



RISK FACTORS FOR THE CONTAMINATION OF WILD *Stomoxys niger niger* MACQUART 1851 (Diptera: Muscidae) WITH THE FOOT-AND-MOUTH DISEASE VIRUS

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ABSTRACT

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The study aimed at detecting the foot-and-mouth disease virus (FMDV) RNA in *Stomoxys niger niger* and cattle. Flies were collected using a Vavoua trap pitched 50m from the center of the herd and by net-catches on clinically sick (n=5) and symptomless cattle (n=5). Vesicular Epithelia Tissues (VETs) from sick cattle were analyzed by real time reverse transcriptase polymerase chain reaction (rt RT PCR) and serum from randomly selected animals without clinical signs was examined by serological test (NSP-ELISA Kit) for the presence of FMDV antibodies. Of a total of 568 *Stomoxys* trapped using the Vavoua trap, two species were identified namely 196 *S. niger niger* (34.51 %, 9.33 Snn/t/d), 101 *S. omega* (17.78%, 4.80 so/t/d) and 271 non-biting *Musca* sp. (47.71%, 12.90 m/vavoua and day). The dissected mouth and legs of each fly caught were screened for FMDV using the rt RT PCR. FMDV RNA was found in the epithelial vesicles of all clinically sick animals (n=5, with mean Ct of 27.98456) and 3 out of 5 were serologically positive in the clinically in-apparent cattle group. The overall *S. n. niger* (most abundant species) contamination rate with the FMDV irrespective of collection method was 40.3 % with females (49.0 %) being slightly more contaminated than males (21.7%) (OR =0.45, P= 0.1506) and legs being more often positive than mouth-parts (P=0.02002). Flies were contaminated less frequently on animals without clinical signs than those on animals with clinical signs, but this difference was not significant (P=0.69680).

Contribution/Originality: This study is one of very few studies which have investigated on the mechanical transmission potential of the Foot-and-Mouth Disease by an African stable fly (*Stomoxys niger niger*). The FMD virus carrier status of wild *S. n. niger* was associated with the fly collection method, sex and anatomical part.

1. INTRODUCTION

Foot-and-mouth disease (FMD) is one of the most contagious diseases of mammals and has a great potential for causing severe economic loss in susceptible cloven-hoofed animals such as cattle, pigs, small ruminants and wild animals. Due to its severe impact on trade in animals and animal products, it is the most important Transboundary Animal Disease (TAD) in the international context. Livestock production provides 30% of the income of the rural population and accounts for 8% of the GDP of Cameroon, and the national development strategy. The average cost of FMD treatment in Cameroon is estimated at 80 000 F CFA/herder/year spent on drugs to manage FMD and the average annual expenditure on drugs for the treatment of FMD in Cameroon is estimated at 32 000 000 000 F CFA (32 Billion F CFA). Furthermore, each herder reportedly loses 1 adult cattle and 2 calves to FMD per year, costing the economy 30,000,000,000 F CFA (30 Billion FCFA) annually. Therefore, the total average annual direct cost of FMD in Cameroon is circa 62 Billion F CFA. This estimate does not include production losses or decreased opportunities for international trade.

Stomoxys niger niger (M.) commonly known as the African stable fly is common in most environments in Africa. Both males and females are blood feeders and some species are considered significant economic pests of livestock and other warm-blooded animals in many parts of the world [1]. They are known to mechanically transmit several pathogens such as protozoans, viruses, bacteria, helminths and Rickettsia [2]. Much has been published on the ecological aspects of *Stomoxys calcitrans* and its vector capacity, but little is known about *S. n. niger* with equal pest potential. Gilles [3] studied the dynamics and population genetics of *S. calcitrans* and *S. n. niger* in the Re-union Island to classify the two as separate species under the family Muscidae. Mavoungou [4] observed the impact of landscape on the eco-distribution of *Stomoxys* spp. in Gabon and went forward to identify their breeding sites [5]. Little is known about this fly in Cameroon but the preliminary work of Sevidzem, et al. [6] led to the identification of four *Stomoxys* spp: *S. calcitrans*, *S. niger niger*, *S. niger bilineatus* and *S. sitiens* of the savanna of North Cameroon.

Different traps are manufactured as vital tool for fly control and survey. Dia and Desquesnes [7] reported on the efficiency of various traps and revealed that the most efficient traps for mechanical vectors (tabanids and stomoxiines) included: Nzi [8] (catches tabanids and stomoxiines in large numbers) while Vavoua [9] (catches tabanids and stomoxiines in large numbers); Biconical traps are efficient for glossines and less specific for tabanids and stomoxiines. The use of artificial orlfactants such as Octenol and Acetone as well as natural odorant like Cow urine [10] have been supplemented to trap's visual (blue/black) cues in order to boost their attractivity. Sevidzem, et al. [6] reported that Vavoua was the most efficient trap and sensitive to glossines, tabanids and stomoxiines. The use of nets to survey mosquitoes, *Simulium* flies and other smaller dipterans is a well-known collection method. Species of the families-Muscidae, Calliphoridae, Bombylidae, Tabanidae, Simulidae and Ceratopogonidae were captured directedly on cattle by Lloyd and Dipeolu [11] using nets, in their collection, biting muscoids (*S. calcitrans* and *S. n. niger*) and non-biting muscoids (*M. lusoria* and *M. domestica vicina*) were the most abundant. This reveals the suitability of nets in the collection of muscoids for experimental purposes.

