Transboundary and Emerging Diseases

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ORIGINAL ARTICLE

Serotype Specificity of Antibodies against Foot-and-Mouth Disease Virus in Cattle in Selected Districts in Uganda

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Summary

Uganda had an unusually large number of foot-and-mouth disease (FMD) outbreaks in 2006, and all clinical reports were in cattle. A serological investigation was carried out to confirm circulating antibodies against foot-and-mouth disease virus (FMDV) by ELISA for antibodies against non-structural proteins and structural proteins. Three hundred and forty-nine cattle sera were collected from seven districts in Uganda, and 65% of these were found positive for antibodies against the non-structural proteins of FMDV. A subset of these samples were analysed for serotype specificity of the identified antibodies. High prevalences of antibodies against non-structural proteins and structural proteins of FMDV serotype O were demonstrated in herds with typical visible clinical signs of FMD, while prevalences were low in herds without clinical signs of FMD. Antibody titres were higher against serotype O than against serotypes SAT 1, SAT 2 and SAT 3 in the sera investigated for serotype-specific antibodies. Only FMDV serotype O virus was isolated from one probang sample. This study shows that the majority of the FMD outbreaks in 2006 in the region studied were caused by FMDV serotype O; however, there was also evidence of antibodies to both SAT 1 and SAT 3 in one outbreak in a herd inside Queen Elizabeth national park area.

Introduction

Foot-and-mouth disease virus (FMDV) is classified within the *Aphthovirus* genus as a member of the *Picornaviridae* family (Andrewes et al., 1978) and is a highly infectious disease agent that causes severe vesicular disease. Foot-and-mouth disease (FMD) affects all cloven-hoofed animals including domesticated ruminants and pigs and more than 70 wildlife species (Thomson, 1994).

The epidemiology of FMDV has been described as complex in Africa (Vosloo et al., 2002) as six of the seven possible FMDV serotypes have been isolated, including serotypes O, A, C, SAT 1, SAT 2 and SAT 3; thus, only Asia 1 has never been reported on the African continent (Vosloo et al., 2002; Rweyemamu et al., 2008). Of the six serotypes, serotype C has most likely been eradicated from Africa and was last reported in Ethiopia in 1983 (Ayelet et al., 2009) and in Kenya in 2004 (Roeder and Knowles, 2008).

Economically, FMD is a very important disease, especially to countries that export and import animals or animal products. Trade barriers set up to protect FMD-free countries have impacted negatively on the gross domestic product (GDP) of many FMD endemic countries that depend on agriculture (FAO-OIE, 2004).

In Uganda, FMD is endemic and outbreaks including serotypes O, A, SAT 1 and SAT 2 (Vosloo et al., 2002) have occurred annually in the cattle population, which is estimated to amount to 11.4 million head of cattle (Anon, 2009). Efforts to control FMD in this country mainly consist of vaccination and restriction of animal movement. However, the success of these efforts is severely

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impeded by lack of proper infrastructure and appropriate FMD vaccines matching the circulating FMDV field strains (Perry and Sones, 2007). Uganda mainly imports Kenyan and Botswana FMDV vaccines that are not purified of non-structural proteins (NSP) (Rutebarika, personal communication) and has from 2003 to 2005 used vaccines including serotypes O, SAT 1 and SAT 2. Historically, serotyping of Ugandan FMDV outbreaks has been performed abroad, because cell culture and PCR, a few of the recommended diagnostic techniques for the diagnosis of FMD (OIE, 2009), have not been available in Uganda. Recently, an in-house system of seven serotype-specific solid-phase blocking ELISAs (SPBE) for the detection of antibodies against each of the seven serotypes of FMDV set up at Lindholm, Denmark (Have and Holm Jensen, 1983; Sørensen et al., 1998; Balinda et al., 2009) was implemented at the National Veterinary Diagnostics and Epidemiology Laboratory (NVDEL), Ministry of Agriculture, Animal Industries and Fisheries (MAAIF) in Uganda. The aim of this study was to determine the serotype specificity of the circulating antibodies against FMDV in cattle in selected districts in Uganda during the major FMD outbreak in 2006.

