# INTRODUCTION

Ticks are one of the most important ectoparasites of livestock. They are transmitters of diseases important to livestock farming throughout the world. Tick infestation on animals can lead to direct effects such as discomfort, soreness, self-inflicted injuries from scratching, irritation and inflammation, allergic reactions, blood and in turn weight loss. Ticks have been observed to act as vectors of diseases like anaplasmosis, babesiosis, cowdriosis, theileriosis, and Rickettsiosis. In West Africa, *Amblyomma variegatum* and *Rhipicephalus. microplus* are among the most prevalent tick species of economic importance (Heylen et al., 2023).

The life stage of a tick is known to cause similar but different levels of effect on their hosts. Ticks are vectors of diseases of and their transmission potential has been found to differ by life stage. Adult ticks are generally bigger than their nymphal counterparts which would take up more blood from the host and cause significantly more discomfort to it. This is the case for the sexes of ticks. Female and male ticks have been observed to contain slightly too widely different microbiota, causing different levels of disease infliction to their host. Furthermore, Female ticks are more likely to cause significant damage to the host due to their large feeding sites, which can lead to irritation, inflammation, and secondary infections (Van Treuren et al., 2015). Their larger numbers would intensify future tick infestations and increase the potential for disease transmission.

Ticks have been seen to have a preference for certain predilection areas. Differences in their choices for attachment may be largely due to the tick's ability to attach to the cattle host's skin, the cattle species, hair density, body temperature, blood vessel proximity, species-specific evolutionary adaptations, and environmental and microclimatic conditions.

In the southwest region of Nigeria, most of the cattle consumed are raised in the northern part of the country. These cattle are brought in from various northern states and occasionally from neighbouring countries like Niger and Chad. Cattle are a source of meat, hides and milk in these areas. In Nigeria and much of Sub-Saharan Africa, cattle also hold social significance, symbolizing status and playing a part in cultural and religious practices. Cattle farming in rural areas is vital, supporting diverse livelihoods and offering alternative ways to make money.

The attachment of ticks to the cattle ends up being of high economic loss for the farmers. They have the capacity for long-term attachment and would act as vectors of microbes that are of veterinary importance. Interestingly, regardless of the tick burden on their livestock, the Fulani pastoralists do not usually employ acaricides (Bayer & Maina, 1984). The variations in tick biodiversity and abundance in cattle ruminants have been poorly studied in Nigeria. Interestingly, many studies in the region study the abundance or prevalence of ticks at predilection sites, but no study has been done to evaluate species-specific tick preference at the different predilection sites of cattle.

For most cattle sold in the southwest region of Nigeria, they have been imported from the northern states. Since these cattle are mostly reared in pastoral conditions, it is important to understand the ticks’ diversity, as this would give credence to how much these ectoparasites can adapt in the dynamic climatic conditions across the country (and beyond). Enriching already existing information would be needed for designing control approaches by pest regulatory agencies, and for equipping animal health authorities for potential tick-borne disease (TBD) management.

Research on ticks in Nigeria has yet to explore co-infestation patterns in cattle. Most studies have only focused on single-, double-, or triple-species infestations, which provide limited information on the specific species commonly found at preferred predilection sites. This highlights the need for more comprehensive community-level studies using multivariate approaches.

The ticks are of important concern in Nigeria. Very few surveys have been published on ticks in Edo state, Nigeria. Peculiarly, there has been no study on tick infestation in cattle in Edo state, except for the first being recorded in 2019, by Adane et al. (2019).

Ticks remain a critical concern in Nigeria, with few surveys conducted in Edo state, the most recent being by Adane et al. (2019). This study aims to address the current knowledge gap by [1] estimating tick diversity and abundance on a cattle ranch in Edo state, Nigeria, and further, [2] examining the community-level structure of ticks that infest different predilection sites on cattle, and [3] investigating whether certain tick population, life stage and sexes show a preference for specific predilection areas.

