biosecurity, including the immediate removal of all infectious pigs, is exercised. The temporal association between ASF outbreaks and slaughter/pork sales, as well as free-roaming pig management systems seen in study I, support the theory that ASF transmission in domestic pigs in the study areas is driven by the practices of the actors in the value chain, such as unsafe slaughter and sale. Even though the results do not necessarily demonstrate causality between the events and ASF outbreaks (i.e., one event could drive any of the other), the associations support that safe slaughter could be an effective way to limit disease transmission.

In study III, the capacity to detect cases and estimate case fatality risks were derived from three different surveillance methods. The mentioned socio-cultural surroundings at meso- and micro-levels affect disease surveillance and its evaluation. For example, case fatality risk is difficult to estimate because sick pigs are sold or slaughtered (studies I and II), and because reporting rates in passive surveillance will be affected by factors relating to infrastructure, norms and traditions (de Balogh *et al.*, 2013; Elbers *et al.*, 2010). Case fatality risk estimates by the three methods were rather similar, with the highest for report-driven outbreak investigations, followed by the PRAs, and the household survey. All three surveillance methods detected large numbers of outbreaks. The three methods have different characteristics and the method of choice will depend on the objective of the surveillance. The initial investigations of the report-driven outbreak investigations in study III correspond to the current passive surveillance of ASF, outlined in the Ugandan Animal Disease Act (*The animal diseases act*, 1918). DVOs, however, are hindered in fulfilling their statutory tasks due to limited budgets and lack of infrastructure for reporting and for making laboratory referrals (Nantima *et al.*, 2015a; de Balogh *et al.*, 2013). Providing the DVOs with necessary resources can dramatically increase the number of detected and investigated ASF outbreaks as demonstrated by the large number of investigated outbreaks during the study.

Only the outbreaks described in study II and those in the report-driven outbreak investigations of study III were laboratory confirmed. Many previous studies have demonstrated that farmers are generally able to identify animal diseases that are of importance to them (Catley *et al.*, 2012; Mariner *et al.*, 2012; Hussain *et al.*, 2008; Mariner *et al.*, 2003). More specifically, the ability of smallholder farmers in northern Uganda to correctly identify outbreaks of ASF was investigated, and to a certain degree established, in study I. Neither classical swine fever nor porcine reproductive and respiratory syndrome (PRRS) are present in the concerned setting (Muhangi *et al.*, 2015). This leaves ASF as one of the few differential diagnoses for infectious pig diseases with very high mortality, further underlining the credibility of farmer-reported ASF outbreaks in Uganda (Muhangi *et al.*, 2015). However, farmer reports of more complex issues, such as detecting emerging disease, making differential diagnosing, including differentiating “sick due to ASF but recovered” from “sick from something else but recovered”, will naturally have lower specificity (Queenan *et al.*, 2017). This could have led to mis-classifications, especially in the household survey of study III. As the true number of outbreaks in the area during the study period was unknown, study III could not compare the sensitivity, specificity or individual disease detection capacity of the different surveillance methods. However, the evaluation of each method shows that ASF outbreaks can be efficiently detected using farmer reports and that real-time large-scale surveillance can be done using a smartphone interactive data-collection tool.

The uncertainty of disease estimates increases in a setting where official animal registration does not exist, animal owners might be illiterate, farm records are generally not kept, and where pigs are not individually marked and often free-roaming. For the PRAs, disease estimates were obtained by proportional piling and group consensus. With this tool it is not the number of markers used, but the proportion between the piles (pigs that died, were sick but recovered, or that never were sick during each outbreak on village level) that matters. In a context of many uncertainties, this degree of vagueness may make the final answers more useful than misleadingly over-exact measurements. It was further observed that the proportions changed during the process of reaching a group consensus. The capacity to capture the collective group knowledge – which is sometimes larger than the sum of the knowledge of all the participating individuals – is one of the advantages of the PE methodology used in the PRAs (Grace, 2003). By letting the participants choose the subjects without strictly following a questionnaire their interests are prioritised, although it can be difficult to obtain quantitative results that can be evaluated with standard statistical models. This can be overcome by using standardised PE tools such as piling, scoring and ranking (Catley *et al.*, 2012; Jost *et al.*, 2010; Mariner *et al.*, 2003). Much attention in the development of PE has been devoted to finding robust ways to validate the results. Acceptance by the conventional research community has been sought through combining PE with conventional scientific methods, and producing quantitative or semi-quantitative results that can be analysed using statistical methods (Catley *et al.*, 2012). In contrast to traditional rapid rural appraisals, PE has thus mainly been influenced by natural science perspectives and quantitative methods. Therfore it possibly missed out on discussions that participatory research as a field within the social sciences has been subjected to (Fischer *et al.*, 2016; Jacobson, 2013; Pain, 2004; Kapoor, 2001). Some further limitations in the methodology are the biases linked to group dynamics, time requirement, and the difficulties in covering large geographical areas (Catley *et al.*, 2012; Chambers, 1983). Problems with coverage and remoteness bias will, however, be present for all surveys performed in remote rural areas of low-income countries, unless somehow specifically addressed (Chambers, 1983). Even if PE sometimes offers limited participation and thus has shortcomings in involving local people (Allepuz *et al.*, 2017), it has been successful in extracting data in contexts where other forms of data have been difficult to collect (study I).