Materials and methods

Study areas, sampling strategy, sample collection and handling

This study was a post-outbreak study carried out in seven districts of Uganda including Bushenyi, Isingoro, Mbarara, Kasese, Mpigi, Kiboga and Kiruhura, where there were suspected outbreaks of FMD during the year 2006 (Fig. 1). Cattle were kept under the communal grazing system except for Bushenyi district where animals were in fenced farms. The herds were selected based on consultation with field veterinary officers in the respective districts on investigation into recent FMD outbreaks. Twenty-two herds with visible clinical signs of FMD and six herds without clinical signs were visited in seven districts in the months of July and August 2006. The farmers were interviewed about management practice, size of herd, previous exposures to FMDV and vaccination history.

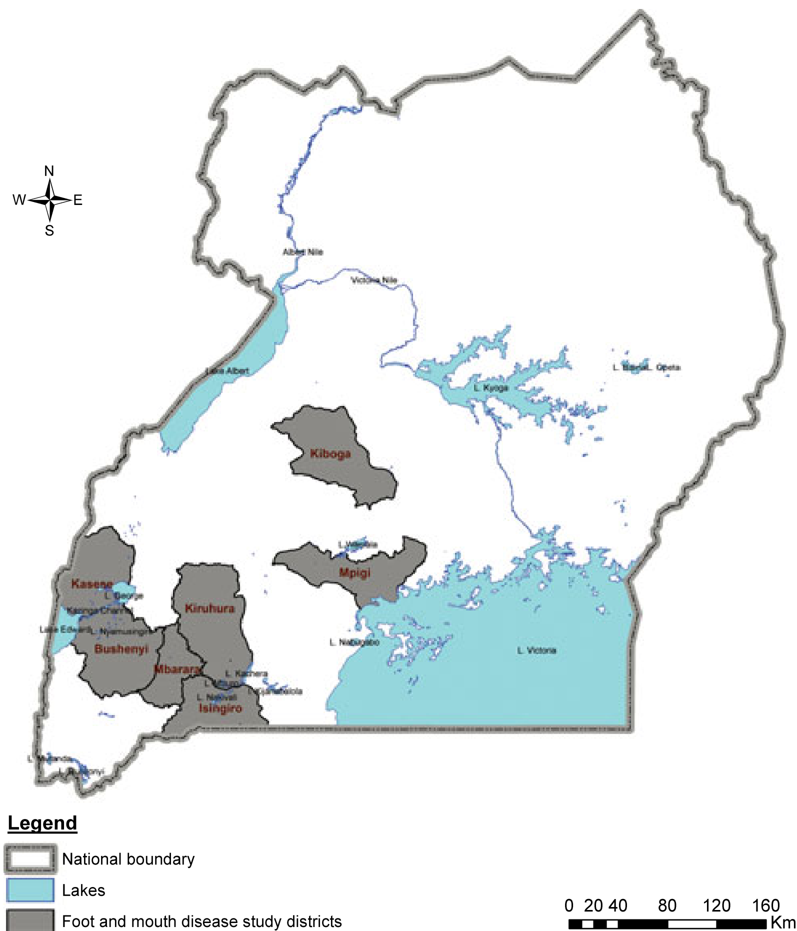

Fig. 1. Map of Uganda showing the districts included in this study.

Vaccination of cattle was last performed in the area in 2005, at least seven months before samples were collected. On the consent of the farmers, blood samples and in some cases oropharyngeal probang (OP) samples were taken, and cattle in herds with suspected FMD outbreak and/or a history of recent occurrence of the disease were examined for lesions in the mouth and feet with focus on the hooves and the interdigital areas.

The serum was extracted in the field within 24 h of sampling using a Mobilespin 12-V field centrifuge (Mobilespin, Vulcon Technologies, Gransview, MO, USA). Aliquots of approximately 4.5 ml of sera were collected and kept on ice until stored at −20°C at NVDEL, MAAIF. The OP samples were collected in sterile phosphate-buffered saline (PBS), stored in liquid nitrogen in the field and thereafter at −80°C at NVDEL, MAAIF, for later analysis at the National Veterinary Institute of the Technical University of Denmark (DTU), Lindholm, Denmark (Lindholm).

