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Distribution of Microsporidia spp. in tick species in cattle in parts of Ogun State, Southwestern Nigeria

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ABSTRACT

Frequent contacts between microsporidia-infected cattle and vectors, enables the role cattle plays in the transmission of Microsporidia spp. among the animals. This cross-sectional study evaluated the presence of Microsporidia spp. in tick species on traded cattle in six cattle farms in some parts of Ogun State, South-western part of Nigeria. A total of five hundred and thirty-five (535) ticks were hand-picked from various parts of the body on cattle from the study area. The hand-picked ticks were identified and screened for the presence of Microsporidia spores. Chi-square and logistic regression model performed using R software with p-value < 0.05 being set as significance. Results showed that out of the 535 ticks sampled, an overall 280 (52.3%) were infected with Microsporidia spp. Among the tick species, Amblyomma variegatum 103 (55.1%) and Dermacentor nitens 24 (42.1%) had the highest and lowest prevalence rates for microsporidiosis respectively. Majority of the infections (59.7%) were found in cattle in Abeokuta-South Local Government Area with p-value < 0.05 across the cattle farms in the study area. In relation to the developmental stages of ticks, 168 (60%) of the ticks positive for Microsporidia spp. belonged to the lymph stage. Lastly, Microsporidia spp. was twice as likely to infect ticks on cattle in Ikenne and Ijebu-North Local Government Areas (p-value <0.05). In conclusion, the study revealed that tick species found on cattle in Ogun State are home to Microsporidia spp. and it is recommended that sensitization programs on the menace of ticks to cattle traders be embarked on by stakeholders and veterinarians in Ogun State. without exacerbating public health challenges.

This study serves as a critical reminder that the intersection of aquaculture and public health requires a coordinated approach to ensure sustainable development in malaria-endemic regions like Mongu District.

KEYWORDS: *Cattle, Distribution, Microsporidia spp., Nigeria, Ogun State, Ticks.*

INTRODUCTION

Ticks, known to be ecto-parasites, are tiny arachnids belonging to the families Argasidae and Ixodidae [1]. They have been recognized as the second largest vectors after mosquitoes. These ecto-parasites practice hematophagous as they feed on the blood of mammals and amphibians. Through the use of their flexible mouthparts called chelicerae which is specialized for both piercing the skin and imbibe liquid food including blood, tick infestations have been linked to harmful consequences [2], [3]. With over 50 species known to infest domestic animals, Amblyomma, Hyalomma and Rhipicephalus spp. have the greatest detrimental consequences on the health of cattle [4]. One of the major veterinary and public health issues in the world are ticks which have an impact on 80% of the world's cattle population [5]. Nigeria is one of the largest livestock-raising countries in Africa as a large part of their cattle come through cross-border movements through neighbouring countries such as Niger, Chad and Cameroon. Based on their potential to directly or indirectly result in significant financial losses, ticks are also significant to the Nigerian livestock industry [6], [7]. In terms of significance, they cause serious harm to hides and skin and pose a number of obstacles to Nigeria's cattle output [8].

Microsporidia spp. considered a diverse group of intracellular parasites and formerly classified as protozoans, are now grouped under fungi based on their microscopic and spore characteristics [9], [10]. They are known to cause the global and agriculturally significant infection, called microsporidiosis, having a wide host range that includes insects, fish, ruminants, primates and humans [11]. The spores which are the infective stage usually appear typically between 1—4 µm in size, possessing unique organelles like the polar tubule and prokaryotic-size ribosomes [9]. Globally, over 187 genera and around 1700 species have been reported. Notable, veterinary and medical-important species of microsporidia are Enterocytozoon bieneusi (E. bieneusi), Encephalitozoon intestinalis (E. intestinalis), Encephalitozoon hellem (E. hellen) and Encephalitozoon cuniculi (E. cuniculi), out of which E. bieneusi accounts for more than 90% of human and animals' microsporidian cases [12]. Transmission typically occurs through ingestion of contaminated food or water, and other routes such as aerosol inhalation, contact with infected animals, sexual contact, organ transplantation and vertical transmission. Human clinical manifestations are dependent on the infecting species and host immunity. For example, while Han et al. [9] reported that in HIV-infected patients, microsporidiosis is most common in those severely immunocompromised (CD4 < 100/µL), seroprevalence rates ranging from 1.3% to 22% with 13% prevalence of E. bieneusi infection were also detected in non-HIV-infected populations [10]. Furthermore, varied prevalence within countries have been documented; in Nigeria, for instance, microsporidiosis in humans was 23.3% in Lagos State and 7.5% in Ibadan, Oyo State [10].

