Transboundary and Emerging Diseases

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ORIGINAL ARTICLE

Prevalence Estimates of Antibodies Towards Foot-and-Mouth Disease Virus in Small Ruminants in Uganda

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Summary

Foot-and-mouth disease (FMD) is endemic in Uganda with control strategies focusing on vaccination of cattle, while small ruminants are largely ignored. In order for Uganda to establish effective control strategies, it is crucial that the epidemiology of the disease is fully understood. This study summarizes results of serological investigations of sheep and goats for antibodies to FMDV from four districts in 2006 following an FMD outbreak in the region and from an attempted comprehensive random sampling in two districts in 2007. Antibodies were quantified and serotyped using competitive ELISA for antibodies towards non-structural proteins (NSP) and structural proteins towards serotype O, and blocking ELISA for antibodies towards the seven serotypes of FMD virus (FMDV). In 2006, sheep and goats in Bushenyi and Isingiro districts were free from antibodies towards FMDV, while herds in Kasese and Mbarara districts excluding Kahendero village were all positive for antibodies towards NSP and SP-O. In 2007, mean prevalence estimates of antibodies towards FMDV NSP was 14% in goats and 22% in sheep in Kasese district, while Bushenyi was still free. The difference between these two districts probably reflects different levels of FMDV challenge attributed to the variation in exposure rates which again in part may be as a result of the differing husbandry practices. Contrary to 2006, with clear antibodies towards serotype O, the serotype-specificity of the antibodies was less clear in 2007, as antibodies towards both serotype O and SAT serotypes were identified. Our results show that goats and sheep are infected during FMD outbreaks, and that they may be useful for determining the serotype of FMD outbreaks in Uganda, if they are sampled shortly after an outbreak.

Introduction

Foot-and-mouth disease (FMD) is one of the most important livestock diseases worldwide because of its economic impact (James and Rushton, 2002; Chenard et al., 2003). It affects many species including cattle, sheep, goats and pigs, and results in reduced productivity (Alexandersen and Mowat, 2005). This is mainly through reduced milk yields, loss of weight, abortions and delayed conception, perinatal mortality and lameness in draught animals (James and Rushton, 2002). The severity of the disease depends on the virus strain and the type of animal affected (Geering, 1967; Dunn and Donaldson, 1997; Kitching and Hughes, 2002). Usually cattle and pigs are more severely affected while the disease has less impact in goats and sheep (Donaldson and Sellers, 2000), however,

some strains can cause severe disease in small ruminants (Alexandersen and Mowat, 2005; Barnett and Cox, 1999; Kitching et al., 2005).

In addition to the loss of income to farmers as a result of restrictions on animal movement, local purchase and sale, the gross domestic income of the affected countries is greatly reduced because of loss of revenue attributed to heavy restrictions on trade of animals and animal products with FMD-free countries (Thompson et al., 2002). This can ultimately result in restrictions on export of other products such as vegetables and fresh fruit (James and Rushton, 2002; Kock et al., 2002). In sub-Saharan Africa, FMD is endemic and the constant heavy restrictions on exports undermine poverty alleviation programs put in place to improve the livelihood of the population (Kock et al., 2002).

In an effort to reduce and/or eradicate the disease with the objective of participating in animal product export, FMD endemic countries have embarked on several control measures. Within Africa, South Africa adopted the zoning system which comprises of a control zone, a buffer zone and a free zone (Brückner et al., 2002). Similarly, Zimbabwe employed the zoning system with success in the 1970s until the 1990s, and this resulted in exports to the international market including the European Union (Anderson et al., 1993; Rweyemamu et al., 2008).

In Uganda, vaccination of cattle coupled with zoosanitary measures such as animal movement restrictions, quarantine and restriction in animal product trade during FMD outbreaks are carried out but with some limited success. FMD is still a problem with 25–38 outbreaks reported annually between 2000 and 2006 as opposed to 1–15 between 1996 and 1999 (Anonymous, 2006). As in most countries in the sub-Saharan region, in Uganda, small ruminants are not included in FMD virus (FMDV) control strategies/measures. Currently, only cattle are vaccinated to limit the spread of FMD outbreaks. This is mainly because the disease is perceived as not affecting small ruminants because of the subclinical nature of the disease in sheep and goats. However, the role of small ruminants in maintaining FMD epidemics has over time attracted more attention, and it has been established that sheep and goats can also become carriers for 9 and 4 months respectively (Zhang and Kitching, 2001; Blanco et al., 2002; Kitching, 2002).

In Uganda, FMD outbreaks have mainly been reported in cattle and the major serotypes involved have been O and SAT 2. Other serotypes recorded in the past have included A, C, SAT 1 and SAT 3. Type C was last recorded in the 1970s while SAT 3 has been isolated only in the African Buffalo sampled in Queen Elizabeth National Park (QENP) in 1970 and 2005 (Vosloo et al., 2002; Kalema-Zikusoka et al., 2005).