Investigation into antibodies against FMDV

All sera were screened for antibodies against FMDV NSP using Ceditest® FMDV NS kit (Cedi Diagnostics BV, Lelystad, The Netherlands) and against structural proteins (SP) of FMDV serotype O (SP-O) using Ceditest® FMDV type O kit (Cedi Diagnostics BV). Briefly, Ceditest® FMDV NS kit is a blocking ELISA that detects antibodies against the non-structural 3ABC protein of FMDV of all seven serotypes, and it may be used to detect infection of vaccinated animals (Sorensen et al., 2005). Standard protocol procedures were followed according to manufacturer's instructions. Optical density values (OD) were measured with a Multiskan Ascent spectrophotometer (Thermo Labsystems Oy, Helsinki, Finland) using dual wavelengths of 620 nm and 450 nm and Ascent Software, version 2.6. Ceditest® FMDV serotype O test was also performed according to the manufacturer's instructions. For both Ceditest® kits, the results were expressed as percentage inhibition (PI) as follows;

$PI = 100 - (\text{Test serum } (OD_{450}–OD_{620})/\text{Mean negative control } (OD_{450}–OD_{620})) \times 100$

PI <50% was interpreted as negative, while a PI value of ≥50% was positive.

Based on the results of the NSP test, 108 sera from 18 farms with a history of recent clinical signs of FMD were selected for screening for antibodies against all the seven FMDV serotypes (O, A, C, Asia 1, SAT 1, SAT 2 and SAT 3) at a fixed dilution of 1/5 using an in-house SPBE system (Have and Holm Jensen, 1983; Sørensen et al., 1998; Balinda et al., 2009). In addition, two samples with PI just below the cut-off in the NSP test and four samples negative in the NSP test, but positive in the SP-O test, were examined.

Evaluation of the results was based on computation of the OD per cent (ODP), expressed as the OD value of the well including a given test sample in per cent of the mean OD value of four wells including a known negative control serum. The cut-off values varied between serotypes. Sera were considered positive if the ODP was <50% for serotypes O, SAT 1, SAT 2, SAT 3, <45% for type A and <35% for serotypes C and Asia 1 (Balinda et al., 2009).

In cases where serotype screening showed reactivity for multiple serotypes in the same herd, representative sera were 2-fold diluted from 1/5 to 1/640 for one or more serotypes as appropriate. The antibody titre was based on the last positive well in the dilution series and calculated as the reciprocal value of this dilution.

FMD Virus isolation

Virus isolation from the OP samples was adopted from the standard procedure described by the World Organisation for Animal Health (OIE, 2009). Briefly, 50 μl of undiluted sample and a 1 : 10 dilution of the sample were each inoculated into five wells of a 96-well microtitre plate with monolayers of primary bovine thyroid (BTY) cells and 100 μl of Eagles media with 2% foetal calf serum. A row with negative control wells including buffer was inserted between each sample. The cell cultures were incubated at 37°C and examined for cytopathic effect (CPE) for 2–4 days. Cultures with CPE were harvested and typed using an in-house antigen ELISA (C. Ayebazibwe, F. N. Mwiine, K. Tjørnehøj, C. Masembe, S. N. Balinda, V. B. Muwanika, A. R. Ademun-Okurut, G. J. Belsham, P. Norman, H. R. Siegismund, and S. Alexandersen, unpublished data) set up at Lindholm, Denmark, while negative cultures were frozen, thawed and passaged altogether two times onto fresh BTY monolayers. The antigen ELISA was modified from the description in the OIE Manual of diagnostics and vaccines for terrestrial animals (OIE, 2009).

Statistical analysis

Descriptive statistics were used and frequency distributions calculated (Thrusfield and Bertola, 2005). Prevalences of positive animals were determined by dividing the number of positive serum samples by the total number of samples tested. A herd was considered positive for a given serotype if one or more serum samples had antibody titres ≥160 in the serotype-specific SPBE.

Results

Screening for antibodies against NSP and SP-O

A total of 349 cattle sera were collected from 28 herds in seven districts. One of these herds (Ks12) was based

within Queen Elizabeth National Park (QENP). Two hundred and eighty-five of the sera originated from 22 herds with visible clinical signs of FMD, distributed in six districts, while the remaining 64 sera were collected from six cattle herds without clinical signs of FMDV infection in three districts (Table 1). Thirty OP samples were collected from nine herds distributed in Kasese and Mbarara districts. Lesions were acute–subacute in one of the six herds sampled in Kasese, while the remaining five Kasese herds and the three herds sampled in Mbarara districts had healing lesions. Farmer interviews and records kept at MAAIF showed that a vaccination campaign in cattle in these areas was last performed in 2005, at least seven months before the samples were collected.

