



The Prevalence of Ectoparasites of Livestock and Dogs in Edo State (South-South), Nigeria

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SUMMARY

The presence of ectoparasites on animals could cause significant economic losses. Hence, profiling of these parasites on hosts in any location is the first step to planning prevention and control activity. Livestock (cattle, goats, sheep and chickens) and dogs were screened for ectoparasites (ticks, lice and fleas) in some local government areas in Edo state, Nigeria. Using standard methods, samples were collected from animals, identified and counted. Only ticks were recovered from cattle with *Amblyomma variegatum* (66%) being the most prevalent species. On goats, the different groups of ectoparasites were geographically isolated. Only tick species were seen in Oredo, lice in Esan-West and fleas in Owan-West; while on sheep, ectoparasites were relatively scanty across study locations. Only lice species were seen on chickens with *Menopon gallinae* (70.37%) most occurring. For dogs, ectoparasites diversity was richer than sampled livestock as ticks, lice and fleas were recovered. The possible significance of recovered ectoparasites in the study areas is thus discussed.

Key words: Livestock; Dog; Ectoparasites; Edo state

INTRODUCTION

In Nigeria, one-third of the country's agricultural gross domestic product is derived from the livestock sub-sector. Meanwhile, for economic gains, most Nigerian rural households and farmers are somewhat involved in livestock husbandry of which the management system is mainly by free range/extensive system or semi-intensive system. Consequently, livestock are at increased risk of

acquiring ectoparasites from their local environment. The economic losses due to ectoparasitism either directly (damage to the skin) (Lehmann, 1993) or indirectly (disease transmission) (Leeflang and Ilemobade, 1977) are noteworthy (Morgan, 1988; Byford *et al.*, 1992). Environmental drivers to parasite distribution have not been satisfactorily understood, partly

because of the intricacies associated with host-parasite systems (Diez and Pullian, 2007).

Generally, geographical distribution of the species of ectoparasites (ticks, fleas and lice) on livestock and dogs as it relates to species prevalence, composition and density/rate of infestation are not uniform because of their dependence on the prevailing environmental and climatic conditions for respective areas (Urquhart *et al.*, 2001). Considering the vastness of Nigeria with regards to the land mass and vegetation types including the dynamics of the climatic condition across the country, the information on the distribution and prevalence of ectoparasites on a range of livestock in given locations should be known; and in areas with such data, an update would often be necessary. On face value, comparative assessments of ectoparasites data on livestock by location have shown differences in the species composition and prevalence (James-Rugu and Jidayi, 2004; Ofukwu *et al.*, 2008; Tongjura *et al.*, 2012; Adang *et al.*, 2015; Lorusso *et al.*, 2016; Ona *et al.*, 2017). Therefore, attempts to identifying the most abundant ectoparasites species on livestock in respective geographical locations is a necessity because aside enriching existing information, it is a possible demonstration of the evidence of adaptation (or otherwise) to the local environment and the first step towards a more effective control activity.

Edo is one of the states in Nigeria that is partly savannah in the far north and largely rainforest; and generally, these are ^{the} two main ecological/vegetation zones in Nigeria. Information on ectoparasites prevalence and distribution on livestock and dogs in the state is largely non-existent. To our knowledge, one attempt in determining the ectoparasite prevalence was carried out on goat in an abattoir (Odogu and Okaka, 2016). We believe that the results from this study were presented to possibly demonstrate the fauna species on goat in the state. But these results could be misleading as there was a huge possibility that the screened ruminant may have been sourced from within and outside the state. In

another work, screening for ectoparasites on dogs was limited to ticks species (Isaac *et al.*, 2016). In this study therefore, roaming and free-range livestock (goat, sheep, cattle and chicken) and dogs domiciled and managed within the state across selected locations were screened for the presence of a range of ectoparasites (ticks, lice and fleas). The implication of our findings is thus discussed.

MATERIALS AND METHODS

Study Area

Edo State (05°44'-07°34'N 06°04'-06°43'E) is in South-South, Nigeria with 18 local government areas (LGAs). Edo is predominantly agrarian with a landmass of about 19,187Km² of which about 70% is cultivatable land for agricultural production. It is estimated that agriculture accounts for about 40% of the State's gross domestic product. The climate of Edo state is tropical with two distinct seasons: rainy (April and October) and dry (November to March) seasons. Average rainfall is between 150cm in the far north in Akoko Edo and Etsako East LGAs and 250cm in the south. Average temperature is 25°C in the rainy season and 28°C in the dry season. Generally, climate is humid tropical in the southern areas and sub-humid in the north. The northern fringes of Esan plateau have savannah vegetation, while the southern part is mainly rainforest with areas of secondary growth and elephant grass.