The keeping of livestock is a key agricultural activity in Uganda with the majority of the animals being grazed under agro pastoral systems which involve extensive livestock intermingling through communal grazing resulting in easy disease spread. These areas also harbour large FMD susceptible wildlife populations. However, in some districts modern agriculture practices that involve fencing have been adopted thereby limiting animal mixing which could have a positive impact in reducing disease spread.

This study was aimed at determining the prevalence of antibodies against FMD in small ruminants in an area that experienced FMD outbreaks in cattle and attempts to assess the influence of husbandry practices on the distribution of FMD in Uganda.

Materials and Methods

The study area in Uganda

A total of four districts; Bushenyi, Isingiro, Kasese and Mbarara in the south-western and western regions of Uganda, with three of these districts having experienced recent outbreaks of FMD in cattle, were studied (Fig. 1). In Bushenyi district, the predominant husbandry practice involves having small ruminants tethered within paddocks, grazing alongside cattle. In some cases, the goats and sheep are tethered close to the households. The most common cattle breed in the area is the Friesian type. Despite the close proximity to QENP, the livestock and

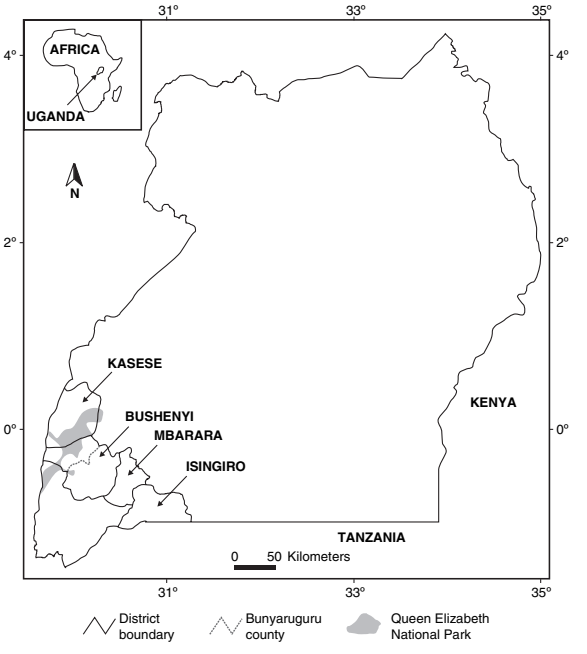

Fig. 1. Map of study area. The study took place in the South-Western and Western regions of Uganda, and included four districts; Bushenyi, Isingiro, Kasese and Mbarara.

wildlife populations generally do not mix. This is achieved through a buffer area (Bunyaruguru) inhabited by crop cultivators.

On the other hand, communal grazing is predominant in Kasese district with almost all the livestock (cattle, goats and sheep) allowed to graze in QENP in close proximity to the African buffalo, wild antelope and other FMD susceptible animal species. Local breeds of cattle like the Ankole comprise the majority of the stock. A few fenced farms exist in the district.

In addition, Isingiro and Mbarara districts, which are part of Greater Mbarara, were also studied. Fenced farming is the principal farming type in Mbarara district while communal grazing is predominant in Isingiro district, particularly close to the Tanzanian border. Friesian and Ankole breeds comprise the animal stock in this region. The livestock populations (Anonymous, 2005) in the respective districts are summarized in Table 1.

The sampling strategy

In 2006, 26 serum samples were obtained from asymptomatic goats and sheep in Kasese and Mbarara following FMD outbreaks in cattle in both districts, and five goat serum samples were obtained from Isingiro district during an active FMD outbreak with the majority of the cattle on the farms clinically affected, while the goats were asymptomatic. An additional 15 serum samples were obtained from goats in Bushenyi district because of close proximity to affected districts.

In 2007, a simple comprehensive random study was attempted in Bushenyi and Kasese districts. Survey Toolbox program (Cameron, 1999) was used to compute the numbers required for a two stage random sampling of the respective districts, however, because of limited number of animals, particularly the sheep, together with weather and time restraints, it was not possible to sample the calculated number of villages and animals during the actual field sampling. Instead the sampling included 1–11 goats on 26 farms in 15 villages distributed in Ryeru, Kichwamba, Bumbaire, Kyeizoba, Mutara, Mitooma, Kagango and Kitagata sub-counties of Bushenyi and 2–20 goats on 14 farms in seven villages distributed in Karusandara, Kisinga, Lake Katwe, Muhokya, Mukunyu and Nyakiyumbu sub-counties in Kasese. Similarly for sheep, in Bushenyi the sampling comprised 1–4 sheep on seven farms in four villages distributed in Ryeru, Bumbaire, Kyeizoba and Kitagata sub-counties and 2–15 sheep on seven farms in five villages distributed in Bukunyu, Karusandara, Kisinga, Lake Katwe and Muhokya sub-counties of Kasese. Altogether, 346 sera were collected from about 31% of the desired number of animals on about 15% of the expected farms in about 73% of the targeted villages.

