Volume 20 Issue 5
Originally issued as K1

Global Journal of Medical Research: K
Interdisciplinary
Volume 20 Issue 1 Version 1.0 Year 2020
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

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GJMR-K Classification: *NLMC Code: QY 60.S8*

QUANTITATIVEOUTCOMESOFAONEHEALTHAPPROACHTOINVESTIGATETHEFIRSTOUTBREAKOFAFRICANSWINEFEVERINTHEREPUBLICOFSIERRALEONE

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Quantitative Outcomes of a One Health Approach to Investigate the First Outbreak of African Swine Fever in the Republic of Sierra Leone

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Summary- African swine fever (ASF) outbreaks have been reported in Sub-Saharan countries, including West Africa states, but has never been notified in the Republic of Sierra Leone. This is the first report describing field epidemiological and laboratory investigations into the outbreak of fatal pig disease in western rural and urban districts, Freetown. A preliminary finding indicated that pigs exhibited clinical and necropsy signs suggestive of ASF. Serological (ELISA) and molecular (qRT-PCR) methods used to confirm and investigate the outbreak yielded three positive results for the ASF antibody and all negative for Swine flu; thus, confirming ASF as the etiology agent. The One Health Multisectoral approach adopted during this investigation involving the Ministry of Agriculture and Forestry, the Ministry of Health and Sanitation, the University of Makeni, Njala University, and other partners proved to be an effective outbreak response strategy and alleviate fear and panic through community engagement and sensitization.

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I. Introduction

African swine fever (ASF) is a significant disease of domestic pigs caused by a DNA arbovirus virus belonging to the family *Asfaviridae* (Dixon et al., 2005). To date, there are 22 different genotypes described based on the p72 sequences, all of which circulate on the Africa continent (Boshoff et al., 2007). Due to its devastating economic impact in affected countries, the World Organization for Animal Health (OIE) listed ASF as a notifiable disease.

Outbreaks of ASF have been reported in Sub-Saharan countries, including West Africa states particularly in Benin, Burkina Faso, Cote d'Ivoire, the Gambia, Ghana, Guinea, Guinea-Bissau, Mali, Nigeria, Senegal, and Togo; however, the Republic of Sierra Leone never reported an epidemic of ASF [Bastos et al., 2003; Brown et al., 2018; Penrith et al., 2013]. Nonetheless, the Ministry of Agriculture and Forestry (MAF), together with the Ministry of Health and Sanitation (MoHS) jointly announced on 23 October 2019 that an outbreak of an unknown disease was killing pigs in western rural and urban districts, Freetown. Preliminary investigation jointly conducted by MAF and the Food and Agriculture Organization of the United Nations (FAO) in the affected areas, indicated that pigs exhibited clinical signs matching suspect case definition for ASF as defined by OIE which included; high fever, depression, loss of appetite, hyperemia and cyanosis (seen as reddening) of the skin, particularly the ears and snout and eventually death.

Recognizing that pigs host various zoonotic pathogens, One Health (OH) approach aligned with global recommendation was established and implemented to investigate the etiological agents responsible for the epidemic, and institute prevention and control measures. OH approach promoted interdisciplinary collaboration and coordination, bringing

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together health scientists and practitioners at the human, animal, and environment to strengthen emerging and re-emerging infectious disease surveillance and outbreak response.

Consequently, a OH rapid response team composed of epidemiologists from MAF, MoHS, FAO, and scientists from the University of Makeni, Njala University and CDC/AFFENET/FELTP intermediate students was deployed in affected communities to gather epidemiological information (morbidity, mortality and cases fatality rates, risk factors) in affected and unaffected farms, collect biological samples for laboratory confirmation and institute prevention and control measures to contain the outbreak.

Here we report preliminary field epidemiological and laboratory investigations findings into the first outbreak of ASF in the Republic of Sierra Leone.

