

Study on seroprevalence and serotyping of foot and mouth disease in Chad

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Summary

Foot and mouth disease (FMD) is a highly contagious viral disease that affects all Artiodactyla. Seven immunologically distinct serotypes of FMD virus (FMDV) exist. In Chad, although FMD is included in the list of diseases monitored by the Chadian Animal Disease Surveillance Network (REPIMAT), the epidemiological situation remains unclear. A serological survey was conducted in the cattle population in eight of the nine administrative regions of the country (those regions with the highest cattle densities), to evaluate the prevalence and serotypes of circulating FMDV. A total of 796 sera from randomly selected cattle were analysed at the World Organisation for Animal Health/Food and Agriculture Organization of the United Nations FMD Reference Laboratory at the *Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna* (IZSLER), in Brescia (Italy). An enzyme-linked immunosorbent assay (ELISA), called 3ABC ELISA, was used to detect antibodies against non-structural proteins (NSPs), as well as a series of six competitive ELISAs to detect and serotype antibodies against the structural proteins of FMDV serotypes O, A, SAT 1, SAT 2, Asia 1 and C.

Based on the detection of anti-NSP antibodies, the animal-level seroprevalence was 35.6% (95% confidence interval [CI]: 32.2–38.9) and the herd-level seroprevalence was 62.3% (95% CI: 53.0–71.5). FMD was present in all livestock administrative divisions surveyed, with a higher prevalence in southern regions, which are characterised by higher rainfall and humidity and more important transboundary animal movements. Cattle aged more than four years had a higher seroprevalence, which may be due to repeated exposure. Semi-

sedentary farming and transhumance were also risk factors. Antibodies against serotypes A, O, SAT 1 and SAT 2 were detected.

Keywords

Cattle – Chad – FMD – Foot and mouth disease – Seroprevalence – Serotype – Virus.

Introduction

Livestock are an important source of income in most developing countries. In Africa, they often contribute up to 10–20% of the gross national product (1). In Chad, livestock contribute 80% of the agricultural capital stock (US\$ 6,912 million in 2007), i.e. 59.1% of livestock fixed assets, 10.4% of livestock inventory, and 10.7% of structures for livestock (2). In Chad, the rural population represents 72.4% of the whole population and livestock is the second-largest source of foreign exchange earnings after oil (3). The livestock sector provides a livelihood for 40% of the rural population and accounts for 30% of Chad's exports (over 50%, excluding oil). Annual revenue streams in this sector are estimated at US\$ 257 million (4). The livestock system is pastoral and based on mobility. This system is used for 80% of the cattle population (3). Animal diseases remain one of the major constraints to the development of this sector, including foot and mouth disease (FMD) (5, 6).

Foot and mouth disease is a highly contagious, transboundary, viral disease, affecting both domestic and wild Artiodactyla (7, 8, 9). There are seven serotypes (O, A, C, SAT 1, SAT 2, SAT 3 and Asia 1) and several subtypes with a worldwide distribution (10, 11, 12). Only five of the seven serotypes occur on the African continent. Serotype Asia 1 has not been observed in Africa and serotype C is extinct, in that it has not been seen for more than 14 years (13).

Foot and mouth disease is one of the major transboundary diseases, responsible for causing considerable economic losses, not only when it (re-)emerges in previously disease-free areas but also in areas to which it is endemic (9, 13, 14, 15, 16). Clinically, FMD is

characterised by pyrexia (up to 42°C), dullness, the presence of blisters and inappetence. In cattle, FMD is characterised by fever, a drop in milk production, and vesicles or blisters that appear in the mouth, on the teats and between the hooves. Hypersalivation, difficulty in swallowing, acute lameness and/or difficulty in walking accompany the lesions.

In epidemiological terms, FMD reaches morbidity of close to 100% and a mortality rate of about 5% when it (re-)emerges in disease-free areas. Mortality usually takes place in young animals as a result of myocarditis, so-called 'tiger heart disease' (17).