# MATERIALS AND METHOD

## 2.4. Statistical analysis

For this study, we used the R software (Version 4.4.0) and Paleontological Statistics (PAST) Version 4.03 software (Hammer & Harper, 2024) to analyse the tick data we collected. All data aggregation and manipulation was done in R.

Margalef indices at each predilection site for individual cattle were calculated using PAST software as a measure of the species richness of each tick community.

Margalef index: *D = (S - 1) / ln(N)*

Where: D is the Margalef Index; S is the total number of species; N is the total number of individuals; ln is the natural logarithm.

Overall, species richness was estimated using the Chao 2 and Jacknife 2 estimators. In addition, a species accumulation curve (SAC) was used to visually show how much sampling effort influences the observed species richness.

A negative binomial model compared ticks' mean abundance and species richness across all cattle predilection sites. This was computed using the ‘glm.nb()’ function of the “MASS” package in R (Venables & Ripley, 2002) . As a post hoc to assess significant differences in abundance and across each predilection site, Tukey tests (α = 0.05) for multiple comparisons were computed using the ‘glht()’ function from the "multcomp" package (Hothorn et al., 2008), given the existence of five distinct habitat levels. Also, a Kruskal-walis test was computed on the calculated Margalef index to compare its difference across all predilection sites, using the ‘kruskal.test()’ function in the “dunn.test” package.

Non-metric Multidimensional Scaling (NMDS) was used to analyze and visualize the overall dissimilarity of the tick community at the cattle predilection sites, using the Bray-Curtis coefficient to create similarity matrices. Two dimensions adequately captured the pattern in the data. We employed Permutative Analysis of Variance (PERMANOVA) to check for significant changes in the tick community compositions for Sex, life stage and predilection sites, determined through 9999 permutations using the ‘adonis2()’ function from the “Vegan” package in R. As a post hoc, we used the ‘pairwise.adonis()’ function to check for significant differences (α = 0.05) between communities.

# RESULTS

In this survey,75 of the 95 sampled cattle were infected with at least one tick species, revealing a prevalence of 78.95%. Each tick-infested-cattle had a median of 3 distinct species of ticks. Overall, 1930 ticks consisting of 1679 females and 251 males were collected in the surveys. From this, 1689 were adults while 241 were nymphal stage ticks. Ticks were collected from all 6 predilection sites: belly (432), head (356), leg (417), Neck (282), shoulder (99) and tail (344). Four (4) genera of ixodic ticks were identified genera (*Amblyomma, Boophilus, Rhipicephalus and Haemaphysalis)*, consisting of twelve (15) species of ticks as shown in Table 1. The most prevalent tick species were *B. annulatus* (65.26%)*, B. decoloratus* (72.63%)and *B. geigyi* (66.32%)*.* Similarly, thesetickswere the most abundant ticks sampled*,* with relative abundance of42.38%, 29.38% and 20.93% for *B. annulatus*, *B. decoloratus*, and *B. geigyi,* respectively. Furthermore, these three species of ticks made up over 92% of ticks collected from all tick-positive cattle.

Relatively rare species, including *R. quilhoni*, *H. laechi*, and an unidentified species from the *Boophilus* genus, were observed. Each of these tick species was found on a single cattle, with a prevalence of 1.05% for each species.

Table 1: Tick species prevalence (%)

