

Alighting Dipterous Insects on Cattle are Associated to Contaminative Transmission of Foot-and-Mouth Disease During Epidemics in Ngaoundere-Cameroon

Keywords: haematophagous flies, Foot-and-Mouth Disease Virus, cattle

Abstract

This study was designed to identify the landing preference sites of common hematophagous symbovine Dipterans and relate it to the FMD shedding sites. Three sets of zebu Goudali (from the DFG-COBE project herd) of different colors (black, brown and white) of same ages were restrained to sticks. Observations were made in October/November 2016 (seven days consecutively) and January 2017 (7days consecutively). Data from literature on FMDV infectious doses (TCID₅₀/ml) shedding areas in clinical cases was used to map such sites on cattle and associate it to the landing predilection sites of hematophagous dipterous insects. The total number of observed biting insects on cattle was 26779 and the following fly-groups were identified in order of magnitude: *Stomoxys* (17453), culicids (8925), *Simulium* (293), *Chrysops* (74) and *Tabanus* (34). *Chrysops* preferred biting front legs. Culicids preferred biting around legs and neck. *Tabanus* preferred biting around head and legs. *Stomoxys* preferred biting around neck and legs. The neck and legs were body parts with the highest insect-vector frequency. The alighting predilection of each insect-group differed statistically ($P < 0.05$). From the association test, an important number of each insect group was associated to at least one of the FMD contamination spots on cattle, but *Stomoxys* and culicids had a higher propensity of being contaminated as compared to others based on their high landing numbers on the exposed animals.

Introduction

Foot-and-Mouth Disease is an *Aphovirus* of the family Picornaviridae and is a highly contagious virus disease of even-toed domestic and wild ungulates. It is caused by seven serotypes notably O, A, C, Asia 1, SAT 1, SAT 2 and SAT 3, Bertram et al identified sequences of three serotypes (topotype O Africa/lineage East Africa, A/Africa and SAT2 topotype and sub-lineage Lib-12) from the Oropharyngeal Fluids (OPFs) and epithelia tissues of clinical and subclinical cattle from Ngaoundere during the 2015 FMD epidemic. Transmission pathways include contact with infected animals, fomites, soil, air and animal secretions, but the role of invertebrates in the spread of FMD is not clearly defined. USDA: APHIS: VS categorized invertebrates especially biting insects as high hazards in the spread of FMDV. This can be confirmed by the reports of Carn, Ferris et al. and Hyslop who reported that viruses of the family Picornaviridae as well as vesicle-forming viruses (like the vesicular stomatitis virus-VSV and FMD) can be mechanically transmitted by tabanids, *Stomoxys* and mosquitoes. FMD is an economically important disease because it causes high morbidity and mortality rate in calves but low in adults as well as precludes international trade between endemic and non-endemic countries [1-7].



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Based on the abundant nature of tabanids and muscids especially *Stomoxys* in the absence of tsetse flies, it has been reported that such biting flies are responsible for the spread of dangerous diseases such as FMD in cattle herds in Ngaoundere. The recovery of the FMDV RNA from *S. niger* body parts around the cattle market in Ngaoundere raised an alarm on the implication of biting flies in the spread of dangerous pathogens. The experiment of Arzt et al. revealed the transmission of FMDV from persistently infected cattle to naïve cattle recipients via mechanical transfer of unprocessed Oropharyngeal fluids (OPFs) and Vesicular Epithelial Tissues (VETs). The occurrence of high anti-FMD antibodies in brown cattle as compared to other color coat of cattle has been reported by Dickmu et al, but there is no link between FMD infection and color of host. However, high cases in brown animals can be related to the contaminative transmission caused by insect vectors which are mostly attracted to color of host and preferably brown and black colors. Moreover, if cattle were to be used as live attractive targets or traps to reduce biting flies and other blood-feeding arthropods of cattle, there is need to know the landing dynamics of these fly-groups on live cattle and associate it to the risk of picking infective agents like FMDV as baseline information for their control in pasture areas. The present objective was to associate the landing dynamics data from live cattle exposed to blood sucking insects and associate it with an FMD risk cattle map to show the implication of the different fly-groups in the contaminative transmission of the disease in Ngaoundere [8-13].