The results in study I demonstrate that not only farmers but all the actors in the smallholder pig-production value chain are affected by ASF outbreaks. Such negative impacts on the entire pig value chain have been shown to occur in poor rural areas also for events other than infectious disease, i.e. political crises (Dewey *et al.*, 2011). Value-chain actors such as middlemen and butchers are often accused of spreading ASF to maximise their profit, but study I demonstrated that they too experienced mostly negative effects from outbreaks. This is further supported by the results from study IV where, in contrast to previous information, no differences in pig sell prices could be detected between households that described ASF and those that did not. In addition, most value chain actors in study I acted at several levels of the value chain, including keeping and breeding of pigs. Thus, they would have a strong interest in disease control. Most of the value chain actors agreed that they were responsible for conducting their business so as to minimise spread of disease.

The frequent outbreaks recorded in study IV led to an increased number of pig mortalities and smaller pig herds for affected smallholders. By using the two different economic outcome variables and two different herd-size variables, economic impact of ASF outbreaks before the second and third interview occasion could be detected at both interview occasions. The results from the third interview occasion, which indicated a higher gross margin for households describing ASF outbreaks before that interview compared to those that did not, underline the difficulties to evaluate economic impact in systems that are largely non-monetary, and not driven by traditional business economy models (Rich & Perry, 2011). These households had lost pigs due to the described outbreaks, but this did not transfer into immediate, significant reductions in the gross margin. The low-input system could be one of the reasons behind this, as could other coping strategies such as trade or consumption of dead pigs (study I). The results indicate that these coping strategies might however have limited duration, because positive economic effects were only seen from recent outbreaks. More long-term effects, in the form of effects of outbreaks at the previous interview occasion, were associated with negative economic impact. In general, the models explained only a limited part of the variation in the data and predictions are therefore unreliable.

With the current lack of impact studies on household level in low income countries, it has often been repeated that the poorest pig keepers, the smallholders, are the ones who suffer most from ASF outbreaks. Poor people are more vulnerable to shocks such as animal diseases (Wagstaff, 2006), and the tangible impacts of ASF on smallholders are demonstrated in studies I and IV. However, study IV also indicates that the impact was aggravated with increasing herd size. Study II further confirmed that the negative impact of ASF was not restricted to only smallholders. As a conclusion, smallholder systems are created and maintained not only as a sheer necessity due to lack of resources, but also as a way of coping in a setting with many uncertainties and uncontrollable risks.

The social impact of ASF outbreaks recorded with the semi-qualitative methods in study I could not be reliably confirmed using the quantitative methods in study IV. That does not mean that the ASF outbreaks did not cause such impact, only that the impact could not be measured using the chosen methods. Quantitative methods are attractive as the results can be analysed using standard statistical tests and impact predicted using regression models. However, qualitative methods are especially potent for capturing social impact of diseases in low-income areas (Fischer *et al.*, 2016). The short-term positive economic impact of outbreaks could be another reason behind the lack of significant differences and tendencies for positive impact on some of the social variables. Small group sizes and low frequency of measured events could have led to difficulties in detecting associations. As in study I and II, these results also indicate that consumption of meat from pigs dead from ASF is common, and that healthy pigs are in fact seldom slaughtered for household consumption.