Sample Collection

Before the commencement of the survey, permission to conduct the study was sought and obtained from livestock owners. All animals were strictly handled in accordance with OIE guidelines for sample collection. Screenings for ectoparasites were carried out on cattle, goats, sheep, dogs and chickens between February and August 2017. Data on the breed, sex and age of examined livestock and dogs were noted. This study examined 150 cattle [breed: N'dama: 25; Keteku: 125]; (sex: males: 119; females: 31); (age: 1 year to 9 years)] across three LGAs (Esan

North-East (Uromi), Esan South-East (Ubiaja) and Esan-West (Ekpoma). For goat, a total of 115 [(breed: Red Sokoto: 76; West African dwarf: 39); (sex: males: 23; females: 92); (age: 2 months to 4 years)] were randomly selected across four LGAs: Esan-West (Ekpoma), Owan-West (Ozalla), Oredo (Aduwawa) and Etsako-West (Auchi). The number of sheep examined in the three LGAs: Esan-West (Ekpoma), Oredo (Aduwawa) and Etsako-West (Auchi) were 96 [(breed: Balami: 37; Uda: 50; Yankasa: 9); (sex: males: 41; females: 55); (age: 4 months to 5 years)]. In addition, three LGAs: Igueben (Idemuka and Afuda), Esan-West (Ikhido and Opoji) and Ovia North-East (Igbogo) were surveyed for ectoparasites on 108 local-breed chickens [sex: male: 53; females: 55]; (age: 4 months to 2 years)]. Also, dogs (local dogs) (sex: males: 60; females: 57); (age: 4 months to 3 years) were sampled for ectoparasites in Esan North-East (Uromi, Efandion and Amedokhan), Etsako-West (Iyakpi, Igbei and Abotse) and Esan-West (Ujoelen, Emaudo and Ujemen).

All parts of the body were screened for ectoparasites using forceps, comb and soft brush and ectoparasites were collected on white cloth material laid at the base of every livestock. The collected ectoparasites were preserved in labelled collection bottles containing 70% ethanol.

Ectoparasites Identification

Preserved ectoparasites were transported to the laboratory for identification using macroscopic and microscopic identification keys (Taylor *et al.* 2007). Before identification, ticks, fleas and lice were cleared, dehydrated and mounted (Borror *et al.* 1989). For ticks, length of the mouthparts (palps, in relation to the basis capituli), presence or absence of eyes, presence or absence of festoons, colour or markings on the dorsal shield, and shape and orientation of the anal groove were considered; while for fleas identification features like the presence or absence of comb and location of various setae and bristles, structure of various plates on the body, and male genitalia were examined. For lice, shape and size of the body, size of the tarsal claws on the meso- and meta-

thoracic legs amongst other morphological features were assessed for identification (Kim, 1991).

Data Analysis

Data were analysed by frequency distribution (frequency counts and percentages). Average ectoparasite density was estimated by dividing the total number of ectoparasite recovered by the number of livestock/dog per study area.

RESULTS

A total of three genera (*Rhipicephalus*, *Amblyomma*, *Hyalomma*) and six species of ticks were recovered from cattle. *Amblyomma variegatum* was predominant followed by *Rh. microplus* and *Rh. decoloratus*. Meanwhile, least prevalent was *H. truncatum* and was recovered in Esan North-East and Esan West (TABLE 1). On average, the infestation density per cattle ranged between 0.02 (for *H. truncatum*) and 2.42 (for *A. variegatum*).

Overall, only three louse species, eight tick species and three flea species were respectively isolated from goats in Esan-West, Oredo and Owan-West (TABLE 2). In Etsako-West, no ectoparasites were seen. Comparatively, the prevalence and average infestation density of fleas were higher than infestations with ticks and lice. On sheep ticks were absent in all LGA except Oredo. In contrast, louse (*Bovicola ovis*) was encountered in all LGA except Oredo. (TABLE 3). On the whole, the prevalence and density of ectoparasites were relatively low.