The prevalence of antibodies against NSP within herds with visible clinical signs of FMD ranged between 33% and 100% (median value 84%, interquartile range 70–98%), while antibodies elicited against SP-O ranged

Table 1. Prevalence of antibodies against Foot-and-mouth disease virus (FMDV) non-structural proteins (NSP) and structural proteins of FMDV serotype O (SP-O) in cattle from 28 herds with and without clinical signs of foot-and-mouth disease (FMD) dispersed in seven districts

						FMD exposure	
District	Village	Herd no.	No. of samples tested	NSP Positive (%)	SP-O Positive (%)	Infection[e]	Vaccination[f]
Bushenyi	Kitwe	Bs6	9	9 (100)	9 (100)	Healing	+
	Kitwe	Bs5	10	9 (90)	10 (100)	Healing	+
	Rwenjeru	Bs3	11	10 (91)	11 (100)	Healing	+
	Kashozi	Bs1	10	0 (0)	0 (0)	–	–
	Busheregyenyi	Bs2	10	0 (0)	0 (0)	–	–
	Mashonga	Bs4	9	0 (0)	0 (0)	–	–
Isingiro	Kagogo	Is1	5	5 (100)	5 (100)	Acute	+
Kasese	Kahendero	Ks2[a]	6	5 (83)	6 (100)	Healing	+
	Kahendero	Ks3[a]	8	7 (88)	7 (88)	Acute–subacute	+
	Kahendero	Ks4	27	22 (81)	25 (93)	Healing	+
	Kahendero	Ks13	2	1 (50)	1 (50)	–	+
	Kahendero	Ks15	3	1 (33)	3 (100)	Subacute	+
	Kasomoro II	Ks5[a]	21	15 (71)	14 (67)	Healing	+
	Kasomoro II	Ks6	16	7 (44)	15 (94)	Healing	+
	Nyabubale	Ks7[a]	4	4 (100)	4 (100)	Healing	+
	Nyabubale	Ks8	14	13 (93)	11 (79)	Healing	+
	Nyabubale	Ks9	13	13 (100)	13 (100)	Healing	+
	Nyabubale	Ks10[a]	9	9 (100)	9 (100)	Healing	+
	Nyabubale	Ks11[a,b]	19	16 (84)	18 (95)	Healing	+
	National park	Ks12	30	19 (63)	2 (7)	Acute	–
Kiboga	Butembe	Kb1	6	4 (67)	4 (67)	Acute	+
Mbarara	Ishanyu	Mb1	8	8 (100)	8 (100)	Healing	+
	Kafunjo	Mb2[a]	21	11 (52)	18 (86)	Healing	+
	Muko	Mb3[a]	12	10 (83)	12 (100)	Healing	+
	Muko	Mb4[a,c]	10	7 (70)	10 (100)	Healing	+
Mpigi	Madu	Mp1	23	18 (78)	21 (91)	Acute–subacute	+
Kiruhura	Rurambira	Kr3	15	0 (0)	1 (7)	–	–
	Kazo	Kr4[d]	18	2 (11)	2 (11)	–	?
Total			349	225 (65)	239 (69)		
Total for herds with clinical signs of FMD			285	222 (78)	235 (82)		
Total for herds without clinical signs of FMD			64	3 (5)	4 (6)		

[a]Oropharyngeal probang samples taken for virus isolation.
[b]Isolated FMDV type O Gene Bank accession number EF611987.
[c]Isolated FMDV type O.
[d]Sera were obtained from slaughter cattle originating from Kiruhura district in a Kasese abattoir.
[e]acute–subacute, raw blisters and/or healing wounds, i.e. up to two-month-old clinical signs of FMD, observed during sampling; healing, healed wounds, i.e. 2- to 4-month-old clinical signs of FMD, observed during sampling; –, no clinical signs of FMD observed during sampling.
[f]+, vaccinated with FMD vaccines against serotypes O, SAT 1 and SAT 2 at least 7 months before the sampling; –, no previous vaccination; ?, vaccination history not known.

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between 7% and 100% (median value 100%, interquartile range 88–100%) (Table 1). Nineteen of the 22 farms with visible clinical signs of FMD had comparable and high prevalences of antibodies elicited against NSP and SP-O, while two Kasese farms (Ks6 and Ks15) had substantially higher prevalence of antibodies against SP-O than against NSP, and the reverse was found on one Kasese farm (Ks12).

Herds without visible clinical signs of FMD showed much more sporadic presence of antibodies against NSP and SP-O with prevalences ranging between 0% and 50% (medians 0% and 3.5%, respectively, and interquartil ranges 0–8.25% and 0–10%, respectively) (Table 1). In Kiruhura district, two of 18 cattle sera from Kazo County were positive for antibodies against both NSP and SP-O, and one serum from Rurambira tested positive for antibodies against SP-O, while in Kasese district, one of two serum samples from Kahendero village was positive in both SP and NSP tests. All sera from three herds in Bushenyi district were negative in both NSP and SP-O ELISAs. Altogether herds without a history of a recent FMD outbreak had much lower prevalences of antibodies against NSP and SP-O than herds recovering from FMD outbreaks. As farmers in these districts are known culturally to receive and/or exchange animals as gifts, this may have lead to acquisition of these positive animals on the farms.