FMD is produced by an *Aphthovirus* of the family *Picornaviridae* and characterized by the formation of vesicles in, and around, the mouth and on the feet [12] as well as around mammary glands of infected females. In Cameroon, the disease is colloquially known as 'Njobu', 'Safaa' (burnt tongue) as well as 'Bauru' by the Peulhs [13]. This disease is caused by a virus capable of great antigenic plurality because it possesses 7 different serotypes: A, O, C, SAT1, SAT2, SAT3 and Asia 1 [14] that cause indistinguishable clinical disease [15]. There is no cross protection between serotypes [16]. Protection within serotypes varies based on the antigenic similarity of the strains. Subsequently, any vaccine must be carefully matched with the field strain to be effective. FMDV can enter

and infect animals because of oral ingestion, inhalation, and entry through a break in skin or artificial insemination [17]. FMDV can be transmitted between herds or flocks, countries and continents by a wide variety of mechanisms. These transmission pathways include: contact between infected and susceptible hosts and indirectly by the airborne route, contaminated animal products as well as mechanically by people, vehicles, wild animals, birds and fomites Sellers [18]. Rozov [19] reported the persistence of FMDV in the gut (72 days) and on (15 days) *Musca* spp. Mechanical transmission by arthropod vectors is suspected and not yet included as one of the important factors of the FMDV transmission pathway as well as in the control of FMD.

Arthropod-borne disease transmission in farmed animals mainly involves members of the class Insecta [20]. Some viruses of veterinary importance have been reported to be transmitted by *Stomoxys* spp. including Equine Infectious Anaemia Virus (EIAV) [21], African Swine Fever Virus (ASFV) [22] West Nile Fever Virus (WNFV) [23, 24] Rift Valley Fever Virus (RVFV) [25] Lumpy Skin Disease Virus (LSDV) [26] Bovine Herpes Virus (BHV) [27] and Bovine Leukosis Virus (BLV) [28]. Vesicular Stomatitis Virus (VSV) is a vesicle forming virus of the family Rhabdoviridae closely related to the Aphthovirus (FMDV) of the family Picornaviridae. The VSV have been reported by Ferris, et al. [29] to be transmitted by *Stomoxys* spp. Thomson, et al. [30] reported that *Haematobia thirouxi potans* (Bezzi) a biting muscoid in the same family (Muscidae) with stomoxiids failed to mechanically transmit FMDV in his experiment. The current experiment of Arzt, et al. [31] showed the possibility of the mechanical transmission of FMDV from persistently infected steers to naïve counterparts with unprocessed Oropharyngeal Fluids (OPFs) and VETs. Krinsky [2] reported the possibility of mechanical transmission of viruses including FMDV by *Stomoxys* spp. Bouyer, et al. [32] reported a list of pathogens that can be transmitted by *Stomoxys* and the FMDV was among. However, no work has yet been carried to prove natural transmission of the FMDV by Stomoxiinae.

Although little evidence has been found to suggest a role for arthropods in general, or biting flies, in the epidemiology of FMDV [33] there are several features of the biology of the African stable fly (*S. n. niger*) which predisposes it to act as a mechanical vector of FMDV. These features include: large numbers per cattle head [>100 flies/cow [34] searching behaviour occurring between 24-30hrs after the previous blood meal [35] and intra and inter-herd biting (through interruptive feeding). The flies oviposit on dry cow dung and other decaying vegetable matter [36] and the FMDV is capable of persisting in manure for up to 24 weeks as reported by Kindiakov [37]. After oviposition, the fly must find a new host and may travel kilometres ($>8\text{Km}$ [38]). Other factors that make mechanical transmission by *S. n. niger* likely include: high apparent densities around game reserves [39] biting wild animals (FMD reservoir hosts) and cattle, scramble over open sores of symptomatic and symptom free hosts [24] and the persistence of the FMDV in the gut (72 days) and on the exoskeleton (15 days) of a typical muscoid [19]. It is therefore probable that cattle will occasionally have contact with an African stable fly which has recently fed upon viraemic cattle. This is in conjunction with the observation of viremias up to 10^4 - 10^5 TCID₅₀ [40] in infected cattle suggesting that the African stable fly may be involved in contaminative mechanical transmission of the disease between cattle and other ruminants (sheep and goats).

This present study aims at identifying and characterizing a potential mechanical vector capable of carrying the FMDV and possibly disseminating it to the surrounding through contaminative mechanical spread within their active and passive flight limits.

2. MATERIAL AND METHODS

2.1. Description of the Study Area

The study area is in Mbidjoro in Ngaoundere II, Vina Division of the Adamawa Region. It is about 15Km away from the town of Ngaoundere along the Ngaoundere/Tignere motorable highway. Geographically, the study herd is situated between Latitude $07^{\circ} 21'452''$ N and Longitude $13^{\circ} 32'366''$ E with an average altitude of 1523m *a.s.l.* The study herd is about 500m from the Ngaoundere cattle market and $>500\text{m}$ from the Ngaoundere airport. The

