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SHORT COMMUNICATIONS

A serological survey for antibodies against foot-and-mouth disease virus (FMDV) in domestic pigs during outbreaks in Kenya

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Abstract Foot-and-mouth disease (FMD) is endemic in Kenya and has been well studied in cattle, but not in pigs, yet the role of pigs is recognised in FMD-free areas. This study investigated the presence of antibodies against FMD virus (FMDV) in pigs sampled during a countrywide random survey for FMD in cattle coinciding with SAT 1 FMDV outbreaks in cattle. A total of 191 serum samples were collected from clinically healthy pigs in 17 districts. Forty-two of the 191 sera were from pigs vaccinated against serotypes O/A/SAT 2 FMDV. Antibodies against FMDV non-structural proteins were found in sera from 30 vaccinated and 71 non-vaccinated pigs, altogether 101/191 sera (53 %), and 91 % of these (92/101) also had antibodies measurable by serotype-specific ELISAs, predominantly directed against SAT 1 with titres of 10–320. However, only five high titres against SAT 1 in vaccinated pigs were confirmed by virus neutralisation test (VNT). Due to high degree of agreement between the two ELISAs, it was concluded that positive pigs had been infected with FMDV. Implications of these results for the role of pigs in the epidemiology of FMD in Kenya are discussed, and in-depth studies are recommended.

Keywords Domestic pigs · Eastern Africa · Endemic areas · Foot-and-mouth disease · SAT 1

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Introduction

Foot-and-mouth disease (FMD) is endemic in eastern Africa (Kenya included), where four of the seven serotypes of the virus have recently been isolated (O, A, SAT 1 and SAT 2) (Namatovu et al. 2013). FMD affects all cloven-hoofed animals (Alexandersen and Mowat 2005); however, recent East African studies concern cattle (Balinda et al. 2010; Habiela et al. 2010; Kasanga et al. 2012; Mwiine et al. 2010; Sangula et al. 2011; Tekleghiorghis et al. 2013), small ruminants (Balinda et al. 2009; Raouf et al. 2012) and buffalo (Ayebazibwe et al. 2010), while little information is available on pigs.

The role of pigs in the epidemiology of FMD has been recognised in outbreaks in FMD-free countries (Chen et al. 2008; Gibbens 2011; Hayama et al. 2012; Knowles et al. 2001), but not in FMD-endemic eastern Africa, where a recent sub-Saharan African survey indicated low prevalences of

antibodies against FMD virus (FMDV) (Fernandez-Pacheco et al. 2012), and similarly, an outbreak study in FMD endemic northern Thailand concluded that pigs were not involved (Chamnanpood et al. 1995). However, FMDV was recently isolated from pigs in neighbouring China (Yang et al. 2011) and in Uganda (Kerfua et al. 2013).

Kenya has a limited pig population (≈300,000) compared to 62 million ruminants (Anonymous 2010), and pig samples are rarely submitted to the national Foot-and-Mouth Disease Laboratory, Embakasi (FMDL-Embakasi), for diagnosis. However, in 2009, unvaccinated pigs at Nairobi University's research farm had clinical symptoms of FMD during a SAT 1 outbreak in cattle (FMDL-Embakasi annual reports).

This study investigated the presence of antibodies against FMDV in sera from domestic pigs collected in 17 Kenyan districts during a countrywide random serological survey to study the circulation of FMDV serotypes and to determine the risk factors for spread, prompted by a widespread SAT 1 FMDV outbreak in cattle in 2010.

Materials and methods

One hundred ninety-one sera from pigs (6–36 months old) collected in April–May 2010 from 26 villages in 17 of Kenya's then 72 districts (Fig. 1, Table 1) were obtained from FMDL-Embakasi in Kenya. The samples were derived from a countrywide serological survey for FMD in cattle performed under the Somali Ecosystem Rinderpest Eradication Coordination Unit (SERECU) project, which coincided with a countrywide SAT 1 FMDV outbreak in cattle. Pigs were only sampled when they were found at cattle sampling sites. Sampled pigs were examined for clinical signs of FMD, and the date of serum collection, age, sex, clinical signs, owner, location and history of FMD outbreaks and vaccinations were recorded.

