




# Investigation of Ebolavirus exposure in pigs presented for slaughter in Uganda

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## Abstract

In 2008, an outbreak of *Reston ebolavirus* (RESTV) in pigs in the Philippines expanded our understanding of the host range of ebolaviruses. Subsequent experimental infections with the human-pathogenic species *Zaire ebolavirus* (EBOV) confirmed that pigs are susceptible to African species of ebolaviruses. Pig keeping has become an increasingly important livelihood strategy throughout parts of sub-Saharan Africa, driven by increasing demand for pork. The growth in pig keeping is particularly rapid in Uganda, which has the highest per capita pork consumption in East Africa and a history of sporadic human outbreaks of Ebola virus disease (EVD). Using a systematic sampling protocol, we collected sera from 658 pigs presented for slaughter in Uganda between December 2015 and October 2016. Forty-six pigs (7%) were seropositive based on ELISA tests at two different institutions. Seropositive pigs had antibodies that bound to Sudan NP ( $n = 27$ ), Zaire NP (Kikwit;  $n = 8$ ) or both NPs ( $n = 11$ ). Sera from 4 of the ELISA-positive pigs reacted in Western blot (EBOV NP = 1; RESTV NP = 2; both NPs = 2), and one sample had full neutralizing antibody against *Sudan ebolavirus* (SUDV) in virus neutralization tests. Pigs sampled in June 2016 were significantly more likely to be seropositive than pigs sampled in October 2016 ( $p = .03$ ). Seropositive pigs were sourced from all regions except Western region. These observed temporal and spatial variations are suggestive of multiple introductions of ebolaviruses into the pig population in Uganda. This is the first report of exposure of pigs in Uganda to ebolaviruses and the first to employ systematic abattoir sampling for ebolavirus surveillance during a non-outbreak period. Future studies will be necessary to further define the role pigs play (if any) in ebolavirus maintenance and transmission so that potential risks can be mitigated.

## KEYWORDS

antibodies, East Africa, Ebola, ebolaviruses, ELISA, pigs, Uganda

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## 1 | INTRODUCTION

Ebolaviruses cause sporadic outbreaks of Ebola virus disease (EVD) in humans in sub-Saharan Africa. Outbreaks involving a large number of human cases have mostly been caused by two species—*Zaire ebolavirus* (EBOV) and *Sudan ebolavirus* (SUDV)—with case fatality rates (CFRs) ranging from 40% to 100% (Feldmann & Geisbert, 2011; Feldmann, Jones, Klenk, & Schnittler, 2003; Muyembe & Kipasa, 1995; Shoemaker et al., 2012). Fewer human cases have been associated with *Bundibugyo ebolavirus* (BDBV) and *Tai Forest ebolavirus* (TAFV) (Formenty et al., 1999; Wamala et al., 2010). A new species (*Bombali ebolavirus*) was recently identified in bats in Sierra Leone (Goldstein et al., 2018), Kenya (Forbes et al., 2019) and Guinea (Karan et al., 2019), although its pathogenicity in humans is unknown.

Uganda has experienced numerous human outbreaks of EVD in recent history. Most outbreaks have been caused by SUDV, while one outbreak was caused by BDBV to date (Albarino et al., 2013; Bausch et al., 2007; Okware et al., 2002; Shoemaker et al., 2012; Towner et al., 2004; Wamala et al., 2010). Most recently (2019), cases of EBOV were reported following incursion from the Democratic Republic of Congo outbreak. In almost all outbreaks, the source of infection remains unknown (Kortepeter, Bausch, & Bray, 2011; MacNeil et al., 2010; Wamala et al., 2010), although in two outbreaks it was speculated, but not confirmed, to be contact with a monkey (Butagira, Bogere, & Mugisha, 2007; ProMED-mail, 2012).

