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Serological evidence of exposure to ebolaviruses in domestic pigs from Guinea

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Abstract

The genus *Ebolavirus* comprises several virus species with zoonotic potential and varying pathogenicity for humans. Ebolaviruses are considered to circulate in wildlife with occasional spillover events into the human population which then often leads to severe disease outbreaks. Several studies indicate a significant role of bats as reservoir hosts in the ebolavirus ecology. However, pigs from the Philippines have been found to be naturally infected with Reston virus (RESTV), an ebolavirus that is thought to only cause asymptomatic infections in humans. The recent report of ebolavirus-specific antibodies in pigs from Sierra Leone further supports natural infection of pigs with ebolaviruses. However, susceptibility of pigs to highly pathogenic Ebola virus (EBOV) was only shown under experimental settings and evidence for natural infection of pigs with EBOV is currently lacking. Between October and December 2017, we collected 308 serum samples from pigs in Guinea, West Africa, and tested for the presence of ebolavirus-specific antibodies with different serological assays. Besides reactivity to EBOV nucleoproteins in ELISA and Western blot for 19 (6.2%) and 13 (4.2%) samples, respectively, four sera recognized Sudan virus (SUDV) NP in Western blot. Furthermore, four samples specifically detected EBOV or SUDV glycoprotein (GP) in an indirect immunofluorescence assay under native conditions. Virus neutralization assay based on EBOV (Mayinga isolate) revealed five weakly neutralizing sera. The finding of (cross-) reactive and weakly neutralizing antibodies suggests the exposure of pigs from Guinea to ebolaviruses or ebola-like viruses with their pathogenicity as well as their zoonotic potential remaining unknown. Future studies should investigate whether pigs can act as an amplifying host for ebolaviruses and whether there is a risk for spillover events.

KEYWORDS

antibodies, Ebola, ebolaviruses, ELISA, neutralization test, pigs, serology, West Africa

1 | INTRODUCTION

Ebolaviruses (family *Filoviridae*) have been reported to circulate in Africa and Asia with varying pathogenicity for humans depending on the virus species. Besides non-specific symptoms such as fever, headache, vomiting and nausea, infections with ebolaviruses have led to severe disease outbreaks of haemorrhagic fever in humans and non-human primates with fatality rates between 25% and 90%

(Feldmann & Geisbert, 2011). The zoonotic origin of ebolaviruses has been widely discussed with reported infections of incidental hosts such as chimpanzees, gorillas and other primates that caused sporadic spillover events into the human population (Georges et al., 1999; Leroy et al., 2004; Pourrut et al., 2005). However, our understanding of the virus ecology including virus transmission and maintenance in nature is still limited. Although virus isolates from bats are still lacking, molecular and serological evidence points towards these animals as the most likely natural virus reservoir (De Nys et al., 2018; Goldstein et al., 2018; Leroy et al., 2005; Ogawa et al., 2015; Pourrut et al., 2009). The recent discovery of a full-length ebolavirus genome, designated Bombali virus (BOMV), in insectivorous bats from Sierra Leone (Goldstein et al., 2018) and the finding of partial BOMV nucleotide sequences in bats from Kenya (Forbes et al., 2019) and Guinea (Karan et al., 2019) underline the complexity of ebolavirus ecology and the potential role of bats in it. Moreover, the finding of BOMV adds further support to the assumed circulation of as yet undiscovered ebolaviruses with unknown zoonotic and/or pathogenic potential. Asymptomatic infections with less or non-pathogenic ebolaviruses have been discussed to account for the presence of ebolavirus-specific antibodies in humans from areas with no record of previous Ebola virus disease (EVD) outbreaks (Formella & Gatherer, 2016; Kuhn & Bavari, 2017; Mulangu et al., 2018).