All sera were screened using PrioCHECK® FMDV NS kit (Prionics AG, Switzerland) (non-structural protein (NSP) ELISA) for antibodies against the NSPs of FMDV. This test detects antibodies induced by all seven serotypes (Ayebazibwe et al. 2012; Sorensen et al. 1998) and in all species (Brocchi et al. 2006) and was performed according to the manufacturer's instructions except expressing results as a percentage of the mean negative control (optical density percentage (ODP)) (Balinda et al. 2009). ODP of <50 % was considered positive.

All sera were screened (1:10) using serotype-specific solid-phase blocking ELISAs (SPBEs) (Balinda et al. 2009) for antibodies against serotypes O/SAT 1/SAT 2/SAT 3, while only anti-NSP-positive sera were screened in SPBEs for A/C/Asia 1. Results were expressed as ODP, and samples were considered positive, if ODP was <50 % for O/SAT 1/SAT 2/SAT 3, <45 % for A and <35 % for C/Asia 1.

Positive SPBE reactions were titrated (1:10–1:1,280) and titres expressed as the reciprocal of the last positive dilution. All sera with SPBE titres of ≥40 and selected sera with lower titres against SAT 1 and SAT 3 were assayed using the virus neutralisation test (VNT) according to the OIE Terrestrial Manual (OIE 2012). Briefly, quadruplicate twofold dilution series of sera were reacted for 1 h with 100 $TCID_{50}$ of FMDV (O Manisa, SAT 1 BOT 1/68, SAT 2 ZIM 5/81 and SAT 3 ZIM 4/81) in equal volumes and were subsequently incubated with a suspension of primary swine kidney (SK) cells for 3 days. Controls included SK cells, titration of a standard positive serum and a tenfold titration of the virus. Final end point titres were calculated (Reed and Muench 1938), and titres of ≥45 were considered positive, 16–44 doubtful and <16 negative.

The SAT 1 VNT was re-run using a recent Kenyan SAT 1 isolate (SAT 1 K85/10), but the results were not significantly different from the 1968 strain (BOT 1/68) (P=2.000, data not shown).

Results were recorded, and descriptive statistics were calculated in MS Excel 2007 (Microsoft Corporation). Seroprevalences were compared using Survey Toolbox (Cameron 1999), while odds ratios (ORs), to determine the impact of reported outbreak and of vaccinations on seroprevalences, were calculated in MS Excel (McHugh 2009).

Results

Though 10/17 sampled districts reported FMD outbreaks in cattle (one, O and A; nine, SAT 1 (three overlapping with O or A)), during the sampling or shortly after (Fig. 1), none of the sampled pigs had clinical signs suggestive of FMDV infection. Forty-two pigs from five districts (Embu-Manyatta, Kieni East, Kieni West, Tetu and Nyeri) had been vaccinated against FMDV (aqueous, non-purified, Kenyan, trivalent vaccine: O/A/SAT 2) 1.5 to 7 months before the sampling (Table 1).

Overall, 101/191 sera (53 %; 95 % confidence interval (CI) 50.3–57.4) from 14 districts (82 %; 95 % CI 74.2–92.5) had antibodies against FMDV NSPs (Table 1) with a higher overall seroprevalence in vaccinated (71 %, 30/42) than in non-vaccinated (48 %, 71/149) pigs. However, seroprevalences did not differ significantly between vaccinating and non-vaccinating districts (P=0.94) or between those with reported outbreaks and those without (P=0.810), and district seroprevalence was not related to reported outbreaks (OR=0.94).

All NSP ELISA-negative samples were also negative for antibodies against O, SAT 1, SAT 2 and SAT 3 by SPBE (data not shown), and all NSP-positive samples were negative for antibodies against serotypes C, Asia 1 and SAT 2, with nine of the NSP positives being negative in all SPBEs. Two (2 %), 10