Zoonotic transmission, especially from animals to humans is likely. The phylogenetic analysis of microsporidia trees, revealed that Encephalitozoon cuniculi and Encephalitozoon hellem detected in non-human mammals and birds, respectively share genetic similarities with strains found in humans, indicating possible transmission from animals [11].

Cattle play a major role as reservoir hosts in the transmission of Microsporidia spp. and this is because of the close contact the animal have with humans [12]. In a systematic review by Ruan et al. [10], the overall prevalence rate in cattle was 16.6% (2216/12,175). In Nigeria, the presence of Microsporidia spp. in cattle has been reported in parts of South-western, Nigeria. Ajagbe et al. [13] and Ojuromi et al. [14] reported 31.4% and 12% among cattle in some parts of Abeokuta, Ogun and Lagos States, respectively. Studies on Microsporidia spp., in animals are still limited despite the possibilities of its zoonotic transmission. Presently, there is limited data on the distribution of Microsporidia spp. among cattle in Nigeria. Therefore, this study aimed to provide more information on the prevalence of microsporidiosis among tick species harbored by cattle in cattle farms in some parts of Ogun State, Nigeria.

MATERIALS AND METHODS

Description of Study Area

Ogun State is located in the southwestern region of Nigeria and some of the agricultural activities carried out in the State are cultivation of crops such as yam, maize, cassava, plantain and rearing of livestock (sheep, poultry, goat and cattle). This study was carried out in four randomly selected six Local Government Areas (LGAs) (Figure 1), across six cattle farms- Olomore and Lafenwa (Abeokuta-North LGA), Oke-Ibukun and Gbangban (Abeokuta-South LGA), Ijebu-Igbo

(Ijebu-North LGA) and Ogere (Ikenne LGA).

Ethics

An introductory letter from Olabisi Onabanjo University, Ago-Iwoye, Ogun State, Nigeria was taken to each of the cattle farms. Approval was sought from the cattle farm managers after enlightenment on the purpose and nature of the study was done.

Sample Study and Sample Size

This study was a cross-sectional survey conducted between January and May 2022, during which a total of 535 ticks were collected from cattle across six purposively selected farms. Farms were chosen based on their role in supplying major markets and their cattle population, with only those having at least 50 cattle considered for inclusion. Local intelligence guided the identification of these farms, and between one and three farms were sampled per Local Government Area (LGA), covering approximately 150 cattle per LGA. All selected farms practiced open grazing. To ensure representative sampling, all available cattle at the time of visit were included in the study. However, the sample size per farm varied depending on the total number of cattle present.

Sample Collection

Tick samples were hand-picked from different parts of the body of cattle that had been carefully and properly restrained using anti-kick and ropes with the help of the farms' staff. The retrieved ticks were put clearly-labeled universal sample bottles and preserved in 70% ethanol. All the preserved samples were transported to the Laboratory of the Department of Zoology and Environmental Biology, Olabisi Onabanjo University, Ogun State Nigeria for further analysis.

Identification of Ticks collected from the Cattle

The identification of the sex, developmental stage and species of the ticks was performed according to a published taxonomical key using a stereo- and a light microscope as described by Walker et al. [15]. Isolation and Identification of Microsporidia spp.