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Species** | **Belly** | **Head** | **Leg** | **Neck** | **Shoulder** | **Tail** | **Overall** |
| ***A. coharenses*** | 0.23 | 0.56 | 0.48 | 0.71 | 0 | 0.58 | 0.47 |
| ***A. variegatum*** | 1.62 | 1.69 | 3.6 | 1.42 | 0 | 4.36 | 2.44 |
| ***B. annulatus*** | 49.31 | 37.36 | 41.97 | 41.84 | 21.21 | 45.93 | 42.38 |
| ***B. decoloratus*** | 30.32 | 28.09 | 33.57 | 25.89 | 44.44 | 22.97 | 29.38 |
| ***B. geigyi*** | 17.36 | 20.79 | 19.18 | 27.3 | 28.28 | 20.35 | 20.93 |
| ***Boophilus sp.*** | 0 | 0 | 0 | 0 | 0 | 0.87 | 0.16 |
| ***H. laechi*** | 0 | 0.28 | 0 | 0 | 0 | 0.58 | 0.16 |
| ***R. gulhoni*** | 0 | 3.09 | 0 | 1.06 | 2.02 | 0.87 | 0.98 |
| ***R. lunulatus*** | 0.46 | 2.53 | 0.96 | 0 | 4.04 | 2.03 | 1.35 |
| ***R. muhsame*** | 0.23 | 1.4 | 0.24 | 0.35 | 0 | 0.58 | 0.52 |
| ***R. quilhoni*** | 0 | 0.28 | 0 | 0 | 0 | 0.29 | 0.1 |
| ***R. sanguineus*** | 0 | 3.09 | 0 | 1.06 | 0 | 0 | 0.73 |
| ***R. senegalensis*** | 0.23 | 0.56 | 0 | 0.35 | 0 | 0.58 | 0.31 |
| ***R. fanguineus*** | 0.23 | 0 | 0 | 0 | 0 | 0 | 0.05 |
| ***R. gemma*** | 0 | 0.28 | 0 | 0 | 0 | 0 | 0.05 |

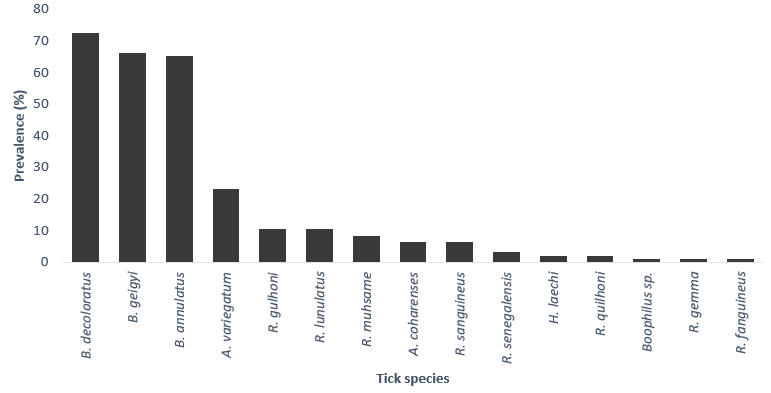


Figure \_: Tick infestation overall prevalence (%) across all 95 cattle sampled.

**Tick community composition at the predilection areas**

The tick community structure of ticks at the predilection sites is represented in the NMDS plot (Figure 1). Visually, the plot shows that the six predilection areas have a high overlap in composition and level of dispersion of the ticks. In alliance with the NMDS plot, the PERMANOVA test revealed no significant difference (P> 0.05) in the overall community structure of ticks at the predilection areas. Nonetheless, notable no significant differences between the ticks at the cattle Shoulders and some other predilection areas, such as the Belly, Tail, Neck, and Head, as shown in Table 2. Furthermore, the test for homogeneity of dispersion showed no significant difference (P> 0.05) amongst the predilection sites.