Screening and titration in serotype-specific antibody ELISAs

Representative sera positive in the NSP-ELISA from 18 of the 22 herds with visible clinical signs of FMD were further screened in the SPBEs. In addition, two samples with PI just below the cut-off in the NSP test and four samples negative in the NSP test, but positive in the SP-O test, were examined. For each of the 18 investigated herds, 28–80% of the sera were tested in at least one of the SPBEs. When screened in SPBE at dilution 1/5, altogether 88% tested positive in the O SPBE, 73% in the SAT 1 SPBE, 58% in the SAT 2 SPBE and 93% in the SAT 3 SPBE, while the numbers of sera testing positive in the A, C and Asia 1 SPBEs were 22%, 24% and 10%, respectively (Table 2).

Titration of representative SPBE screening–positive sera in the relevant antibody ELISAs and using cut-off of ≥160 eliminated reactions in the serotype A, C and Asia 1 SPBEs. A number of sera with titres above this cut-off were identified in the SPBE for serotypes O (38%, 22/58), SAT 1 (11%, 5/45), SAT 2 (2%, 1/49) and SAT 3 (9%, 7/79) (Table 3).

Our data show that investigated sera from nine herds in the three districts Bushenyi (Bs3, Bs5, Bs6), Kasese (Ks7, Ks8, Ks9, Ks10) and Mbarara (Mb3, Mb4) only had high serotype-specific titres against serotype O(≥80), while sera from seven herds in the four districts of Isingiro (Is1), Kasese (Ks4, Ks6, Ks11, Ks12), Kiboga (Kb1) and Mpigi (Mp1) had high antibody titres (≥160) to more than one FMDV serotype, including O, SAT 1, SAT 2 and SAT 3 (Table 4).

Antibody titres of 160 and above against serotype O were detected in 14 of these 16 herds, while the only herd located within a national park, QENP in Kasese district (Ks12), had evidence of antibodies against serotypes SAT 1 and SAT 3, but not against serotype O.

Seven of the 21 investigated herds had serotype-specific antibody titres of ≥160 against more than one serotype (Table 4). Thus, sera from five of these herds either had highest antibody titres against serotype O or had not been titrated for antibodies against serotype O (Is1, Ks6, Ks11, Kb1 and Mp1), while one herd had clear evidence of exposure to two serotypes, O and SAT 1 (Ks4) Ks12 differed from the other herds, as it only had evidence of exposure to SAT 1 and SAT 3. Ks12 differed from the other herds, as it only had evidence of exposure to SAT 1 and SAT 3. Sera from the two herds Mb4 and Ks11 where FMDV serotype O was isolated had high antibody titres of 80 and 160, respectively, against serotype O. This level of antibodies was also found on the seven OP-sampled

Table 2. Screening of sera for serotype-specific antibodies against Foot-and-mouth disease (FMDV)

District	No. of sera	Proportion of positive sera per serotype						
		O	A	C	Asia 1	SAT 1	SAT 2	SAT 3
Bushenyi	13	13/13	2/10	1/13	0/10	5/9	3/10	10/13
Isingiro	4	4/4	0/3	1/4	1/3	2/2	2/3	4/4
Kasese	75	61/75	14/68	10/45	3/39	53/70	42/72	69/73
Kiruhura	2	1/1	0/0	0/2	0/0	1/1	0/0	0/0
Kiboga	1	1/1	0/0	1/1	0/0	1/1	1/1	1/1
Mbarara	14	14/14	4/10	3/12	1/6	5/10	7/11	11/12
Mpigi	5	5/5	1/5	4/5	1/5	5/5	4/5	5/5
Total	114	100/114 (88%)	21/96 (22%)	20/82 (24%)	6/63 (10%)	72/98 (73%)	59/102 (58%)	100/108 (93%)

Sera were screened in a dilution of 1 : 5 in SPBE.