Ngaoundere cattle market consist of a large park where commercial cattle are kept overnight on transit for the next market day, two of such parks are located 156m and 200m respectively from the sedentary herd under investigation. Cattle from the whole of grand North as well as from neighboring countries such as Nigeria, Chad, Central African Republic and Sudan are usually brought to this market. The influx of cattle from other regions and neighboring countries makes the study area a FMDV risk area throughout the year as >5 outbreaks are reported by sedentary herds around this market. The presence of potential fly breeding substrate such as manure mixed with decomposing vegetable material spotted around cattle pens may be the reason for the abundant Stomoxyinae biting cattle during seasons of FMDV outbreaks, increasing transmission risk. The study site is a pasture area with Goudali as the most dominant cattle breed and others like red Fulani, white Fulani, Charlorais and their crosses (metis) are also common. The hydrographic network is made up of two all season rivers one located in the East and the other in the west that provides drinking water for cattle and other stocks (sheep and goats). Mixed farming is common, and manure is used to cultivate maize and other vegetables. The climate of this area is a typical Soudano-sahelian type with vegetation consisting of savanna grasses grazed upon by cattle. The mean annual temperature is 23.1°C, mean annual humidity is 63.2 %, mean annual rainfall is 1176.9 mm (rainy season) and wind usually blowing in the South East (SE) direction. Ecologically, the area consists of gallery forest, primary forest, secondary forest and open grass savanna.

2.2. Blood and Vesicular Tissue Collection from the Study Cattle Herd

Blood of the animals (n=5, with no clinical signs) was collected through the jugular vein into anticoagulant free sterile tubes and labeled. The blood was transferred to the laboratory where it was centrifuged at 4000revs per minute. The sera were transferred into well labeled cryotubes prior to serological analysis. Vesicular tissue [from cattle with clinical signs (n=5)] from mouth and foot areas was carefully dissected using sterile forceps and scissors and then immediately transferred into cryotubes containing 1ml RNA-later (Ambion®, made in USA) as well as in impinger fluid prepared following the media preparation sheet prepared by the Pirbright Institute with the following reagents: 370ml of Glasgow Eagle's Medium, 5ml of Pen/Strep, 5ml Amphotericin B, 10ml of 5% Bovine Serum Albumin and 10ml of 1M Hepes with storage temperature of 2 to 3°C and shelf life of 3months. The sample solutions were stored at -80°C at the National Veterinary Laboratory (LANAVET) in Garoua, prior to molecular and serological analyses. Animal use protocols were approved by the Ohio State University Animal Care and Use committee.

2.3. Fly Collection

Stomoxys spp. were trapped beside a herd around the Ngaoundere cattle market where this herd consisted of cattle showing FMD clinical signs (foot/mouth lesions and lameness that started one day before our arrival for this study as reported by the veterinarian who regularly check the general health of cattle in the herd). Capture was made using two different methods notably trapping with odor-baited (Octenol) Vavoua trap [9] and a modified heavy-duty sweep net, consisting of a white mosquito net, light aluminum circular (with diameter of 35cm) handle and bag depth of 75cm. The Vavoua trap was pitched at 50m away from the experimental cattle. Octenol was placed inside a sterile urine tube and placed at the foot of the Vavoua trap. Traps were exposed from 6:00 am to 6:00 pm. The Vavoua trap cages were emptied every evening (6:00pm). Trap and animal total exposure duration was 21 days because whenever an animal in any of the infected and non-infected group showed early clinical signs, flies were trapped for at least five days while waiting for the appearance of fresh FMD cases. In this study we started with five clinical case animals (CSA, n=5) and randomly selected animals (RA, n=5) that represented the non-clinical sign group with collection from both groups carried out using sweep nets (n=2) and Vavoua trap (n=1) pitched at 50m from both exposed cattle groups. Follow up days and collection of blood, VET and flies were carried out during a primary FMD outbreak in Ngaoundere as shown in Figure 1.