The potential role of domestic animals and livestock for human transmission has further emerged with the detection of RESTV in sick pigs from the Philippines in 2008 (Barrette et al., 2009). However, the observed pathogenicity and histopathological findings might have been biased due to the co-infection of pigs with porcine reproductive and respiratory syndrome virus (PRRSV) (Barrette et al., 2009). Experimental infection of 5-week-old piglets with RESTV alone confirms virus replication and shedding in the absence of clinical signs (Marsh et al., 2011). While infection of macaques with RESTV is characterized by generalized rashes and/or sudden death, the virus appears to cause asymptomatic infections in humans (Burk et al., 2016; Demetria et al., 2018). The finding of RESTV-specific antibodies in healthy pig farm workers from the Philippines indicated several transmission events between pigs and humans (Barrette et al., 2009), highlighting the need to investigate the potential role of pigs in the ebolavirus transmission cycle. Subsequent experimental infection of pigs with Ebola virus (EBOV) confirmed susceptibility of pigs to this highly pathogenic virus which resulted in clinical symptoms, oronasal shedding as well as transmission to co-housed piglets and non-human primates (Kobinger et al., 2011; Weingartl et al., 2012). In addition, a more recent study identified several factors such as domestic pig habitat overlap with assumed wildlife reservoir hosts as well as reported human–pig–wildlife interactions that might contribute to potential zoonotic transmission of ebolaviruses from pigs under field conditions (Atherstone, Smith, Ochungo, Roesel, & Grace, 2017). Overall, serological field studies investigating pigs for the presence of ebolavirus-specific antibodies are sparse. In the Philippines, the prevalence of RESTV antibodies in pigs from RESTV-affected farms has been reported to be 70% while pigs sampled in a non-epizootic region displayed no detectable serum reactivity (Sayama et al., 2012). Interestingly, another study performed in Shanghai, China, revealed molecular evidence for RESTV infection in 4 out of 137 tested pigs that were co-infected by PRRSV (Pan et al., 2014). In Africa, we recently detected a seroprevalence for ebolavirus nucleoprotein-specific antibodies in 400 pigs from Sierra Leone of below 1% indicating that rare exposure to ebolaviruses or ebola-like viruses has occurred in the sampling area (Fischer et al., 2018). To determine whether pigs from Guinea have also been exposed to ebolaviruses, we collected 308 serum samples from pigs in local pig farms around Conakry between October and December 2017 and tested for the presence of ebolavirus-specific IgG using various serological assays.

2 | MATERIALS AND METHODS

2.1 | Study area and sampling

A total of 308 porcine serum samples were collected from local pig farms around Conakry, Guinea, between October and December 2017. Available information on sex, age and weight was recorded. We summarized information about housing conditions within the pigsties and the surrounding habitats in Table 1. With the exception of animals under the age of 3 months as well as pregnant or lactating females, all other pigs were randomly selected for blood collection. For serum collection, animals were handled according to a protocol submitted to and reviewed by the Comité National d'Ethique pour la Recherche en Santé (CNERS) of Guinea under the number 040/CNERS/17. We collected a total blood volume of 3–5 ml per pig from the right jugular vein into a clot activator vacutainer. All samples were placed in a cooler and then transported back to the laboratory at the Institut Pasteur, Conakry. Serum was separated by centrifugation at 2,000× *g* for 20 min and stored at −80°C until further processing.

Before further analysis, sera were heat inactivated for 30 min at 56°C.

2.2 | Control serum samples and antigens

Swine sera from experimentally EBOV-infected animals (pigs P21, P23; (Kobinger et al., 2011)) as well as from an experimentally Sudan virus (SUDV)-infected pig (P28; unpublished data; serum kindly provided by Dr. Hana Weingartl, Canadian Food Inspection Agency, Winnipeg, Canada) were collected on day 21 and/or 28 post-infection and served as positive controls. In addition, serum samples from Ebola virus-like particles (eVLPs) immunized pigs were utilized as previously described (Fischer et al., 2018). The origin of different antigens used in this study is summarized in Table 2.