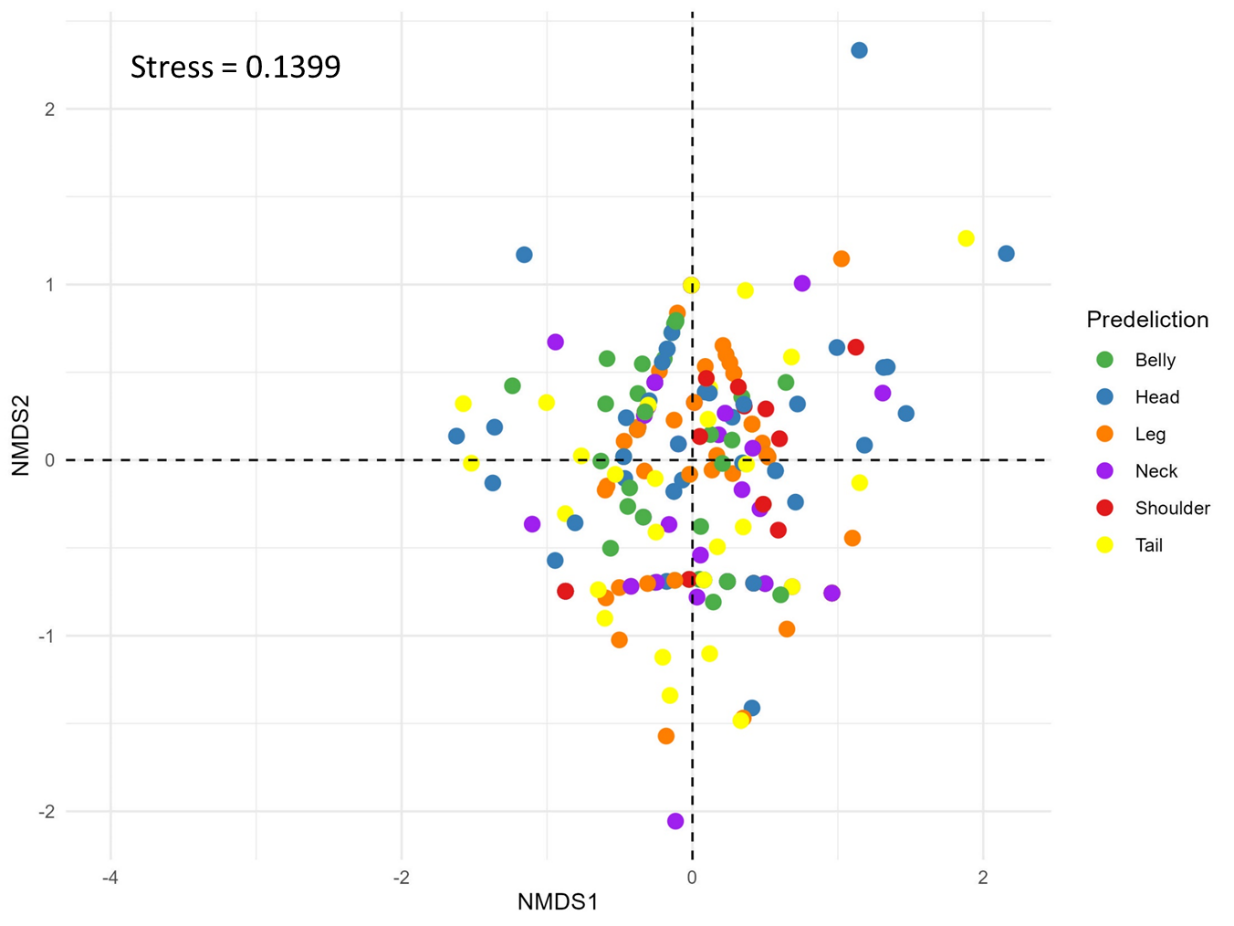


Figure 1: non-metric multidimensional scale (NMDS) plot showing points representing the tick community in predilection sites. Points are coloured according to predilection sites.