Table 1. Livestock populations in the study area population size of the different livestock species estimated by the Ministry of Agriculture Animal Industry and Fisheries in 2005 (Anonymous, 2005)

	Animal species		
District	Cattle	Goats	Sheep
Bushenyi	160 000	60 000	23 000
Kasese	55 000	25 000	2000
Mbarara	807 000	573 000	47 000

At the time of sampling, there was no FMD outbreak and all animals sampled were not clinically affected. None of the sampled sheep and goats had previously been vaccinated against FMD. With the exception of two farms out of 26 (7%) in Bushenyi and three farms out of 14 (21%) in Kasese, the farms visited in the two districts also had cattle. Unlike the small ruminants, the majority of the cattle in Kasese area are vaccinated against FMD as a control measure following frequent outbreaks of FMD. The vaccines used during 2005–2007 include serotypes O, SAT 1 and SAT 2.

Laboratory Methods

Measurement of antibodies against the non-structural proteins (NSP) of FMDV by NSP ELISA

This assay was performed using a commercially available kit, Cedi® FMDV NS ELISA kit (Prionics, Zurich, Switzerland) as described by the manufacturer. Briefly, 96 microtitre plates pre-coated with recombinant 3ABC protein were set up with test sera in single wells, and duplicates of negative control serum and two positive control sera, all at a 1 : 5 dilution. After overnight incubation at room temperature (20–25°C), the plates were washed, and conjugate comprising MabL74D5 linked to horseradish peroxidase was added and incubated for 1 h at room temperature. After washing, substrate [tetramethyl benzidine (TMB) and H_2O_2] was added and the reaction stopped after 15 min with 0.5 M H_2SO_4. Optical density (OD) was measured at 450 and 620 nm after 15 min on a spectrophotometer (Thermo Electron Corporation/Thermo Fisher Electron, Waltham, MA, USA), and results were expressed as a percentage of the mean negative control; $[(OD_{450} - OD_{620}\ \text{sample})/OD_{\text{mean negative control}}] \times 100$. Sera with ODP ≤ 50% were scored as positive (Sorensen et al., 1998, 2005).

Measurement of antibodies against the structural proteins of FMDV serotype O (SP-O) by SP-O ELISA

The Cedi® FMDV type O ELISA kit (SP-O ELISA) is a ready to use commercial kit currently from Prionics and the manufacturer's instructions were followed. In brief, reference sera 1–4 (provided with the kit and used in

duplicate) and samples (dilution 1 : 5) were added to 96 well microtitre plates pre-coated with inactivated FMDV O_1 Manisa (approximately 80 ng/well) and incubated for 1 h at room temperature (20–25°C). After washing, the plates were incubated with horseradish peroxidase-conjugated monoclonal antibody (MAb99), which recognizes an epitope on VP1-protein of FMDV type O, for 1 h at room temperature. Then the plates were washed and incubated with substrate (TMB and H_2O_2) for 15 min. Finally the reaction was stopped by adding 0.5 M H_2SO_4. The plates were read on a spectrophotometer (Thermo Electron Corporation) at 450 and 620 nm, and the results were expressed as a percentage of the OD of the mean negative control as follows;

$$((OD_{450} - OD_{650}\text{sample})/\text{ODmean negative control}) \times 100$$

Sera with ODP ≤ 50% were positive (Chenard et al., 2003).

Measurement of antibodies by serotype-specific solid phase blocking ELISA

Sera positive in either the NSP ELISA or SP-O ELISA kits were subsequently screened in serotype-specific solid phase blocking ELISA developed at the Department of Virology of the National Veterinary Institute of the Technical University of Denmark (Lindholm).

