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ORIGINAL RESEARCH

African swine fever among slaughter pigs in Mubende district, Uganda

Adrian Muwonge · Hetron M. Munang'andu · Clovice Kankya · Demelash Biffa · Chris Oura · Eystein Skjerve · James Oloya

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Abstract Owing to frequent reports of suspected outbreaks and the presence of reservoir hosts and vectors (warthogs, bushpigs and *O. moubata* ticks), African swine fever (ASF) is believed to be an endemic disease in Uganda. There have, however, been very few studies carried out to confirm its existence in Uganda. This study was carried out to describe the prevalence of ASF based on pathologic lesions and analysis of serum samples from slaughtered pigs during a suspected outbreak in the Mubende district of Uganda. The study was based on visits to 22 slaughterhouses where individual pigs were randomly selected for a detailed ante-mortem and post-mortem inspections. Sera were also collected for laboratory analysis. A total of 997 pigs (53.7% male and 46.3% female) were examined for lesions suggestive of ASF and sero-positivity of sera for ASF antibodies. The sera were tested using enzyme-linked immunosorbent assay (ELISA) and positive samples were further confirmed with an immunoblot assay. The results showed that 3.8% (38/997) of the pigs examined had clinical signs and post-mortem lesions suggestive of ASF. Two of 997 (0.2%) sera analysed were positive for ASF antibodies. Of the sub-counties investigated, Bagezza (12%) and Kiyuni (11%) had the highest prevalence of lesions suggestive of ASF based on ante- and post-mortem examination results, while Mubende town council (1.7%) had the lowest. This study found a low number of pigs (3.8%) with lesions suggestive of ASF at slaughter and an even lower number of pigs (0.2%) that were seropositive at slaughter, however a significantly higher number of pigs were slaughtered during the outbreak as a strategy for farmers to avoid losses associated with mortality.

Keywords Sero-prevalence · African swine fever · Pigs · Uganda

A. Muwonge (✉) · D. Biffa · E. Skjerve
Department of Food Safety and Infection Biology,
Norwegian School of Veterinary Science,
P.O. Box 8146 Dep., 0033, Oslo, Norway
e-mail: adrian.muwonge@veths.no

H. M. Munang'andu
Department of Basic Sciences and Aquatic Medicine,
Section of Aquatic Medicine and Nutrition,
Norwegian School of Veterinary Science,
P.O. Box 8146 Dep., 0033, Oslo, Norway

C. Kankya
Department of Veterinary Public Health and Preventive Medicine,
Faculty of Veterinary Medicine, Makerere University,
P.O. Box 7062, Kampala, Uganda

C. Oura
Institute for Animal Health, Pirbright laboratory,
Ash Road, Pirbright,
Surrey GU24 ONF, UK

J. Oloya
Department of Epidemiology and Biostatistics/Population Health,
College of Public health, 132 Coverdell Centre,
University of Georgia,
Athens, GA 30602-7396, USA

Background

African swine fever (ASF) is a viral disease with a devastating impact on the pig industry in sub-Saharan Africa (Feran and Bastos 2009). The disease is caused by double-stranded DNA virus with an icosahedral symmetry that belongs to genus *Asfivirus* and family Asfarviridae (Penrith et al. 2005).

It can manifest as a lethal per acute to acute haemorrhagic fever or a less virulent chronic disease. Infected pigs can live long enough to produce antibodies that are measurable 3–10 days after exposure (Penrith et al. 2005). Depending on the strain and intensity of exposure, clinical signs are visible within 5–15 days (Lubisi et al. 2007; Penrith et al. 2005).