Finally, the chicken-and-egg-aspect of ASF control and poverty in Uganda cannot be neglected. The poverty in the rural areas affects people’s livelihoods and thus their practices related to pig keeping and to ASF. Subsequently, these practices affect ASF epidemiology. Some examples of poverty related drivers for ASF spread on micro-level are difficulties to invest in biosecurity (Leslie *et al.*, 2015), feed or housing for the pigs (studies I and IV); and the necessity to trade in, or eat, sick/dead pigs to prevent loss of investments and animal protein (studies I, II and IV). On the meso-level poverty affects ASF epidemiology in terms of lacking infrastructure for ASF-safe slaughter and failing control efforts. Conversely, the repeated outbreaks, sustained by the practices shaped by poverty, push people deeper into poverty (Krishna *et al.*, 2004). To break this cycle, disease control efforts on the micro-, meso- and macro-levels need to be combined with other efforts to reduce poverty. To improve implementation of disease control, control advice on the local level, as well as policy advice at the global and national levels, need to be flexible and better adopted to local circumstances. In formulating such advice, the participation of stakeholders on all levels will be key to successful implementation.

6 Conclusions

This thesis reports on the epidemiology of ASF in the smallholder setting in northern Uganda. It elucidates determining factors for control, compares surveillance methods and estimates social and economic impact of outbreaks. The following are the main conclusions.

- ASF was endemic in domestic pigs in the study area, with a yearly household incidence between 15 and 20%. Outbreaks were characterised by high case fatality risks (between 64 and 100%). The frequent outbreaks and numerous pig deaths had negative social and economic impact on pig production value-chain actors, regardless of their different roles in the pig production value chain.
- Outbreaks were temporally associated with human activities, such as trade in pigs and pig products and free-range management systems. Human practices drove the circulation of ASF, and the transmission was characterised as belonging to the domestic transmission cycle.
- ASF transmission in the studied smallholder setting was not mainly driven by lack of knowledge. Smallholder farmers in the study area were well aware of the clinical signs of ASF, routes for disease spread, and measures for disease control. However, awareness of the control measures did not guarantee their implementation. Farm-level biosecurity was insufficient for ASF protection both on the smallholder farms and on the larger farm in study II.
- Cultural circumstances, taboos, and poverty on micro-, meso- and macrolevels affected ASF ecology and epidemiology. To be successfully implemented, biosecurity advice must be simple, adaptable, acceptable and cost effective.
- The continuous circulation of ASF in smallholder settings created biosecurity challenges also for larger farms and disease impact was aggravated with increasing herd size. Consequently, smallholder systems are created and maintained not only as a sheer necessity due to lack of resources, but also as a way of coping in a setting with many uncertainties and uncontrollable risks.
- Pig businesses were of low-input–low-output character with low gross margins. This had implications for the interpretation of the results, for formulating disease control advice, and for a deeper understanding of both the farming context and the disease epidemiology. Despite small inputs, and thus low losses connected to mortalities, pigs had a value that could be realized if needed.

- Report-driven outbreak investigations, participatory rural appraisals, and a household survey using a smartphone application all had good disease-detecting capacity, (better than that of official reporting), establishing that ASF outbreaks can be detected using farmers' reports both retrospectively and in real-time.
- Trade and consumption of sick and dead pigs were coping strategies used to minimize losses of capital and animal protein. Dead pigs were sold, slaughtered or eaten and could therefore still bring income or serve as a source of important animal protein, but also contribute to disease transmission. Such habits, as well other elements of a non-monetary economic system not driven by traditional business economy models, made evaluation of economic impact difficult.

7 Future perspectives

In 2030, we are expected to be 9 billion people in the world. Meanwhile, the proportion of the population that belongs to the middle class is increasing, with millions of people being able to leave poverty (United Nations, 2015). The growing middle class consumes more and more meat and dairy products, creating market opportunities for livestock producers in low-income countries (Delgado, 2003). Unfortunately, increased animal density and intensified trade can lead to increase in infectious disease occurrence, shattering the window for poverty reduction created by the increased demand. To break this pattern and unleash the potential of pig keeping, ASF needs to be controlled. Likewise, to minimize the global presence of ASF virus, its transmission needs to be reduced at its source – the smallholder settings in sub-Saharan Africa.

Even if under-reporting continues to blur insights needed about ASF in Uganda, many positive steps have been taken in regards to surveillance in recent years. Several alternatives to traditional surveillance have been piloted, a participatory epidemiology network has been created and district veterinary officers are supported with tools to report disease outbreak in real time (Pinto *et al.*, 2014). However, these efforts are all project based and not sustainable without external funding. Policymakers now need to make decisions on how to reform surveillance with the ultimate goal of disease control and mitigation of the negative consequences of ASF (and other diseases). To achieve this goal with the limited resources at hand, it is important to use all existing data sources, even if imperfect and incomplete.