Table 3. Titration of serotype-specific antibodies against seven Foot-and-mouth disease (FMDV) serotypes

Titre*	O	A	C	Asia 1	SAT 1	SAT 2	SAT 3
<80	19/58	15/15	8/8	2/2	37/45	44/49	67/79
≥80, <160	17/58	0/15	0/8	0/2	3/45	4/49	5/79
≥160	22/58	0/15	0/8	0/2	5/45	1/49	7/79

*Expressed on $\log_{10}$ and cut-off ≥160.
Sera were titrated in serotype-specific SPBE for the seven FMDV serotypes to enable differentiation between true reactivity and cross-reactivity.

farms, where FMDV could not be isolated. Age of lesions was comparable on OP-positive and six of the seven OP-negative farms.

Probang samples

Oropharyngeal probang samples were obtained from nine cattle herds as indicated by ([a]) in Table 1. Only two of thirty OP samples collected from the districts of Kasese and Mbarara were positive for FMDV by culture. Antigen ELISA showed that these viruses belonged to serotype O. A nearly complete genome sequence of one of these samples can be accessed in the Gene Bank accession number EF611987.

Discussion

The observed high prevalences of 78% (222/285) for antibodies against NSP and 83% (235/285) for antibodies against SP-O in the herds with recent clinical signs of FMD confirm that the majority of the animals in these herds had been infected by FMDV. The proportion of cattle positive for antibodies against NSP and SP-O in herds with no recent clinical signs of FMD was 5% (3/64) and 6% (4/64), respectively, which could be remaining antibodies from previous vaccinations and/or infections or in some cases could probably be from animals acquired as gifts or for improving the herds in the area.

In the majority of herds, prevalence of antibodies against SP-O was equal to or greater than that of antibodies against NSP. Nine of 16 herds with 2–4-month-old lesions and two of six herds with acute–subacute lesions had more animals positive in the SP-O ELISA than in the NSP-ELISA. For the herds with older lesions, this may indicate faster decline of antibodies against NSP than against structural proteins, while in herds with fresh lesions it probably reflects the delayed onset of antibodies against NSP (Sorensen et al., 2005). In this study, prevalences of antibodies against NSP was very high compared to a study by Gelaye et al. (2009), which showed prevalences of 12.08% ($n = 273$) of antibodies against FMD NSP in cattle in Ethiopia. The recurring use of non-purified vaccines in cattle in Uganda might have increased the prevalence of antibodies against NSP in the tested sera, because previous studies (Sutmoller et al., 2003) have shown that repeated vaccinations with non-purified vaccines would raise antibodies against NSPs without the presence of FMDV infection.

Most herds sampled in Kasese and the four herds sampled in Mbarara districts had had clinical signs of FMD within the preceding 2–4 months of the sampling and had high levels of antibodies against NSP and SP-O, confirming that the districts had suffered from a widespread outbreak of FMD. Similarly, high prevalences of antibodies against NSP and SP-O in herds with a history of recent clinical signs of FMDV in Mpigi, Kiboga and Isingiro districts confirm that these districts were involved in the 2006 outbreak; however, because of the limited number of herds sampled, it is not possible to conclude on the extent of the FMD outbreak in these districts.

The Bushenyi-outbreak reported in this paper was geographically small, identified early and immediately contained. The investigation into this outbreak showed that it was most likely caused by farmers in the villages of Kitwe and Rwenjeru who introduced cattle bought from Isingiro district where FMD had been reported to MAAIF. The general freedom from FMD in this area may be attributed to the highly organized dairy farming system, which is based on good farming practices including fencing, sufficient income to allow veterinary assistance and awareness of contagious diseases.

The demonstration of high antibody titres against FMDV serotype O in eight cattle herds from Bushenyi, Mbarara and Kasese districts, coupled with a wide geographical distribution in villages and districts, and the recovery of serotype O virus (Gene Bank accession number: EF611987) from two probang samples from Kasese and Mbarara districts, together indicate that the major 2006 outbreak in southern Uganda was because of a FMD serotype O virus. High antibody titres of ≥320 against FMDV serotype O in sheep and goats sampled in parallel with the cattle in Kasese district (Balinda et al., 2009) confirm that this outbreak was indeed widespread in the investigated region, and that it affected other species than cattle. The very low recovery rate of the virus despite healing lesions suggestive of FMDV was probably due to long transportation times for samples and difficulties in maintaining the cold chain, during both transportation and storage.