II. Materials and Methods

a) Localization of the study

Investigations were carried out from 29 October to 1 November 2019 by three sub teams derived from the One Health rapid response team. Each team was assigned communities where reports of death pigs had occurred to undertake active disease searches to identify cases using case definition for ASF provided by FAO. The case definition used was any pig herd with one or more age groups affected with high fever, depression, and loss of appetite, hyperemia, and cyanosis of the skin, particularly at the ears and snout and eventually death within 2-10 days in the affected areas from 10th September 2019 to present.

Areas visited included Monkey Bush, Campbell Town, Samuel Town in Waterloo, Maburieh, Bengumah, Ibo Town, Bomeh in waterloo, Bomeh/Kingtom, Kroobay, Susan Bay, Moawharf and Racecourse Clintown (Figure 1). Each investigation team received forms/questionnaires for collecting epidemiological and laboratory data, and case definition checklist to assist them to identify cases.

b) Sample Collection

Infected and non-infected farms were visited for inspection and specimen recovered from more than 60 pigs of ages between 4 -12 months. Moreover, each team recorded GPS coordinate of various communities visited during the five days of field activities. For each pig, oral pharyngeal swabs, nasal mucosae swabs were collected using sterile swab collection kits and placed in 2 mL cryovial containing RNAlater (Ambion Inc., Austin, TX, USA). Cardiac puncture method was used for blood sample collection into a vacutainer tube with or without containing EDTA and plasma or serum recovered and stored until analysis. Fecal samples and tissues (spleen, liver, lymph nodes, lungs, heart, and kidneys) from dead pigs at postmortem were also collected.

During field expedition, scientists kept all samples on dry ice, and upon returning to the laboratory they were transferred to a - 20 °C freezer then – 80 °C until further analysis.

All specimens were collected in duplicate and transported to Central Veterinary Laboratory (CVL), Teko Makeni for testing for ASF, and to the University of Makeni Infectious Diseases laboratory (IDRL) for Swine Flu and other potential zoonotic diseases analysis.

c) Serology assay for detection of ASF

All serum and blood samples were analyzed using a Multi-antigen indirect ELISA kit for the detection of ASF antibodies against P32, P62, and P72 ASF antigens manufactured by ID Vet diagnostics (ID Vet, 2019). Briefly, samples were added to the antigen pre-coated plate and incubated at room temperature, after washing to eliminate excess serum, a specific conjugate was added and incubated. The plates were further washed, and upon addition of substrate and stop solution respectively, incubated and optical density read on a Multiskan Sky Microplate Spectrophotometer (ThermoFisher Scientific).

d) Detection of Swine Flu

i. *RNA Extraction and qRT-PCR*

RNA from oral swabs, nasal swabs, serum, and plasma was extracted using QiaAmp Viral RNA Mini Kit (Qiagen, Hilden, Germany) following manufacturer's instruction and material eluted in 60μL of AVE buffer and stored at – 80 °C until needed. Quantitative reverse transcription real-time PCR (qRT-PCR) for the detection of Human Influenza A H1 and H3 viruses was performed on a Light Cycler® 96 Instrument (Roche, Life Science) using a Primer Design™ genesig kit (Southampton, United Kingdom) following the manufacturer's instruction with cut-off Ct set at 38. The PCR amplification reaction was performed in a 20 μL reaction mix as follows; 50 °C for 600 s (reverse transcription) and 95 °C for 15 min (DNA polymerase activation), followed by 50 cycles at 95 °C for 10 s (denaturation), and finally 60 °C for 60 s (annealing and extension). Positive results obtained were characterized by a sigmoid curve, showing an initial, rapid, exponential increase in fluorescence signal followed by a plateau. Negative reactions did not show any increase in fluorescence signal. A standard curve was created automatically with the Light Cycler® software in each run by plotting the cycle threshold number against the copy numbers of each standard, and quantification of unknown samples were inferred from the regression line.

e) Data Analysis

Microsoft excel was used for all analyses and animal-related factors characterizing animals or herd such as breed, age, herd size, grazing system, morbidity, mortality, and breeding system were established through univariate analysis.