Qualitative research methods are especially potent for increasing understanding of the whys and hows in the people part of epidemiology, and for capturing social impact of diseases in low-income areas (Fischer *et al.*, 2016). This has become increasingly recognised, and there is a general trend in global ASF research towards cross-disciplinary research including qualitative aspects of social science. So far, however, the involvement of social scientists has been very limited (Fischer *et al.*, 2016). Hitherto, the most commonly used method for researchers that want to include aspects of social science in ASF research has been participatory epidemiology (PE). At the core of PE is the idea that participatory research and development work achieve outcomes that are locally relevant, and produce more accurate findings, because they fully include local people's perspectives and knowledge (Chambers *et al.*, 1989). At the same time, Chambers *et al.* (1989) point out that many researchers in veterinary medicine claim to engage in PE, but that the participatory component is practically non-existent or only used for extracting local information. An example of the importance of community involvement, true participation, and the need for profound involvement of social science approaches in disease control became painfully evident during the recent Ebola outbreak in West Africa (Richardson *et al.*, 2015). A number of studies describe how human behaviour drove transmission of Ebola virus and how a multi-disciplinary, or biosocial, bottom-up, community-centred approach drawing on social science competence was fundamental in achieving control of the disease (Abramowitz *et al.*, 2015a; Abramowitz *et al.*, 2015b; Roca *et al.*, 2015; Ravi & Gauldin, 2014). Along these same lines, to move forward in ASF research, and ultimately its control in Uganda, a deeper involvement of social science and true participation on community level are needed.

Research about ASF immunology and the associated search for a safe and efficient vaccine has been ongoing for decades (Neilan *et al.*, 2004), still without success. Lack of a vaccine is often mentioned as the main limiting factor for ASF control, both by farmers (study I) and researchers (Sanchez-Vizcaino *et al.*, 2012; Costard *et al.*, 2009). A vaccine would, of course, aid in achieving control, but it should not be seen as a holy grail. Ideally, a vaccine needs to be available in large quantities, cheap, multivalent, thermostable and give rise to a long protection period. For species with short reproduction cycle such as pigs, the costs versus control effect equation is not guaranteed to be in favour of vaccination. Achieving control of ASF by vaccination is further complicated by the complex epidemiology involving both wild vertebrate hosts and arthropod vectors (Thomson *et al.*, 2015; Dowdle, 1998). In low-income countries such as Uganda, resource scarcity affects animal health work so that vaccination is often carried out in response to outbreaks, not as a planned, preventive measure. In combination with late or missing reporting, this creates an overhanging risk that vaccinated animals are already incubating disease. If vaccination is done in response to an outbreak there is also a biosecurity risk with vaccination teams going from farm to farm. Despite these complicating factors, an effective vaccine would be a useful tool for achieving ASF control. Other tools are improved biosecurity on all levels in the value chain. With improved biosecurity, several diseases will be controlled simultaneously.

Much evidence is available concerning risk factors for ASF in smallholder settings, and subsequently, how ASF could be controlled by avoiding them (Penrith *et al.*, 2013). Given the nature of ASF, most risk factors concern biosecurity. Currently missing, however, is knowledge concerning which biosecurity routines can be successfully, and sustainably, implemented on micro- and meso levels, and, their respective effect in reducing ASF incidence. Thus, an important field for future research is implementation of biosecurity routines (Eccles & Mittman, 2006). Other knowledge gaps include how different interventions promote successful implementation (Tumwebaze & Mosler, 2015). The human dimensions of epidemiology are central in research on these aspects of disease control (Brugere *et al.*, 2016). It is very challenging to design and perform observational studies in smallholder settings evaluating the effect of specific biosecurity routines, measured as a reduction in disease incidence. Furthermore, for many such potential biosecurity routines we already know that if fully implemented, disease transmission will be reduced or prevented. Thus, future research should instead evaluate and optimise the implementation of biosecurity routines.