For more than a decade, the hypothesis that fruit bats are the natural reservoirs of ebolaviruses has dominated (Leirs et al., 1999; Leroy et al., 2005; Olson et al., 2012; Pourrut et al., 2005; Swanepoel et al., 1996), largely based on serological surveys conducted in these animals (Bausch & Schwarz, 2014; Leroy et al., 2005; Ogawa et al., 2015; Olival & Hayman, 2014; Pourrut et al., 2009; Reed, 2012). Epidemiological evidence of links between fruit bats and human index cases is sparse (Atherstone, Smith, Ochungo, Roesel, & Grace, 2017; Leendertz, Gogarten, Düx, Calvignac-Spencer, & Leendertz, 2016; Leroy et al., 2009), suggesting ebolavirus maintenance might involve multiple host species (Atherstone et al., 2017; Feldmann & Geisbert, 2011; Groseth, Feldmann, & Strong, 2007; Leendertz et al., 2016).

In 2008, an outbreak of *Reston ebolavirus* (RESTV) in pigs in the Philippines (Barrette et al., 2009) expanded our understanding of the host range of ebolaviruses. Six individuals who worked on pig farms or with swine products developed RESTV IgG antibodies following the outbreak. This species of ebolavirus is not known to cause disease in humans; however, the outbreak resulted in the culling of more than 6,500 pigs in efforts to stop the spread of RESTV (Floro, 2009; Mogato, 2009). Subsequent experimental infections with the human-pathogenic species EBOV confirmed that pigs are susceptible to African species of ebolaviruses (Kobinger et al., 2011). EBOV replicated to high virus titres in pigs; in addition, naïve pigs in direct contact with experimentally infected pigs also became infected (Weingartl

et al., 2012). In a further study, experimentally infected pigs transmitted EBOV to non-human primates without direct contact, suggestive of airborne transmission (Weingartl et al., 2012). EBOV infection in these pigs caused respiratory signs consistent with severe lung pathology. Infected pigs shed EBOV in nasal, oral and rectal fluids, had detectable RNA in multiple tissues and developed an antibody response at 5 days post-infection (Kobinger et al., 2011). Most recently, serological evidence of natural EBOV exposure in pigs in Sierra Leone (Fischer et al., 2018) and Guinea (Fischer et al., 2019) has been reported following the West Africa outbreak in humans. To date, no studies have demonstrated the ability of (clinically or sub-clinically) ebolavirus-infected pigs to transmit the virus to humans.

Pig keeping has become an increasingly important livelihood strategy throughout parts of sub-Saharan Africa, driven by increasing demand for pork (FAO Statistics Division, 2017). The growth in pig keeping is particularly rapid in Uganda, which has the highest per capita pork consumption in East Africa (FAO Statistics Division, 2017; Ouma et al., 2013). A recent risk assessment found a number of factors that support potential zoonotic transmission from pigs in Uganda including habitat overlap between pigs and fruit bat hosts; interactions at the human-pig-wildlife interface that could support transmission; and temporal association of EVD outbreaks with peak pork consumption periods (Atherstone et al., 2017). The aims of the present study were to determine whether pigs in Uganda are exposed to ebolaviruses and to identify risk factors and spatial determinants for exposure.

## 2 | MATERIALS AND METHODS

### 2.1 | Study area and rationale

This study was conducted at a large urban abattoir. Pigs are sourced from throughout Uganda to meet the urban demand for pork in the city and its surroundings. The large volume of pigs and the wide geographic area these pigs are sourced from make it an ideal location for investigation of ebolavirus exposure in the domestic pig population in Uganda.

### 2.2 | Study design and sample size

Four sampling periods were chosen corresponding with national holidays, when pork consumption increases (Roesel et al., 2019) and more pigs are presented for processing to meet this demand (Atherstone et al., 2017; Roesel, Holmes, & Grace, 2016). These four sampling periods were Christmas/New Year (December 25, January 1), Easter (March 27), Martyr's Day/Hero's Day (June 3, June 9) and Independence Day (October 9). The sampling was conducted from December 2015 to October 2016, for 5–8 days during each of these holiday periods. A minimum sample size of 628 pigs was determined ( $n = 157/\text{period}$ ) to detect the presence of ebolaviruses with 95% confidence, assuming

not more than 2% of the domestic pig population are seropositive and a diagnostic sensitivity of 95% (Pickering et al., 2018).