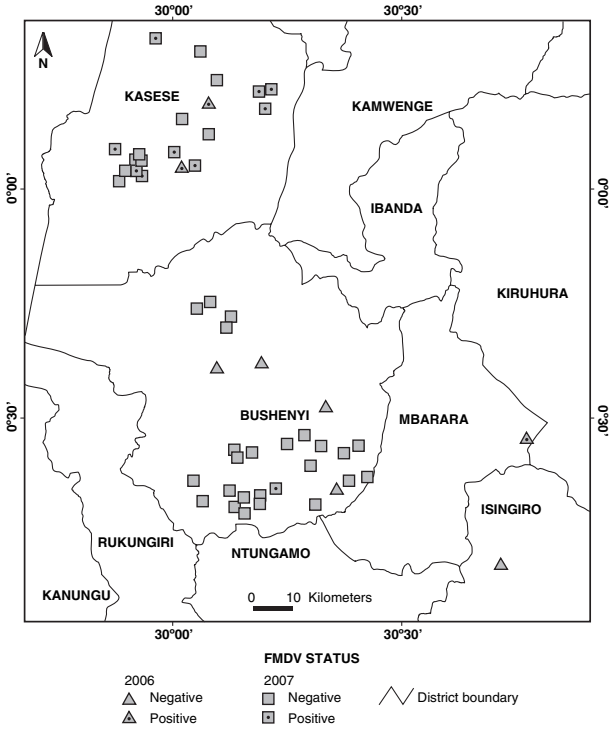

Fig. 2. Location of farms sampled during the sampling in 2006 and the attempted comprehensive random sampling in 2007. The sampling in 2006 (triangles) included Bushenyi, Isingiro, Kasese and Mbarara districts, while the attempted comprehensive random sampling in 2007 (squares) included Kasese and Bushenyi Districts. Location of infected farms (dots) and uninfected farms (plain) are depicted.

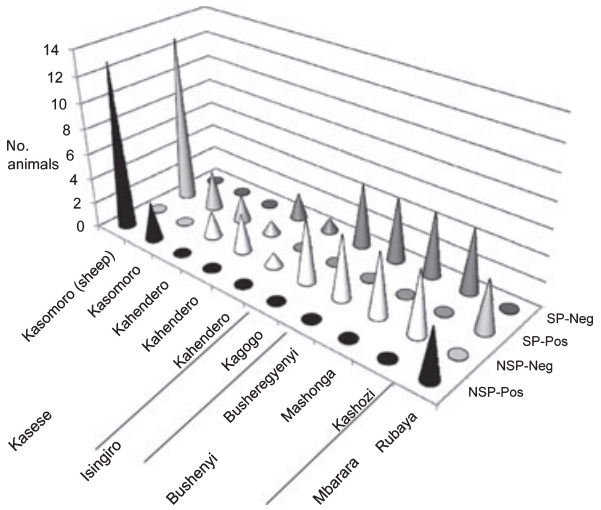

Fig. 3. Result of 2006 sampling in Bushenyi, Isingiro, Kasese and Mbarara districts. Number of small ruminants positive and negative in NSP and SP-O ELISA at farm and village level.

The FMD virus strains O Manisa, A Iraq 96, C Noville, Asia 1 Shamir, SAT 1 (BOT 1/68), SAT 2 (ZIM 5/8) and SAT 3 (ZIM 4/81) were kindly provided by WRL, Pirbright, UK, and propagated in primary or secondary calf kidney (CK) cells or baby hamster kidney (BHK) cells at Lindholm. Virus harvests were inactivated using ethylenimine and used as antigens.

Guinea pig and rabbit immune sera towards O Manisa, A Iraq 96, C-Turup/Denmark/61 (a Danish FMDV strain) and Asia 1 Shamir were produced at Lindholm, while guinea pig and rabbit sera towards SAT 1 (BOT1/68), SAT 2 (ZIM 5/8) and SAT 3 (ZIM 4/81) were purchased from WRL, Pirbright, UK.

All incubations were at room temperature (20–25°C), and plates were washed three times between steps using ELISA buffer (0.015 M Na_2HPO_4, 0.0025 M KH_2PO_4, 0.5 M NaCl, 0.05% Tween-20), except the last wash step which consisted of five rinses. Serotypes were run on separate plates, and plates were coated with serotype-specific guinea pig sera diluted at an optimum dilution in coating buffer (0.035 M $NaHCO_3$, 0.015 M $Na_2CO_3 \cdot 10H_2O$) for 1 h and subsequently reacted with the corresponding antigen diluted in ELISA buffer for 1 h.

Each plate included four wells with 10% negative calf serum, two wells with a weak positive control serum and two wells with a strong positive control serum from animals vaccinated with monovalent vaccines.

For serotype screening, test and control sera were added to the plates in 1/5 dilution in ELISA buffer with 10% normal calf serum (NCS) and 0.05% NaN_3. Following an

overnight incubation, serotype-specific rabbit antisera at an optimum dilution in ELISA buffer with 10% NCS were added to the appropriate plates and incubated for 1 h. Horseradish-peroxidase conjugated to swine anti-rabbit IgG antibody (Dakopatts P0217) diluted 1/1000 in ELISA buffer with 10% NCS and 1% normal guinea pig serum, was added and incubated for 30 min. The ELISAs were completed using substrate, sulphuric acid and calculation of OD% as described for the NSP/SP-O methods above. Cut-offs varied between serotypes: OD% ≤ 50% were considered positive in the tests for serotypes O, SAT 1, SAT 2 and SAT 3, while OD% ≤ 45% were positive in the serotype A-test and OD% ≤ 35% were positive in the serotype C and Asia 1 tests (Have and Holm-Jensen, 1983; Sorensen et al., 1992).