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Recent and past exposure to ASF was determined by the presence of ASF specific antibodies, and seroprevalence defined as the proportion of positive ASF IgG/IgM antibodies among the sampled pigs, and molecular assay data analysis performed on the LightCycler® 96 Application Software Version 1.1.

III. Results and Discussion

Pig production in Sierra Leone is mainly small-scale traditional using indigenous breeds of pigs, which are small, short, and sturdy with little hair covering on the skin. Some farmers keep exotic breeds, mainly large white race, and Duroc. There has been a lot of cross-breeding between the local and the exotic breeds, giving rise to hybrids. The country has an estimated pig population of 57,877 pigs representing 1% of the total livestock population in the country (Table 1). Western Urban and Western areas have an estimated pig population of 4,343 and 6,603 respectively representing about 20% of the total pig population in the country.

The OH teams visited 48 households in 12 communities in Monkey Bush, Campbell Town, Samuel Town in Waterloo, Maburieh, Bengumah, Ibo Town, Bomeh in waterloo, Bomeh/Kingtom, Kroobay, Susan Bay, Moawarf and Racecourse. The majority of the pig farmers visited were keeping pigs on the free-range system, and they were roaming and scavenging under very poor hygienic conditions. Also, the farmers were dumping dead pigs in the nearby streams/tributaries where other pigs were scavenging, which increased the risk for ASF spreading to others districts such as Port Loko and Tonkolili; thus, representing a major risk of contamination in those districts.

Studies have highlighted that indiscriminate disposal of pig viscera, waste materials, poor biosecurity measures and salvage sale of survivor and sick pigs without testing contribute to in-country maintenance and spread of ASF (Kabuuka et al., 2014; Muhangi et al., 2015; Nantima et al., 2015).

The estimated herd mortality in the sampled households ranged from 0 to 99.4%. The mortality rate was highest in local communities within the Western Urban district compared to the Western Rural district (Table 2). Kroobay in Western Urban had the highest herd mortality rate of 99.4% while Colvert and Benguima in Western Rural districts had 0% herd mortalities. These mortality disparities are concordant with previous studies indicating that ASF mortality varies between 30-70% for moderately virulent strains and 100% in naïve herds of domesticated pigs (Spickler, 2019).

Three hundred and thirty-nine (339) specimens were collected during investigation including, whole blood, serum, oral swabs, nasal swabs, fecal samples, and organs. The samples collected included; whole blood, serum, nasal and oral pharyngeal swabs as well as fecal samples. A post-mortem was undertaken on dead pigs, and organs collected, including spleen, lymph nodes, kidneys, liver, and heart.

Among the samples collected during the investigation, ASF ELISA detected antibodies in three samples from sick pigs (2 URC serum and 1 UKT (blood and serum)), indicative of exposure to the ASF virus (Table 3). Currently, there is no vaccine against ASF; therefore, the presence of antibodies in sick pigs is a result of exposure to the ASF virus. The behavior of farmers quickly selling off sick pigs to mitigate economic impact compromised the detection of virus and antibodies. By the time the response team arrives for investigation, most of the farmers didn't have sick animals. Also, most pigs sampled may not have been exposed to ASF, reason for a large number of negative samples. Considering the epidemiologic features of communities where the massive death of pigs occurred, the most probable source of infection suggested was through virus-contaminated pork products. OIE terrestrial manual for diagnostics of ASF recommends that investigation of new outbreaks should include the detection of specific antibodies in serum or extracts of tissues (OIE, 2019). These results confirmed the initial suspicion of ASF being the etiology agent responsible for the outbreak.