### 2.3 | Selection of pigs and biodata collection

A systematic random sampling strategy was used in which every 3rd pig brought through the door of the slaughter building was selected for inclusion in the study and a tag was placed in the ear to identify the animal. Biodata were collected using a standard form (see Supplemental Materials 1) that captured the date of sampling, ear tag number, rectal temperature, pig breed (based on visual classification as local, cross or exotic), sex, whether the male pigs were intact or castrated, visible clinical signs of illness and source location of the pig (reported at the county or district level by the trader supplying the pig).

### 2.4 | Specimen collection and handling

Blood was collected in a vacutainer (clot-activated) at the time of slaughter. All samples were placed on ice in an icebox, stored for the duration of sampling for the day (2–3 hr) and transported to Makerere University, College of Veterinary Medicine, Animal Resources and Biosecurity, where they were refrigerated until processing for storage the following day. Serum was separated by centrifugation, aliquoted and stored in duplicate at  $-80^{\circ}\text{C}$  until shipment.

### 2.5 | Laboratory analysis

One set of serum samples was shipped to Friedrich Loeffler Institute (FLI), Germany, where they were tested in duplicate using the EBOV (Kikwit) ELISA described previously (Pickering et al., 2018). Sera with an optical density (OD) value  $\geq 0.290$  (three standard deviations above the mean) in both ELISA runs were considered positive. Positive serum was confirmed using Western blot against EBOV nucleoprotein (NP), SUDV NP and RESTV NP antigens as described previously (Fischer et al., 2018).

Serum samples with a positive determination using ELISA at FLI were transferred to the Canadian Food Inspection Agency (CFIA) for confirmatory analysis. At CFIA, serum was tested in duplicate using an in-house ELISA for both EBOV (Kikwit) (Pickering et al., 2018) and SUDV (Gulu) NP-specific antibodies. Serum with an optical density  $\geq 0.325$  cut-off value (three standard deviations above the mean) in the EBOV NP (Kikwit) ELISA or  $\geq 0.301$  (three standard deviations above the mean) in the SUDV NP ELISA was considered positive. To test whether ELISA-positive serum samples contained neutralizing antibodies, virus neutralization tests using both EBOV (Kikwit) and SUDV (Gulu) were performed as described elsewhere (Pickering et al., 2018). Serum was diluted at 1/10 and 1/20 for the virus neutralization tests.

In addition, due to concerns about antibody stability, the second set of serum samples was transferred from Uganda directly to CFIA

for analysis using the assays described above. Serum with a positive determination was analysed in duplicate in virus neutralization tests using both EBOV (Kikwit) and SUDV (Gulu) as described above.

### 2.6 | Data analysis

True prevalence was calculated in EpiTools using a test sensitivity of 95% and test specificity of 100%. Standard descriptive analyses were conducted for categorical and continuous variables. For purposes of analysis, serum was considered positive if the OD value exceeded the cut-off for the ELISA used at both institutions (FLI and CFIA). Serum with OD values greater than the cut-off in duplicate runs of the ELISA used at one institution, but not the other, was considered seroreactive. Univariable logistic regression was performed to determine which variables were predictive of ebolavirus seropositivity. Explanatory variables with  $p$ -value of  $<.25$  were considered for inclusion in a multivariable logistic regression model. The model was fitted using backward stepwise regression, and each model was tested for overall significance using the likelihood ratio test. Three-way and two-way interactions between explanatory variables were tested using the interaction term as part of the model building. Interactions that were statistically significant ( $p < .05$ ) were retained in the model.

For mapping, shapefiles for district boundaries were obtained from Uganda Bureau of Statistics (2018 edition, published March 2019). Due to changes in district boundaries since the time of sampling, the dissolve tool was used in ArcGIS 10.5.1 (Environmental Systems Research Institute, Redlands, CA, USA) to generate a map approximating district boundaries around the time of sampling (2016). Pig source locations (reported at the district level) were then joined to the centroid of each district polygon and the distribution of Ebola seropositive and seronegative pigs visualized using pie charts.