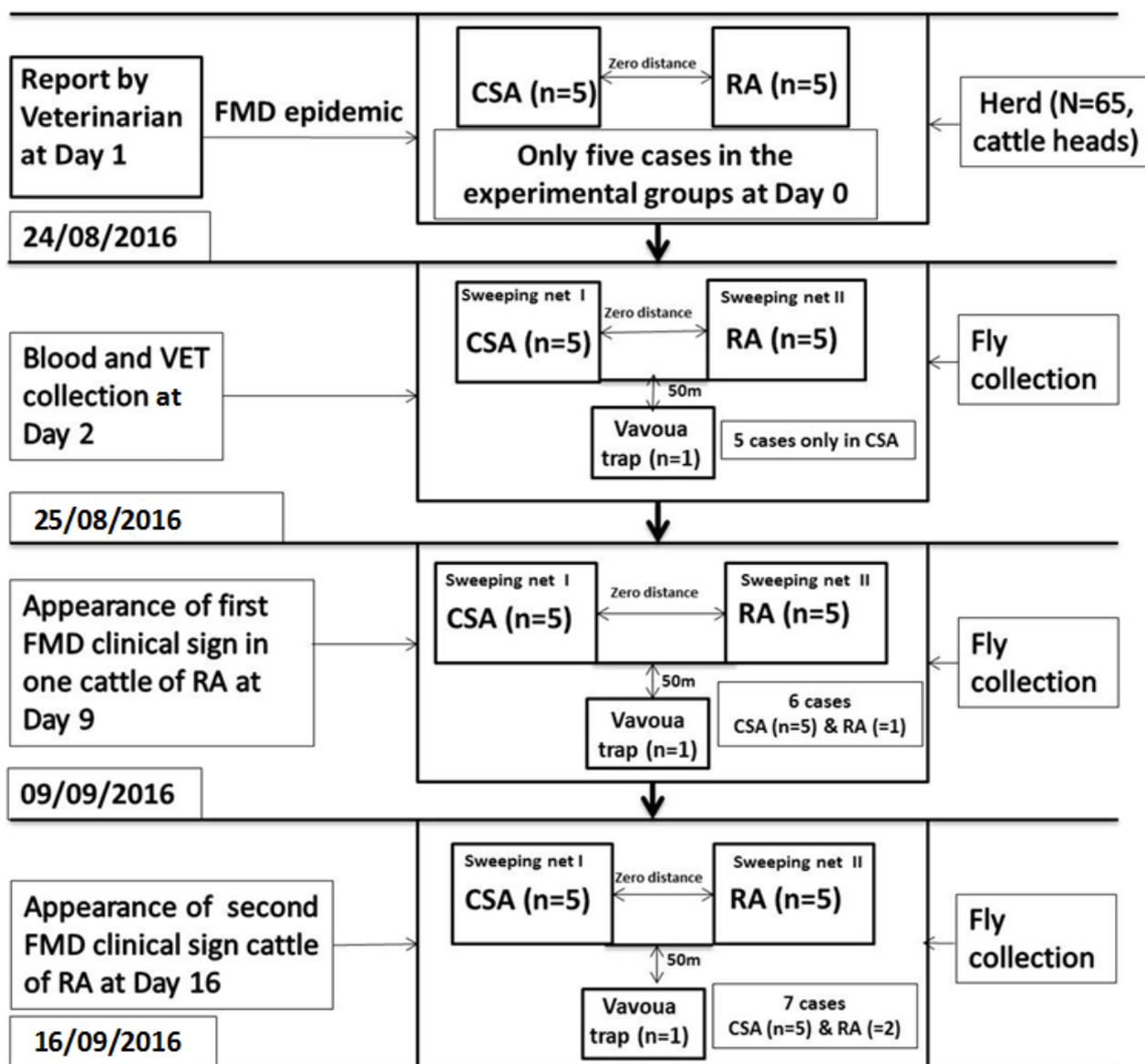


Figure-1. Monitoring a cattle herd during the 2016 FMD epidemic in Ngaoundere: CSA- clinical sign cattle group, RA, randomly selected cattle group with no visible signs of FMD, VET-vesicular epithelia tissue.
Source: Field survey, 2016.

2.4. Fly Identification

The captured flies from both methods were first identified at species level using the taxonomic keys of Zumpt [1] and *Musca* genus was identified using the morphological key of Gregor, et al. [41]. Sex of *S. n. niger* was determined using the frontal index criterion [42].

2.5. Isolation of Mouth and Legs of *S. n. niger*

Isolation of *S. n. niger* anatomical parts (legs and proboscis) by dissection was carried out in the field using Suture Removal Kit (made in China) containing the following items-11ittauer Scissors, Metal Forceps, 4" and 1 gauze sponge. One kit was used per fly sample. For each fly caught either the leg or mouth part were carefully pulled out using forceps and then immediately transferred into a 1ml RNA-later containing cryotube. Samples were stored at -80 °C freezer at the LANAVET. A pilot survey had revealed that *S. n. niger* was the dominant *Stomoxys* spp.; therefore, this species was targeted for this investigation.

2.6. Scanning Electron Micrograph (SEM) of Leg and Mouth part of *S. n. niger*

The Scanning Electron Micrograph (SEM) of leg and mouth of *S. n. niger* was carried out using the LaB6 (Lanthanhexaborid 6)-Cathode (Zeiss Evo LS10), following the protocol established in the SEM laboratory of Pr Dr Oliver Betz in the University of Tuebingen, Germany.

2.7. Serological Analysis

The cattle sera (from non-clinical animal group) were screened using the FMDV NSP-ELISA kit (PrioCHECK®, Prionics™) according to the manufacturer's instructions. Optical density of test samples was read at 450 nm on a MultiScan-Ex ELISA spectrophotometer (Thermo Fisher Scientific OY, Vantaa, Finland) and results were expressed as index derived by dividing the absorbance value of test serum by cut-off control value, according to manufacturer's instruction. The test was valid if the optical density value was greater or equal to 0.8 in wells of negative controls. The positive control serum was expected to give a Percentage Inhibition (PI) equal to or greater than 90%. The sample $PI \geq 50\%$ was classified positive and samples with $PI < 50\%$ were considered negative.