Even though findings confirmed ASF as the leading etiology of the outbreak, concerns raised about pigs been co-infected with other zoonotic disease were legitimate given that pigs are known to be susceptible to zoonotic diseases such as Ebola virus disease (*Reston ebolavirus*) and Swine flu (CDC, 2019; Nelson et al., 2019). Accordingly, a total of 204 RNA derived from oral swabs, nasal swabs, and serum/plasma were screened by qRT-PCR for the detection of Human Influenza A H1 and H3 gene. Results reveal that no amplification was produced in the no-template control reaction wells while positive control used as standard at various concentration upon amplification, yielded a typical sigmoidal curve; thus, validating the assay. However, all samples tested were negative, with the amplification curve failing to display sigmoidal curves as positive control. Presently, the laboratory diagnosis of influenza virus infection in pigs typically relied upon the detection of the virus in nasal swabs. Serology to detect antibodies is of low value for swine Influenza virus surveillance because vaccination against the disease rely on inactivated H1N1 and H3N2 vaccines, and current serologic tests do not differentiate between vaccinated and infected animals (Detmer et al., 2013). Therefore, virological assays are currently preferred over serology for surveillance. In the current study, Human influenza A virus H1 and H3 weren't detected in the various samples analyzed; thus, supporting that pigs weren't co-infected with the Swine flu virus. Nevertheless, the non-detection of these viruses was crucial as appropriate information was tailored and provided to decrease public fear of the zoonotic

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potential of the outbreak in Freetown. The intensive community sensitization by the OH teams was satisfactory as farmers were engaged about issues of implementing biosecurity measures and had to bury and burn dead pigs in some localities. Some farmers that still had few pigs surviving was advised on the benefit of isolating sick pigs even though most farmers reported to have lost all their pigs.

Sierra Leone has never reported an outbreak of African swine fever. During the epidemiological investigations, some farmers revealed that they experienced an outbreak similar to this one about three years ago. It is possible that outbreaks have been occurring without being reported to Livestock and Veterinary Services Division of MAF, or no response was provided by the weak Veterinary service of Sierra Leone.

The major risk factors that could have exacerbated the spread of ASF during this outbreak were selling sick and dead pigs by the affected farmers, disposal of dead pigs in the streams/tributaries where other pigs were scavenging, and inadequate biosecurity measures. These could have been among the reasons why the disease was more severe in localities in Western Urban compared to Western Rural district.

There is no specific policy for controlling ASF in Sierra Leone. The control of ASF is governed under the Animal Diseases Ordinance (1948). The challenge is that most of the legislation is outdated and therefore lacks relevant provisions in controlling the disease. The old law is currently being revised, and hopefully, the new law will address ASF control. The country does not have a compensation policy to support farmers that have lost pigs to ASF. Also, there is no surveillance study related to ASF, and other pig diseases currently being carried out in the country.

IV. Conclusion

Our findings confirmed ASF as the etiology of the reported outbreak of ASF in the republic of Sierra Leone. We demonstrated that rapid response and community engagement following the One Health approach is an effective means to alleviate fear and panic during an outbreak.

Recommendations

1. African Swine Fever is a notifiable disease in Sierra Leone, and the Government should urgently notify OIE and RECs about the outbreak
2. A program to train farmers on implementation of biosecurity practices should immediately be undertaken by Government
3. Adequate community engagement in future should be undertaken before investigations to improve compliance by the farmers and communities
4. There should be a rapid response to outbreaks by Government to mitigate the impact from such outbreaks
5. A strategy for ASF control should be developed and implemented
6. The One Health Multisectoral approach adopted during this investigation involving; MAF, MOHS, UNIMAK, Njala University, and other partners should be continued and cascaded to other outbreak investigations.
7. Security personnel should be included especially while investigating an outbreak associated with high mortality rates
8. Training of investigation teams in basic skills of Biosafety and biosecurity, use of PPEs, sample labeling, collection, transportation, processing, and storage should be provided to the teams in future before investigation
9. The government should provide feedback to the farmers after testing the samples to improve compliance in future
10. Assembly of emergency sampling equipment for a timely and quick response
11. Emergency funds for rapid response to an outbreak

Ethics Statement

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. No ethical approval was required as this was the Sierra Leone government investigation into the outbreak. However, animal handling and sample collection respected the rules formulated under the Animal Welfare Act by the United States Department of Agriculture (USDA) and by adopting ARRIVE guidelines (Kilkenny et al., 2011).