To investigate spatial clustering of seropositive pigs, Moran's spatial autocorrelation and local indicator of spatial autocorrelation (LISA) were calculated. Moran's spatial autocorrelation describes the relationship between a variable of interest associated with a spatial location (Ward & Carpenter, 2000); in this case, ebolavirus seroprevalence at locations aggregated to the district level. District locations ( $n = 6$ ) with less than 5 sampled pigs were removed from Moran's spatial autocorrelation analysis. While Moran's spatial autocorrelation measures overall (global) clustering of the spatial data, LISA assesses clustering in individual units (e.g. districts), identifying rates above or below those expected in a random distribution (Anselin, 1995). Only clusters with high rates of ebolavirus seropositive pigs were identified.

### 2.7 | Ethics approval and permits

Human and animal ethics approval for this research was obtained from the International Livestock Research Institute, Nairobi, Kenya (ILRI-IREC2015-01), the Ugandan National Council for Science &

Technology (A499) and Makerere University College of Veterinary Medicine, Animal Resources and Biosecurity, Kampala, Uganda (SBL.S.CA.2016). The Animal Ethics Committee at The University of Sydney, Australia, was also notified of external ethics approval (2015/891). Export permits were granted by the Ministry of Agriculture, Animal Industries and Fisheries of Uganda, and in accordance with access and benefits sharing as detailed in the Nagoya Protocol.

### 3 | RESULTS

#### 3.1 | Ebolavirus seroprevalence

Of the 658 sera tested at both FLI and CFIA, 46 (7.0%; 95% CI: 5.6, 9.7) were considered positive by ELISA at both institutions. True prevalence ranged from 4.2% (95% CI: 2.1, 8.8) in October 2016 to 11.1% (95% CI: 7.5, 17.8) in June 2016. Of the 46 seropositive pigs, twenty-seven (58.7%) were seropositive on the Sudan ELISA, 8 were seropositive on the Zaire ELISA (17.4%) and 11 were seropositive on both the Sudan and Zaire ELISAs (23.9%) (see Table 1). Of these seropositive samples, four reacted in Western blot (see Figures 1 and 2). One of the sera samples reacted with EBOV NP, one with RESTV NP and two with both EBOV and RESTV NP in Western blot. One sample (539) had full neutralizing antibody at 1/10 and 1/20 against SUDV, but not EBOV (Kikwit), in the virus neutralization test. An additional three sera were seroreactive at CFIA, and another 52 sera were seroreactive at FLI.

#### 3.2 | Risk factors for ebolavirus seroprevalence

Table 2 shows the characteristics of sampled pigs. Seropositive pigs were detected in both sexes and local, exotic and cross-breed pigs. Exposed pigs may or may not have had fever or signs of illness at

time of slaughter. Seropositive pigs were sourced from all regions except Western region (see Figure 3) and were detected in all sampling periods. In univariable analysis, pigs sampled in June 2016 were nearly 3 times more likely to be seropositive than pigs sampled in October 2016 (OR: 2.88; 95% CI: 1.17–7.08,  $p = .02$ ; see Table 2). During model building, a significant association between pig sex and fever was found (likelihood ratio: 7.68;  $p < .01$ ). As pig sex explained more of the variance in seroprevalence than fever, pig sex was retained in the final model. The final model included pig sex, breed and sampling period. After adjusting for other covariates, pigs sampled in June 2016 were significantly more likely to be seropositive than pigs sampled in October 2016 (OR: 2.78; 95% CI: 1.11–6.93;  $p = .03$ ; see Table 2).

#### 3.3 | Spatial clustering of ebolavirus seropositive pigs

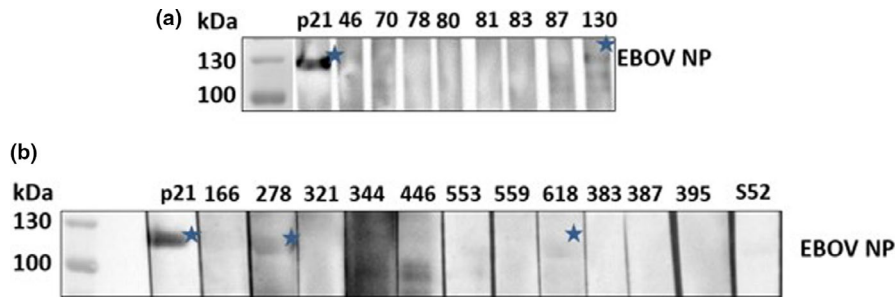
Spatial analysis (LISA) indicated a significantly ( $Z$ -score:  $-0.84$ ,  $p$ -value:  $.02$ ) high seroprevalence in Lira district, which shares one boundary with a low seroprevalence (0%) district.