Materials and Methods

Description of the study area

The cattle paddock where the experiment was carried out falls between Latitude 7° 11'N and Longitude 13° 34'E. It was elevated at about 1000m *a.s.l.* The site was a cattle breeding zone of the Adamawa plateau in Cameroon. The dominant cattle breed of this area was

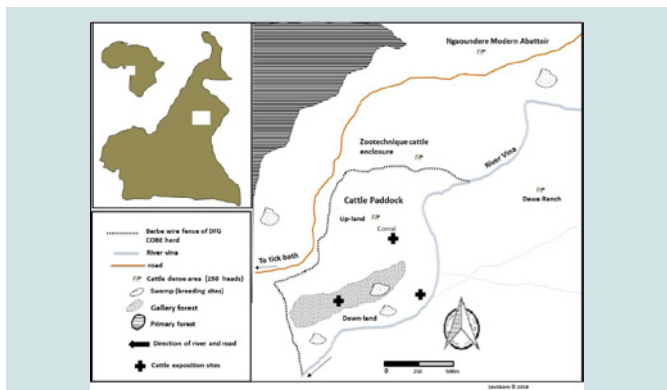


Figure 1: Map of the DFG-COBE cattle paddock showing cattle exposition sites.

Goudali, but others like White Fulani, Red Fulani, Bokolodji, Charlorais and their cross breeds (metis) prevailed in low numbers in some herds. Cattle breeds of this region had different color coats ranging from brown, white, black and a mixture of colors (red+brown, brown+white, black+white etc.). Greater than 90% of herds in this region are sedentary. The climate was the Soudan Guinean type with landscape dominated by gallery forest and open grass savanna. The main water body of this area consisted of river Vina du Sud (Figure 1).

Field exposure experiment to observe boophilic flies

Three animals from the DFG-COBE cattle herd were used for the experiment, i.e. animal type 1 (red color), animal type 2 (white color) and animal type 3 (black color) with minimum 80% color coverage. The age of the animals was between 2 and 6 years. Animals were restrained on fixed wood poles and kept at equi-distances of 5m. Cattle exposition was carried out in the potential breeding sites (i.e. in the marshy low land beside river Vina du Sud, in the gallery forest and around the cattle overnight park) of flies. The animal body-parts destined to indicate the fly predilections sites were mapped on a bovine model. Three well trained observers (to distinguish the flies up to the genus level) were close as 50 cm to the animal to identify and count the flies per lateral side of each animal. Observation was carried out following that of Hansen [14,15] (Figure 2).

Fly identification

Identification was carried-out on the spot up to genus level by well-trained observers by strictly applying the criteria found in already published taxonomic keys. For tabanids, the key of Odroyd was used. *Stomoxys* were identified using the identification key of Zumpt. Anophelinae were identified using the key of Gilles and De Meillon and that of Jupp was used for Culicinae identification. For *Simulium*, the key of Freeman and De Meillon was used [16-20].

Data on FMDV excretion or secretion dose

Data from 32 published scientific articles concerning FMDV infection experiments was exploited by consulting the published document of Bravo de Rueda. Data on FMDV in secretions and excretions were collected from 32 scientific articles published between 1965 and 2007 found in internal databases and through

the electronic (external) databases Scopus and PubMed in 2010, all reporting experimental trials involving FMDV infection. The quantify of the FMDV excreted or secreted was maintained in Tissue Culture Infectious Dose 50 per milliliter (TCID_{50/ml}) unit. The infectious dose ranged from 0.95 to 10.15 TCID_{50/ml} was considered. The data was from cattle, sheep and pigs [21].

Data analysis

Data analysis was carried out using the R-software (R version 3.4.0). The Principal Component Analysis (PCA) test was used to associate the biting sites of various fly-groups to the FMD shedding sites on cattle. The Kruskal Wallis non-parametric test was used to compare the number of alighting biting insects with respect to predilection sites. The significant level of all tests was kept at p<0.05.

Ethical Statement

Animal use protocols were reviewed and approved by the Ohio State University Institutional Animal Care and Use Committee (Protocol Number: 2012A00000154). Restrained cattle were supplied with water and fresh grass ad libitum. Animals were changed and a fresh set recruited to avoid stressing the animals. Experimental herders were present to carefully restrain the animals. Written authorization was received from the project Director of the DFG-COBE project to use the animals.