2.8. RNA Extraction and Detection of FMDV using rt RT PCR

Insect anatomical parts (legs and mouth) and VET sample homogenates were prepared according to the OIE diagnostic Manual [16] where samples were transferred into a sterile mortar with small volumes of tissue culture medium (with antibiotics). The samples were ground using a sterile pestle and then medium added until 10% suspension was obtained and centrifuged at 2000g for 10 minutes prior to FMD viral RNA extraction. RNA was extracted (from homogenized *S. n. niger* isolated anatomical parts and VET) using the RNeasy® Mini Kit following manufacturer's instructions. Amplification was performed using the rt RT PCR kit (Qiagen, Germany) with forward 5'GACAAAGGTTTTGTTCTTGGTCA-3' and reverse 5'TGCGAGTCCTGCCACGGA-3' and the probe 6FAM-TCCTTTG CACGCCGTGGGAC (Plums Island, OIE reference Laboratory) [16] universal primers that target the 3D^{pol} of the FMDV genome. The reaction mixture consisted of 2XRT-PCR Buffer with reaction volume of 12.5 µL, forward primers (with initial conc of 50 µM, final conc of 400nM and 0.2uL reaction volume), reverse primers (with initial conc of 50 µM, final conc of 400 nM) and 0.2uL reaction volume, fluorescent probe (initial conc 10 µM, final conc of 100nM and 0.25µL reaction volume), 25XRT-PCR enzyme with reaction volume of 1.0µL and sterile distil water with reaction volume of 8.35µL. Once the reaction mixture was prepared, 22.5 µl of this reaction mixture was dispensed into each of the wells of the 96-well micro plate, and each well then received 2.5 µl of a sample. The microtiter plate was then coated with an adhesive film and introduced into the thermal cycler (Applied Biosystems™, 7300). The settings for this unit regarding the temperature, duration and number of amplification cycles were as follows: 50 ° C for 10 min for reverse transcription of viral RNA, 95 ° C for 10 mins for inactivation of reverse transcription of viral RNA, 95 ° C for 15 seconds for denaturation of the cDNA, 60°C for 60 seconds for hybridization and elongation. The fluorescence was read, at each cycle, at the end of the elongation step, which is one minute at 60°C.

2.9. Statistical Analysis

Data was analysed using the R-statistical software (R version 3.4.0). Univariate analysis of *S. n. niger* related risk factors such as sex, isolated fly parts, collection type compared with the screening results of rt RT PCR was made using the Fisher's Exact Test and significant factors were included in a Generalized Linear Regression Model (GLRM). Box plots were constructed for positive samples to compare the ct values of parameters such as sex, collection types and parts isolated. All statistical tests were stated at $P < 0.05$ significant level.

Trap Apparent Density (ADT), defined as Stomoxyinae caught per trap per day (s/t/d) and mathematically expressed as:

$$ADT(f/t/d) = \frac{\text{Number of Stomoxys captured}}{\text{number of traps} \times \text{number of trapping days}}$$

The *S. n. niger* contamination rate with the FMDV was determined using the following formula:

$$\text{Contamination rate (\%)} = \frac{\text{Number of positive legs or mouth parts of } S.n.niger}{\text{Number of legs or mouth parts dissected}} \times 100$$

3. RESULTS

The FMDV RNA was found in the epithelial vesicles of all clinically sick animals (n=5, with mean ct of 27.98456) and 3 out of 5 were serologically positive in the clinically in-apparent cattle group.

The overall stomoxiines fly captured during the survey with the Vavoua trap was 568 and all flies were identified upto species level. Two species of *Stomoxys* were identified as *S. n. niger* 196 (34.51 %, 9. 33 Snn/t/d) and *S. omega* 101 (17.78%, 4.80 so/t/d). Non-biting *Musca* sp. 271(47.71%, 12.90 m/vavoua and day) was also caught. *S. n. niger* was the most abundant biting muscid in the collection. *S. n. niger* was the most nuisible biting muscoid, the reason for their choice to verify their role as mechanical vectors of FMDV [Figure 2](#).

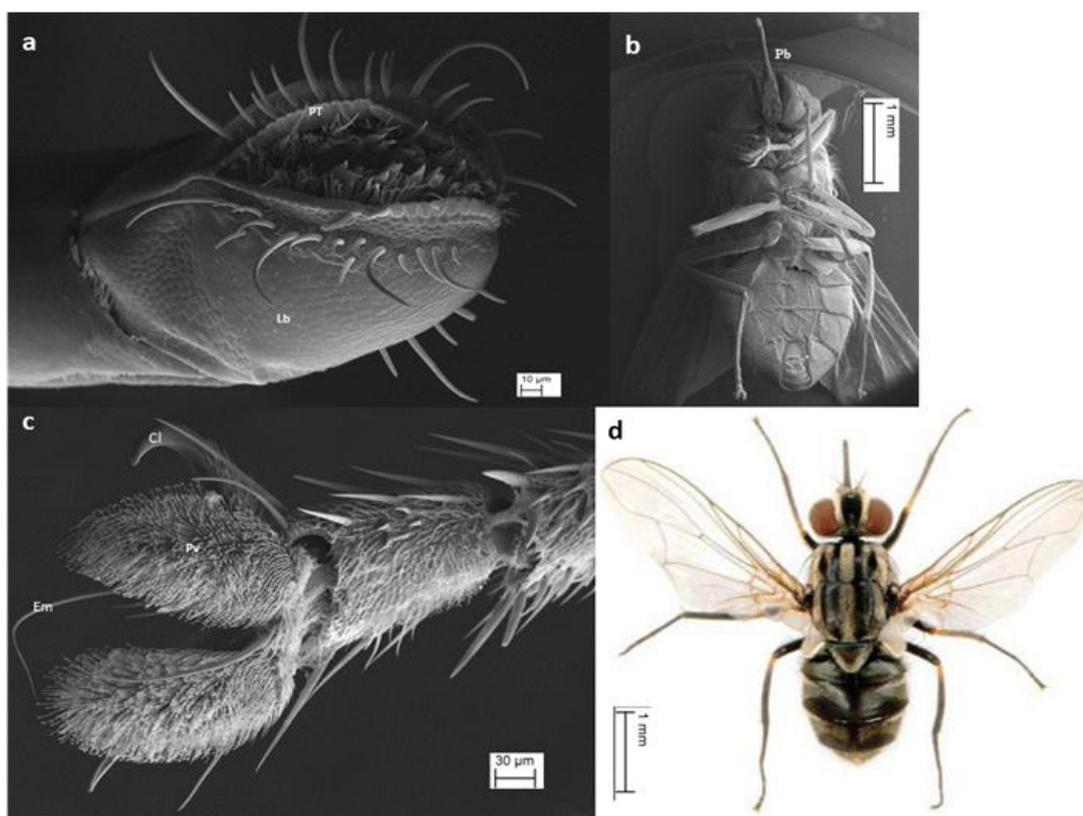


Figure-2. SEM and macroscopic photos of the anatomical parts of *S. n. niger*.