Acknowledgments

We thank the Western Urban and Rural districts community stakeholders for their support and for allowing us to perform sampling; officers of the Ministry of Health and Sanitation, Ministry of Agriculture and Forestry; the Food and Agriculture Organization of the United Nations including Dr. Mohamed Vandi, Dr. Amadu Tejan Jalloh, Sorie Kamara, Bayoh Musa, Tommy Josephine; Minkailu, field teams including Mary, Satta, Zainabu, Leigh, Kadijja Kamara, Bintah Bah, Bangura, Ishmail, Lamin Bangura, Mohamed Jalloh, Hajja Bah, Saidu AB Turay, Christian Scoff, Fafia Bona, and Alhassan Jalloh for participating in community engagement and supporting sample collection.

Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Funding Statement

This work was supported by the Food and Agriculture Organization of the United Nations, Sierra Leone.

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Table 1: Livestock population data in Sierra Leone

District name	Province	Poultry	Cattle	Donkeys	Goats	Pigs	Sheep
Kailahun	Eastern	363,690	3,289	48386	75,090	8,645	36,173
Kenema	Eastern	284,730	2,308	33193	27,272	2,493	19,799
Kono	Eastern	233,799	40,051	26793	69,082	4,765	45,637
Bombali	Northern	355,298	47,592	17025	78,727	3,190	56,684
Kambia	Northern	402,805	16,375	34167	63,983	1,755	50,719
Koinadugu	Northern	374,373	309,291	22298	212,634	2,892	191,788
Port Loko	Northern	602,327	20,105	57607	92,740	4,801	68,581
Tonkolili	Northern	384,659	9,852	56268	68,086	4,380	41,473
Bo	Southern	307,955	4,931	23511	30,643	6,166	13,303
Bonthe	Southern	191,121	1,584	13719	20,369	1,588	9,967
Moyamba	Southern	346,812	2,464	21434	42,286	4,918	15,206
Pujehun	Southern	220,030	720	18563	15,598	1,338	10,206
Western Area/Rural	Western Area	203,106	5,241	16950	13,876	6,603	10,763
Western Area/Urban	Western Area	65,644	2,014	6189	3,883	4,343	4,407
Total		4,336,349	465,817	396,103	814,269	57,877	574,706

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Table 2: Morbidity and Mortality in affected communities

District	Community	Number of Suspect Cases				Number of deaths				Total Herd Size	Mortality (%)
		Adults		Young		Adults		Young			
		Males	Females	Males	Females	Males	Females	Males	Females		
Western Urban	Kanikay	1	1	1	2	5	5	15	10	40	88%
Western Urban	Kroo Bay	0	0	0	0	4	3	0	0	7	99.4
Western Urban	Susans Bay	0	1	0	0	4	3	0	0	8	88%
Western Rural	Benguima	2	12	10	21	0	0	0	0	45	0%
Western Rural	Bomeh	86	93	97	80	86	89	87	65	685	48%
Western Rural	Bomeh	2	9	0	1	0	0	2	2	16	25%
Western Rural	Colvert	0	0	2	6	0	0	0	0	8	0%
Western Rural	Ibu Town	1	1	1	0	2	2	0	0	7	57%
Western Rural	Last Banking	6	9	10	7	3	3	3	2	36	31%
Western Rural	Mabureh	0	1	0	0	2	7	0	0	10	90%
Western Urban	Kingtom	40	58	30	28	60	75	31	35	415	48.4%
	Total	**138**	**185**	**151**	**145**	**166**	**187**	**138**	**114**	**1277**	**52.3%**