On chicken, more lice were encountered than ticks (*Haemaphysalis spinigera*) and flea (*Ceratophyllus columbae*) with the most

TABLE 1: Prevalence and density of ticks on cattle in the surveyed locations

Tick	Esan-North-East n=50		Esan-South-East n=50		Esan-West n=50		Total N=150	
	Number infested (% prevalence)	Number recovered (average density per cattle)	Number infested (% prevalence)	Number recovered (average density per cattle)	Number infested (% prevalence)	Number recovered (average density per cattle)	Number recovered (% prevalence)	
<i>Amblyomma hebraeum</i>	6(12)	7(0.14)	-	-	11(22)	14(0.28)	17(11.33)	21(0.14)
<i>Amblyomma variegatum</i>	32(64)	97(1.94)	45(90)	198(3.96)	27(54)	69(1.38)	99(66)	364(2.42)
<i>Hyalomma truncatum</i>	7(14)	2(0.04)	-	-	1(2)	1(0.02)	8(5.33)	3(0.02)
<i>Hyalomma rufipes</i>	5(10)	5(0.1)	-	-	5(10)	5(0.1)	10(6.66)	10(0.06)
<i>Rhipicephalus decoloratus</i>	1(2)	2(0.04)	7(14)	17(0.34)	11(22)	27(0.54)	19(12.66)	46(0.2)
<i>Rhipicephalus microplus</i>	16(32)	66(1.32)	-	-	12(24)	21(0.42)	28(18.66)	87(0.58)

preponderant being *Menacanthus stramineus*, *Menopon gallinae* and *Lipeurus caponis* (Table 4).

Fifteen ectoparasite species (ticks: 9; lice: 2; Fleas: 4) were recovered from dogs across the study locations. Most prevalent were *Rh. sanguineus* and *Ctenocephalides canis* (Table 5). In all, the average infestation density per dog was highest with *Rh. sanguineus* and *C. felis*.

DISCUSSION

In this investigation, the only group of ectoparasites seen on cattle were ticks (*Rhipicephalus*, *Amblyomma*, *Hyalomma*). While the aforementioned genera are commonly reported in Nigeria from previous surveys on cattle (Bayer and Maina, 1984; Lorusso *et al.*, 2013; Kamani *et al.*, 2017), at species level, *A. variegatum* followed by *Rh. microplus* and *Rh. decoloratus* were most occurring. In Nigeria, the preponderance of *A. variegatum* on cattle has also been reported elsewhere (Bayer and Maina, 1984; Kamani *et al.*, 2017) but being least prevalent in a

certain surveyed location in northern Nigeria (Lorusso *et al.* 2013). The presence of *A. variegatum* could pose a challenge to animal health because it is a vector to an array of pathogenic organisms (Hoogstraal, 1956; Uilenberg, 1981; Saidu *et al.*, 1984). Similarly, the recovery of *Rh. microplus* on cattle could be of veterinary importance as it is a known vector of *Brucella ovis* (Bock *et al.*, 2004). *Rhipicephalus microplus* was the most prevalent tick species in parts of eastern Nigeria (Eyo *et al.*, 2014) but was found completely absent in a survey in central Nigeria (Lorusso *et al.*, 2013). In some parts of Nigeria, *Rh. decoloratus* on cattle was recorded as the most abundant (Dipeolu, 1975; Lorusso *et al.*, 2013) unlike in the surveyed areas were on the average, it was the third most occurring. *Rhipicephalus decoloratus* is a one-host tick that entirely maintains its development on cattle with its presence being a risk factor to acquiring bovine anaplasmosis and babesiosis (Bock *et al.*, 2004; Aubry and Gaele, 2011).