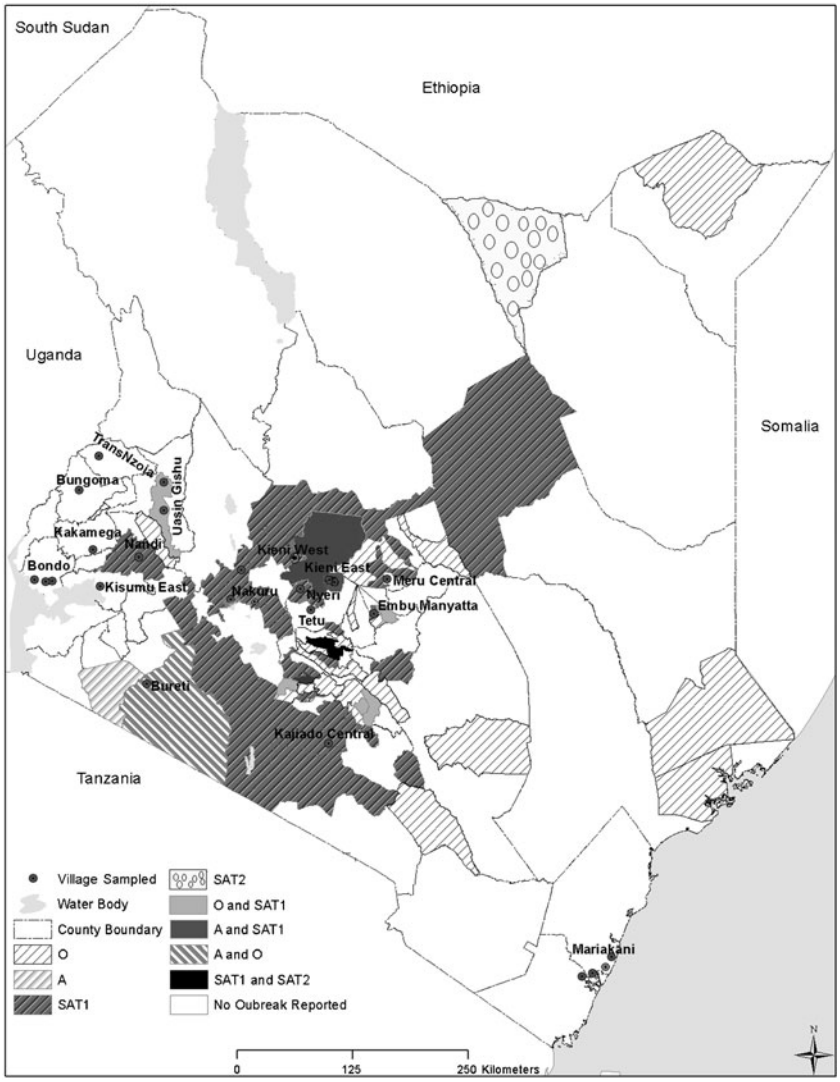

Fig. 1 Map of Kenya showing villages where pigs were sampled (*black dots*). *Shading* indicates FMDV serotype diagnosed by FMDV antigen ELISA on cattle samples submitted from Kenyan districts to FMDL-Embakasi between January and July 2010

(10 %), 77 (76 %) and 23 (23 %) sera positive in NSP ELISA were positive in the SPBEs for antibodies against O, A, SAT 1 and SAT 3, respectively (Table 1), but when titrated, only 35 of these 112 positive reactions were of titre ≥40 (O, 2/2; A, 2/10; SAT 1, 29/77; SAT 3, 2/23). The prevalences of titres ≥40 were comparable between vaccinated and non-vaccinated pigs (P=1).

The majority of high titres against SAT 1 (21/29) were 40, while eight were higher (80, 4/29; 320, 4/29). High titres against other serotypes were only found in a few vaccinated pigs from Embu-Manyatta (A, 40/40; SAT 3, 160/320) and in two Mariakani sera (O, 80/160) (data not shown).

Only the vaccinated pigs from Embu-Manyatta were positive in VNT with titres of ≥320 against SAT 1 in five out of eight pigs and titres of 67 against SAT 3 in two of these (Table 1). Moreover, in Mariakani, the two SPBE O titres 80 and 160 were doubtful in VNT (titres of 28), while low SPBE SAT 1 titres were not confirmed by this assay. The three sampled Nakuru villages had one out of seven, six out of six and eight out of eight animals with antibodies against NSP and SAT 1 (low titre), but five of these were negative in VNT (<16) (Table 1).