Positive samples for each of the seven serotypes were then titrated by 2-fold dilution in the same serotype-specific tests, and titres were recorded as the reciprocal of the last positive dilution.

Titres ≥40 were considered positive in non-vaccinated animals like the sheep and goats, while values <40 were considered cross reaction (in presence of high titres to other serotypes) or evidence of historical infection (in absence of high titres to any other serotypes).

Statistics

Software packages employed in data analysis included STATA 8 (State Corp., College Station, TX, USA) for the overall number of antibody-positive goats and sheep in Bushenyi and Kasese districts.

Results

FMDV antibodies found in sheep and goats following the 2006 FMD outbreaks in cattle in four districts

Figure 2 summarizes the distribution of infected and uninfected farms for both the 2006 and 2007 samplings. The distribution of infected and uninfected animals per farm in villages of Bushenyi, Isingiro, Kasese and Mbarara districts during the FMD outbreak in 2006 are summarized in Fig. 3. In Kasese district, all sheep (13/13) and goats (3/3) sampled in Kasomoro village were positive for antibodies towards both SP-O and NSP of FMDV, and in Kahendero village, two out of three farms had goats with positive reactions in the SP-O ELISA (2/2 and 1/3). In Rubaya of Mbarara district, all the goats (4/4) were positive in both the SP-O and the NSP ELISA. Sampling in Kagogo village in Isingiro district took place during an active FMD outbreak featuring acute lesions in cattle, however, there were no clinical signs of FMD in goats, and all (5/5) sampled goats were negative in both the SP-O and the NSP ELISA. Similarly, the goats from Mashonga, Busheregyenyi and Kashozi villages in Bushenyi district were negative, according well with no reported cases of FMD in these villages prior to the sample collection.

Serotype-specific antibodies against FMDV detected in sheep and goats from Kasomoro village in Kasese during the 2006 post-outbreak sampling are summarized in Table 2.

Nine of ten goats and sheep had antibodies towards serotype O with titres ranging from 80 to 1280 and a geometric mean value of 549. Lower reactions towards serotypes A, C, Asia1, SAT 1, SAT 2 and SAT 3 were recorded in three, one, zero, four, two and six of the 10 tested goats and sheep respectively.

Prevalence estimates for antibodies towards FMDV in Bushenyi and Kasese districts in the 2007 attempted comprehensive random sampling

Bushenyi

All 146 serum samples collected from goats and sheep in 15 villages in Bushenyi district were negative for antibodies towards NSP, and only one goat from Katunda village was

Table 2. Post-outbreak sampling in Kasese District in 2006: serotype-specificity of antibodies towards foot-and-mouth disease virus (FMDV) detected in goats and sheep in Kasomoro village

Species	O	A	C	Asia 1	SAT 1	SAT 2	SAT 3	Conclusions
Goats	80	–	5	–	–	–	–	O
	320	–	–	–	40	160	160	O and SAT-serotypes
	640	10	–	–	10	–	–	O
Sheep	1280	–	–	–	10	10	20	O
	1280	–	–	–	–	–	40	O
	640	20	–	–	–	–	–	O
	640	–	–	–	–	–	–	O
	640	–	–	–	–	–	10	O
	640	–	–	–	10	–	40	O
	–	10	–	–	–	–	40	SAT 3

Sera with antibodies towards non-structural proteins were screened for serotype-specific antibodies towards the seven FMDV serotypes. Sera from all positive goats and seven of the 13 sheep were titrated in the relevant serotype-specific ELISA. (–) negative at serotype screening in 1/5 dilution.

found positive for antibodies towards SP-O (data not presented). Screening and titration of this sample as described above identified antibodies to SAT 1 with a titre of 160.

Kasese

Estimates of prevalences of antibodies towards NSP in Kasese was 14% [95% confidence interval (CI): 10–18%] in 143 serum samples from goats and 22% (95% CI: 12–32%) in 56 serum samples from sheep. At farm level, prevalence estimates of the goats and sheep were as summarized in Table 3.

Animals belonging to three fenced farms in three Kasese villages (Busunga, Rwentutu and Rwembyo) were negative for antibodies towards NSP. For comparison, in Busunga, a high proportion of communally reared goats and sheep were positive in the NSP ELISA (6/10 goats and 2/2 sheep). However, two Kasese farmers practicing communal grazing also had sero-negative animals (Kisasa: 0/3 goats; Kayanja: 0/20 goats) despite sharing grazing with neighbours whose animals were sero-positive (Kisasa: 4/6 goats, 3/8 goats and 6/10 sheep; Kayanja: 1/11 goats and 1/12 goats). Two fenced farms in Kabaka had animals with antibodies towards NSP (3/11 goats, 2/13 goats and 3/13 sheep), probably because of the public watering of the cattle on these farms in River Mobuku.