### 4 | DISCUSSION

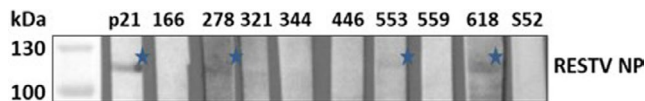
While pigs are the only livestock species known to be susceptible to infection with EBOV (Barrette et al., 2009; Fischer et al., 2018), their role (if any) in the ecology of ebolaviruses in sub-Saharan Africa remains unknown. This study is the first to report evidence of ebolavirus exposure (i.e. presence of antibodies against ebolavirus in blood) in the pig population of Uganda, a country where there is a growing pig industry and history of sporadic human outbreaks of EVD. Using systematic abattoir sampling of 658 pigs at slaughter, we detected an overall seroprevalence of 7.0% (95% CI: 5.6, 9.7), based on ELISA tests at two different institutions.

**TABLE 1** Sampling period, source region and ebolavirus ELISA results of 658 pigs sampled at an urban abattoir in Uganda, 2015–2016

	Number (n)	Number of seropositive animals (%)	Positive ELISA (n = 46)		
			Sudan NP ELISA (n = 27)	Zaire NP ELISA (n = 8)	Both ELISAs (n = 11)
<i>Region</i>					
Central	356	24 (6.7)	13	5	6
Eastern	112	5 (4.5)	3	1	1
Northern	14	2 (14.3)	1	0	1
Western	24	0	0	0	0
Not recorded	152	15 (9.9)	10	2	3
<i>Sampling period</i>					
December 2015	168	13 (7.7)	8	3	2
March 2016	160	8 (5.0)	3	3	2
June 2016	162	18 (11.1)	14	1	3
October 2016	168	7 (4.2)	2	1	4



**FIGURE 1** Western blot analysis of serum reactivity against EBOV NP (3  $\mu$ g EBOV NP per lane). P21 serves as a positive control (sera from EBOV-infected pig) and S52 as a negative control (German pig). All serum samples were diluted 1:20. A star denotes seroreactive serum from pigs sampled at the abattoir. Weak positive reaction observed for sample 618 [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]



**FIGURE 2** Western blot analysis of serum reactivity against RESTV NP (10  $\mu$ l cell lysate loaded per lane). P21 serves as a positive control (sera from EBOV-infected pig) and S52 as a negative control (German pig). All serum samples were diluted 1:20. A star denotes seroreactive serum from pigs sampled at the abattoir. Weak positive reaction observed for samples 278 and 553 [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

Seropositive pigs had antibodies that bound to Sudan NP ( $n = 27$ ), Zaire NP (Kikwit;  $n = 8$ ) or both NPs ( $n = 11$ ) and virus neutralization tests (SUDV Gulu;  $n = 1$ ). Temporal and spatial variation in seropositivity was observed, suggestive of multiple introductions into the pig population.

Natural exposure of pigs under field conditions has only been reported twice previously in sub-Saharan Africa, specifically in areas of Sierra Leone (Fischer et al., 2018) and Guinea (Fischer et al., 2019) that were impacted by the West African outbreak in humans (EBOV; 2013–2016). In Sierra Leone, 3/400 (0.75%) pig sera samples collected from 3 different districts were found to be positive, none of which neutralized the virus. Two of the ELISA-positive sera reacted in Western blot—one to RESTV and the other to SUDV, RESTV and EBOV. In Guinea, 19/308 (6.2%) sera samples collected from farms around Conakry were found to be positive, five of which showed weak neutralization against EBOV. Fourteen of the ELISA-positive sera reacted in Western blot—eight to EBOV, three to both EBOV and SUDV and one to SUDV. These studies confirm ebolavirus-reactive antibodies in pigs in Sierra Leone and Guinea in proximity to areas with recorded EVD cases in humans. In contrast, pigs in our study were not sampled during a recognized human outbreak in Uganda.