There are three cycles of ASF transmission documented in Africa; (1) the sylvatic cycle in which the Ornithodoros moubata soft tick gets infected after feeding on viraemic warthogs/bushpigs. These then become sources of infection for domestic pigs. (2) The tick cycle in which the virus is transmitted between ticks and domestic pigs in the absence of the reservoir hosts and (3) the domestic cycle, which occurs in the absence of ticks where the virus is transmitted by direct contact from infected to non-infected domestic pigs (Penrith et al. 2005; Solenne et al. 2009). The sylvatic and domestic pig cycles exist in East Africa and are known to play an important role in existence of multiple genotypes that cause both the virulent and chronic types of the disease (Fasina et al. 2010). Enzyme-linked immunosorbent assay (ELISA) can detect antibodies as early as 3–4 days after infection, while immunoblotting has been used to confirm results obtained by ELISA (Penrith et al. 2005). These two methods are reported to be the most dependable laboratory tools for studying endemic ASF in African countries (Penrith et al. 2005). They have also been used to support eradication and restocking programs in Spain, Belgium and the Netherlands (Penrith et al. 2005; OIE 2010). The office of international epizootic (OIE) also recommends the use of these two methods for diagnosis of ASF (OIE 2008).

The media speculation of ASF outbreaks and OIE confirmed outbreaks of ASF between 2006 and 2010 have consistently identified Mubende district as one of the epicentres of ASF emergence (El Swalhy et al. 2008). Recent studies have shown a steady increase in pork consumption in Uganda, especially in urban areas. A large proportion of these pigs is transported from rural areas (Waiswa et al. 2009; Muwonge et al. 2010). Mubende is the poorest among the sources of urban consumed pork, and here pigs are considered low-input livestock which grow to market size on minimal feed inputs (Waiswa et al. 2009). There are speculations that outbreaks are associated with a boom in pig sales to slaughterhouses to avoid losses linked to mortality. This is thus believed to trigger a massive transportation of pigs to local and nearby markets and districts. Such a phenomenon could highly influence the spread of ASF. Therefore, this study was aimed at describing dynamics in slaughter population, prevalence of ASF suggestive lesions and serological status during an outbreak in Mubende district.

Materials and methods

Study area

Mubende is located in the central region (00°33′27″N, 31°23′42″E) of Uganda. It is divided into two counties, Buwekula and Kassanda, which are further sub-divided into ten sub-counties. Mubende is estimated to have 35,195 head of cattle, 80,000 pigs, 12,238 goats, 5,955 sheep and 360,157 chickens. Madudu and kiyuni sub-counties are home to the biggest proportion of the pig population in the district (Mugisha 2007). Pigs are often kept on free range, feeding on swill and scavenging on crop residues. It should be noted that a quarantine due to ASF imposed by the Ministry of Agriculture Animal industry and fisheries (MAAIF) was in effect during the entire period of the study. This outbreak was also reported by the OIE and the Pan-African animal health yearbook 2008 (OIE 2010; El Swalhy et al. 2008). Pig slaughtering within the district continued regardless of the quarantine.

Study design and sample size estimation

A cross-sectional convenient-random sampling strategy was used to examine pigs and collect sera from 22 slaughterhouses in the ten sub-counties of Mubende district. Slaughterhouses were conveniently selected depending on accessibility. At the slaughterhouses, individual pigs were randomly selected for sampling. The number of pigs inspected depended on the total slaughtered per slaughter house. Based on a similar study carried out in Tanzania (Swai et al. 2005), a 14.9% mean expected sero prevalence of ASF was assumed to calculate the sample size. The pig population was estimated at 80,000 in the district (Mugisha 2007). Desired precision ($\acute{a}$) and power of the test were set at 0.05% and 95%, respectively. Using these assumptions, the minimum sample size required to detect a case of ASF in the sampled pig population was 195 pigs. Sample size was calculated using web-based software (http://www.ausvet.com.au/). This sample size was divided among the ten sub-counties which required that at least 18 pigs be sampled from each sub-county. In order to increase precision of the estimator, we examined total of 997 during the study period. Information on age, breed, sex, source and date of sample collection was recorded in a data recording sheet.