Antibodies are found in a large proportion of animals kept under extensive husbandry, a production system characteristic of most countries in western and central Africa. Since FMD causes more losses in intensive husbandry systems, such as intensive dairy farms on the periphery of towns, these farms are usually targeted for vaccination campaigns (18).

A more accurate knowledge of the epidemiology and impact of FMD in traditional farming systems in sub-Saharan Africa is a prerequisite when evaluating and adapting control programmes, such as those advocated by the World Organisation for Animal Health (OIE) and the Food and Agriculture Organization of the United Nations (FAO) (19, 20). According to the World Reference Laboratory for FMD (WRLFMD) at the Pirbright Institute (www.wrlfmd.org), there are few historical accounts of FMD outbreaks in Chad: serotype A was first reported in 1973 and serotype SAT 1 in 1963, 1964 and 1972. FMD has been included in the list of monitored diseases of the Chadian Animal Disease Surveillance Network since the Network's creation in 1995 (the *Réseau d'Épidémiosurveillance des Maladies Animales au Tchad* or REPIMAT). REPIMAT surveillance of FMD (6, 21) is passive and primarily targets cattle because of their importance to the country's economy (21). FMD is the most reported disease to REPIMAT (5). However, the national laboratory is unable to analyse suspected FMD samples, due to a lack of trained personnel and the necessary equipment and materials. For this reason, FMD

notification is limited to clinical suspicion without laboratory confirmation (6). The epidemiological situation of the disease thus remains unclear in the absence of any identification of FMDV serotype(s) circulating in the country. There has never been any FMD vaccination in Chad.

This present retrospective aims to evaluate the seroprevalence of FMD and assess the presence of specific antibodies directed against FMDV serotypes in Chad in early 2010, based on randomised historical serum samples.

Materials and methods

Survey area and sampling design

Chad has three main regions: a desert zone in the north, an arid Sahelian belt in the centre and a more fertile Sudanian savanna zone in the south. The survey area is depicted in Figure 1. Most herders are mobile pastoralists. Among the breeds of cattle raised in Chad are the white Fulani, red Fulani, white Lake Chad, Tupuri and Sokoto (22).

Two-stage random sampling was conducted to gather samples. The primary unit was the village (an epidemiological unit equivalent to the herd in the African context; i.e. with an absence of fences) and the secondary unit was the individual animal.

A total of 106 villages were randomly drawn from the 3,297 villages in the study area (involving eight breeding delegations) (Fig. 1). These villages were distributed over various geographic areas which were covered by 34 active REPIMAT surveillance posts. This should make it possible to detect herd seroprevalence of FMD of 50% (no *a priori* knowledge is available), with an absolute precision of 10% and a confidence level of 95% (plus a reserve of 10% of samples in case some breeders declined to take part). The regional livestock delegation from the north was not included in this survey because the surveillance posts were no longer functional due to a lack of agents. In addition, this area is largely desert and cattle breeding here is of relatively low importance compared to in other delegations.

An average of seven to eight cattle per village were randomly selected from animals aged one year and over, of both sexes. No vaccination against FMD has been carried out in Chad so far. A total of 796 randomly selected cattle were sampled without gender stratification; 628 were between one and four years old and 168 were more than four years of age.

Blood collection

Blood sampling was conducted between December 2009 and February 2010. Jugular vein blood was collected in 5-ml Vacutainers[®]. Samples were kept at an ambient temperature for two to three hours to induce clotting. After removal of the coagulate, the serum was preserved on ice until its arrival at the *Institut de Recherche en Élevage pour le Développement*, in N'Djamena (Farcha Laboratory). The samples were centrifuged at 3,000 revolutions per minute (rpm), aliquoted in cryotubes (Nalgenes[®]) and stored at -20°C until transportation to the laboratory where analysis was carried out. The international requirements imposed on the transport of dangerous infectious materials were strictly adhered to during transportation from Chad to the analysing laboratory in Italy.

Serological testing

The samples were analysed at the *Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna* (IZSLER), in Brescia, Italy, an OIE/FAO FMD Reference Centre.