In our study, antibodies were detected in pigs regardless of sex, breed, location (except Western region) and season, suggesting multiple introductions of ebolaviruses into the domestic pig population in Uganda. Bats are distributed throughout Uganda, and so, it is expected that spillover of ebolaviruses from bat to pigs could result in exposure of pigs at multiple locations. Multiple independent spillover events were also reported to occur between 2001 and 2003

in Gabon and the Republic of Congo where eight different EBOV strains were found circulating during outbreaks in humans and apes (Groseth et al., 2007; Leroy et al., 2004).

The higher frequency of SUDV exposure in pigs is consistent with historical outbreaks of SUDV in Uganda. Detection of antibodies to EBOV is curious and may suggest cross-reactivity as observed in other serological studies in pigs (Fischer et al., 2018, 2019), bats (De Nys et al., 2018; Hayman, 2012) and humans (Macneil, Reed, & Rollin, 2011). An additional possibility is that EBOV is circulating in Uganda. Locally acquired human outbreaks have not yet been detected in Uganda, although three patients infected with EBOV in neighbouring Democratic Republic of Congo crossed the border resulting in the first notifications of EBOV in Uganda in 2019 (World Health Organization Regional Office for Africa, 2019). The Democratic Republic of Congo has reported numerous EVD outbreaks in humans caused by EBOV (Centers for Disease Control & Prevention, 2018). In the present study, EBOV exposure was found in pigs sourced from the Central, Eastern and Northern regions and during all four sampling periods, suggesting circulation in multiple regions during the sampling period. While spatial analysis identified Lira as a district with a higher than expected seroprevalence, this district only had one sampled neighbouring district. Follow-up sampling in this district is warranted to determine the risk of exposure, and in neighbouring districts to confirm this apparent cluster. Additionally, future studies should prioritize identifying and describing risk factors for exposure in pigs throughout Uganda.

The serum antibody reactivity against RESTV in the Western blot is consistent with findings in pigs from Sierra Leone (Fischer et al., 2018). RESTV is thought to be of Asian origin, but antibodies to RESTV have recently been found in African straw-coloured fruit bats (*Eidolon helvum*) in Zambia (Ogawa et al., 2015) suggesting RESTV or Reston-like viruses exist in Africa. The presence of neutralizing antibodies strongly supports exposure to SUDV but does not exclude the possibility of exposure to another closely related virus.

Pigs sampled in June 2016 were nearly 3 times more likely to be seropositive than those sampled in October 2016. June had the highest number of SUDV seropositive pigs of all sampling time periods which may indicate a spillover event involving this virus preceding this particular time period. June is the start of the dry season in Uganda (World Weather & Climate Information, 2016). Several



**TABLE 2** Risk factors for ebolavirus seropositivity in 658 pigs sampled at an urban abattoir in Uganda, 2015–2016

Explanatory variables	Number (n)	Number of seropositive animals (%)	Outcome variable: ebolavirus seropositive on ELISAs at both FLI and CFIA			
			Unadjusted odds ratio (95% CI)	p-value	Adjusted odds ratio (95% CI)	p-value
<i>Sex</i>						
Female	384	31 (8.1)	1.48 (0.78, 2.80)	.23	1.54 (0.80, 2.99)	.20
Male	268	15 (5.6)	1.00		1.00	
Not recorded	6	0	NA			
<i>Male status</i>						
Intact	92	4 (5.4)	0.95 (0.31, 2.86)	.93		
Castrated	175	10 (5.7)	1.00			
Not recorded	1	0	NA			
<i>Breed</i>						
				.12 <sup>a</sup>		
Cross	251	23 (9.2)	1.31 (0.63, 2.71)	.47	1.31 (0.63, 2.74)	.47
Exotic	230	10 (4.3)	0.59 (0.25, 1.40)	.23	0.54 (0.22, 1.30)	.17
Local	168	12 (7.1)	1.00		1.00	
Not recorded	9	0	NA			
<i>Fever at time of sampling</i>						
Febrile (>39.8°C)	46	6 (13.0)	2.12 (0.85, 5.29)	.11	*	
Afebrile (<39.8°C)	604	40 (6.6)	1.00			
Not recorded	8	0	NA			
<i>Clinical signs at time of sampling</i>						
Yes	16	2 (12.5)	1.93 (0.42, 8.74)	.40		
No	637	44 (6.9)	1.00			
Not recorded	5	0	NA			
<i>Sampling period 0.06<sup>a</sup></i>						
December 2015	168	13 (7.7)	1.93 (0.75–4.96)	.17	2.36 (0.90–6.18)	.08
March 2016	160	8 (5.0)	1.21 (0.43–3.42)	.72	1.44 (0.50–4.12)	.50
June 2016	162	18 (11.1)	2.88 (1.17–7.08)	.02	2.78 (1.11–6.93)	.03
October 2016	168	7 (4.2)	1.00		1.00	