Discussion

The overall pig anti-NSP seroprevalence (53 %) was comparable to that in unvaccinated pigs (48 %), and both were much higher than the 2 % seroprevalence found in 869 pig sera collected in sub-Saharan African countries (Fernandez-Pacheco et al. 2012). Our findings concur with data from FMD-free countries, where pigs are known to acquire FMDV infection (Alexandersen and Mowat 2005), and with isolation of FMDV from naturally infected pigs in Uganda and China (Kefua et al. 2013; Yang et al. 2011), however, our district seroprevalences were not related to the presence of FMD outbreaks in cattle (OR=0.94). This may be due to under-reporting of FMD or be a result of a too small sample size to give a comprehensive and comparable quantitative picture of

Table 1 Districts and number of villages sampled in this study indicating vaccination and outbreak history and FMDV antibody results in NSP ELISA, in SPBEs for antibodies against serotypes A, SAT 1 and SAT 3 and in VNT for antibodies against O, SAT 1 and SAT 3

District	No. of villages sampled	Vaccine serotypes	Vaccination (months preceding sampling)	FMD[a] outbreak serotypes (days from sampling[b])	NSP ELISA No. positive/no. tested (%)	SPBE titres (no. of positive with titre)			VNT titres (no. of positive with titre)		
						A	SAT 1	SAT 3	O	SAT 1[c]	SAT 3
Embu-Manyatta	1	O, A, SAT 2	1.5	O and SAT 1 (0)	8/8 (100 %)	10 (2) 20 (1) 40 (2)	40 (2) 320 (4)	10 (3) 20 (3) 160 (1) 320 (1)	–	381 (4) 320 (1)	<16 (2) 17 (1) 24 (1) 67 (2)
Kieni East	2	O, A, SAT 2	7	A and SAT 1 (>60)	7/14 (50 %)	–	10 (1) 20 (4)	–	–	<16 (2)	–
Kieni West	1	O, A, SAT 2	3	SAT 1 (<60)	7/8 (88 %)	–	20 (2)	10 (5)	–	–	<16 (1)
Nyeri	1	O, A, SAT 2	3	SAT 1 (>60)	4/8 (50 %)	–	10 (1)	–	–	<16 (2)	–
Tetu	1	O, A, SAT 2	4	None	4/4 (100 %)	10 (1)	20 (2)	10 (3)	–	–	<16 (2)
Bondo	3	NV	NA	None	13/16 (81 %)	–	10 (1) 20 (10) 40 (2)	–	–	<16 (2)	–
Bungoma	1	NV	NA	None	2/7 (29 %)	–	40 (2)	–	–	–	–
Bureti	1	NV	NA	A and O (>60)	0/3 (0 %)	–	–	–	–	–	–
Kajiado Central	1	NV	NA	SAT 1 (>30)	0/8 (0 %)	–	–	–	–	–	–
Kakamega	1	NV	NA	None	8/9 (89 %)	–	10 (1) 20 (1) 40 (3) 80 (2)	–	–	<16 (5)	–
Kisumu East	1	NV	NA	None	4/8 (50 %)	–	20 (2) 40 (1)	–	–	–	–
Mariakani	4	NV	NA	None	14/32 (44 %)	10 (1) 20 (1)	10 (1) 20 (7) 40 (7) 80 (1)	–	28 (2)	<16 (3)	<16 (1)
Meru Central	1	NV	NA	SAT 1 (>30)	8/8 (100 %)	–	–	10 (4) 10 (2)	–	–	<16 (4)
Nakuru	3	NV	NA	SAT 1 (0)	15/21 (71 %)	10 (1)	10 (2) 20 (12) 80 (1)	10 (1)	–	<16 (5)	<16 (1)
Nandi	1	NV	NA	SAT 1 (<60)	0/10 (0 %)	–	–	–	–	–	–
Trans Nzoia	1	NV	NA	None	2/8 (25 %)	–	–	–	–	–	–
Uasin Gishu	2	NV	NA	O and SAT 1 (0)	5/19 (26 %)	10 (1)	20 (1) 40 (4)	–	–	–	–
Total	26				101/191	10	77	23			

–, no sample here was qualified for the particular test

NV not vaccinated, *NA* not applicable

[a] Outbreak serotypes reported in cattle and detected using antigen ELISA

[b] Days between districts' report of outbreak and sampling (0, sampled during outbreak; >60/<60/>30, sampling took place more than 60 days, less than 60 days or more than 30 days after outbreak)

[c] VNT titres are against a recent Kenyan FMDV SAT 1 isolate (K85/10)

the presence of antibodies against FMDV, since for 12/17 districts, only between three and ten samples were collected from one village, while in the remaining districts, seroprevalences varied from 0 to 100 % in the two to four villages sampled (data not shown).