After identification, each tick was wrapped in foil paper and frozen for two hours. With the aid of a clean mortar and pestle, the frozen tick was crushed before being placed in sterile micro-centrifuge tubes. Distilled water was added to the crushed tick until a thin transparent layer was created. 20µl of homogenate was placed on a clean glass slide, spread into a smear and the smear allowed to air dry. Following fixing the dried smear in absolute 70% methanol for five minutes, the fixative was washed off, and then saturated in dilute Giemsa's stain and left for 20 minutes. After staining, running tap water was applied to the glass slide to make the stain float off and avoid precipitation. Finally, all the slides were air-dried and examined under a compound microscope using 10x and 40x. Using the modified trichrome stain procedure by Garcia [16], Microsporidia spp. spores were confirmed.

Data Analysis

Data obtained from this study were initially entered into an excel spreadsheet, cleaned up imported into R software for further statistical analysis. Chi-square was carried out with p-value set at <0.05. Logistic regression models (multivariate and univariate) was used to calculate the relationship between the explanatory (study location, specie of ticks, sex of ticks) and outcome variables (prevalence of Microsporidia spp.). The estimated 95% Confidence Intervals (CI) and regression estimations were then given with a level of significance set at p-value < 0.05.

RESULTS

Morphological identification of tick spp. by location and sex across parts of Ogun State, Nigeria

Out of the 535 ticks harvested from the cattle, 261 (48.8%) were of adult stage while 274 (51.2%) belonged to the lymph stage. Five (5) tick species were identified morphologically across the 6 cattle farms in the study area (Figure 2). Rhipicephalus microplus (R. microplus) was the most common tick species with 252 (47.1%) and Amblyomma tapirellum (A. tapirellum) being 14 (2.6%). In relation to sex, there were more female ticks for all the species identified, except Amblyomma variegatum (A. variegatum) which had more male ticks of 124 (66.3%) (Figure 3).

Prevalence of microsporidiosis in tick species across parts of Ogun State, Nigeria

A total of 280 (52.3%) of the 535 ticks collected from cattle across the study area had microsporidia (Figure 4). The majority of the infections (59.7%)

were found in ticks from Abeokuta-South LGA and the least in Ikenne LGA having a prevalence of 49.8%. The incidence of microsporidia varied significantly (p-value =0.014) among the sampled LGAs.

Prevalence of Microsporidia spp. across tick species in the parts of Ogun State, Nigeria

In Table 1 the prevalence of Microsporidia spp. in relation to tick species across the study area showed that, of the 5 species of tick observed, Microsporidia spp. was more associated with A. tapirellium (64.3%) and Dermacentor nitens (D. nitens) had the least prevalence of 42.1%. There were no significant differences in the proportion of Microsporidia spp. infection in the ticks recorded across the various LGAs in this study (p-value > 0.05) (Table 1).

Prevalence of Microsporidia spp. in relation to sex among tick species in parts of Ogun State, Nigeria

Microsporidia spp. infection was more among the female tick species observed across all the 4 LGAs (Figure 5). Statistically, there were also no significant differences in the proportion of Microsporidia spp. infection among the ticks in relation to their sex (p-value > 0.05) as p-value = 0.21 for male ticks and p-value = 0.09 for the female ticks.

Prevalence of Microsporidia spp. in relation to stages of tick species in parts of Ogun State, Nigeria

In Table 2 among the ticks positive for Microsporidia spp. infection, the adult stage (40%) was detected to be the least infected.

Logistic regression model between Microsporidia spp. infection and variables

The univariate and multivariate logistic regression relationships between the presence of Microsporidia spp. infection and other factors such as species of the ticks, gender and location of cattle farms are shown in Table 3. Analysis showed that among the three variables, only LGA was linked with Microsporidia spp. infection in the model. Lastly, Microsporidia spp. infection rates were twice as likely to occur in Ikenne and Ijebu-North LGAs (OR: 2.32; 95% CI: 1.18-4.72, p=0.02 and 0.03, respectively).