According to MAAIF records (2006), previous FMD outbreaks had occurred in Kasese district in May 2005 and April 2006 and the situation was not any different in the other five districts (Kiboga, September 2004 and July 2005; Isingiro, March 2006; Mbarara, December 2004;

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Table 4. Serotype-specific antibodies against Foot-and-mouth disease (FMDV) serotypes in sera from selected cattle farms

Herd no.	Number of sera tested	Titres of antibodies towards serotypes				Conclusion on titration data	FMDV exposure	
		O	SAT 1	SAT 2	SAT 3		Infection[a]	Vaccination[b]
Bs3	3	80	nd	–	10	O	Healing	+
		320	p	20	20			
		320	–	nd	P			
Bs5	3	80	5	–	5	(O)	Healing	+
		40	10	–	–			
		40	nd	p	P			
Bs6	3	160	20	5	20	O	Healing	+
		160	5	–	10			
		160	–	–	5			
Is1	3	320	20	40	40	O, SAT 3	Acute	+
		p	nd	nd	160			
		P	p	80	40			
Ks2	3	160	–	–	10	O (SAT 3)	Healing	+
		p	p	–	80			
		20	10	–	10			
Ks3	3	40	p	10	80	(O, SAT 3)	Acute–subacute	+
		80	20	10	40			
		p	10	–	–			
Ks4	11	80	160	–	20	O, SAT 1	Healing	+
		P	10	5	40			
		20	–	–	20			
		80	–	–	5			
		80	P	80	5			
		P	–	5	20			
		P	10	–	40			
		160	20	5	10			
		320	20	5	20			
		20	nd	nd	10			
		40	20	10	40			
Ks5	6	P	10	5	20	O (SAT 1)	Healing	+
		80	80	5	40			
		P	20	–	10			
		80	5	5	40			
		P	40	–	20			
		160	40	20	P			
Ks6	7	P	160	–	10	O, SAT 1	Healing	+
		160	5	5	10			
		20	20	10	P			
		40	40	5	20			
		80	10	–	20			
		P	5	10	10			
		160	p	P	10			
Ks7	3	80	–	–	40	O	Healing	+
		160	10	5	20			
		20	–	–	10			
Ks8	4	40	5	–	P	O	Healing	+
		80	–	–	10			
		P	5	–	5			
		160	–	5	20			
Ks9	4	20	–	5	10	O	Healing	+
		40	10	–	10			
		160	5	–	10			
		160	5	–	10			

Table 4. Continued

Herd no.	Number of sera tested	Titres of antibodies towards serotypes				Conclusion on titration data	FMDV exposure	
		O	SAT 1	SAT 2	SAT 3		Infection[a]	Vaccination[b]
Ks10	4	80	–	5	10	(O)	Healing	+
		P	–	–	10			
		40	–	–	10			
		80	–	–	5			
Ks11	9	80	20	–	20	O, SAT 3	Healing	+
		20	10	40	20			
		40	–	10	5			
		160	p	–	5			
		P	40	10	160			
		P	40	20	40			
		P	40	5	40			
		160	nd	P	20			
		–	p	40	20			
Ks12[c]	3	–	320	20	10	SAT 1, SAT 3	Acute	–
		–	5	80	160			
		–	5	40	160			
Kb1	1	320	80	160	80	O, SAT 2	Acute	+
Mb1	1	320	80	40	80	O (SAT 1, SAT 2)	Healing	+
Mb2	1	20	P	P	10	?	Healing	+
Mb3	3	80	10	–	20	(O)	Healing	+
		80	–	–	5			
		P	nd	20	10			
Mb4	3	20	–	–	–	(O)	Healing	+
		80	–	–	5			
		40	nd	nd	P			
Mp1	4	320	p	20	160	O, SAT 3, SAT 1	Acute–subacute	+
		640	160	80	320			
		P	160	40	160			
		320	P	40	40			

[a]acute–subacute, animals in herd with raw blisters to healing wounds up to two-month-old clinical signs of foot-and-mouth disease (FMD) during sampling; healing, animals in herd with healed wounds of 2- to 4-month-old clinical signs of FMD during sampling; –, herds without clinical signs of FMD during sampling.
[b]+, vaccinated with FMD vaccines against serotypes O, SAT 1 and SAT 2 at least 7 months before the sampling; –, no previous vaccination.
P, strong positive in dilution 1/5 (OD ≤ 20); p, weak positive in dilution 1/5 (OD ≥ 40,<50).
[c]only herd sampled within a national park, QENP.
(–) negative in dilution 1/5.
nd, not done.