The tests, developed at IZSLER, consisted of:

- an ELISA for the detection of antibodies against NSPs, abbreviated in the text as the '3ABC ELISA'. Antibodies are elicited after an infection and are common to all FMDV serotypes;
- a series of six 'in-house' competitive ELISAs for the detection and serotyping of antibodies against the SPs of FMDV serotypes O, A, SAT 1, SAT 2, Asia 1 and C, to indirectly identify the viral serotypes circulating in Chad. IZSLER did not test for SAT 3.

Non-structural protein enzyme-linked immunosorbent assay

The serological assay used to detect anti-NSP antibodies was the previously validated 3ABC-trapping ELISA (23), in the form of a ready-to-use kit, with pre-sensitised ELISA immunoplates, peroxidase-conjugated anti-ruminant immunoglobulin G (IgG), control sera, and the 3,3',5,5'-tetramethylbenzidine chromogen, produced at IZSLER. The specificity of this NSP ELISA exceeds 99%, while sensitivity varies, depending on the time lapse after infection and on previous vaccination status. Sensitivity of 100% can be achieved for non-vaccinated cattle exposed to infection, while 86.4% can be achieved for the detection of carriers among vaccinated cattle (23).

Monoclonal antibody-based, solid-phase, competitive, enzyme-linked immunosorbent assay

The principle behind and the procedure for the monoclonal antibody (MAb)-based, solid-phase, competitive ELISA (SPCE) is described below. SPCEs were first used to quantify antibodies specific to the European FMDV subtypes O1, A5 and C1 (24), and later applied to other infectious diseases (25, 26). Afterwards, the test reagents used for serotype O and A ELISAs, i.e. FMDV-specific MAbs and antigens, were adapted and validated to detect antibodies more closely related to currently used vaccine strains or circulating serotypes and variants. In particular, the updated ELISAs used FMDV antigens from the vaccine strains O1 Manisa and A22 Iraq. Each was respectively associated with a competing detector MAb characterised by broad intra-type reactivity. Similar ELISAs for the detection of antibodies against serotypes Asia 1, SAT 1 and SAT 2 have been described more recently (27, 28).

Briefly, the assay procedure for each SPCE was as follows: immunoplates were coated with 50 µl per well of a type-specific catching MAb at a saturating dilution in carbonate/bicarbonate buffer of pH 9.6 by overnight incubation at 4°C. After three washes, 50 µl of FMDV antigen of the homologous serotype was placed in each well at a predetermined optimal dilution.

After 1 h of incubation at 37°C and washing, 50 µl of a series of three-fold dilutions of test and control sera was incubated with the trapped antigen for 1 h at 37°C. Then, 25 µl per well of the homologous peroxidase-conjugated competitor/detector MAb was added to each well and plates were incubated at 37°C for a further 1 h. After a last series of washes, the colorimetric reaction was developed by distributing 50 µl per well of the substrate solution: 0.5 mg/ml of *o*-phenylenediamine dihydrochloride (OPD) in phosphate-citrate buffer, pH 5, containing 0.02% H₂O₂.

The reaction was stopped after 10 min by adding 50 µl of 2N H₂SO₄ and the optical density values (ODs) were read at a 492-nm wavelength. The optimal working dilution of antigen and peroxidase-conjugated MAb, giving a spectrophotometric reading of 1.5 OD, was predetermined by checker-board titrations. The screening dilution for sera was 1/10; the endpoint titres of positive sera were expressed as the reciprocal of the highest serum dilution providing 50% inhibition.

Statistical analysis

Data were entered into a spreadsheet using the program Microsoft Excel®2010. Statistical analysis was carried out in Stata/MP 14.2 (29). A Poisson regression (or a negative binomial regression, when required, due to extra-binomial variability) was used, followed by a Bonferroni corrected multiple test, if appropriate. A chi-square test was used to test the association between management type and seroprevalence. A level of significance of 5% was used throughout (divided by the number of tests performed for the Bonferroni correction).