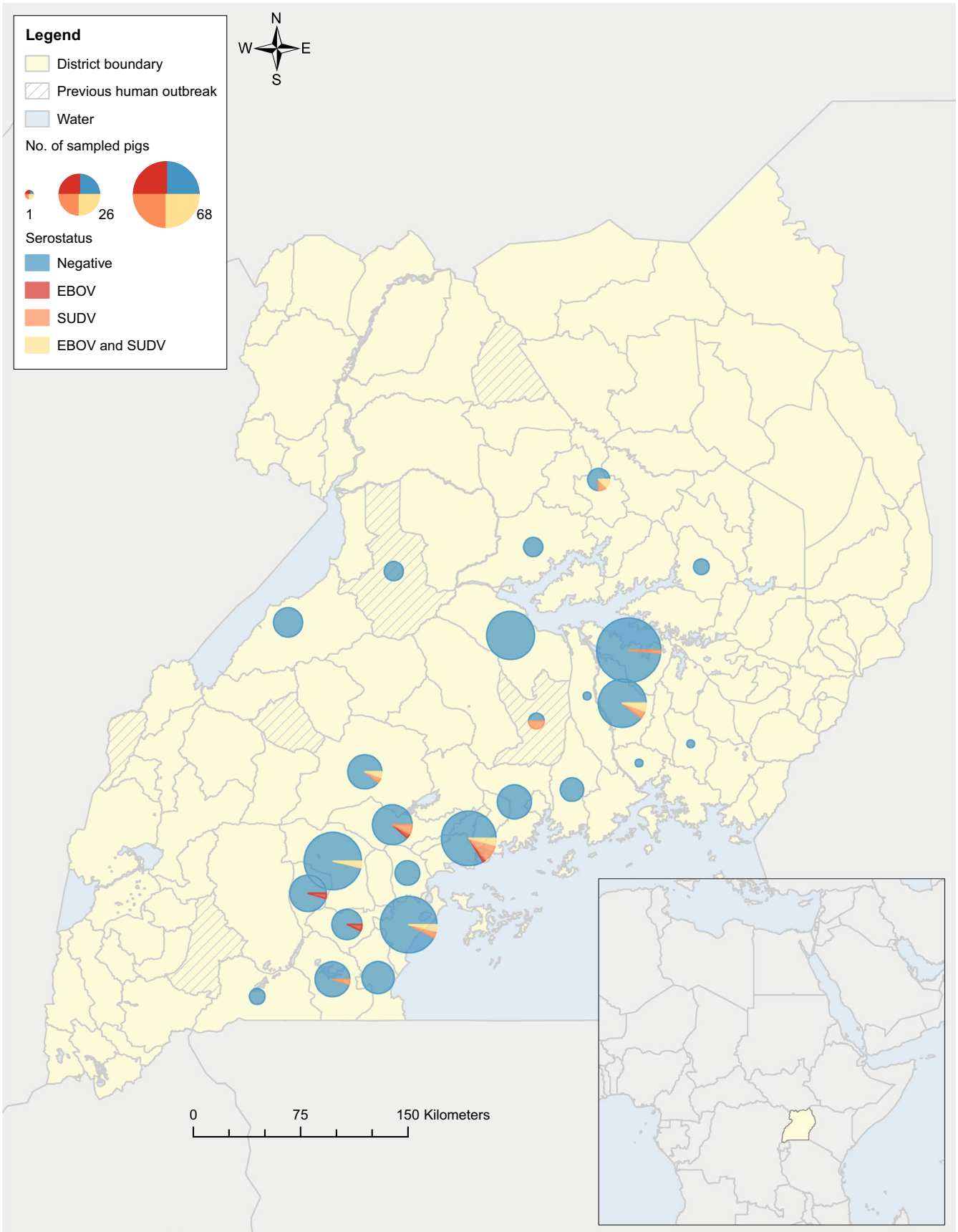
Note: Explanatory variables with  $p < .25$  in the univariable analysis were included in the final multivariable logistic regression model.