Table 3: Detection of ASF antibodies in samples using iELISA

Sampling Area	Code	Type of Sample	Positive	Negative	Total samples
Urban Racecourse	URC	Serum	2	21	23
Urban Kingtown	UKT	Serum	1	2	3
Urban Kingtown	UKT	Blood	1	2	3
Urban Kanikay	UKN	Serum	0	1	1
Rural Monkey Bush	RMB	Serum	0	14	14
Urban Kroobay	UKB	Serum	0	1	1
Rural Campbell Town	RCT	Serum	0	3	3
Rural Bomeh	RBe	Serum	0	6	6
Rural Ibo	RIB	Serum	0	1	1
Bengumah	BE	Serum	0	7	7
Bengumah	BE	Blood	0	13	13
Mabureh	MA	Serum	0	8	8
Mabureh	MA	Blood	0	13	13

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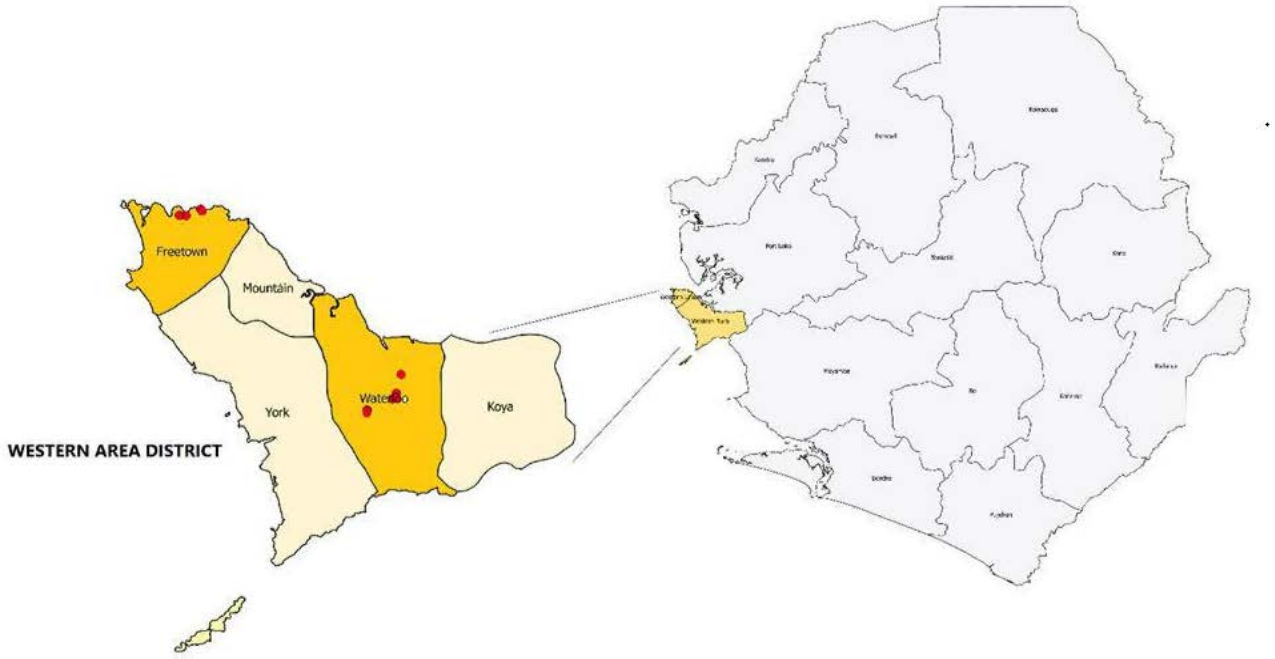


Figure 1: Localities where investigations were conducted in the Republic of Sierra Leone

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