8 Populärvetenskaplig sammanfattning

När fler och fler människor lämnar fattigdom och får möjlighet att leva bättre och mer hälsosamma liv ökar efterfrågan på kött. Som svar på denna positiva utveckling har grisproduktionen ökat globalt. Uganda har den näst största, och den snabbast växande, grisproduktionen i Afrika. Produktionen sker till största delen i småskaliga familjejordbruk. Grishållning utgör en inkomstmöjlighet för fattiga småbrukare, och skulle kunna vara ett bra verktyg för fattigdomsbekämpning. Tyvärr begränsas denna möjlighet av flera produktionsfaktorer, liksom av ett rikt sjukdomspanorama. Afrikansk svinpest (ASF) är en av de viktigaste grissjukdomarna i många afrikanska länder. I Uganda är den ständigt förekommande med många årliga utbrott. När en besättning drabbas blir nästan alla grisar sjuka och de flesta dör. Därför har ASF beskrivits som en av de faktorer som kan driva fattiga småbönder in i kronisk fattigdom.

ASF drabbar tamgrisar och europeiska vildsvin med akut, blödarfeber som i de flesta fall leder till döden. Sjukdomen orsakas av ett virus och inget vaccin eller behandling finns. Historiskt cirkulerar viruset på den afrikanska kontinenten mellan vilda afrikanska grisar (framför allt vårtsvin) och mjuka fästingar, utan att de vilda grisarna uppvisar kliniska tecken på sjukdom eller sprider smittan vidare till andra grisar. Via fästingarna kan viruset överföras till tamgrisar som då de smittas blir sjuka, oftast dör, och kan sprida smittan vidare till andra grisar eller fästingar. I de flesta områden där ASF idag förekommer hos tamgrisar överförs virus dock vanligast via direkt eller indirekt kontakt mellan grisarna, utan medverkan av vårtsvin eller fästingar. Detta gäller de flesta områden där sjukdomen förekommer, inklusive Uganda. ASF-virus kan förbli livskraftigt väldigt länge i infekterade kroppsvävnader, bearbetade grisprodukter och rått, fruset, saltat eller rökt kött. Även indirekt kontakt via nedsmittade djurutrymmen och utrustning kan bidra till smittspridning.

I avhandlingen presenteras fyra studier som alla genomförts i norra Uganda. Grisproduktionen i området är extremt småskalig, den genomsnittliga besättningsstorleken i studie I, III och IV var mindre än fyra djur, inklusive smågrisar. Området hör till de fattigare i landet. Detta beror dels på ett generellt förhållande där landsbygden är fattigare än städerna, men också på en ännu icke officiellt avslutad väpnad konflikt mellan regeringsmakten och rebellgrupper. Konflikten var som mest aktiv mellan 1986 och 2006. Sedan 2007 pågår en återuppbyggnad av civilsamhället, landsbygdsbor återvänder till sina byar och återupptar jordbruksaktiviteter.

I den första studien utvärderades kunskaper, attityder och beteende rörande ASF hos olika aktörer i grisproduktionsvärdekedjan genom deltagaraktiverande gruppintervjuer. Resultaten visade att deltagarna väl kände till de kliniska tecknen på ASF, hur sjukdomen sprids och åtgärder för sjukdomskontroll. Medvetenhet om kontrollåtgärder garanterade däremot inte genomförande. En majoritet av deltagarna tillstod att de hade sålt levande grisar eller fläsk som de trodde var smittat med ASF. Studien visade hur utbrott av ASF hänger samman i tid med säsongsmässigt ökad försäljning och slakt av grisar och att det är människors handlingar som driver spridningen av ASF. Vidare visade studien på en rad negativa konsekvenser av utbrott. Grisarna används ofta som kapitalreserv för att betala skolavgifter, arbetskraft till jordbruket och sjukvård. När grisar dör får det direkta följder i form av minskad konsumtion av protein, utebliven skolgång, minskad produktion från jordbruk samt svårigheter att betala för vård.

I den andra studien detaljstuderades ett utbrott av ASF på en i sammanhanget mellanstor gård med 140 grisar. Samtliga grisar dog eller slaktades inom tre månader från utbrottets start. I studien kunde konstateras att brister i yttre och inre biosäkerhet ledde till omfattande spridning av ASF i miljön på gården. Virus identifierades i jord, vatten och foder, från en slaktplats, samt från sjuka och döda grisar. I studien visas att det är viktigt att biosäkerhetsåtgärder och smittskyddsråd anpassas till lokala förutsättningar för att de ska genomföras. Vidare konstaterades det att i områden med ständigt förekommande smitta utgör små självhushållsgårdar med dåliga möjligheter till sjukdomskontroll en risk för större företag som vill satsa på kommersiell grisproduktion.