Table 2: PERMANOVA test comparing the community structure of ticks at the predilection sites.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **pairs** | F-Model | R2 | P-value | Adjusted P-value |
| **Belly vs Head** | 1.899 | 0.016 | 0.082 | 1 |
| **Belly vs Leg** | 0.711 | 0.005 | 0.553 | 1 |
| **Belly vs Tail** | 0.329 | 0.003 | 0.832 | 1 |
| **Belly vs Neck** | 0.686 | 0.006 | 0.592 | 1 |
| **Belly vs Shoulder** | 3.321 | 0.039 | 0.011 | 0.165 |
| **Head vs Leg** | 0.774 | 0.006 | 0.547 | 1 |
| **Head vs Tail** | 0.941 | 0.008 | 0.442 | 1 |
| **Head vs Neck** | 0.936 | 0.009 | 0.427 | 1 |
| **Head vs Shoulder** | 1.054 | 0.013 | 0.404 | 1 |
| **Leg vs Tail** | 0.700 | 0.006 | 0.592 | 1 |
| **Leg vs Neck** | 0.741 | 0.006 | 0.544 | 1 |
| **Leg vs Shoulder** | 0.955 | 0.011 | 0.424 | 1 |
| **Tail vs Neck** | 0.414 | 0.004 | 0.817 | 1 |
| **Tail vs Shoulder** | 2.536 | 0.034 | 0.035 | 0.525 |
| **Neck vs Shoulder** | 2.249 | 0.032 | 0.072 | 1 |

**Male and Female Tick community composition.**

Based on Sex, the tick community structure of ticks is represented in the NMDS plot (Figure\_). Visually, the plot shows that the male ticks have higher dispersion and heterogeneity compared to the female ticks which looked relatively homogenous. the PERMANOVA test revealed a significant difference (P< 0.001) in the community structure of males and ticks. Furthermore, the test for homogeneity of dispersion showed significant differences (P< 0.001) amongst both sexes.