Serum collection and analysis

Sampling was done between September 2008 and February 2009. During this period pigs showed typical clinical signs suggestive of ASF prior to slaughter. These included the following: huddling together, dropped snout, wobbling hind legs leading to a swaying gait and cyanosis of the snout, ears abdomen and tail in exotic breeds. At post-mortem, pigs exhibited lesions such as generalised oedema, congestion, petechiation on organs, frothing in the trachea and spleen enlargement. Pigs that exhibited at least three of these clinical signs at ante-mortem and had pathological lesions at post-mortem examination were deemed to be positive for ASF based ante and post-mortem examination. A blood sample was collected from each individual pig in sterile vacutainer tubes. The samples were kept in icebox and transported to the Veterinary Public Health laboratory at Makerere University,

Kampala, Uganda. Serum was separated after spinning at 2,500 rpm for 10 min and later shipped to the Institute for Animal Health laboratory at Pirbright, UK, for serological testing. Detection of ASF antibodies was carried out on all 997 sera samples using a blocking ELISA using a purified protein extract from ASF virus (VP73) as antigen (Ingezim PPA Compac 1.1.PPA K3 Elisa kit, Ingenasa, Spain). Samples that were positive on ELISA were further confirmed by immunoblotting technique as described by the European Community Reference laboratory (CRL) for the diagnosis of ASF (CISA-INIA 2008). Briefly pre-coated strips were blocked with 2% milk in phosphate buffered saline (PBS) at 37°C, with constant agitation. Thereafter, the strips were incubated for 45 min in the test and control sera at 37°C, with constant agitation. After washing four times in PBS, the strips were incubated for 45 min in Protein A–peroxidase conjugate at 37°C with constant agitation. Diaminobenzidine (DAB) substrate was added after washing four times in PBS and the strips were observed for 15 min. The reaction was stopped by washing the strips in running tap water.

Data collection and analysis

Sera test results together with lesion presence/absence for each individual case were entered into Microsoft Excels spread sheet® 2003. Subsequently all the data inclusive of the animal, time factors and weather condition records from Mubende metrological station were merged and transferred to Stata/SE 10 for windows; StataCorp, College Station, TX, USA) for statistical analysis. Descriptive summary statistics were used to explore the data. A mixed-effects logistic regression with sub-counties as the random effect (clustering) was used to identify the risk factors associated with prevalence of ASF based on ante-mortem and post-mortem lesions at slaughter. The model was developed using the backward elimination of biologically and statistically plausible variables (the variable inclusion criteria was set at $P \leq 0.25$)

Results

Of the 997 sera tested, only two (0.2%) were positive for ASF. These two samples were further subjected to Immunoblotting to confirm true positivity. Sex, breed and age structure of the sampled pigs, and their corresponding sero status, ante- and post-mortem findings are shown in Table 1.

Among the inspected pigs, 3.8% (38/997) had lesions suggestive of ASF at ante- and post-mortem inspection (Table 1). This prevalence was highest in old exotic sows (11%, 14% and 4.3%, respectively). The sub-counties of Bagezza (12%) and Kiyuni (11%) had the highest rate, while the Mubende town council (1.7%) had the lowest prevalence of lesions suggestive of ASF. In addition, both Kiyuni and Kasambya each had an adult and young local breed boar that test sero-positive for ASF antibodies, respectively. Majority of the pigs deemed positive exhibited ante-mortem clinical signs like dropped snout, wobbling hind legs presenting as a sway gait and cyanosis of the snout, ears and abdomen. At post-mortem, these showed generalised oedema, congestion, petechiation on the liver and spleen enlargement. The study found a temporal trend associated with lesions suggestive of ASF as shown in Table 3. There was a dramatic increase in the number of pigs with lesions suggestive of ASF and number of pigs inspected in the months of October and November. The association between breed, study month, precipitation and the prevalence of lesions suggestive of ASF at slaughter is shown in Table 2. There was a significant difference in prevalence of lesions between the three different breeds with the exotic breeds being four times more likely to have these lesions than the local breeds (odds ratio [OR]=4; P=0.05). The model also showed that precipitation was a protective factor in the outbreak of ASF (OR=0.9; P=0.02). Furthermore, the variance estimate (VE) of 0.78 indicates that the difference in prevalence of lesions suggestive of ASF was wider between sub-counties than within sub-counties. Therefore, geographical locations influenced prevalence.