I den tredje studien jämfördes tre olika metoder för sjukdomsövervakning och sjukdomsupptäckt. De tre metoderna var: undersökningar av rapporterade sjukdomsutbrott, deltagaraktiverande gruppintervjuer och individuella kortintervjuer med direktrapportering via mobiltelefon. Alla tre metoderna hade god förmåga att upptäcka sjukdomsutbrott, och var och en upptäckte fler utbrott än vad som rapporterades till Världsorganisationen för djurhälsa (OIE) under tidsperioden.

I den fjärde studien genomfördes intervjuer med tvåhundra småbrukare vid tre upprepade tillfällen, med syfte att mäta sociala och ekonomiska effekter av ASF-utbrott. Studien visade att ASF-utbrott ledde till ökad dödlighet bland grisarna och till minskad besättningsstorlek. Vidare sågs att grisföretagen investerade extremt lite i grisproduktionen, ofta ingenting alls utöver inköp av grisar. Grisarna fick sedan gå lösa och själva finna sin föda. Frigående uppfödningssystem innebär ökad energiåtgång då grisarna rör sig över stora ytor, liksom ökad risk för smittspridning. Däremot kan det ändå vara ekonomiskt fördelaktigt med ett system som tillåter så minimal investering, eftersom ASF är ständigt förekommande och det är svårt att under rådande förutsättningar förhindra att grisarna smittas. I studien jämfördes det ekonomiska utfallet för hushåll som haft, respektive inte haft, ASF-utbrott. Både nettoinkomst och ett värde som baserades på besättningens antal grisar och deras försäljningsvärde användes. Det senare värdet användes för att ge ett hypotetiskt värde till grisar som inte hade medfört mer än inköpspriset i investering, men som ändå hade ett värde vid försäljning. Om en sådan gris dör blev alltså den ekonomiska förlusten mätt i nettoinkomst liten, medan förlusten mätt i den variabel som inkluderar grisen hypotetiska värde vid försäljning blev större. Resultaten visade att det ekonomiska utfallet ökade med besättningsstorleken, liksom skillnaden i utfall mellan besättningar som hade, respektive inte hade, haft ASF-utbrott. Dessutom kunde man med nettoinkomstvariabeln se kortsiktiga vinster av utbrott. På lång sikt var dock effekterna alltid negativa. Studien visade att det var svårt att kvantifiera ekonomiska förluster i en ekonomi som till stora delar är informell. Likaledes var det svårt att mäta sociala effekter av ASF-utbrotten, dessa uppskattas troligen bättre med andra metoder. Resultaten pekar också på att småbruk som driftsform kan vara ekonomiskt fördelaktigt.

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Acknowledgements

The work presented in this thesis was performed at the Department of Biomedical Sciences and Veterinary Public Health at the Swedish University of Agricultural Sciences and the Department of Disease Control and Epidemiology at the National Veterinary Institute. The studies were financed by the Swedish research council/U-forsk under contract no. 348-2013-146. The work would not have been possible without the help and support from many. I would like to especially acknowledge some of them.

My four *super* supervisors: I have felt extremely privileged to have (almost) unlimited access to the treasure of your collected knowledge and experience, thank you for so generously sharing this with me. Thank you for giving me the opportunity to be enrolled in this project, and to pursue the PhD-education. **Sofia Boqvist**, my main supervisor, for always having full control over all the administrative procedures involved from application to defense (the administrative hurdles sometimes felt higher than the research ones) and for having a relaxed attitude towards the ISP! **Ulf Emanuelson**, co-supervisor, for stringent discussions, for not agreeing, and for making sure the introductions did not transform into review-papers. **Susanna Sternberg-Lewerin**, co-supervisor, for your fantastic enthusiasm and for the energy you create in every project you participate in and every room you enter! I also thank you for being such an excellent proof reader, with endless patience regarding “there” and “where”. **Karl Ståhl**, co-supervisor and project investigator, that answered my first e-mail expressing interest for animal health research in low-income countries on a positive note. Thank you for always being available, disregarding the hour or geographical location! Thank you also for sharing your passion about ASF, and for introducing me to all the important partners and places in Uganda as well as the international ASF-community.

All the **farmers** and **value-chain actors** in Gulu and **staff** at the farm in Lira, that participated, gave of their time, opened their homes and shared their experiences.