Results

Out of 796 samples tested in the 3ABC ELISA, 283 yielded a positive result, amounting to an animal seroprevalence of 35.6% (95% confidence interval [CI]: 32.2–38.9). The seroprevalence was geographically heterogeneous, varying significantly according to the livestock administrative division. The southern and central divisions showed a higher seroprevalence than the others. In addition, the East

and Central East divisions showed a lower seroprevalence (less than 20%) than the other divisions (Table I). The overall average herd-level seroprevalence was 62.9% (95% CI: 53.0–71.5).

As shown in Table II, a significantly higher seroprevalence was recorded in older animals ($p < 0.001$).

Table III shows seroprevalence by production type. A significantly higher seroprevalence (chi square = 4.12, $p = 0.042$) was recorded in transhumant and semi-sedentary herds, compared to that found in sedentary herds.

Out of the 796 sera tested for antibodies against the SPs of six FMDV serotypes, 480 (60%) scored positive for one or more serotypes. In 278 positive sera, the FMDV serotype responsible for the infection was identified as type O, A, SAT 1 or SAT 2, based on the detection of antibody against a single serotype or antibody titres that were significantly higher against one serotype. Another 200 samples showed similar levels of antibodies against two or more serotypes from O, A, SAT 1 or SAT 2 (Table IV). In contrast, the seropositivity levels for serotypes Asia 1 and C were very low (22 out of 296 sera tested weakly positive for FMDV Asia 1 and only six out of 796 tested borderline positive for type C) and could reasonably be attributed to cross-reactive antibodies.

Discussion

There is a clear need for FMD risk-based surveillance to determine the status and factors that influence disease dissemination in areas where FMD is endemic, such as African countries, and to target appropriate control strategies. Under the auspices of the FAO and OIE, some African regions have embarked on a progressive control pathway (PCP) for the control of FMD (20, 30). The success of any FMD control campaign depends on many factors (13):

- the specific epidemiology of the disease in areas of interest
- the local and regional surveillance programmes monitoring circulating FMDV

- a vaccine that includes strain(s) that antigenically match the circulating strain(s)
- the potency of the vaccine
- vaccine coverage
- rapid vaccine implementation
- the planning and management of the vaccination campaign by a well-resourced Veterinary Service
- the involvement and cooperation of livestock farmers.

Chad's current status along the PCP for FMD is stage 0 (on a scale ranging from 0 to 5), corresponding to an uncontrolled risk of FMD, with no reliable information (13, 20). Consequently, there is a crucial need for reliable epidemiological information on FMD in Chad, where the disease is endemic. As a result of the lack of local diagnostic laboratory expertise and the cost of reagents for FMD diagnosis, only clinical suspicions without laboratory confirmation are recorded by the surveillance network, REPIMAT (6).

The overall seroprevalence estimated in 2010 at the individual level (35.6%) and at the herd level (62.3%) highlights the gravity of FMDV circulation in Chad. Considering this high level of FMD seroprevalence and the common transhumant system in Chad (involving 80% of cattle), there is an important risk of spreading FMD through cattle movements over long distances (FMD 'trans-pool' movement). In consequence, at least two main options must be evaluated when attempting to control FMD. First, animal movement controls must be imposed to try to prevent the movement of FMDV. However, the practicality of this option is questionable as transhumance is an ancestral practice and increases the resilience of livestock (through the search for nutritive resources due to climatic conditions) and their associated population health. The second course of action is a vaccination campaign for livestock, but this requires a much better knowledge of the FMDV serotypes currently circulating throughout Africa, especially in countries that practise transhumance, to select the appropriate vaccine (thus requiring a regional approach to

FMD control). In addition, more field information is needed on persistently infected carriers after vaccination. More transdisciplinary research (e.g. in anthropology, sociology, infectiology, epidemiology and economics) is also required to produce evidence-based information that will help decision-makers to choose the best option(s) for controlling FMD in Africa.