Though generally severe in pigs (Yoon et al. 2012), clinical signs of FMD were neither observed in the sampled pigs nor by farmers in their pigs. This is consistent with FMD not being commonly reported in Kenyan pigs and with observations from studies of pigs during outbreaks in cattle in Thailand (Chamnanpood et al. 1995) and China (Yang et al. 2011; Zhang et al. 2008).

In contrast, in this study, based on the high level of agreement between the two independent ELISA systems (91 % of positive and 100 % of negative samples), we have concluded that the pigs had indeed been infected with SAT 1 FMDV and in Mariakani district with O FMDV. This is supported by earlier studies demonstrating that the 3ABC-based NSP ELISA tests and SPBEs can be used to detect antibodies against FMDV with high sensitivity and specificity for both NSP ELISA (Brocchi et al. 2006; Chen et al. 2011) and SPBEs for antibodies against SAT 1, SAT 2 and SAT 3 used as screening tests (Sorensen et al. 1992). Moreover, these tests have been used to investigate FMD epidemiology in small ruminants (Balinda et al. 2009), cattle (Mwiine et al. 2010) and African buffalo (*Syncerus caffer*) (Ayebazibwe et al. 2010), where titre cut-offs were set between ≥40 and ≥160.

Lack of clinical signs and neutralising antibodies together with low titres in SPBE could indicate waning of antibodies after past FMDV infection but could also be due to pigs requiring a higher infection dose than cattle and sheep (Alexandersen and Donaldson 2002) with low infection dose potentially leading to subclinical infection and low-level antibody responses in pigs (Kitching and Alexandersen 2002). Moreover, this particular SAT 1 FMDV strain may only recently have jumped from cattle to pigs and may not yet have adapted sufficiently to give clear clinical symptoms in pigs. A similar situation was reported in China, where a 2005 Asia 1 FMDV strain exclusively affected cattle in the field, and in experiments only gave mild clinical symptoms and low-level antibodies in 25–50 % of infected pigs (Zhang et al. 2008) but, in 2006, increased virulence in pigs after natural genetic recombinations (Yang et al. 2011). Furthermore, host susceptibility to certain strains have varied (Yoon et al. 2012), and earlier studies have observed pigs to play significant roles in the spread of serotypes O (Gibbens 2011; Hayama et al. 2012), Asia 1 (Yang et al. 2011) and A (Mohamed et al. 2011) FMDV. According to Kitching and Alexandersen (2002), subclinically infected pigs with low-level antibody responses may have very limited ability to transmit infection. Hence, the findings in this study could indicate infections of pigs acquired from cattle, but at such low levels that they did not develop clinical symptoms.

Vaccination of pigs is not routinely done in Kenya, mainly due to limited resources and lack of clinical symptoms; nevertheless, five districts had vaccinated pigs against serotypes O/A/SAT 2. Vaccinated pigs only had antibodies against SAT 1 and SAT 3 by SPBE, of which only five high titres against SAT 1 and two lower titres against SAT 3 in the very recently vaccinated pigs in Embu-Manyatta (6 weeks) were confirmed by VNT. The lack of antibodies against the vaccine raises concern about its use in pigs and confirms that aqueous vaccines generally give poor, short-lived, antibody responses in pigs (Doel 1999). Moreover, since we found no significant difference in seroprevalence of antibodies against NSPs between vaccinating and non-vaccinating districts, and the pigs were only vaccinated once, it is likely that the observed antibodies were induced by recent or ongoing infection with SAT 1. This is in line with outbreaks of SAT 1 FMDV in this and three neighbouring districts.

In conclusion, we found serological evidence for SAT 1 FMDV infection in pigs without obvious clinical signs during an outbreak in cattle. Thus, although pigs may not play an obvious role in the spread of FMD in the eastern Africa endemic situations, they can be infected and could become important in the epidemiology if virulence or infection dose increased, e.g. through intensified exposure. Therefore, we recommend limiting contact between pigs and cattle. Due to the small sample size and incomplete epidemiological information, this study recommends more in-depth studies of the role of pigs in the epidemiology of FMD in Kenya, including a more comprehensive sample material and information. In addition, the studies should endeavour to isolate and characterise FMD viruses from pigs whenever there are clinical signs.

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