2.3 | Indirect IgG ELISA based on E. coli-derived EBOV NP

The EBOV nucleoprotein (NP) was expressed in *E. coli* and purified via affinity chromatography as specified elsewhere (Fischer et al.,

2018; Groseth et al., 2009). Indirect ELISA was performed as previously described (Fischer et al., 2018). Pig serum samples were diluted for analysis in ELISA 1:100. The absorbance at OD_{405nm} was monitored in a Tecan Sunrise absorbance microplate reader until a pre-determined OD range of 0.7–0.9 for the positive control sera was reached. To account for unspecific binding of the field serum samples tested, *E. coli* extract served as a mock antigen in control wells. The cut-off value was established by calculating the mean value of corrected ODs from 150 German porcine sera plus three standard deviations, resulting in a cut-off OD_{405} value of 0.17.

2.4 | Analysis of ELISA-reactive porcine serum samples by Western blot

For further analysis of all ELISA-reactive pig serum samples, Western blots were performed as described previously (Fischer et al., 2018) using insect cell-derived recombinant EBOV NP and SUDV NP (pABbee-FH-SUDV NP; GenScript) as antigens. Serum samples were used in a 1:20 dilution for Western blot analysis.

2.5 | Native immunofluorescence assay based on ebolavirus glycoproteins GP

To test for serum reactivity against a second ebolavirus protein, immunofluorescence assay (IFA) under native conditions based on EBOV or SUDV GP transfected Vero cells was performed with samples that detected EBOV NP in ELISA. Briefly, Vero cells were transfected with either pCAGGS EBOV GP (Mayinga isolate; (Hoenen et al., 2006)) or pCAGGS SUDV GP (Gulu variant) expression plasmids (kindly provided by Dr. Allison Groseth, Friedrich-Loeffler-Institut, Greifswald, Germany). Mock-transfected cells served as negative control for each serum. Transfection was performed using FuGene (Promega) according to manufacturer's guidelines. Prior to actual staining procedure, serum samples were pre-incubated on non-transfected cells to reduce background staining caused by unspecific binding of antibodies to cellular components. For this, cells were washed once with cold PBS and then blocked (0.25% skim milk in PBS) for 30 min at 4°C. Serum samples were added in a 1:20 dilution and incubated for 30 min at 4°C. Supernatant was then re-collected and centrifuged (3 min, 1,000× *g*, 4°C) for further use in IFA.

For IFA, Vero cells expressing EBOV or SUDV GP and respective non-transfected cells were washed once with cold PBS and blocked (0.25% skim milk in PBS for 30 min at 4°C). Centrifuged supernatants

TABLE 2 Overview of antigens and viruses used in this study

Assay	Antigen/Virus	Origin of antigen/virus variant
ELISA	EBOV NP	*E. coli*
Western blot	EBOV NP SUDV NP	Insect cells (Sf9) Insect cells (Sf9)
Immunofluorescence assay	EBOV GP SUDV GP	Vero cells Vero cells
Virus neutralization test	EBOV	Mayinga isolate

TABLE 1 Summary of housing conditions in the pigsties and surrounding habitat

	Sampling site	Name of sampling site	Holding	Sample number[a]	No. of samples	Thereof reactive in ELISA	Habitat	Housing
	1	Conakry Dabompa	A	GUI 2 - GUI 31	30	0	Close to mangrove	Open air pigsty
	2	Conakry Simbaya Gare	B	GUI 32 - GUI 40	9	0	Urban market	Open air pigsty
	2	Conakry Simbaya Gare	C	GUI 41 - GUI 48	7	1	Urban market	Open air pigsty
	2	Conakry Simbaya Gare	D	GUI 49 - GUI 51	3	0	Urban market	Open air pigsty
	2	Conakry Simbaya Gare	E	GUI 52 - GUI 55	4	0	Urban market	Open air pigsty
	3	Coyah Doumbouya	F	GUI 56 - GUI 159	104	10	rural area, mango trees, frugivorous bats	Open air pigsty
	3	Coyah Doumbouya	G	GUI 160 - GUI 224	65	5	rural area, mango trees, frugivorous bats	Open air pigsty
	4	Dubreka Keninde	H	GUI 225 - GUI 311	86	3	rural area	Open air pigsty
Total number	4		8		308	19		

[a]Samples GUI 1, GUI 46 and GUI 260 were not analysed in any serological assay.

from a pre-absorption plate containing porcine serum in a dilution of 1:20 were incubated on transfected Vero cells for 1 hr at 4°C followed by three washes with cold PBS. Secondary antibodies (goat anti-swine—Alexa Fluor 594; Dianova; 1:500 in PBS) was incubated for 1 hr at 4°C. Nuclei were counterstained with Hoechst 33342. Fluorescence was visualized using an Eclipse Ti-S inverted microscope system. Images were processed with the NIS-Elements BR 4.00.07 software (Nikon).