Table 1 Number of seropositive pigs and pigs with ante/post-mortem ASF lesions recorded during a suspected ASF outbreak in the Mubende district of Uganda

Variable	Label	No of pigs sampled	Number of pigs with clinical & necropsy findings suggestive of ASF	(%)	Number of ELISA+ve and Immunoblot+ve pigs	(%)
Total animal	Sampled pigs	997	38	3.8	2	0.2
Sex	Male	535	18	3.4	2	0.4
	Female	462	20	4.3	–	
Breed	Local breed	846	30	3.5	2	0.2
	Crossbreed	129	5	3.8	–	
	Exotic breed	22	3	14.0	–	
Age	Infant	45	1	2.2	1	2.2
	Adult	864	27	3.1	1	0.1
	Old	88	10	11.0	–	

Infant = (1–8 months), Adults = (9–18 months), Old = (19 and above)

Table 2 Mixed effects logistic regression model for factors associated with ante–post and post-mortem ASF lesion in pigs at slaughter in Mubende District

Variables	Level	SE	Odds ratio	P value	95% CI	Variance estimate
Breed	Exotic vs. local breed	0.74	4	0.05	0.97–18.35	
Months	September		1			
	October	0.88	0.05	0.001	0.009–0.231	
	November	0.86	0.04	0.001	0.008–0.232	
	January	0.80	0.17	0.02	0.03–0.60	
	February	0.43	0.11	0.01	0.02–0.5	
Weather	Precipitation	0.05	0.9	0.02	0.8–0.98	
Random effect	Sub-county	0.58	–	–	0.18–3.36	0.78

Discussion

Historically, ASF has been reported from domestic pigs, wild hogs and soft ticks in Uganda (Carlos 2009; Feran and Bastos 2009; Gallardo et al. 2011). Several factors—(1) domestic pigs are mostly reared using free range or tethering methods (Mugisha 2007; Muwonge et al. 2010), and (2) the presence of the *O. moubata* soft ticks (Penrith et al. 2005), and (3) a high population of wild hogs near Lake Wamala (Kateregga 2010)—could have an influence on the occurrence of ASF outbreaks in the area, leading to this district's designation as an ASF hot spot. Unfortunately, there have been few field-based studies carried out to document dynamics in outbreaks. In this study, a 0.2% (2/997) and 3.8% (38/997) prevalence of ASF was reported based on serological status and gross lesions suggestive of ASF at

Table 3 Temporal distribution of ante–post and post-mortem ASF lesions and number of slaughtered pigs during the outbreak

Sub-county	Slaughterhouse	September[a]	October[b]	November[b]	December[a]	January[a]	February[c]
Madudu	Madudu	5	16	24	10	4	6
	Naluwondwa	2	13	10	2	2	7
	Ngabano	4	6	1	0	0	0
	Kilawula	4	8	6	3	6	2
	Kisamula	0	10	4	0	0	0
	Kabweyakiza	1	3	7	3	1	0
	Kabulamuliro	1	7	4	1	0	0
Kasambya		7	8	12	11	8	23
Kiyuni	Kiyuni	2	13	8	1	0	0
	Kiyuya	1	2	2	2	0	0
	Muwoko	0	4	10	2	0	0
	Kyawoonge	0	3	0	0	0	0
Bagezza	Bagezza	0	2	2	2	0	0
	Kibalinga	0	4	8	0	0	0
Butologo	Kalama	2	0	8	16	5	40
Bukuya	Bukuya	39	18	69	36	54	16
Myanzi	Myanzi	26	12	20	15	16	17
Town council	You and me	1	23	11	8	2	0
	Pork centre	7	31	9	20	2	0
Kiganda	Kiganda	19	1	21	9	3	1
	Kasanda	10	14	23	7	1	0
Kasanda	kigalama	4	2	6	2	3	2
Total inspected		135	200	273	164	111	114
Total ASF lesion		5	8	14	5	4	2