These high seroprevalence results, especially in the southern part of Chad, are probably due to a range of factors. Among them are:

- intense animal movements (80% of cattle are kept under a transhumant husbandry system), resulting in frequent contacts between different herds
- a more humid climate (rainfall of 800–1,200 mm/year), allowing longer virus survival outside the host, and
- the presence of wildlife (e.g. buffalo, antelope and warthogs), which can serve as reservoirs of the virus, in and around the numerous game reserves in the area (which generally have established boundaries but are not fenced).

Wildlife density is more important in the reserves to the south of the country, especially in Zakouma National Park. It is during the hot, dry season (March to April) that contact with livestock occurs frequently. This zone, rich in lush pastures, continuously has a high concentration of livestock, with herds coming from the whole of the Chadian territory, as well as from neighbouring countries (Cameroon and the Central African Republic) (11).

The role of game animals in the epidemiology of FMD has previously been investigated in Chad; in particular, in Zakouma National Park (12). In this study, blood samples from buffalo ($n = 53$) and other wildlife ($n = 203$) were collected from 1999 to 2003. Based on the detection of anti-NSP antibodies, the seroprevalence of FMD was estimated at 23.1% (mainly in Nile buffalo, *Syncerus caffer aequinoctialis*). Subsequently, the presence of specific antibodies against different serotypes of FMDV (A, O, C, SAT 1, SAT 2 and SAT 3) was evaluated in a subset of 30 sera, using SPCE. Positive

responses were observed, in descending order, for serotypes SAT 2 (13/30), O (9/30), SAT 1 and C (2/30) (12).

The same serotypes found in the present study involving cattle were also recorded in a retrospective study by Di Nardo *et al.* (12), involving wildlife species (mainly buffalo). More investigations are needed to better understand the role of wildlife in FMD epidemiology in Chad. In addition, the lower seroprevalence observed in the East and Central East divisions is likely due to the low livestock density and fewer movements of animals in these areas.

The present study also shows a link between age and seroprevalence. Animals aged more than four years have a higher probability of being seropositive, probably due to a higher chance of having come into contact with the virus. A similar trend was observed in Ethiopia (31). While the inherent logic of this observation is obvious (by definition, older animals have had more opportunity to be exposed to FMDV during their lifetime), husbandry practices probably also have some influence. Young animals are kept around the camp while only older animals are trekked over long distances in search of pastures and water. This higher risk of being exposed to FMDV also means that older animals are more likely to become infected with different serotypes.

The results demonstrate the presence of serotypes A, O, SAT 1 and SAT 2, while the very low levels of antibodies that react against serotypes Asia 1 and C are attributable to inter-type cross-reaction. In fact, ELISAs to detect antibodies against SPs suffer from imperfect type specificity, and cross-reactions may occur among all FMDV serotypes (32). However, the significantly higher proportion of sera that cross-react with two or more of the first four serotypes is also suggestive of multiple infections with different serotypes. Indeed, these four serotypes are regularly recorded in Africa (9, 14), where the diffusion of the various serotypes is essentially determined by uncontrolled animal movement (14).

The importance of husbandry is also reflected in the higher seroprevalence values obtained from transhumant and semi-sedentary

herds, when compared to those of sedentary animals (Table III). This higher probability of exposure to the virus also means that older animals harbour antibodies against a larger variety of serotypes, given that infection with one FMDV serotype does not protect against infection with other serotypes.

This is also reflected by the similar percentages of different serotypes observed in the results. In Cameroon, serotypes O, SAT 1 and SAT 2 have been identified (33). The global FMD situation is characterised by seven regional pools of dominant serotypes (11, 13). Chad is classified in the fifth pool, with serotypes A, O, SAT 1 and SAT 2. The authors' results corroborate this classification. FMDV type C had a limited distribution compared to most of the other serotypes and has not been reported since 2004. Serotype Asia 1 has thus far never been reported in Africa (13). Serotype SAT 3 is present only in pools 4 (East Africa) and 6 (South Africa), and geographically close to the countries comprising pool 5 (West/Central Africa), which include Chad (11). Given the uncontrolled introduction of animals and animal products into Chad, the incursion of type SAT 3 cannot be ruled out. Further study of SAT 3 may be warranted, as this serotype has recently been recorded in sera from West and Central Africa (e.g. 34) and from eastern Africa (e.g. 35).