DISCUSSION

The present study has exhibited that 5 different tick species belonging to 3 genera were harbored on cattle in cattle farms in Ogun State, Nigeria. These harbored species are found in the sub-tropic and tropic regions of the world. According to Kyari et al. [8] and de la Fuente et al. [17], various factors that contribute to their distribution are; movement of unrestricted animals crossing country's borders, nomadic or trans-movement, lack of health regulations, unrestricted livestock grazing and favorable climatic changes and conditions which enable the breeding and growth of tick species.

The most dominant tick species among the ticks associated with the cattle in the study area was the R. microplus known to transmit of bovine babesiosis. Also, diseases like anaplasmosis and theileriosis transmitted by ticks have also been reported among cattle in parts of Nigeria [18], [19]. The survival of the R. microplus, is due to the hard nature of the cuticle which can withstand the high temperature of the tropical environment in which they find themselves. A study that has also reported R. microplus as the most dominant tick in terms of infestation of cattle was in Kwara State [20] (Nigeria). Opara et al. [21] detected a prevalence of 9.6 % for D. variabilis in Borno and Yobe States (Northeastern, Nigeria). In relation to sex of the ticks, although there was a variance between study communities (p = 0.001), it was observed that there were more female ticks than male ticks across all the species except A. variegatum. The abundance of female ticks on cattle have also been reported in a cross-sectional study in Sokoto State, Nigeria by Okwuonu et al. [22]. The abundance of female ticks across most of the tick genera detected on the cattle in our study could be attributed to the developmental stage of the ticks collected from the bodies of the cattle at the time of the study. Fully engorged adult female ticks are expected to drop off from the bodies of their hosts in order to lay eggs [23].

Due to frequent interaction between cattle and people, according to Ali et al. [24], cattle play a significant role as animal reservoirs for microsporidia. The findings of this study showed that 280 (52.3%) of the 535 ticks that were harvested and tested were found to be microsporidia-positive. This was in line with the findings of Ajagbe et al. [13] in parts of Abeokuta in Ogun State, South-Western, Nigeria. In other parts of the world, Al-Herrawy and Gad [25], Udonsom et al. [26] and Zhang et al. [27] have reported prevalence of 14.3%, 5% and 17.5% for

E. bieneusi in Egypt, Thailand and China, respectively. Although there were variations in the prevalence among our study populations (p = 0.014), most of the microsporidia-infested ticks were found in Abeokuta-South LGA. The high prevalence in Abeokuta-South LGA could be due to the constant cross-border influx of cattle brought in for sales in the LGA. Other risk factors that could enhance the transmission of the disease could be hygiene of the cattle shed, age of cattle and the season. Interestingly, female ticks across the tick species were more infested with Microsporidia spp. than their male counterparts. Their large size, prolonged feeding habits on the body of their hosts and higher pathogen exposure could contribute to their susceptibility to the disease. The risk of infection is higher in lymph stage than in the adult stage of the ticks. According to Rochlin and Toledo [28], lymph stage of ticks is more dangerous as they are numerous, hard to detect, minute and very active during temperate and tropical climates. Microsporidiosis was likely to be 2 times more in ticks in Ikenne and Ijebu-North LGAs when compared to Abeokuta LGA; this was not surprising as during our interactions with the cattle traders in these two LGAs (Ikenne and Ijebu-North), it was revealed that they did not consider ticks to be a substantial threat to their livestock health.