TABLE II: Prevalence and density of ectoparasites on goat in the surveyed locations

Ectoparasite	Esan-West n=35		Owan-West n=68		Oredo n=11		Etsako-West n=10		Total N=115	
	Number infested (% prevalence)	Number recovered (average density per goat)	Number infested (% prevalence)	Number recovered (average density per goat)	Number infested (% prevalence)	Number recovered (average density per goat)	Number infested (% prevalen ce)	Number recovered (average density per goat)	Number infested (% prevalence)	Number recovered (average density per goat)
Ticks										
<i>Amblyomma variegatum</i>	-	-	-	-	1(9.09)	1(0.63)	-	-	1(0.86)	1(0.008)
<i>Hyalomma dromedarii</i>	-	-	-	-	1(9.09)	1(0.63)	-	-	1(0.86)	1(0.008)
<i>Hyalomma truncatum</i>	-	-	-	-	1(9.09)	3(0.27)	-	-	1(0.86)	3(0.02)
<i>Rhipicephalus appendiculatus</i>	-	-	-	-	1(9.09)	3(0.27)	-	-	1(0.86)	3(0.02)
<i>Rhipicephalus guilhoni</i>	-	-	-	-	1(9.09)	5(0.45)	-	-	1(0.86)	5(0.04)
<i>Rhipicephalus microplus</i>	-	-	-	-	3(27.27)	7(0.63)	-	-	3(2.6)	3(0.02)
<i>Rhipicephalus simus</i>	-	-	-	-	1(9.09)	1(0.09)	-	-	1(0.86)	1(0.008)
<i>Rhipicephalus zambeziensis</i>	-	-	-	-	2(18.18)	14(1.27)	-	-	2(1.73)	14(0.12)
Lice										
<i>Bovicola ovis</i>	1(2.85)	1(0.02)	-	-	-	-	-	-	1(0.86)	1(0.008)
<i>Damalinia caprae</i>	2(5.71)	2(0.05)	-	-	-	-	-	-	2(1.73)	2(0.01)
<i>Linognathus stenopsis</i>	2(5.71)	2(0.05)	-	-	-	-	-	-	2(1.73)	2(0.01)
Fleas										
<i>Ctenocephalid es felis</i>	-	-	23(33.82)	98 (1.44)	-	-	-	-	23(20)	98(0.85)
<i>Ctenocephalid es canis</i>	-	-	23(33.82)	118(1.73)	-	-	-	-	23(20)	98(0.85)

TABLE III: Prevalence and density of ectoparasites on sheep in the surveyed locations

Ectoparasite	Esan-West n=18		Owan-West n=5		Oredo n=73		Total N=96	
	Number infested (% prevalence)	Number recovered (average density per sheep)	Number infested (% prevalence)	Number recovered (average density per sheep)	Number infested (% prevalence)	Number recovered (average density per sheep)	Number infested (% prevalence)	Number recovered (average density per sheep)
Ticks								
<i>Amblyomma variegatum</i>	-	-	-	-	1(1.36)	1(0.01)	1(1.04)	1(0.01)
<i>Hyalomma dromedarii</i>	-	-	-	-	2(2.73)	1(0.01)	2(2.08)	2(0.02)
<i>Rhipicephalus simus</i>	-	-	-	-	1(1.36)	1(0.01)	1(1.04)	1(0.01)
Lice								
<i>Bovicola ovis</i>	2(11.11)	2(0.11)	1(20)	1(0.2)	-	-	3(3.12)	1(0.01)

TABLE IV: Prevalence and density of lice on chicken in the surveyed areas

Ectoparasite	Igueben n=50		Esan –West n=50		Ovia North-East n=8		N=108	
	Number infested (% prevalence)	Number recovered (average density per chicken)	Number infested (% prevalence)	Number recovered (average density per chicken)	Number infested (% prevalence)	Number recovered (average density per chicken)	Number infested (% prevalence)	Number recovered (average density per chicken)
Ticks								
<i>Haemaphysalis spinigera</i>	2(4)	2(0.04)	-	-	1(12.5)	2(0.25)	3(2.77)	4(0.03)
Lice								
<i>Chelopistes meleagridis</i>	3(6)	4(0.08)	8(16)	9(0.18)	4(50)	5(0.62)	15(13.88)	18(0.16)
<i>Gonicotes dissimilis</i>	11(22)	21(0.42)	10(20)	19(0.38)	-	-	21(19.44)	40(0.37)
<i>Lipeurus caponis</i>	30(60)	269(5.38)	18(36)	148(2.96)	1(12.5)	2(0.25)	49(45.37)	419(3.87)
<i>Menacanthus stramineus</i>	30(60)	208(4.16)	15(30)	66(1.32)	3(37.5)	12(1.5)	48(44.44)	286(2.64)
<i>Menopon gallinae</i>	36(72)	192(3.84)	34(68)	110(2.2)	6(75)	10(1.25)	76(70.37)	312(2.88)
Fleas								
<i>Ceratophyllus columbae</i>	20(40)	73(1.46)	4(8)	6(0.12)	2(25)	2(0.25)	26(24.07)	81(0.75)