Results

The total number of biting insect vectors observed on cattle was 26779. The following taxonomic taxa were identified: *Stomoxys* (17453), culicids (8925), *Simulium* (293), *Chrysops* (74) and *Tabanus* (34). The observed insect vectors on the exposed experimental animals differed with biting insect-groups. The predilection sites of *Simulium* was around the udder/scrotum (F1) and there was a statistically significant difference (P<0.05) in their alighting predilection sites on cattle. The predilection sites of *Chrysops* was the front legs (C0 and C1) and there was a statistically significant difference (P<0.05) in their alighting preference on exposed cattle. Culicids were most frequent around legs and neck (C0 and G0), but there was a statistically significant difference (P<0.05) in their alighting sites on cattle. *Tabanus* were most frequent on the head and legs (A2 and G0) with a statistically significant difference (P<0.05) in their alighting sites. *Stomoxys* were most frequent around the neck and legs (C0 and G0) with a statistically significant difference (P<0.05) in their landing sites. It occurred that most biting insect-groups had preference for the legs and neck region and there was an overlap of some biting

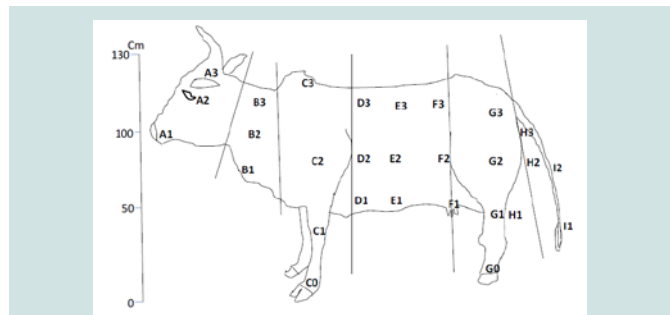


Figure 2: Map of cattle model showing potential predilection sites of flies.

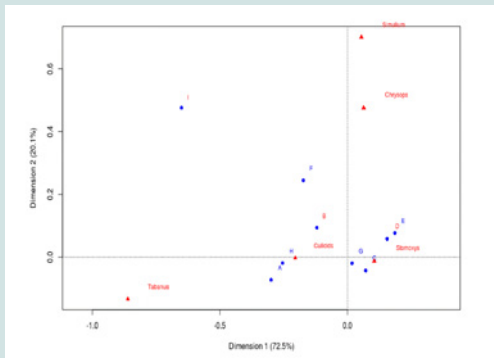


Figure 3: Analysis of correspondence showing the association of FMD risk grading and fly-groups preferred body-parts of cattle. *Letters in blue signify body part regions with high FMDV excretion potential while those in red signifies body regions with low FMDV excretion. Letters A to I represent biting preference sites of the various fly-groups as annotated on the cattle model where: A-head regions (high FMD risk region), B-neck region (low FMD risk), Co/Go-lower-leg regions (high risk of FMD), D-the trunk region is a low FMD excretion/secretion zone, EF-belly region (high risk only around E1 and F1, around the udder (female) and testes (bull)), GH-back regions (highly close to urine or faecal discharge hence high risk of contamination with FMDV) and I-tail region is a low FMDV risk region.

insect groups for some body parts like legs (Table 1).

The FMD discharge doses (TCID_{50/ml}) was gotten from already published experimental works. According to Bravo de Rueda, excretion of FMD occurred in unequal concentration in excretions from the different body parts of domestic animals (cattle, sheep and pigs). For the Upper Respiratory Tract (URT) consisting of Oropharyngeal Fluid (OPF) swabs, salivary and nasal discharges had mean infectious dose of 5.70±1.66, Vesicular Epithelia Tissues (VETs) around the mouth, tongue, udder, scrotum and interdigital spaces had mean infectious dose of 5.6, milk with infectious dose of 4.48±1.46, semen with infectious dose of 4.55±1.33, urine with infectious dose of 1.93±0.87 and faeces with infectious dose of 1.55±0.10 [21,22] (Table 2).