As long as the inter-relationships between humans, animals, and tick vectors exist, a multidisciplinary approach linking human, animal and environmental health within a "One Health" framework is essential [17]. Furthermore, the presence of a strain named Microsporidia MB (regarded as a symbiont) in mosquitoes could serve as a possible biological control against the vector by hindering the transmission of Plasmodium parasites, although this symbiont has been reported to have no effect on the development and fecundity of the infected mosquitoes [29]. Herren et al. [30], Akorli et al. [29] and Moustapha et al. [31] have affirmed Anopheles spp. to be associated with Microsporidia MB in parts of East and West Africa. Currently, there is no documented data concerning the presence of Microsporidia MB in mosquitoes and its Plasmodium transmission blocking potentials in Nigeria.

CONCLUSION

Cattle raised in Ogun State, Nigeria are susceptible to tick infestation which in turn could be Microsporidia spp. infected. It is believed that further research in humans and animals in relation to tick infestations will help to improve our knowledge on the transmission and host reservoirs in our environment as well as to also pose as a benefit in the control of primary malaria vectors in Nigeria on the other hand. Therefore, we recommend the development of programs in educating cattle traders on issues including ticks' propensity to spread disease to humans and animals, and effective pest control be embarked on by the local and national stakeholders.

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APPENDICES

Appendix A. Figures

Appendix A.1

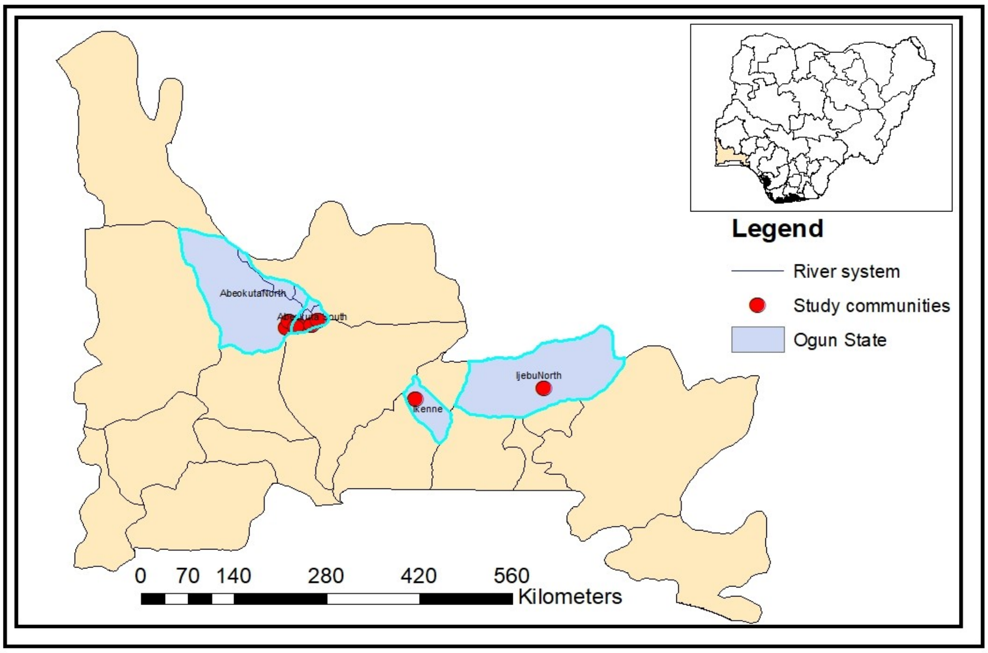

Figure 1: Map showing the cattle farms where the study was carried out across Ogun State, Nigeria