The results of screening sera with antibodies towards NSP and/or SP-O in tests for antibodies towards all seven FMDV serotypes, and subsequent titration of sera screened positive, are summarized in Table 4.

Only a few goats reacted in the screening tests for antibodies towards serotypes A (6/40), C (8/40) and Asia 1 (1/40). Titration of these sera showed that nearly all antibody reactions towards serotypes A, C and Asia 1 were cross-reactions, since the titres were below 40 and all but one serum had higher titres towards other serotypes. This serum had a titre of 20 for antibodies towards serotype A and even lower titres towards the SAT serotypes.

One goat and one sheep in Busunga had serological evidence of exposure to serotype O. Similarly, sheep and goats in Kisasa except those belonging to farmer F showed serological evidence of exposure to serotypes O, SAT 1, SAT 2 and SAT 3 with the highest titres registered towards serotype O. In contrast, in Kabaka and Kayanja, goats and sheep did not have serological evidence of exposure to serotype O, while there was scattered evidence of exposure to SAT 1 (one goat in Kayanja) and SAT 2 (one goat in Kabaka). Besides this, there were low-level titres towards other serotypes.

Table 3. Attempted comprehensive random sampling in Kasese District in 2007: prevalence estimates of antibodies towards foot-and-mouth disease virus (FMDV) non-structural proteins (NSP) and FMDV SP-O

Villages	Farm type	No. sera tested	No. NSP (+) sera (% positive)	No. SP (+) sera (% positive)
Goats				
Kahendero	Communal	3	0 (0)	0 (0)
Busunga	Fenced	11	0 (0)	0 (0)
	Communal	5	3 (60)	1 (20)
	Communal	5	3 (60)	0 (0)
Rwentutu	Fenced	19	0 (0)	0 (0)
Kabaka	Fenced[a]	11	3 (27)	0 (0)
	Fenced[a]	13	2 (15)	0 (0)
Kayanja	Communal	20	0 (0)	0 (0)
	Communal	11	1 (9)	1 (9)
	Communal	12	1 (8)	0 (0)
Kisasa	Communal	6	4 (67)	3 (50)
	Communal	3	0 (0)	0 (0)
	Communal	8	3 (38)	4 (50)
Rwembyo	Fenced	16	0 (0)	0 (0)
Total/average		143	20 (14)	9 (6)
Sheep				
Kahendero	Communal	5	1 (20)	2 (40)
Busunga	Fenced	6	0 (0)	0 (0)
	Communal	2	2 (100)	1 (50)
Kabaka	Fenced[a]	13	3 (23)	10 (23)
Kisasa	Communal	10	6 (60)	4 (40)
	Fenced	4	0 (0)	1 (25)
Rwembyo	Fenced	15	0 (0)	0 (0)
Total/average		55	12 (22)	18 (33)

All sera from the district were screened for antibodies towards both NSP and SP-O using commercial kits supplied by CEDI Diagnostics. (+) Positive sera on respective method.

[a]Fenced, but watering the cattle at the same farms in the river.

Discussion

Goat and sheep samples collected after FMD outbreaks in cattle in four Ugandan districts showed estimated prevalences of antibodies towards NSP approximating 100%. Attempted comprehensive random sampling of sheep and goats in 2007 in one of these, Kasese district, gave much lower estimated prevalences of antibodies towards NSP (14% and 22% respectively) as well as towards SP-O. The lower estimates of antibody-prevalences in 2007 than in 2006 probably reflects the different sampling methods, and is most likely also a result of time, since the last outbreak of FMD in cattle (2 months in 2006 and 5–11 months in 2007). However, the difference in sample sizes may also be partly responsible for the variation.

Similar studies in Morocco following an FMD outbreak in 1999 identified a prevalence of 13% among sheep (Blanco et al., 2002). In contrast, an earlier Kenyan study showed high antibody prevalences of 89% (type O) and 56% (SAT 2) in small ruminants (Anderson et al., 1976). Based on the observations in the present study, it may be argued that these differences could have been explained

Table 4. Attempted comprehensive random sampling in Kasese District in 2007: serotype-specific antibodies towards foot-and-mouth disease virus (FMDV) detected in goats and sheep