2.6 | Virus neutralization assay

Apart from five serum samples of insufficient volume, all ELISA-reactive pig serum samples were tested for EBOV-neutralizing activity. All EBOV infection experiments were performed in the biosafety level 4 (BSL4) laboratory at the Institute of Virology, Philipps University of Marburg, Germany. Virus neutralization assays using EBOV (Mayinga isolate) were performed as reported previously (Agnandji et al., 2016).

3 | RESULTS

A total of 308 pigs (152 female, 155 male, one unspecified) were sampled at four sampling sites around Conakry including eight different local pig farms (Table 1). All pigs were kept in stationary pigsties covered by laterally open roofs that allowed for fresh air intake and potential entry and roosting of bats. However, no bats were present within the pigsties during sample collection. The animals had no history of previous migration, and selected areas were known for their high record of Ebola virus disease (EVD) patients during the recent epidemic from 2014 to 2016 in West Africa. At the time of sampling between October and December 2017, no pig had visible clinical symptoms. The calculated average age (mean) of the pigs was 10 months, while the median was 8 months. Only 11 pigs were reported with an age of 24 months or older meaning that they were alive during the late phase of the EVD outbreak. However, none of these 11 sera recognized recombinant *E. coli*-derived EBOV NP in our in-house ELISA. Instead, we detected 19 porcine serum samples from 6- to 18-month-old pigs to exceed the determined threshold of OD_{405} 0.17 (Figure 1). The majority of these reactive samples (15 out of 19) was collected at sampling site 3 (see Table 1) which is characterized by a rural setting with surrounding mango trees and the circulation of frugivorous bats.

In order to confirm ELISA reactivity, we established several consecutive analyses (see Figure S1). For the 19 serum samples that were reactive in ELISA twice, Western blot analysis was performed using nucleoproteins from EBOV and SUDV produced in an insect cell-based expression system (Table 2). The strongest signal against both antigens was clearly observed for the control serum samples P21 and P28 derived from experimentally EBOV- or SUDV-infected pigs (Figure 2a). From 19 serum samples that tested positive in EBOV NP ELISA, a total of 13 samples showed binding to insect cell-derived EBOV NP in Western blot with two sera (GUI 78 and 122) that only weakly recognized EBOV NP. Four samples detected SUDV NP. Five EBOV NP ELISA-positive serum samples (GUI 87, 114, 190, 196 and GUI 287) were not confirmed to react with any of the ebolavirus NP used in Western blot analysis. One serum (GUI 246) tested positive in EBOV NP ELISA but failed to react with EBOV NP in Western blot, while it recognized SUDV NP antigens. Taken together, these results suggest the presence of serological cross-reactivity between the *Ebolavirus* species in pig serum samples.

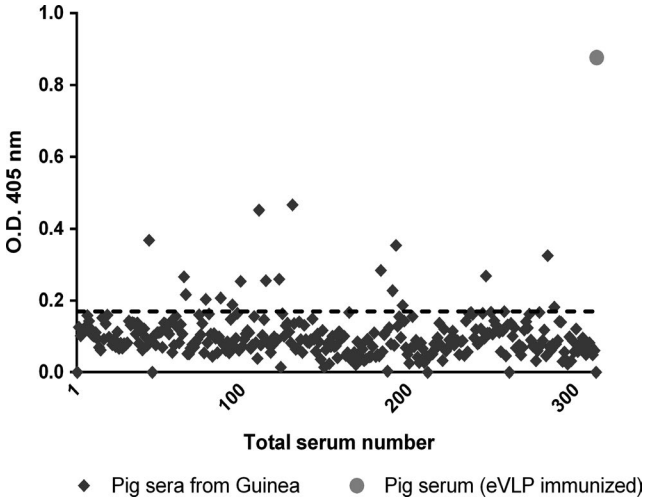

FIGURE 1 Analysis of pig serum samples from Guinea in indirect ELISA based on EBOV NP. Optical densities (OD_{405}) of all tested serum samples are displayed. Serum from an Ebola virus-like particle (eVLP) immunized pig was used as positive control. Vertical dashed line indicates the cut-off at 0.17