Appendix A.2

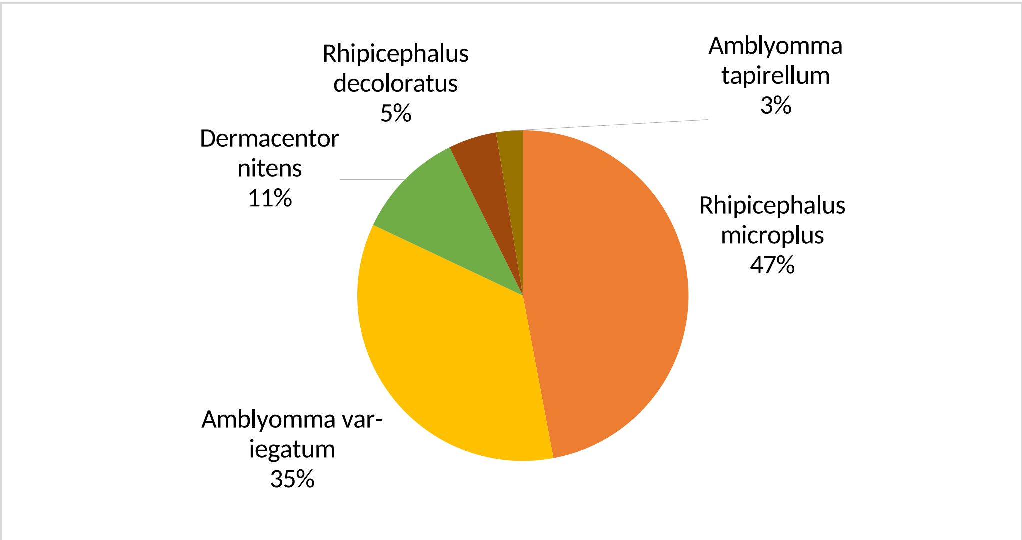

Figure 2: Morphological identification of tick species on cattle across parts of Ogun State, Nigeria

Appendix A.3

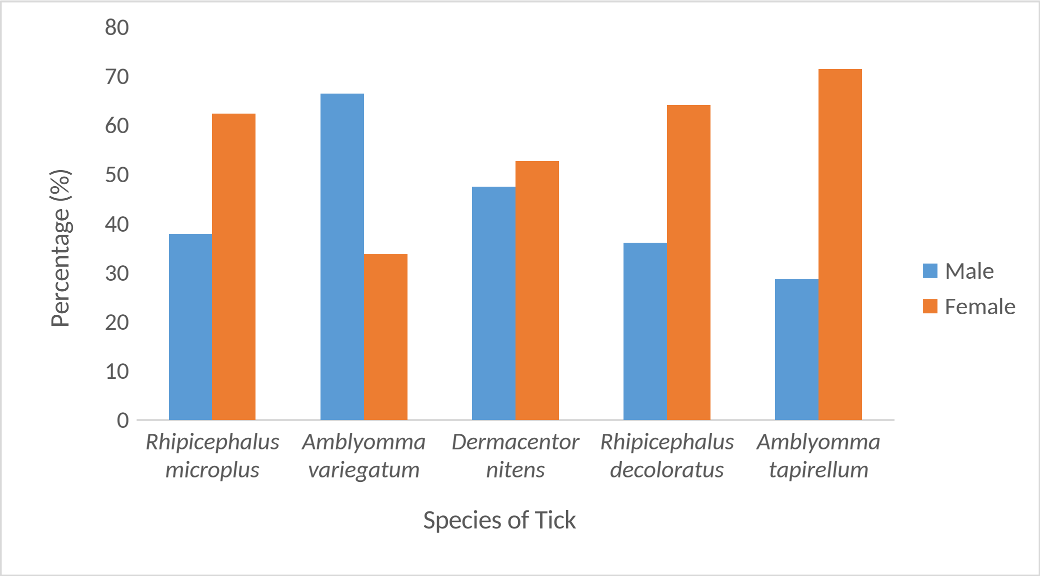

Figure 3: Distribution of tick species in terms of sex across the study area in Ogun State, Nigeria