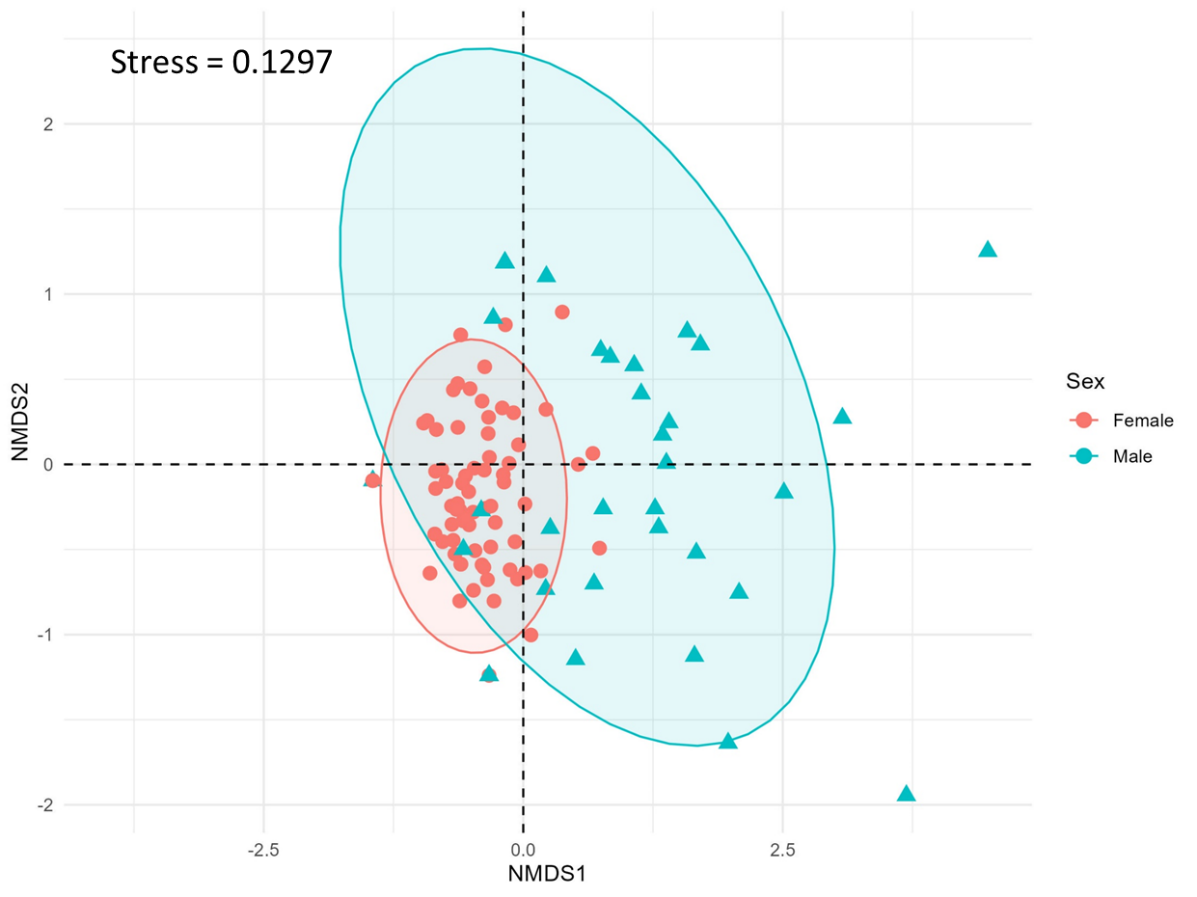


Figure 1: non-metric multidimensional scale (NMDS) plot showing points representing the tick community in predilection sites. Points are coloured according to predilection sites.

**Tick stage and community composition.**

Two tick stages were identified: nymph and adult. The NMDS plot in Figure \_ shows the community structure of both tick stages. However, the NMDS of both communities shows non-significant dissimilarity (p>0.05) in terms of its homogeneity of dispersion. PERMANOVA test shows a significant difference (p<0.001) in the community composition of both tick stages.