TABLE V. Prevalence and density of ectoparasites on dogs in the surveyed locations

Ectoparasite	Esan North-East n=55		Etsako-West n=31		Esan-West n=31		Total N=117	
	Number infested (% prevalence)	Number recovered (average density per dog)	Number infested (% prevalence)	Number recovered (average density per dog)	Number infested (% prevalence)	Number recovered (average density per dog)	Number infested (% prevalence)	Number recovered (average density per dog)
Ticks								
<i>Haemaphysalis elliptica</i>	-	-	5(16.12)	12(0.38)	-	-	5(6.83)	12(0.10)
<i>Haemaphysalis leporispalustris</i>	8(14.54)	12(0.21)	-	-	-	-	8(6.83)	12(0.10)
<i>Hyalomma dromedarii</i>	-	-	7(22.55)	10(0.32)	-	-	7(5.98)	10(0.08)
<i>Rhipicephalus annulatus</i>	-	-	4(12.9)	8(0.25)	-	-	4(3.41)	8(0.06)
<i>Rhipicephalus appendiculatus</i>	1(1.81)	1(0.018)	-	-	-	-	1(0.85)	1(0.008)
<i>Rhipicephalus decoloratus</i>	-	-	-	-	4(12.9)	7(0.22)	4(3.41)	7(0.05)
<i>Rhipicephalus microplus</i>	10(18.18)	23(0.41)	19(61.29)	50(1.61)	1(3.22)	1(0.03)	34(29.05)	74(0.63)
<i>Rhipicephalus pravus</i>	-	-	-	-	1(3.22)	1(0.03)	1(0.85)	1(0.008)
<i>Rhipicephalus sanguineus</i>	30 (54.54)	68(1.23)	25(80.64)	78(2.51)	9(29.03)	23(0.74)	64(54.7)	169(1.44)
Lice								
<i>Linognathus setosus</i>	8(14.54)	25(0.45)	1(3.22)	3(0.09)	-	-	9(7.69)	28(0.23)
<i>Trichodectes canis</i>	8(14.54)	15(0.27)	4(12.9)	4(0.12)	1(3.22)	1(0.03)	13(11.11)	20(0.17)
Fleas								
<i>Ctenocephalides felis</i>	31(56.36)	53(0.96)	10(32.25)	16(0.51)	19(61.29)	46(1.48)	60 (5.28)	145(1.23)
<i>Ctenocephalides canis</i>	18(32.72)	33(0.6)	5(16.12)	6(0.91)	22(70.96)	55(1.77)	56(47.86)	94(0.84)
<i>Echidnophaga gallinacea.</i>	4(7.27)	7(0.12)	-	-	-	-	4(3.41)	7(0.05)
<i>Xenopsylla cheopis</i>	-	-	2(6.45)	3(0.09)	-	-	2(1.7)	3(0.02)

In this study, the isolation of certain groups of ectoparasites on goats in the different study locations as well as ectoparasite absence in Etsako-West brings to focus the possible role of sanitary and environmental conditions in the distribution of these parasites on livestock

However, in Oredo and Etsako-West, sample size was relatively small and as such data could be subjective. Areas with available data in Nigeria had reported some common ticks species (*A. variegatum*, *Rh. decoloratus*, *Rh. microplus*, *Rh. appendiculatus* and *H. truncatum*) (Ofukwu et

al., 2008; Tongjura *et al.*, 2012; Uttah, 2012; Adang *et al.*, 2015; Odogu and Okaka, 2016; Ona *et al.*, 2017); which were also recovered in this study. Other species of ticks scarcely reported in Nigeria but seen on goat were *Rh. appendiculatus*, *Rh. guilhoni*, *Rh. simus* and *Rh. zambeziensis*. Notably, *Rh. simus* and *Rh. guilhoni* were previously reported on cattle in parts of northern Nigeria (Mohammed, 1977; Lorusso *et al.*, 2013) but for the first time recovered on goats in Oredo LGA. *Rhipicephalus simus* is a known vector of *Anaplasma centrale* and *A. marginale* (Potgieter *et al.*, 1983; Potgieter and van Rensburg, 1987) but the veterinary significance of *Rh. guilhoni* is unknown. *Rhipicephalus appendiculatus* and *Rh. zambeziensis* are closely related morphologically (Norval *et al.*, 1982) with *Rh. appendiculatus* linked to inflicting severe ear injuries (Norval *et al.*, 1988), while the economic importance of *Rh. zambeziensis* is still being investigated.