Appendix A.4

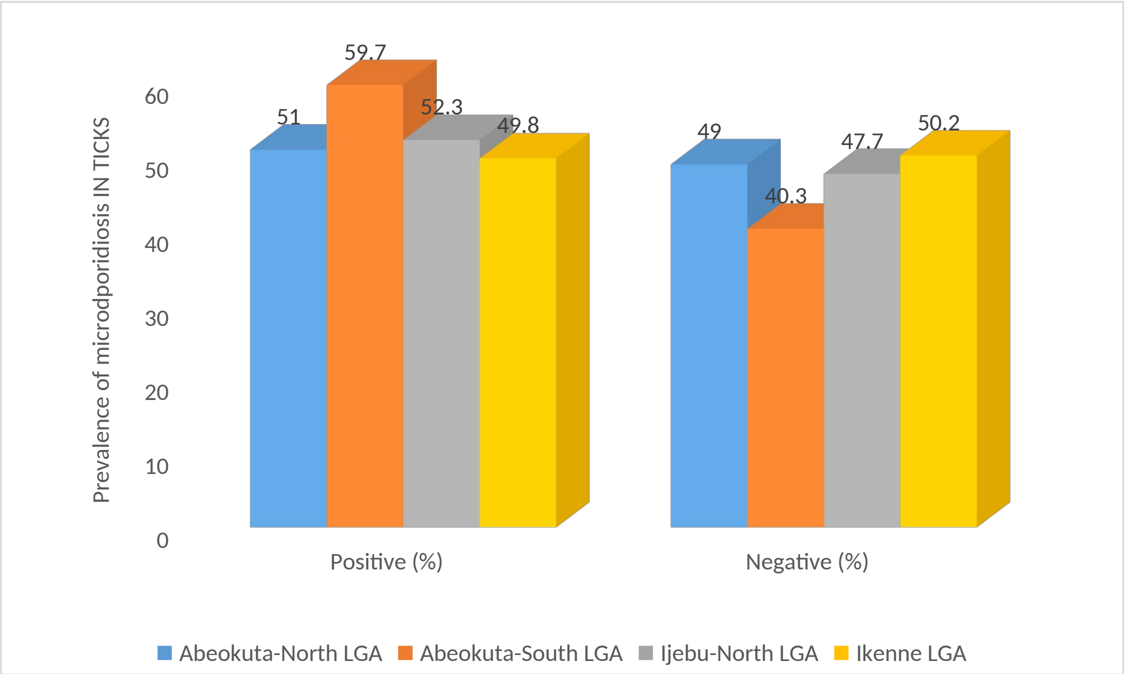

Figure 4: Prevalence of Microsporidia spp. across the study area in parts of Ogun State, Nigeria

Appendix A.5

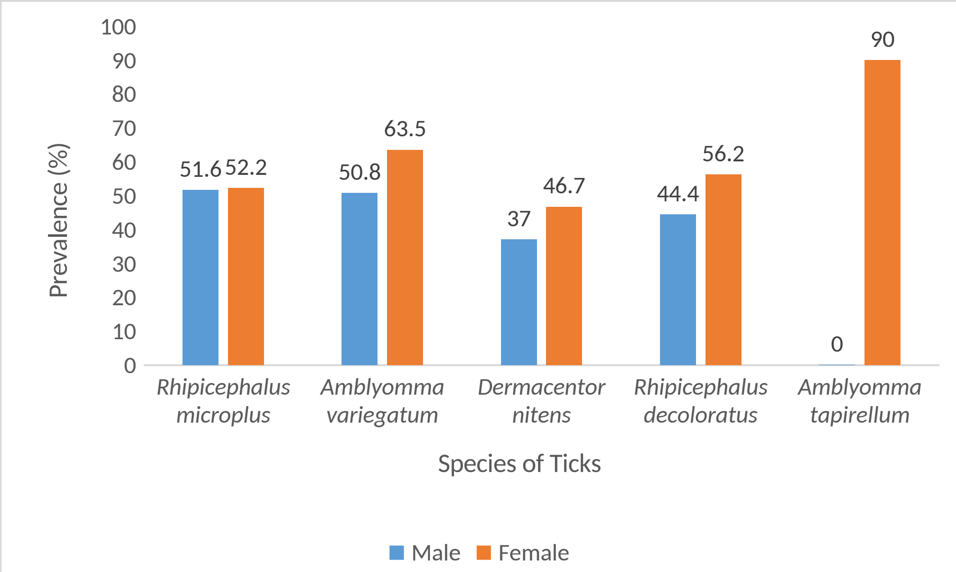

Figure 5: Prevalence of microsporidia in relation to the sex of various tick species