Association of predilection sites of haematophagous flies and FMD discharge spots on cattle

The multiple analyses of variance [Principal Component Analysis (PCA)], where body parts constituted the rows and species constituted the columns was used to associate alighting predilection sites of biting insect vectors with sites of contamination with FMD. The letters in red stood for low FMDV shedding sites while those in blue stood for high FMDV shedding sites. From the association test, all the biting insect vectors had a high probability of landing on FMDV high risk spots [A1, C0, E1, F1, G0 and H3] due to their high alighting propensity for such zones (Figure 3).

The sketch of FMDV excretion/secretion spots on cattle using the published data of Bravo de Rueda can be seen in figure 4. The mouth and nasal pathways are high sources of FMDV contamination, followed by VET around the mouth, legs and testicular/mammary region, milk, semen, urine, faeces and urine. Such spots are FMDV contamination risk areas for alighting boophilic hematophagous insects because of the infectious dose (1.55 to 5.60 TCID_{50/ml}) discharged from those sites [21] (Figure 4).

Discussion

The total number of alighting biting insects on cattle in the present study was alarming and was dominated by culicids and *Stomoxys*. Fly-groups that were likely to be observed or caught on cattle included surface feeders, blood-sucking and myiasis causing flies. The most dominant group observed on cattle constituted of muscids. Such an observation was like that of Lloyd and Dipeolu. The abundant nature of *Stomoxys* in the present study was in line with the report of Mihok and Clausen [23,24]. *Stomoxys* have been reported to adapt in several environments [17, 25]. The second most dominant group constituted of culicids and was like the observation made by Muenworn et al. [26] who showed that culicids preferred biting cattle than humans. However, the biting predilection for cattle body parts by the different fly-groups was different and most of the times overlapped. This biting site-overlap resulted in scramble-feeding which was a possible risk factor for their contamination when feeding on open sores or lesions in the case of vesicle forming diseases like Vesicular Stomatitis Virus (VSV) and FMDV. Biting preferences recorded in the present study revealed alighting preference discrepancies by the biting insect groups identified where *Stomoxys* preferred the legs and belly region, *Anopheles/Culex* preferred legs and head region, *Simulium* preferred belly region, *Chrysops* preferred legs and *Tabanus* preferred legs and head regions. An overlap in the different fly-groups predilection sites in our study has been observed for some groups like muscids and tabanids, scrambling for biting sites around the lower limb [24, 27]. The probable reason for the choice of legs by most biting insects was that the skin there was thinner and blood capillaries were closer to the surface of the skin. Those that preferred the head region like culicids and *Tabanus* might be because they were orientated to CO₂ emissions from this part of the body [28,29].

The implication of color coat in the prevalence of FMD in Cameroon by Dickmu et al. showed that brown cattle were the most infected and this was in line with the present finding that brown cattle were preferably attacked by three out of four fly-groups of biting flies identified. Among the three fly-groups associated with brown color coat was *Stomoxys* where *S. n. niger* was recently shown to be contaminated with infective doses of the foot and mouth disease virus RNA during the 2016 epidemic in the environs of the Ngaoundere cattle market. Arzt et al [9-11]. showed the mechanical transmission of FMD from persistently infected carrier cattle to naïve counterparts via the transfer of oropharyngeal fluid. Based on the association

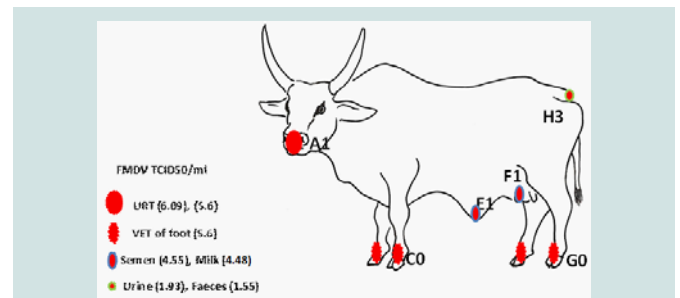


Figure 4: bovine model showing FMDV contamination risk grading based on the excreted doses from the various body parts (data from Bravo de Rueda [21] & Dillon [22]). URT: upper respiratory tract, VET: vesicular epithelia tissue.

Table 1: Number of fly counts with respected to the body part of cattle.