Source: Field survey, 2016.

A) SEM of *S. n. niger* proboscis- PT: prestomal teeth; Lb: Labellae; Pb: Proboscis, where the labium forms the proboscis and the labellae are transformed into a penetrating organ, which quickly pierces the skin during the blood meal. B) SEM of *S. n. niger* from the ventral side C) SEM of *S. n. niger* leg- Cl: Claws, Pv: Pulvillus hairs; Em: Empodium or sensory body: FMDV particles (size 0.03µm) presumably attach to the hairs of the pulvillus, when the fly lands on infected body parts. D) Macroscopy of adult *S. n. niger* (7mm).

There were more *S. n. niger* males 142 (72.28%) than females 54 (27.72%), but for *S. omega*, more females 64 (63.37%) were collected than males 37 (36.63%). The contamination rate with the FMDV irrespective of collection method was 40.3 % with females (49.0 %) being slightly more contaminated than males (21.7%) even though males, like females, had equal chances of being contaminated when in contact with animals in an infected herd as indicated by a non-significant difference (OR=0.45, P=0.1506) recorded with sex. Based on the univariate analysis of the various factors, females were more contaminated than males even though there was no statistically significant difference (OR= 0.45, P=0.1506) with sex. Legs were more contaminated than mouth parts with a statistically significant difference (OR=0.38, P=0.047). The anatomical parts isolated from those flies caught on clinically sick cattle were most infected than those from clinically inapparent cattle and Vavoua trap with a statistically significant difference (OR=0.24, P=0.013).

From our model, mouth parts were significantly less likely to be positive than legs, the contamination rate of the African stable fly caught from cattle with no clinical signs was not significantly different from those caught from their clinically sick counterparts Table 1. Considering the collection method, *S. n. niger* (isolated parts-mouth and legs) from the Vavoua trap were significantly less likely to be contaminated with the FMDV compared with Direct Skin Catches (DSCs).

Table-1. Generalized linear regression model comparing the various *S. n. niger* related risk factors.

Risk Factor	Coefficient Estimate	Standard Error	Z-value	P-value
Intercept	0.2890	0.4819	0.600	0.54866
Fly part (mouth)	-1.1572	0.4975	-2.326	0.02002
Collection type (DSC-RA)	-0.2255	0.5788	-0.390	0.69680
Collection type (Vavoua)	-1.6277	0.5991	-2.717	0.00659

DSC-RA Direct Skin Catch of flies from random animal group with no clinical sign.

The ct values were plotted against sex, part isolated and method of fly collection to show the mean viral RNA titres recovered from them. Females with a mean ct value slightly above 30 were less than that of males which was slightly above 35 Figure 3. Female flies were carriers of larger amounts of virus than males even though their mean amounts were not statistically significantly different. However, female parts isolated recorded the highest FMD viral titres as compared to males.

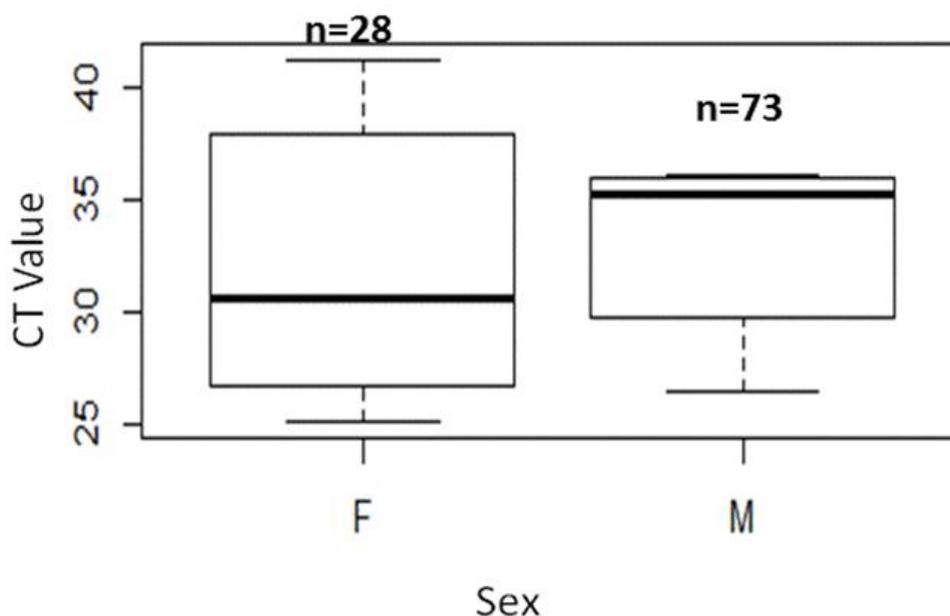


Figure-3. Comparison of ct-value with sex of *S. niger niger*.