ELISA-reactive samples were further analysed in immunofluorescence assay (IFA) under native conditions using EBOV or SUDV glycoprotein (GP) transfected Vero cells to investigate potential serum reactivity towards a second viral protein. One sample (GUI 130) clearly recognized EBOV GP on the Vero cell surface, while three samples (GUI 94, GUI 183, and GUI 202) detected SUDV GP (Figure 2b). Finally, 14 out of the 19 serum samples tested positive in EBOV NP ELISA were tested for their neutralizing activity against EBOV (Mayinga isolate) in a virus neutralization test (VNT). Calculated mean titres of nine serum samples were rather low ranging between 1:5 and 1:8 and are considered negative (Table 3). However, 5 serum samples showed a weak inhibition of EBOV infection with titres of 1:10 to 1:13, including GUI 202 (titre 1:10) which recognized SUDV GP in IFA. Since calculated mean titres ranged above 1:8 but below 1:16, samples were considered indeterminate (Table 3).

4 | DISCUSSION

Within the past 40 years, a number of EVD outbreaks have been traced back to zoonotic spillover events facilitated through contact of a human index case with infected wildlife species such as gorillas or chimpanzees (Leroy et al., 2004; Pourrut et al., 2005).

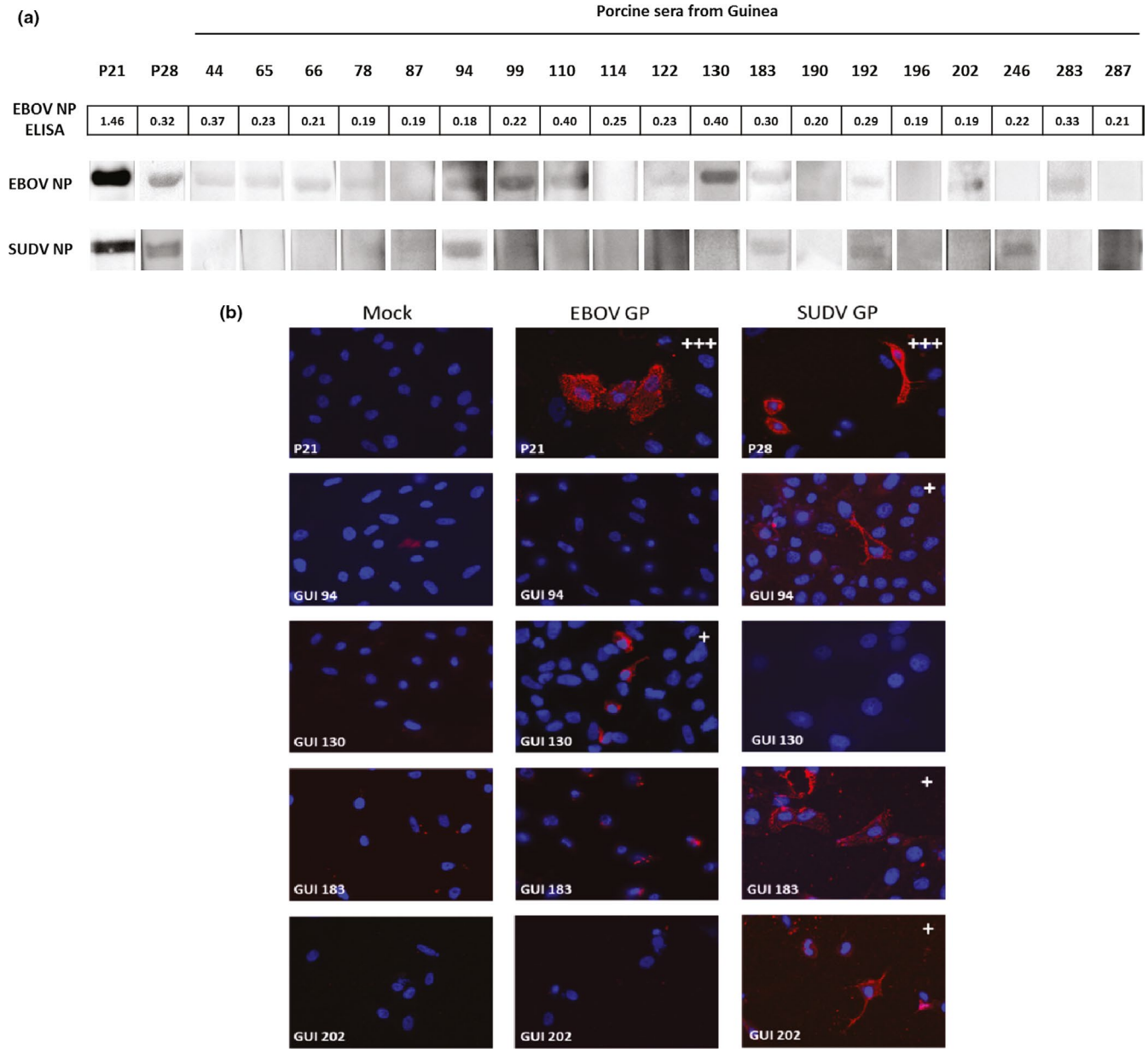

FIGURE 2 Confirmatory testing of pig serum samples from Guinea. (a) Western blot analysis of serum samples classified as reactive in EBOV NP-based ELISA. Specimens were tested against the NP of EBOV and SUDV. Antigens were produced using an insect cell-based expression system. The values noted in squares correspond to the OD_{405} values of the porcine sera in EBOV NP-based ELISA. (b) Immunofluorescence analysis of ELISA-reactive serum