Appendix B. Tables

Appendix B.1

Table 1: Prevalence of Microsporidia in relation to tick species in the study area in Ogun State, Nigeria

	Study LGAs										
Tick species	**Abeokuta-North**		**Abeokuta-South**		**Ijebu-North**		**Ikenne**		**Total**		**p-value**
	NE (NI)	**%**	**NE (NI)**	**%**	**NE (NI)**	**%**	**NE (NI)**	**%**	**NE (NI)**	**%**	
Rhipicephalus microplus	81 (40)	49.4	57 (34)	56.6	53 (24)	45.3	61 (33)	54.1	252 (131)	52	0.45
Amblyomma variegatum	14 (11)	78.6	15 (8)	53.3	79 (45)	57	79 (39)	49.4	187 (103)	55.1	0.23
Dermacentor nitens	24 (10)	41.7	11 (6)	54.5	0 (0)	0	22 (8)	36.4	57 (24)	42.1	0.80
Rhipicephalus decoloratus	25 (13)	52.0	0 (0)	0	0 (0)	0	0 (0)	0	25 (13)	52.0	0.99
Amblyomma tapirellum	5 (2)	40.0	9 (7)	77.7	0 (0)	0	0 (0)	0	14 (9)	64.3	0.57
Total	149 (76)	51.0	92 (55)	59.7	132 (69)	52.3	162 (80)	49.8	535 (280)	52.3	0.44
p-value	0.25		0.81		0.73		0.73		0.44		

NE: Number of ticks examined; NI: Number of ticks infected; LGA- Local Government Area; **(%) –** Percentage of Number Infected

Appendix B.2

Table 2:Prevalence of Microsporidia spp. among the different stages of Ticks spp. harvested in the study area

	Stages of Ticks		
***Microsporidia* spp.**	Lymph (%)	Adult (%)	Total (%)
Positive	168(60)	112(40)	280(100)
Negative	106(41.5)	149(58.5)	255(100)
Total	**274(51.2)**	**261(48.8)**	**535(100)**

% - Percentage

Appendix B.3

Table 3: Relationship between Microsporidia spp. infection in ticks and selected variables

	OR	**(95% CI)**	**p-value**	**OR**	**(95% CI)**	**p-value**
Variables						
LGA						
Abeokuta-North	1	-	-	1	-	-
Abeokuta-South	0.70	0.41-1.18	0.18	1.2	0.53-2.74	0.67
Ijebu-North	0.95	0.59-1.52	0.83	2.17	1.08-4.51	0.03**
Ikenne	1.07	0.68-1.67	0.77	2.32	1.18-4.72	0.02**
Specie of Ticks						
Rhipicephalus microplus	1	-	-	1	-	-
Amblyomma variegatum	0.88	0.60-1.29	0.52	0.79	0.51-1.21	0.27
Dermacentor nitens	1.49	0.84-2.68	0.18	1.36	0.74-2.55	0.32
Amblyomma tapirellum	0.6	0.18-1.79	0.37	1.03	0.3-3.24	0.96
Rhipicephalus decoloratus	1.00	0.43-2.29	1.00	0.61	0.24-1.57	0.31
Sex of ticks						
Male	1	-	-	1	-	-
Female	0.75	0.53-1.05	0.10	0.76	0.53-1.09	0.14

OR: Odd Ratio; AOR: Adjusted Odd Ratio: 95% CI: 95% Confidence Interval; LGA- Local Government Area; **Significant result