Source: Field survey, 2016.

The ct value plot of the various anatomical parts of *S. n. niger* isolated revealed that legs had a higher FMD viral amount than mouthparts even though their mean differences were not statistically significant Figure 4. However, mouth parts possessed the highest amounts of FMDV as compared to legs.

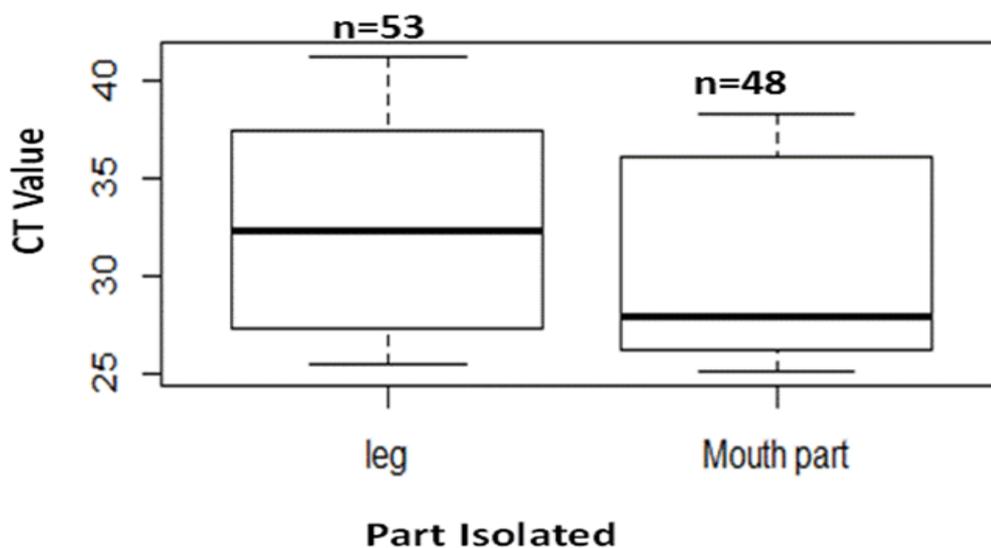


Figure-4. Comparison of ct value with the isolated anatomical parts of *S. n. niger*.
Source: Field survey, 2016.

Based on the collection method, flies caught with the Vavoua trap had the highest ct value followed by those caught with fly-nets on clinically sick animals and finally those from clinically inapparent cattle even though there was no statistically significant difference Figure 5. Flies from clinically in apparent cattle group had the highest FMD viral titer as compared to other collection sources.

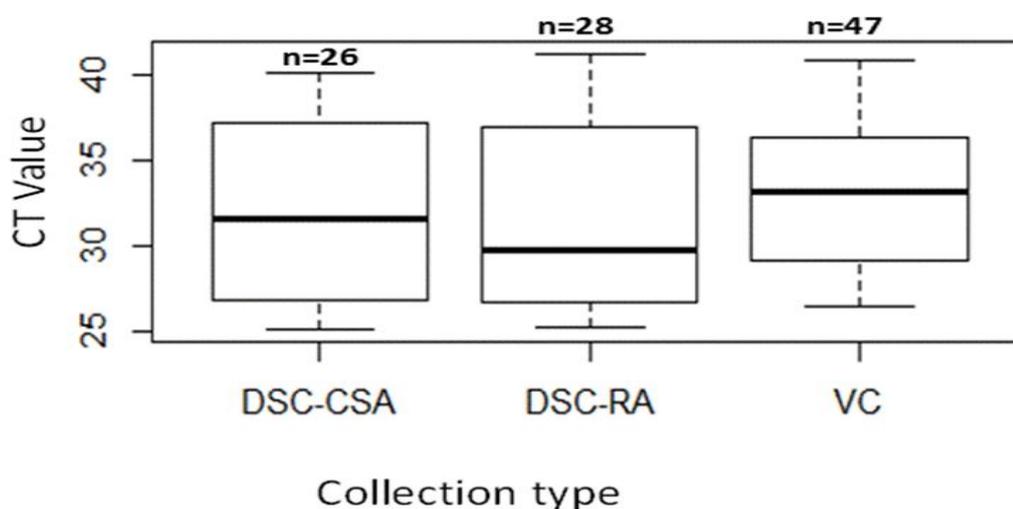


Figure-5. Comparison of Ct-value with method of *S. n. niger* collection.
Source: Field survey, 2016.

DSC-CSA Direct Skin Catch from clinical sign animals, DSC-RA Direct Skin Catch of flies from random animal with no clinical sign, VC Vavoua trap Catch.

4. DISCUSSION

The implication of the African stable fly (*S. n. niger*) in the mechanical transmission of the FMDV has not yet been described. Our present investigation in the field attempts to characterize risk factors for contamination with

FMDV for this fly. The pasture area we studied is a high transmission risk area as it neighbors the Ngaoundere cattle market and several overnight parks which receive cattle from the region and adjacent countries more especially because most of them are brought to the market already infected with the FMD.