Tonny Aliro, without you none of the studies could have been done. Thank you for all the time you devoted to the project, your fantastic organization, network, communication and cooperation skills, and for “it’s OK”.

Charles Masembe, for providing the corner stone of ASF-Uganda, in both scientific and logistic terms, and for chasing after the phone-tracker!

Solomon Alike, Bruce Nokorach, Wokorach Samuel Opiyo, Simon Peter Otema and **Peter Ogweng** from the Gulu district veterinary office for invaluable help with the field work. Thank you for facilitating the different interviews in such professional ways and for all the interesting discussions we had in the car! Everything I know about Gulu I learnt from you. Afoyo matek!

Stella Atim, Martin Esau, Mayega Johnson, Neil LeBlanc, Lihong Liu and **Charles Masembe,** for processing the samples in the labs. Neil LeBlanc and Lihong Liu for explaining many, many times, and for finally, making me understand at least a little bit about the PCR mumbo-jumbo.

Michel Dione, Delia Grace, Johanna Lindahl, Emily Ouma, Danilo Peso and **Tom Randolph** at ILRI in Kenya and Uganda, for sharing your local knowledge, expertise and for always being so generous with your time.

Noelina Nantima and **Rose Ademun Okorut** at Ministry of Agriculture, Animal Industries and Fisheries in Uganda for facilitating the research in such extraordinary ways.

MFS students **Caroline Bössfall, Frida Crafoord** and **Mikaela Klahr Fritz** for the work you did in the field!

Fred Mitala and **Julius Kalema**, for always driving me safely and nicely between Kampala and Gulu and all around Gulu district. On roads, on paths and in the bush!

Co-authors: **Tonny Aliro, Sofia Boqvist, Claudia von Brömsen, Giampaolo Cocca, Frida Craaford, Michel Dione, Ulf Emanuelson, Neil LeBlanc, Lihong Liu, Charles Masembe, Emily Ouma, Susanna Sternberg Lewerin, Karl Ståhl** and **Emma Tejler**.

All fantastic colleagues at **ESS**, especially the present and former **epiz**-team, for your friendship and inspiration. Thank you for taking over all "my diseases" and for supporting me while pursuing this effort. **Linda Svensson**, for help with numerous maps. **Maria Nöremark**, for the cover illustration. The R-gurus **Fernanda Dorea, Stefan Widgren** and **Thomas Rosendal**, for your endless patience with beginner questions, and for helping transform my 1409-variables data set for study IV into something that could at all be analysed. A very special thanks to my present and former managers at epiz and ESS: **Susanna Sternberg Lewerin, Gunilla Hallgren, Karl Ståhl, Cecilia Hultén, Marianne Elvander** and **Ann Lindberg** for your fantastic support all along the way, with the recruitment to epiz and being given the opportunity to pursue the PhD-education while being employed at SVA as extra significant mile stones.

Micke Pettersson, Johnny Ericsson and **Rickard Levén** at IT, for both general computer health care as well as heroic lifesaving emergency contributions during the years!

Anna Rothman at BVF, SLU, for processing heaps and heaps of receipts of un-traditional character, questionable cleanliness and strange smells.

The **SLU library** for finding the historical reports and first publications of ASF.

All colleagues in **Sveriges veterinärmedicinska sällskaps kollegie** och dess **sektion för veterinär folkhälsa**, for providing me with frequent visits to the bigger picture of research and continued education in Sweden, for discussions, and for inspiration.

Anonymous and non-anonymous reviewers as well as language editor **Terese Bergfors,** for making the manuscripts and the thesis so much better than the first versions.

Equines at **Centre Equestre de Corminbeuf** and **Nationales Pferde Zentrum, Bern**, for providing necessary breaks in the thesis work.

Friends from at least three continents, for providing a pleasant place to be outside research, for listening, for discussions, for gossip, for sharing troubles, joy, facebook updates, whatsapp chats, walks, vacations, meals, coffees and far too many bottles of wine.

Mamma och **pappa**, for endless support, for inputs from the Swedish farming community, and for still being interested in the strange things I do for work.

Frédéric, Elise and **Amelie**, for putting up with me being away so much during the last four years, and also for putting up with me at home during thesis and article writing times. Very special thanks to Frédéric for being the constant ground crew at home for these four years. To the three of you for making me happy, for making me angry, for making me laugh, and for being you.