samples. Porcine sera (diluted 1:20) were incubated on Vero cells transfected with EBOV or SUDV glycoprotein (GP) expression plasmids. Mock-transfected cells were used as a negative control. Porcine antibodies were stained with anti-pig Alexa Fluor 594 secondary antibodies. Nuclei were counterstained with Hoechst 33342. Pig serum from experimentally EBOV (P21) or SUDV (P28) infected pigs served as positive controls

Nonetheless, our knowledge on ebolavirus ecology remains relatively limited in terms of its natural reservoir and maintenance in nature since primate species are assumed to only act as incidental hosts (Caron et al., 2018; Gonzalez, Pourrut, & Leroy, 2007). When RESTV was detected in pigs from the Philippines in 2008 (Barrette et al., 2009), it was hypothesized whether pigs might also play a role in EBOV ecology. Subsequently, pigs have been shown to be susceptible to EBOV infection under experimental settings, while evidence of natural EBOV infection of pigs is currently lacking (Barrette et al., 2009; Kobinger et al., 2011; Weingartl et al., 2012).

In this study, we provide the first serological evidence for the exposure of pigs from Guinea to ebolaviruses or ebola-like viruses that are antigenically related to EBOV. To reinforce the significance of a previous study during which we detected ebolavirus-specific IgG antibodies in pigs from Sierra Leone and determined a seroprevalence of <1% (Fischer et al., 2018), we further tested 308 porcine serum samples from a greater area around Conakry, Guinea. In addition, we complemented the search for ebolavirus-specific IgG antibodies by using an expanded panel of serological assays. Seroprevalence was slightly higher in Guinea than in Sierra Leone,