In addition, only lice were recovered on goats in Esan-West, while in Owan-West, only flea species were encountered. Infestation with lice could result in considerable damage to the skin or fleece as infested animals do scratch and rub against fences, trees and other objects. Biting louse (*Damalinea caprae*) feed on skin and scurf, while sucking louse (*Linognathus stenopsis*) could cause more severe damage because they puncture the skin in an attempt to suck blood and tissue fluids. This survey recorded low lice infestation rate and so anaemia being associated with heavy infestation is less likely. The presence of sheep louse (*Bovicola ovis*) on goat could be instructive. The relatively high prevalence of *Ctenocephalides canis* and *C. felis* on goat in Owan-West LGA could be indicative of a close association of this livestock with dogs and cats as they are natural hosts. *Ctenocephalides felis* is a synanthropic flea and as such its presence could be of medical significance (Bitam *et al.*, 2010). Meanwhile, the likely roles of these fleas on animal health have been advanced (Dobler and Pfeiffer, 2011). For ectoparasites on sheep,

prevalence and density were low unlike some surveyed locations in Nigeria where relatively high prevalence were recorded (Tongjura *et al.*, 2012; Adang *et al.*, 2015).

Of the seven lice species seen, three (*Menopon gallinae*, *Lipeurus caponis* and *Menacanthus stramineus*) were most prominent on chicken. The reported lice species on chicken in this study varies with species distribution in other places in Nigeria. For instance, in Anambra state (South-East), *L. caponis* was most prevalent followed by *Menopon gallinae* (Ikpeze *et al.*, 2008), while in Sokoto (North-West), five species of lice were recovered with *Menacanthus cornutus* being most prevalent (Usmana *et al.*, 2012). Meanwhile, in Maiduguri (North-East), three lice species were isolated of which *Goniodes gigas* was predominant, while *L. caponis* was least occurring (Biu *et al.*, 2008). Generally, these lice feed on dry skin scales, feathers and scabs which could lead to skin irritation, reduced feed intake, slowed body growth, decreased fertility and declining egg production.

The diversity of ectoparasites on dogs was richer than livestock with ticks being the most occurring. Clearly, *Rh. sanguineus* and *Rh. microplus* were the main tick parasites seen on dogs, while other tick species were remotely seen and could only be an opportunistic infestation. *Rhipicephalus sanguineus* is known to be the main ectoparasite of dogs; and its relatively wide distribution is due to the fact that all its developmental stages have preference for domestic dogs (Walker *et al.*, 2000). The common lice that potentially cause canine pediculosis are host specific; and are the sucking louse (*Linognathus setosus*) and the chewing or biting louse (*Trichodectes canis*) (Wall and Shearer, 1997). *Linognathus setosus* and *T. canis* were recovered on dogs in most of the study locations with relatively low infestation burden. Low grade infestation may result in relentless itching through hypersensitivity reaction. *Expectedly, the prevalence of Ctenocephalides canis was high but of particular interest as regard future investigation is the*

veterinary health implications of the presence of *Echidnophaga gallinacea* (a natural bird flea) as well as *Xenopsylla cheopis* (a small mammal flea) on dogs.

CONCLUSION

The profiling of ectoparasites of livestock managed at small scale in Edo state has shown high prevalence. The management system being largely semi intensive/free-range is a possible contributor to the level of infestation. We strongly recommend that relevant authorities should ensure that livestock owners in these localities receive orientation on identification and risks associated with these parasites as well as the use of the most appropriate control measures in line with local peculiarities. Similarly, dogs are often kept as pets in homes; it is critical that owners should be wary of the infestation levels on dogs and regularly treat to prevent human infestation. In addition, future research should precisely appraise the impact of ectoparasites infestation to the economy of the livestock owners so as to further drive the need to strengthen prevention and control efforts.

Compliance with ethical standards

Statement of animal rights: This study was conducted in accordance with OIE guidelines for sample collection from live animals.

Conflict of interest: The authors declare that they have no conflicting interest.

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