[a] Pre- and post-outbreak

[b] Outbreak

[c] Endemic

ante- and post-mortem examination, respectively (Table 1). This is lower than the 2%, 9%, 14.9% and 29.4% sero prevalence reported in northern Tanzania, Northern Cameroon, Nigeria and Sardinia, Italy, respectively (Awa et al. 1999; Fasina et al. 2010; Mannelli et al. 1998; Swai et al. 2005). Patho-clinically, the prevalence documented by this study is lower than 20% lesion prevalence documented in North Cameroon (Awa et al. 1999). The findings in this study, however, concurred with the EU report which showed a low prevalence of sero-positive pigs during outbreaks in East Africa and Sardinia (Carlos 2009).

It is likely that most pigs in this study died or were sold for slaughter before they developed antibodies, thus leading to the low sero-prevalence recorded in the present study. This is supported by reports of mortalities during this outbreak by veterinary services. Similarly, ASF strains previously collected from Uganda have been classified as highly virulent and were documented to wipe out most of the infected pigs (Carlos 2009; Lubisi et al. 2007).

On the other hand, surveys carried out elsewhere have shown that countries with low virulent strains like Spain tend to have high ASF endemic situations leading to relatively higher sero-prevalence levels than countries with highly virulent strains (Carlos 2009). By contrast, reports on East Africa showed a higher prevalence of ASF antibodies in pigs from northern Tanzania, indicating that a proportion of pigs survive infection (Carlos 2009;Swai et al. 2005). In terms of clinical signs and gross pathological lesions suggestive of ASF, this study found a significant association between breed and lesion prevalence. Exotic pigs were found to have a higher prevalence of lesions than the local breed (OR=4; P=0.05) (Table 2). This finding is contrary to what is published elsewhere, which indicates that this disease showed no breed preference (Penrith et al. 2009).

The numbers of pigs slaughtered/inspected seem to follow the same trend as the lesions suggestive of ASF (Table 3). This trend is a reflection of the cascade of events that ensue when farmers realise that it is an outbreak of ASF. The sharp increase in sales of both healthy and infected pigs for slaughter is an effort to salvage some economic value. The same trend also reveals that Kiyuni sub-county experienced the outbreak earlier than the rest of the sub-counties because the slaughter numbers increased in October prior to the general surge in the district in November. Mubende urban slaughterhouses also experienced a surge in slaughter numbers in October; this is because their source of slaughter pigs is the rural areas including Kiyuni and Madudu sub-counties (Table 3). Therefore, this could be a spill-over effect from panic sales in Kiyuni. This is qualified by the random effects model (Table 2), which indicated that there is more variation in numbers of slaughter pigs between sub-counties rather than within (VE=0.78). The model also indicated that precipitation was a protective factor in the outbreak of ASF (OR=0.9; P=0.02). In other words, the prevalence was lower in the rain season (Table 2). This is in agreement with the findings of Mannelli et al. (1997) in Sardinia, Italy.

Conclusion

This study found a low sero-prevalence and ASF lesion pigs at slaughter but a significantly higher number of pigs slaughtered during the outbreak as a strategy for farmers to avoid loss associated with mortality. This supports the common notion that farmers sell off sick and healthy pigs during ASF outbreaks in an effort to salvage some economic value in their stock. This response is highly likely to enhance the onward spread of ASF making the control of the disease even more of a challenge.

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