Body parts	<i>Simulium</i>	<i>Chrysops</i>	Culicids	<i>Tabanus</i>	<i>Stomoxys</i>
A1- head low	8	0	1073	1	680
A2-head middle	2	1	314	13	925
A3-head high	2	0	293	0	260
B1-dewlap	19	5	316	1	512
B2-neck low	17	1	222	1	357
B3-neck high	5	1	228	5	314
C0-front legs very low	32	10	1616	0	3715
C1-front legs low	28	14	984	0	2052
C2-front legs middle	10	2	394	0	1072
C3-front shoulder	1	5	352	0	733
D1-breast low	45	1	397	1	1224
D2-breast middle	8	4	219	1	492
D3-breast high	0	0	110	0	301
E1-Umbilicis/belly low	27	7	206	1	583
E2-lower breast middle	3	0	115	0	447
E3-belly high	0	0	59	0	107
F1-udder/scrotum	45	6	329	0	430
F2-belly middle	0	2	111	0	271
F3-hind shoulder	0	0	108	0	14
G0-hind legs very low	19	0	798	9	1928
G1-hind legs low	16	9	406	1	761
G2-hind legs middle	4	4	150	0	168
G3-tail high	0	0	48	0	36
H1-behind low	0	0	32	0	15
H2-behind middle	1	0	13	0	6
H3-anus	0	0	7	0	39
I1-tail low	0	0	4	0	3
I2-tail middle	1	2	21	0	8

of biting preference and FMD risk-graded spots, *Stomoxys* were strongly-positively correlated with legs and belly areas (sub parts C, D, E and G), *Tabanus* (head-sub part A), culicids (hind legs-sub part H), *Simulium* (belly region-F) and *Chrysops* (head-sub parts DE). From the association of cattle fly-frequencies/bionomics with respect to different annotated parts and the FMD risk graded maps, it occurred that all the biting flies had a high probability of biting or landing on FMDV high risk spots due to their high biting propensity for FMD risk zones on cattle. This present result is in line with the report of Carn [5]; Ferris et al. [6]; Hyslop [7] who reported that tabanids, *Stomoxys* and mosquitoes can transmit viruses of the family Picornaviridae through horizontal transmission. However, since all the groups showed equal chances of being contaminated, *Stomoxys* and culicids will have a higher probability of being contaminated as compared to others based on their high alighting densities on the exposed animals.

Conclusion

The total number of observed biting insect vectors on cattle was 26779 and the following insect-groups were identified in order of magnitude: *Stomoxys* (17453), culicids (8925), *Simulium* (293), *Chrysops* (74) and *Tabanus* (34). *Chrysops* preferred front legs (C0 and C1). Culicids preferred biting around legs and neck (C0 and G0). *Tabanus* preferred head and legs (A2 and G0) and *Stomoxys* preferred neck and legs (C0 and G0). From the association test, all the biting insect vectors had a high probability of biting or landing on FMDV high risk spots [A1, C0, E1, F1, G0 and H3] due to their high biting propensity for such zones.

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Table 2: FMDV discharge doses with respect to the source and route of contamination.

FMDV infection variable	Number of observations	Maximum average (range) TCID _{50/ml}	Maximum titre Standard Deviation TCID _{50/ml}
Contamination source	1	5.6 (NA)	NA
VET			
Airborne	9	4.33 (3.88, 5.08)	0.36
Blood	47	4.03 (0.95, 6.20)	1.18
Faeces	5	1.55 (1.50, 1.75)	0.1
Milk	40	4.48 (2.15, 7.35)	1.46
URT (OPF swabs, saliva and nasal discharge)	33	5.70 (1.25, 7.85)	1.66
Nasal discharge only	7	6.09 (2.75, 7.85)	1.61
Probang	68	4.91 (2.20, 8.65)	1.53
Semen	8	4.55 (2.10, 6.20)	1.33
Urine	10	1.93 (1.00, 3.80)	0.87
Contamination route			
Intra-nasal	37	4.68 (0.95, 8.65)	1.76
Parenteral	95	4.75 (1.25, 8.50)	1.63
Contact	88	4.17 (1.00, 8.05)	1.57
Undetermined	1	4.60 (NA)	NA

Total refers to all the maximum titres observations that were encountered. VET, vesicular epithelia tissue NA (not available), *TCID₅₀ per animal per day for airborne excretion; dose of infection and days post infection were divided as above and below the median of the maximum titer calculated using the maximum titres when either the dose of infection or the days post infection was available [21, 22].

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