The spread of the FMDV through direct and indirect contact to the environment is possible during outbreaks. The direct contact can be through infected muscids feeding on infected cattle and immediately feeding on naïve cattle through interruptive feeding. The study area is infested with two species of *Stomoxys* namely *S. n. niger* Macquart 1851 and *S. omega* Newstead 1907. *Musca* sp. (non-blood sucking muscids) was most frequent. *S. niger* was the most frequent biting muscid and this finding is consistent with that of Mavoungou, et al. [43] which revealed that *S. n. niger* was abundant in their collection. Our hypothesis was that 'the African stable fly biting cattle might spread the disease in the environment within their active and passive flight limits'. This hypothesis was verified as 40.3% of the anatomical parts of 101 dissected flies were positive. This result confirms the intelligent guess of Mellor, et al. [22] that apart from Capri pox and African Swine Fever Viruses transmitted by *Stomoxys* spp., FMDV can be transmitted by *Stomoxys* spp. Ticks, non-biting flies and biting flies were categorized by USDA [44] as high hazards, based either on transmission capability or long carrier status (whether mechanically or biologically) of FMDV. House flies can carry FMDV both externally and internally and the persistence of the FMDV on the exoskeleton of the house fly was reported by Rozov [19] to be 15 days. If the house fly can carry the disease for such a long time, *S. n. niger* which is a biting muscid with the same high hazard category and symbiotically coexist with this disease can transmit the disease within its active flight range of >8Km [38].

Clinical and clinically inapparent cattle may play a role in the epizootiology of the disease as flies biting them are exposed to contamination. Stable flies are compulsory blood-feeders, males and females are hematophagous and possess equal chances of being contaminated. Legs are used for landing by adult flies as they are usually seen landing on manure where they breed, permitting them to pick-up viral particles and get contaminated frequently. Mouth parts used for piercing the skin of cattle and biting open wounds of clinically sick as well clinically inapparent cases which might contain viral amounts capable of infecting flies which in turn spreads the virus through landing or during interruptive blood meal. The number of mechanical vectors landing are usually greater than biting due to several host defensive mechanisms permitting the fly to land several times, get driven and continue to search for a less defensive host. These actions permit a fly to land on FMD risk spots (open blister around the foot, mouth and around mammary glands) on the cattle, pick up the viral particles and land around the head of a susceptible case hence contaminating them. This phenomenon justifies the fact that fly-legs were contaminated more frequently than mouth parts. However, mouth parts had more FMDV RNA as compared to legs. This is because the few flies that bite blisters containing vesicles pick up high numbers of viral particles which heavily contaminates their mouthparts. Also, Rozov [19] report high persistence of the FMDV inside *Musca* spp. Higher levels of viral RNA [low ct values (less than 30)] were recovered from anatomical parts of flies caught from clinically inapparent cattle than those from cattle with clinical signs and those circulating and caught 50m away from an infected herd with a Vavoua trap. This finding is parallel to that of Nelson, et al. [45] who reported that pre-clinical sign animals were shedding more FMDV in probang samples, as compared to clinical and recovering animals and this can probably lead to high contamination of fly parts coming in contact with clinically inapparent cases in such a herd. From the experiment of Arzt, et al. [31] carrier steers (donors) with ct value of 31.8 (approximately 10^1 and $10^{2.5}$ TCID₅₀/ml) were able to cause FMD in naïve recipients through mechanical transmission using OPFs and VETs. Such mechanical transmission can equally be done by invertebrates like flies. Similarly, Suttmoller and Casas [46] reported that cattle become infected and excrete the virus despite their non-development of clinical signs of the disease. Equally, two out of the five non-clinical sign group showed clinical signs on day 9th and 16th day of monitoring with possible infection from the infected group since there was zero distance between them during fly-collection. Hence the fact that flies caught from non-clinical sign group were contaminated was not a surprise. A greater fraction of flies caught in traps are usually hungry and come to search

for a susceptible host for blood meal. However, flies can be contaminated by picking viral pathogens from contaminated cow products like milk, urine, fresh/dry manure and hair meaning flies caught with traps might have not necessarily been contaminated through biting or landing on FMD risk spots of cattle. Mouth parts were significantly less likely to be positive than legs. The contamination rate of flies from clinically in-apparent cattle was not significantly different from that of their clinical sign animal counterparts. Based on collection method, flies caught directly from cattle groups under investigation using fly-nets when compared with those collected with the Vavoua trap, revealed that those entering the Vavoua trap were less likely to be contaminated with the FMDV. Therefore, legs of flies encountering clinical sign/clinically inapparent cattle and caught around a herd reporting outbreaks with Vavoua trap will have a greater FMD viral spread than mouth parts of flies coming in contact with clinical/non-clinical and entering the Vavoua trap. Both clinical and non-clinical cattle contribute to *S. n. niger* contamination. However, if high viral amounts were to be recovered, mouth parts will be a suitable anatomical structure to be isolated. Hence, mouth parts may play a perfect role in mechanical spread of the FMDV to surrounding herds during an FMD outbreak.

5. CONCLUSION

From molecular evidence, *S. n. niger* anatomical parts (legs and mouth parts) dissected from those caught directly on cattle with fly nets and at a 50m distance using Vavoua traps were detected with FMD viral RNA. Legs were frequently and significantly contaminated as compared to mouth parts, but contaminated mouth parts had higher amounts of viral RNA as compared to legs. Contaminated *S. n. niger* caught on animals and around the herd with reported outbreak pose a potential mechanical spread to livestock in the surrounding of Mbidjoro. *S. n. niger* is a potential mechanism to spread FMDV to livestock in the surrounding of Mbidjoro. Therefore, *S. n. niger* is a potential mechanical vector for the FMDV and suggests that control measures be put in place for them during the disease outbreaks.

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