Village	Farmer	O	A	C	Asia1	SAT1	SAT 2	SAT 3	Conclusions
Goats									
Kabaka	A	–	–	–	–	–	5	5	SAT-serotype (earlier infection)
		–	–	–	–	–	40	5	SAT 2
Kabaka	B	–	–	–	–	–	5	–	SAT 2 (earlier infection)
		–	–	–	–	–	10	5	SAT-serotype (earlier infection)
		–	–	–	–	–	5	5	SAT-serotype (earlier infection)
Busunga	C	–	–	–	–	–	20	5	SAT-serotype (earlier infection)
		–	–	–	–	–	20	10	SAT-serotype (earlier infection)
		80	–	5	–	80	–	10	O and SAT 1
		5	5	–	–	–	10	5	SAT 2 (earlier infection)
Busunga	D	–	–	–	–	–	10	5	SAT 2 (earlier infection)
		–	–	–	–	–	5	5	SAT-serotype (earlier infection)
		–	–	–	–	10	5	5	SAT 1 (earlier infection)
Kayanja	E	20	–	5	–	40	–	–	SAT 1
Kisasa	F	–	–	–	–	–	–	5	SAT 2 (earlier infection)
		5	–	–	–	–	40	20	SAT 2
		–	–	–	–	–	5	–	SAT 2 (earlier infection)
		–	–	–	–	–	20	5	SAT 2 (earlier infection)
Kisasa	G	20	–	5	–	–	80	20	SAT 2
		640	–	–	–	10	80	320	O > SAT 3 > SAT 2
		5	5	–	–	–	10	5	SAT 2 (earlier infection)
		160	10	–	–	40	40	80	O > SAT 3 > SAT 2 = SAT 1
Sheep									
Kabaka	A	–	–	–	–	–	10	5	SAT 2 (earlier infection)
		–	–	–	–	–	5	5	SAT-serotype (earlier infection)
		5	–	–	–	5	5	10	SAT 3 (earlier infection)
Busunga	C	5	–	–	–	–	10	–	SAT 2 (earlier infection)
		320	5	–	10	5	10	20	O
Kisasa	F	80	–	–	–	40	5	20	O > SAT 1
Kisasa	G	–	20	–	–	5	10	5	A (earlier infection)
		160	–	5	–	5	80	10	O > SAT 2
		20	–	5	–	5	10	20	O = SAT 2 (earlier infection)
		640	10	5	–	5	20	20	O
		160	–	10	–	–	10	40	O
Kahendero	H	–	–	–	–	20	–	–	SAT 1 (earlier infection)
		160	–	5	–	–	–	5	O

Serum samples positive for antibodies towards non-structural proteins and/or SP-O were screened for serotype-specific antibodies towards the seven serotypes of FMDV, and all sera with positive reactions were titrated in the relevant serotype-specific ELISA. (–) Negative at serotype screening at dilution 1/5.

by the rate of decline of antibodies over time after an FMD outbreak. Limited earlier studies have shown that antibodies in sheep, despite a slight decrease after day 10, remain relatively high for at least 147 days (Dellers and Hyde, 1964). Similarly, at Lindholm, sheep experimentally infected with FMDV were positive for antibodies towards FMDV NSP and SP-O for more than 4 months (C. Stenfeldt, unpublished observation).

On the other hand, in the present study, antibody titres among the cattle on the same farms were higher than in the small ruminants (F. Mwiine, unpublished observation), and antibodies towards FMDV have been shown to decline faster in small ruminants than in cattle (Dellers and Hyde, 1964; Cunliffe, 1964; Garland, 1974). The observed longer duration of antibodies in cattle than in sheep and goats in the same villages (F. Mwiine, unpublished observation) is most likely because of priming of the immune defense of the cattle by previous vaccinations with multi-valent FMDV vaccines used to prevent FMD outbreaks in Uganda.

In epidemiological situations such as in Uganda, where vaccination of the cattle population in outbreak areas can hinder the serological diagnosis of on-going outbreaks, confirmation and serotyping of outbreaks depends on isolation of the virus, which may be biased by old persistent infections (Anderson et al., 1976). This

again depends on probang or blood sampling immediately after outbreak reporting. In such areas, unvaccinated sheep and goats can be used as tracer animals to determine the serotype of on-going outbreaks of FMD. This method can be seen as an alternative to virus isolation and sequencing where facilities and expertise for such techniques are not available. In this study, the testing in Kasese and Bushenyi districts in 2007 resulted in very different prevalence estimates of antibodies towards NSP and SP-O, with Bushenyi almost free and the overall prevalence estimates of antibodies towards NSP of 14% in goats and 22% in sheep in Kasese. The single seropositive animal in Bushenyi was probably purchased and introduced on the farm. This difference in prevalence estimate levels most likely reflects the different level of FMDV exposure in the two areas, since Kasese district was experiencing frequent outbreaks, while Bushenyi district had only had one isolated incident of FMD (2006) during the last 10 years.