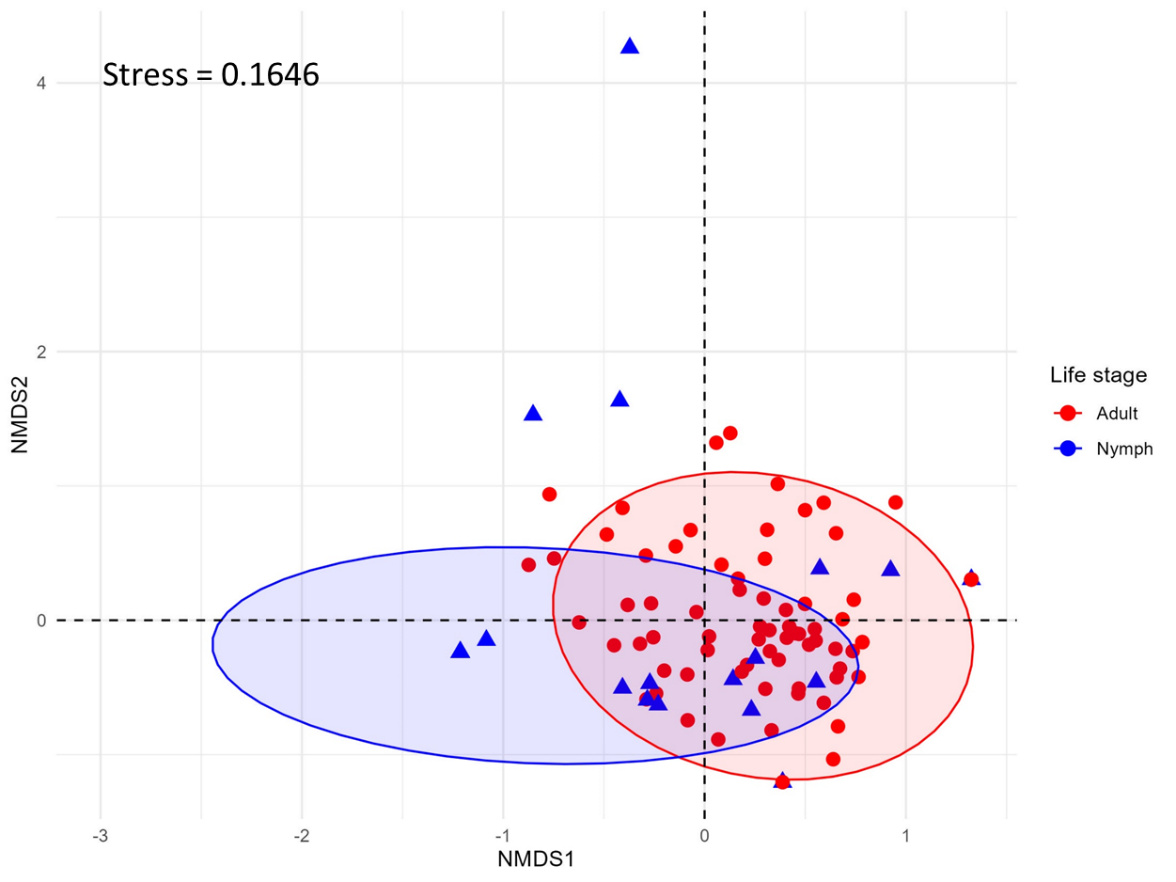
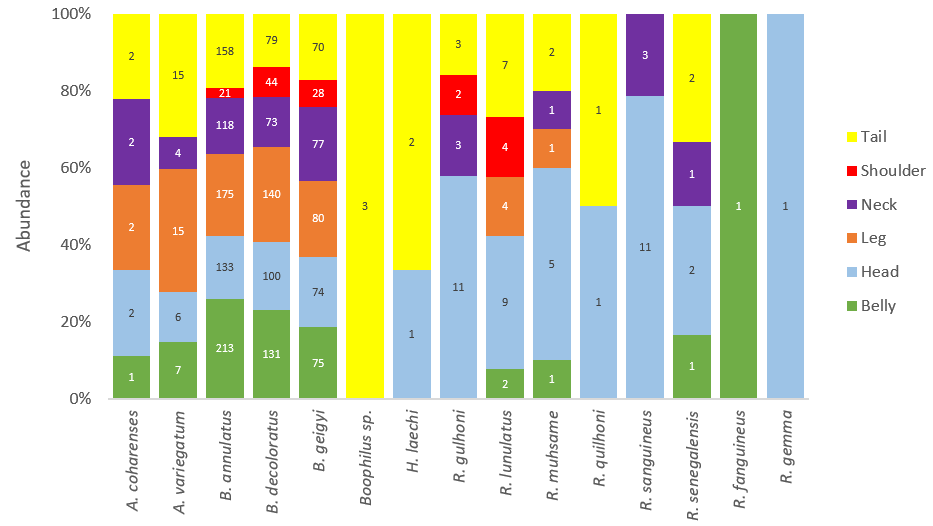


Figure 1: non-metric multidimensional scale (NMDS) plot showing points representing the tick community in predilection sites. Points are coloured according to predilection sites.



**Figure 4**: Relative abundance (%) of tick species at each predilection site of cattle.

Aside from being the most abundant, *B. annulatus, B. decoloratus*, and *B. geigyi* were present at all predilection sites at different levels. Also, *A. coharenses* and *A. variegatum* were observed in all predilection areas except shoulders. All *R. gemma*, *R. fonguineus* and *Boophilus* sp. were found in just one predilection site each – Head, Belly and Tail, respectively. *H. laechi* and *R. sanguineus* were present only in the head and tail of the cattle.

**Species richness of ticks**

The observed number of tick species was 15. Figure 5 is a species accumulation curve (SAC) that estimates the species richness of ticks sampled at the cattle ranch. The SAC shows a mildly upward trend, which suggests that more sampling (of ticks) from more cattle would have revealed additional tick species. Furthermore, the Jackknife 2 and Chao 2 species richness estimates also support this trend. In support of this estimate by the SAC curve, Chao 2 estimates a tick species richness of 16 species, while Jackknife 2 predicts that 19 species are in the area.