<sup>a</sup>Overall  $p$ -value for non-binary variables.

\*This variable was highly correlated with pig sex ( $p < .01$ ). As pig sex explained more of the variance in serostatus than fever (log-likelihood ratio: 313.94, chi-square: 2.73,  $df = 8$ , H&L: 0.95,  $p = .05$  versus log-likelihood ratio: 312.29, chi-square: 7.77,  $df = 8$ , H&L: 0.46,  $p = .01$ , respectively), the latter variable was excluded from the final multivariable model.

studies suggest that EBOV spillover to humans is more likely to occur at the onset of the dry season (Pinzon, Wilson, & Tucker, 2005; Pinzon et al., 2004; Tucker, 2002). In addition, EBOV outbreaks in great apes in Gabon and the Republic of Congo were reported at the start of the dry season (Leroy et al., 2004). Furthermore, in predictive modelling, EBOV spillover intensity was found to be highest at the transitions between wet and dry seasons (Schmidt et al., 2017). These seasonal dynamics in spillover and outbreak intensity are likely driven by changes in resource availability, wildlife migration, reproductive cycles and frequency of host encounters (Schmidt et al., 2017). Other unrecognized emergence factors might also explain why pigs in June 2016 were more likely to be seropositive than pigs sampled at other times.

This is the first study to report findings from systematic abattoir sampling for ebolavirus surveillance in pigs and domestic animals more generally. Nonetheless, there are some important limitations. While the development and application of a standardized protocol for ebolavirus surveillance (see Supplemental Materials 2) in abattoirs in low-income settings is a strength of this study, the requirement for BSL4 laboratory facilities and control sera generated from infection studies necessitated that testing be performed in more equipped laboratories than those currently available in Uganda. Related to this, a decline in antibody stability following transport and laboratory manipulation was noted at both institutions and resulted in some strong positive reactions appearing lowered in subsequent testing. Furthermore, the unreliability



**FIGURE 3** Source location (district-level) and Ebola serostatus of pigs sampled at an urban abattoir in Uganda, 2015–2016. Symbols map to the centroid of the district in which pigs originated, with size/color corresponding to number/serostatus of pigs, respectively. District boundaries approximate administrative boundaries around the time of sampling (2016). Striped districts have experienced previous human Ebola cases. [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

of ageing pigs using dentation alongside the lack of formal traceability mechanisms meant age and source location could not be reliably assessed for all animals. This made it difficult to identify the timing and location of any specific spillover events. However, the district source locations used for mapping in this study are likely to be reasonably reliable as the traders use community-based scouts to identify pigs for sale (Atherstone et al., 2018). Nonetheless, the spatial analysis undertaken was limited by the number of pigs that had no reported source location and the large gaps in the spatial data, particularly in the Northern region.

In conclusion, we provide serological evidence of ebolavirus exposure in pigs from Uganda, in multiple locations and with a temporal association. Overall, this study broadens the current understanding of ebolavirus ecology in Uganda. Future studies will be necessary to further define the role pigs play in ebolavirus maintenance and transmission so that potential risks can be mitigated.

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## CONFLICT OF INTEREST

All authors declare that they have no competing interests.

## DATA AVAILABILITY STATEMENT

Data available on request due to privacy/ethical restrictions.

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#### SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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