TABLE 3 Results of serological analysis of porcine serum samples from Guinea

Animal number	Age (months)	Gender	ELISA EBOV NP	WB EBOV NP	WB SUDV NP	IFA EBOV GP	IFA SUDV GP	VNT (EBOV Mayinga)	
GUI 44	6	m	0.37	+	–	–	–	–	1:8
GUI 65	8	f	0.23	+	–	–	–	(+)	1:13
GUI 66	7	m	0.21	+	–	–	–	(+)	1:11
GUI 78	7	f	0.19	(+)	–	–	–	–	1:6
GUI 87	7	m	0.19	–	–	–	–	n.t.†	–
GUI 94	6	m	0.18	+	+	–	+	–	1:8
GUI 99	8	f	0.22	+	–	–	–	(+)	1:10
GUI 110	8	m	0.40	+	–	–	–	–	1:6
GUI 114	8	f	0.25	–	–	–	–	–	1:4
GUI 122	15	f	0.23	(+)	–	–	–	–	1:6
GUI 130	14	f	0.40	+	–	+	–	–	1:6
GUI 183	7	f	0.30	+	+	–	+	–	1:5
GUI 190	12	f	0.20	–	–	–	–	n.t.†	–
GUI 192	7	f	0.29	+	+	–	–	–	1:7
GUI 196	7	m	0.19	–	–	–	–	n.t.†	–
GUI 202	18	m	0.19	+	–	–	+	(+)	1:10
GUI 246	8	m	0.22	–	+	–	–	n.t.†	–
GUI 283	8	m	0.33	+	–	–	–	(+)	1:13
GUI 287	18	f	0.21	–	–	–	–	n.t.†	–

Note: Samples that ranged above the cut-off of OD_{405} 0.17 in indirect ELISA based on EBOV NP were further analysed in confirmatory tests.
(+), weakly positive; –, negative; +, positive/reactive; EBOV, Ebola virus; GP, glycoprotein; IFA, immunofluorescence assay; n.t., not tested († = due to low amount of serum); NP, nucleoprotein; SUDV, Sudan virus; VNT, virus neutralization test; WB, Western blot.

with a total of 14 samples (4.5%) that were shown to be positive in EBOV NP ELISA and confirmed to react with at least one ebolavirus NP antigen in Western blot. Interestingly, the majority of reactive samples (15/19) were collected at a rural sampling site with two different holdings surrounded by mango trees with the reported circulation of frugivorous bats. Pigs at these holdings are housed under stationary conditions that still allow entry and roosting of bats through a laterally open roof. However, no bats were noticed during sampling activities. Further, seropositive samples exclusively originated from pigs aged between 6 and 18 months. Older animals did not have detectable antibodies indicating that although alive during the EBOV outbreak in Guinea from 2014 to 2016, those animals were either not exposed to EBOV or did not develop a long-lasting antibody response. A decrease in antibody titres was observed in experimentally EBOV-infected pigs at 28 dpi (Pickering et al., 2017) but persistence of antibodies against individual virus proteins in pigs remains unknown. For human survivors, long-lasting antibody responses against EBOV GP and NP have been described with a persistence of up to 40 years (Rimoin et al., 2018). Furthermore, a study analysing the humoral immune response towards SUDV identified neutralizing antibodies in serum of survivors up to ten years post-infection (Sobarzo et al., 2013). In this case, neutralizing activity correlated with reactivity against the SUDV NP, GP and viral protein VP 30.

Interestingly, a few porcine sera in our study bound to GP of either EBOV or SUDV in IFA while some sera weakly neutralized EBOV infection with the Mayinga isolate. Noteworthy, GP binding was not associated with neutralizing activity and vice versa which could be explained by binding to different epitopes. Weak neutralizing activity of the pig sera might either be explained by generally low or progressively decreasing antibody titres over time as recently shown for reconvalescent *Rousettus aegyptiacus* bats (Schuh et al., 2018), or by exposure to an antigenically related but phylogenetically divergent ebolavirus. This hypothesis is supported by the diverse interactions of the 19 serum samples, originally selected for their reactivity for EBOV NP in ELISA, observed in WB or IFA against NPs or GPs from another ebolavirus species. In this respect, it is interesting to note that Bombali virus (BOMV), a new ebolavirus recently discovered in insectivorous bats from Sierra Leone, revealed a sequence similarity of 92% in the NPC1-interacting residues of BOMV GP and the GP of other known ebolaviruses known to be responsible for receptor binding (Goldstein et al., 2018). Thus, exposure of pigs from Guinea to BOMV or another closely related ebolavirus might result in weak but detectable cross-neutralizing activities of the sera. With respect to the very recent finding of partial BOMV nucleotide sequences in *Mops condylurus* bats from Kenya and Guinea (Forbes et al., 2019; Karan et al., 2019), an exposure of pigs to BOMV is conceivable.