This study has some limitations that are worth mentioning. The principal shortcoming is the period of the study (late 2009 to early 2010) when no records on laboratory confirmation of FMD outbreaks could be found in the statistics of the Ministry of Agriculture and Livestock. The only laboratory findings confirming FMD outbreaks in Chad are those of the WRLFMD and the last confirmed date is in 1973. A lack of local diagnostic laboratory expertise, the high cost of reagents for FMD diagnosis, a limited number of field staff, the cost of FMD vaccine, and the lack of a national and/or regional strategy for FMD surveillance and control are reasons frequently invoked (20).

Since the present study, some interesting initiatives have been taken to improve the situation, including the establishment of a laboratory network with support from the European Commission (EC) for the

Control of FMD (EuFMD); the organisation of training seminars for FMD laboratories in Mauritania, Niger and Chad in May 2012; and the provision of diagnostic kits (ELISAs) to each laboratory for local typing (20). Continuing such action (capacity-building, serological survey, isolating circulating FMDV strains) is strongly recommended in Chad and, more generally, across all of sub-Saharan Africa.

Despite such limitations, this study has clearly demonstrated the presence of antibodies directed against NSPs of FMDV in Chad, and shown that serotypes O, A, SAT 1 and SAT 2 were present there in 2010, on the basis of the prevalence of the serotype-specific antibodies identified. Epidemiological information obtained during this study has provided important insights for planning a control strategy. However, further similar studies are needed, including research into virus isolation and sequencing and studies of other highly sensitive species in Chad, including sheep, goats, pigs, camels and buffalo, if we are to come to a fuller understanding of the struggle against FMD.

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Fig. 1
Location of the 106 Chadian Animal Disease Surveillance
Network posts, by livestock administrative division

■ Posts became non-functional in the northern delegation and were not retained ($n = 2$)

● Functional surveillance posts that did not participate in sampling for this study ($n = 72$)

○ Functional surveillance posts that participated in sampling for this study ($n = 34$) from 106 villages, randomly drawn from 3,277 villages in total. These 106 villages are distributed across various geographical areas, covered by 34 active surveillance posts.