This variation cannot be directly attributed to distance from a national park, as this is about the same for the two districts. However, Bushenyi cattle are separated from the national park by Banyaruguru County, which is largely inhabited by crop cultivators, while such a buffer zone does not exist in Kasese district. Consequently, Kasese livestock are routinely grazed in the park area within close proximity to wildlife, including African buffalo, which are known to harbour FMDV as a persistent infection (Hedger, 1972; Condy et al., 1985; Thomson et al., 2003).

Another important factor could be the differences in farm management system in place in the two districts. In Bushenyi, a paddocked animal management system based on rearing cross breeds or entirely exotic (Friesian) cattle has been in existence for over 20 years. Such animals are highly susceptible to tropical animal diseases, and outbreaks of FMD and other infectious diseases are greatly felt by the dairy farmers, who as a consequence are exceptionally well organized into communities with a high level of knowledge about infectious diseases which might affect their dairy industry.

In contrast, in Kasese, local breeds of cattle comprise the majority of the stock, with communal grazing as the main husbandry practice, and farmers experience less adverse effect of FMD outbreaks because of inherently less severe clinical FMD in the local cattle breeds.

In Kasese, some farmers that practice fencing in high-risk areas (Rwembyo, Rwentutu and Busunga) had managed to keep their sheep and goats free from exposure to FMDV, however, so had some farmers practicing communal grazing (Kisasa, Kayanja and Kahendero). During sampling it was observed that some animals were tethered around homesteads. This may explain the absence of antibodies towards FMDV on some farms in the communities practicing communal grazing. This survey has found that fencing of farms may help protect against FMDV infection but this may not be sufficient in high-risk areas.

During the 2007 sampling, the numbers of ruminants, particularly of sheep, found on the majority of the farms were lower than expected and many of the farms were inaccessible because of heavy rains. So we were not successful in collecting the comprehensive number of samples calculated by the Survey Toolbox. However, these experiences will be valuable for planning of future sampling trips to the area.

Despite the low number of small ruminant samples collected from the 2006 outbreak sampling, investigation of serotype-specificity clearly showed that this massive outbreak was caused by a serotype O virus. One sheep without O antibodies had a low level of antibodies towards SAT 3, and a number of other animals showed low levels of antibodies towards the SAT serotypes. These reactions may be evidence of previous infections or simply cross reactions. The overall results from post-outbreak sampling and testing of cattle in 2006 confirmed that this outbreak was caused by a serotype O FMD virus, but also identified some SAT 1 activity in some villages (F. Mwiine, unpublished observation). The SAT 1 reactivity was not evident in the small ruminants in this area, and it is thus likely that these SAT 1 antibodies in cattle were derived from vaccinations or previous outbreaks.

The result of the attempted comprehensive random sampling in Kasese in 2007 gave a less clear result than expected, since there was evidence of exposure to at least two different serotypes in many goats and sheep; e.g. a Kisasa goat with antibody titres of 640 towards serotype O and of 320 towards serotype SAT 3. In such a case, possible recent exposure to both serotypes O and SAT 3 may be likely. With regard to low titre levels (5–40), such titres in a serum with high titres to other serotypes suggest cross reactions, while similar values in the absence of antibodies towards other serotypes may suggest old infections (Hedger et al., 1982). The attempted comprehensive random sampling was carried out between 5 and 12 months after the 2006 FMD outbreak which was serologically typed as O by Ministry of Agriculture Animal Industry and Fisheries, and while the small ruminants belonging to some of the farmers still had convincing serological evidence of this outbreak, sera from other farms were negative for antibodies towards this serotype.

The many cross-reactions recorded in the 2006 outbreak, as well as the very mixed picture in the 2007 sera with low levels of antibodies towards one or several

serotypes, may be a result of waning antibodies from previous outbreaks, but may also signal imperfect specificity of the serotype-specific ELISAs in the face of repeated infections.

Conclusions

Sheep and goats are also infected with FMD virus during FMD outbreaks in cattle in Uganda. In endemic situations where serological diagnosis may be affected by frequent vaccination of cattle, unvaccinated sheep and goats can be used as tracer animals to serotype outbreaks. For definite results, the sampling should be carried out immediately following an outbreak.

Bushenyi district is confirmed free from FMDV which may be as a result of management practices, but is more likely because of decreased risk of exposure (challenge) brought about by a buffer zone and the highly organized farming industry in the district.

Husbandry practices involving less movement and mixing of animals such as paddocking and fencing may protect against exposure to FMDV even in high-risk areas like Kasese district, but do not prevent all cases of FMD infection. The data from sheep and goats tested after the massive FMD outbreak in 2006 indicated that the outbreak was caused by serotype O. The current serotype-specific ELISA may lose specificity in areas with repeated FMDV infections and vaccinations.

Recommendations

Further investigation into the dynamics of FMDV antibodies in sheep and goats in Uganda should be carried out. In addition, more work to validate the serotype-specific ELISA coupled with efforts targeted at increasing their specificity is required.

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