The discovery of BOMV and other filovirus RNA as well as filovirus specific antibodies in different bat species (He et al., 2015; Jayme et al., 2015; Negredo et al., 2011; Olival et al., 2013; Yang et al., 2017) supports the assumed circulation of as yet undiscovered filoviruses with unknown pathogenic potential. Consequently, these filoviruses have been discussed to account for detectable serological cross-reactivity against known ebolaviruses in asymptomatic humans living in regions with no record of previous EVD outbreaks (Formella & Gatherer, 2016; Kuhn & Bavari, 2017; Mulangu et al., 2018) as well as in pigs from Sierra Leone (Fischer et al., 2018) with no report of previous clinical disease.

Confirmatory testing of EBOV NP ELISA-reactive samples was performed by Western blot analysis using recombinant EBOV and SUDV nucleoproteins expressed in eukaryotic insect cells. Three serum samples clearly cross-reacted with SUDV NP in Western blot. The presence of NP-specific antibodies in pigs with limited cross-reactivity between these ebolavirus species has been observed previously (Fischer et al., 2018). Besides the well-known presence of EBOV and SUDV in Africa, RESTV or Reston-like viruses have been described to circulate mainly in Asia (Barrette et al., 2009; Demetria et al., 2018; Jayme et al., 2015; Pan et al., 2014). However, the recent finding of RESTV-specific antibodies in African straw-coloured fruit bats (*Eidolon helvum*) from Zambia suggests the spread of RESTV or Reston-like viruses in a greater geographical range than previously anticipated (Ogawa et al., 2015). Whether RESTV or Reston-like viruses circulate among pigs in Africa remains to be investigated.

In contrast to the study from Sierra Leone, none of the seropositive pigs from Guinea had an age that allowed for EBOV contact during the EVD outbreak from 2014 to 2016. In addition, the pigs from Guinea were not reported to be free-ranging and had no history of migration suggesting a virus exposure other than by contact to acutely infected EVD patients and/or their secretions. The definite route of exposure of the pigs to an ebolavirus eliciting cross-reactive antibodies remains unknown; though their housing conditions allowed for contact to bats. The serological cross-reactivity among ebolavirus NPs observed in pigs might also be affected by the level of antibody titres. While positive control sera from experimentally EBOV-infected pigs displayed the strongest signal and cross-reactivity in Western blot, ELISA and Western blot signals of field serum samples were lower with limited cross-reactivity. However, cross-reactivity between different ebolavirus species using serological assays based on ebolavirus nucleoproteins (NP) has been demonstrated in several studies when testing human and bat sera while glycoprotein (GP)-based assays displayed lower cross-reactivity (Laing et al., 2018; Macneil, Reed, & Rollin, 2011).

Given the detected seroprevalence for ebolavirus NP antibodies and the very weak EBOV-neutralizing activity of sera from relatively young and apparently healthy pigs from Guinea, it is likely that these pigs were exposed to and can be naturally infected with ebolaviruses or ebola-like viruses that are antigenically related but distinct to highly pathogenic EBOV. Although an active epidemiological role of pigs as intermediate or amplifying hosts in EBOV ecology appears less likely, our results suggest that pigs have come into contact with ebolaviruses of unknown pathogenic and/or zoonotic potential that might be circulating in West Africa. Future studies including continuous serosurveillance among pigs are imperative to further understand the role of this species in the ecology of ebolaviruses.

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CONFLICT OF INTEREST

All authors declare that they have no competing interests.

ETHICAL APPROVAL

For pig serum collection, animals were handled according to a protocol submitted to and reviewed by the Comité National d'Ethique pour la Recherche en Santé (CNERS) of Guinea under the number 040/CNERS/17.

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SUPPORTING INFORMATION

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

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