▲ Capital of Chad

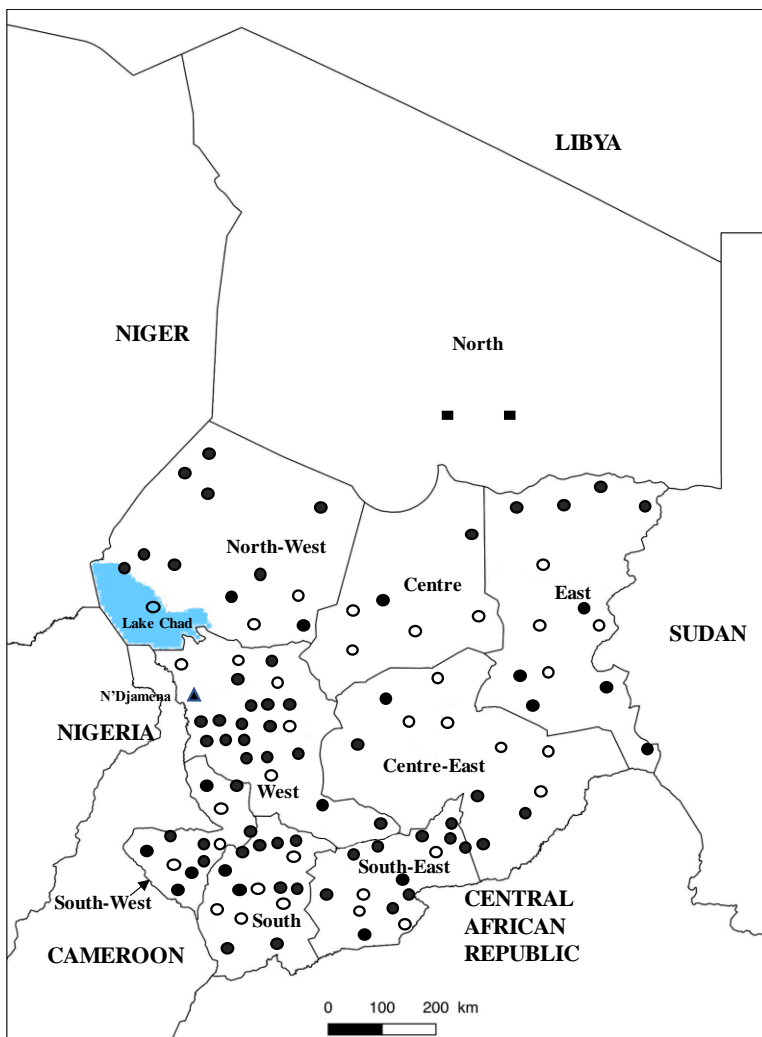


Table I
Seroprevalence of foot and mouth disease in Chad, by
administrative division (antibodies against non-structural
proteins)

Administrative division of Chad	Number of		Prevalence (%)	95% CI	Multi-test ^(a)
	Samples analysed	Positive samples			
Centre	43	19	44.2	(29.4–59.0)	ab
Centre-East	111	17	15.3	(8.6–22.0)	cd
East	83	5	6.0	(0.9–11.1)	D
North-West	126	38	30.2	(22.2–38.2)	cd
West	87	21	24.1	(15.1–33.1)	bcd
South	128	51	39.8	(31.3–48.3)	ab
South-East	138	86	62.3	(54.2–70.4)	A
South-West	80	46	57.5	(46.7–68.3)	A
Total	796	283	35.6	(32.3–38.9)	

a) Regions with different letters are significantly different from each other ($p < 0.05$, with Bonferroni correction)

CI: confidence interval

Table II
Seroprevalence of foot and mouth disease (antibodies against non-structural proteins), according to age of animals

Age stratum	Number of		Prevalence %	95% CI
	Sera tested	Positive sera		
< 4 years	628	191	30.4	(26.8–34.0)
> 4 years	168	92	54.8	(47.2–62.3)
Total	796	283	35.6	(32.2–38.9)

CI: confidence interval

Table III
Seroprevalence of foot and mouth disease (antibodies against non-structural proteins), according to type of farm production

Type of farm production	Number of sera tested	Number of positive sera	Prevalence %	95% CI
Sedentary	690	236	34.2	(30.7–37.7)
Semi-sedentary	70	29	41.4	(29.9–53.0)
Transhumant	36	18	50.0	(33.7–66.3)
Total	796	283	35.6	(32.2–38.9)

CI: confidence interval

Table IV
Prevalence of antibodies against foot and mouth disease serotypes
in Chad, by administrative division

Administrative division in Chad	Number of sera tested	Number of sera positive by serotype ^(a)				Number of sera that cross react with multiple serotypes ^(b)	Number of sera that test negative for all serotypes
		O	A	SAT 1	SAT 2 ^(c)		
Centre	43	13	1	4	2	4	19
Centre-East	111	13	0	20	3	5	70
East	83	7	0	18	4	4	50
North-West	126	8	10	13	10	27	58
West	87	3	11	13	2	8	50
South	128	5	15	16	15	39	38
South-East	138	6	11	5	19	77	20
South-West	80	9	17	2	3	36	13
Total	796	64	65	91	58	200	318
		8.0%	8,2%	11.4%	7.3%	25.1%	40%

a) Positivity for a single serotype or antibody titre that is three-fold higher or more against a single serotype

b) Number of sera showing cross-reactivity for two or more serotypes

c) O, A, SAT 1 and SAT 2 are foot and mouth disease virus serotypes