Kiruhura, December 2004; Mpigi, March 2006; and Bushenyi, May 2006). In a 2004 FMD outbreak in Kiboga district, VP1 sequencing on material collected in a slaughterhouse identified a SAT 2 virus (Balinda et al., 2010). The high titres of antibodies against SAT 2 (160) and O (320) in the one investigated animal from Kiboga district could be as a result of the 2004 and 2006 outbreaks or ongoing outbreaks with either of these serotypes. The lack of high antibody titres against SAT 2 in all other herds signals that the SAT 2 outbreak in 2004 was not widespread.

Herd Ks 12 located in QENP in Kasese district had much higher prevalence of antibodies against NSP (19/30) than against SP-O (2/30), which may indicate that this outbreak was not caused by a serotype O virus. Further analysis of sera from this herd (Ks12) in the SPBE demonstrated antibodies against SAT 1 and SAT 3. The data clearly show that this herd had escaped the outbreak of FMDV serotype O in the district until the time of sampling, and that it had not been vaccinated with the trivalent vaccine including serotype O. The cattle in this herd were grazing freely with buffaloes in QENP at the time of sampling, and it is likely that the source of this exposure was the buffalos in the park.

Direct contact between cloven-hoofed animals and buffaloes is a common occurrence all over sub-Saharan Africa (Vosloo et al., 2002). Buffaloes are known maintenance hosts of FMD (Hedger, 1972; Sutmoller et al., 2000;

Kalema-Zikusoka et al., 2005, Bronsvoort et al., 2008). Previous studies (Kalema-Zikusoka et al., 2005), as well as more recent work (C. Ayebazibwe, F. N. Mwiine, K. Tjørnehøj, C. Masembe, S. N. Balinda, V. B. Muwanika, A. R. Ademun-Okurut, G. J. Belsham, P. Norman, H. R. Siegismund, and S. Alexandersen, unpublished data), indicate high prevalence of FMD antibodies in the buffalo population in QENP.

The districts investigated in this study are regularly involved in FMD outbreaks, and it cannot be excluded that some of the sampled animals were vaccinated with combined O, SAT 1 and SAT 2 vaccines during 2005 or the preceding years. However, vaccinations had most likely not been carried out for at least 7 months preceding the sampling, and it is likely that the antibodies elicited by such vaccinations though present would be of low titre at the time of sampling. Hence, because of the identified high antibody titres towards serotype O and the isolation of FMDV serotype O virus, we still believe that it is reasonable to identify the 2006 outbreak strain as serotype O based on the presented data. Identified titres and antibody reactions (antibody titres ≤ 80) towards other serotypes could either be residual activity from past infections or from vaccinations.

It has previously been shown that cross-reactions are not uncommon between serotype-specific ELISAs for FMDV (Mackay et al., 2001), and analysis of sera from controlled experiments also indicates that this is most likely the case for at least the SPBEs for antibodies towards SAT 3 and Asia 1 used in this study (Tjørnehøj, unpublished observations). The cross-reactivity was most likely due to the history of repeated vaccinations with bi- and trivalent vaccines and presumably repeated infections. This diagnostic challenge may be solved by decreasing the time from reporting of FMD outbreaks to sampling and by focusing the sampling on animals without previous vaccination and without maternal antibodies (4–10-month-old) and animals with acute clinical signs of FMD.

The complications to serological diagnosis of FMD in Uganda are probably due to vaccinations carried out to control ongoing disease outbreaks, and it is imperative for the success of such control measures that the serotype of new outbreaks are readily identified. In the future, emphasis should be put on recovering the involved virus as soon as the outbreak is reported.

The results of this study show high prevalence of antibodies to FMDV serotypes O and SAT 1, SAT 2 and SAT 3 in cattle in seven investigated districts in southern and western regions of Uganda. However, to characterize the circulating FMDV serotypes, more systematic epidemiological and molecular studies need to be carried out not only in cattle but more so in small ruminants and buffaloes at the wildlife–livestock interface to ascertain their roles in the regular FMD outbreaks in Uganda. If wildlife–livestock interaction is proven to play a role in the epidemiology of FMD in Uganda, participatory sensitization of wildlife officials and cattle owners grazing their cattle in the national parks about the potential risk of transmission of FMDV from cattle to wildlife or vice visa should be carried out. Carrier endemic status should be evaluated in districts where FMD outbreaks are frequent. Therefore, for Uganda to properly control FMD, there is a need to invest in scientific valid epidemiological data to help devise a national strategic plan, including re-evaluation of vaccines used and the role of wildlife animals in the transmission and maintenance of FMD.

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