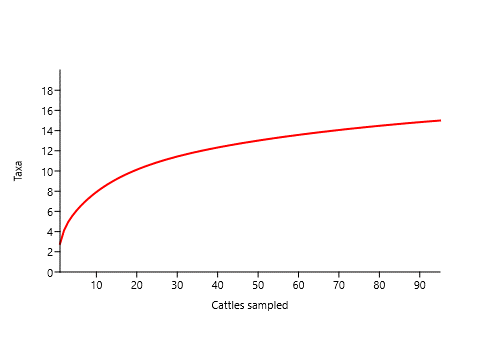
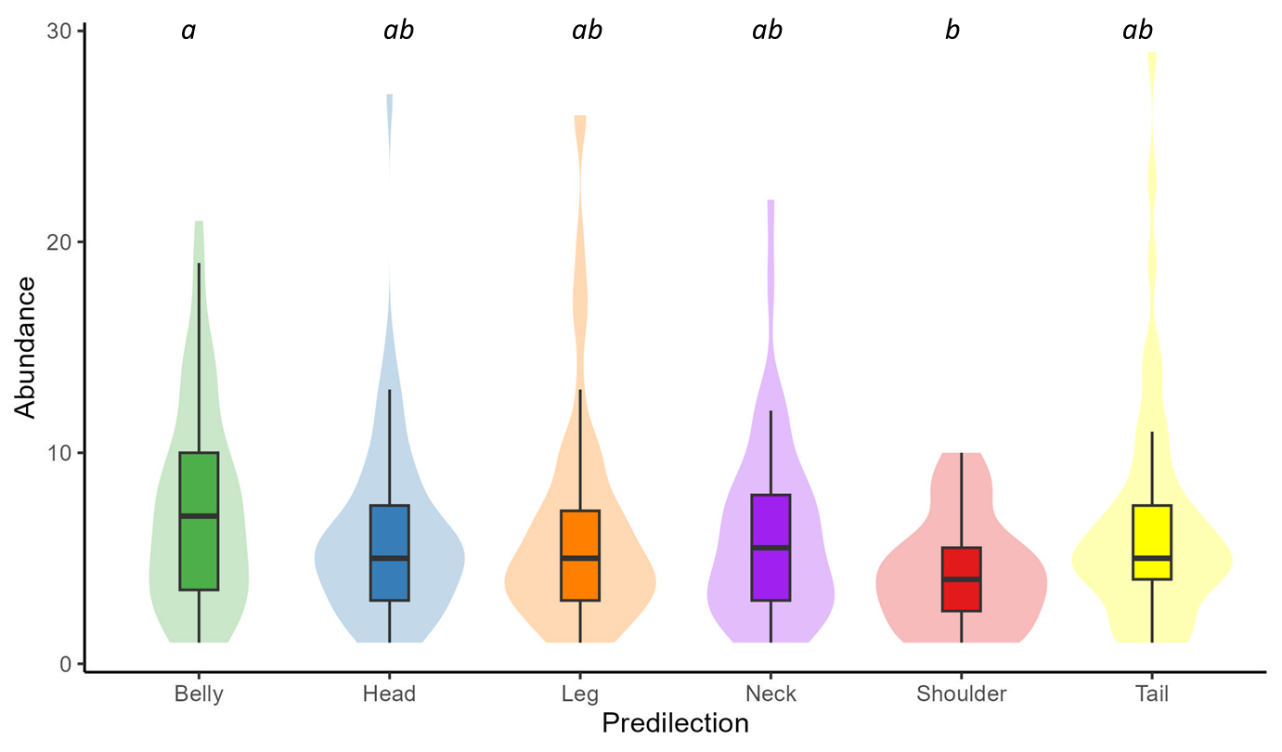
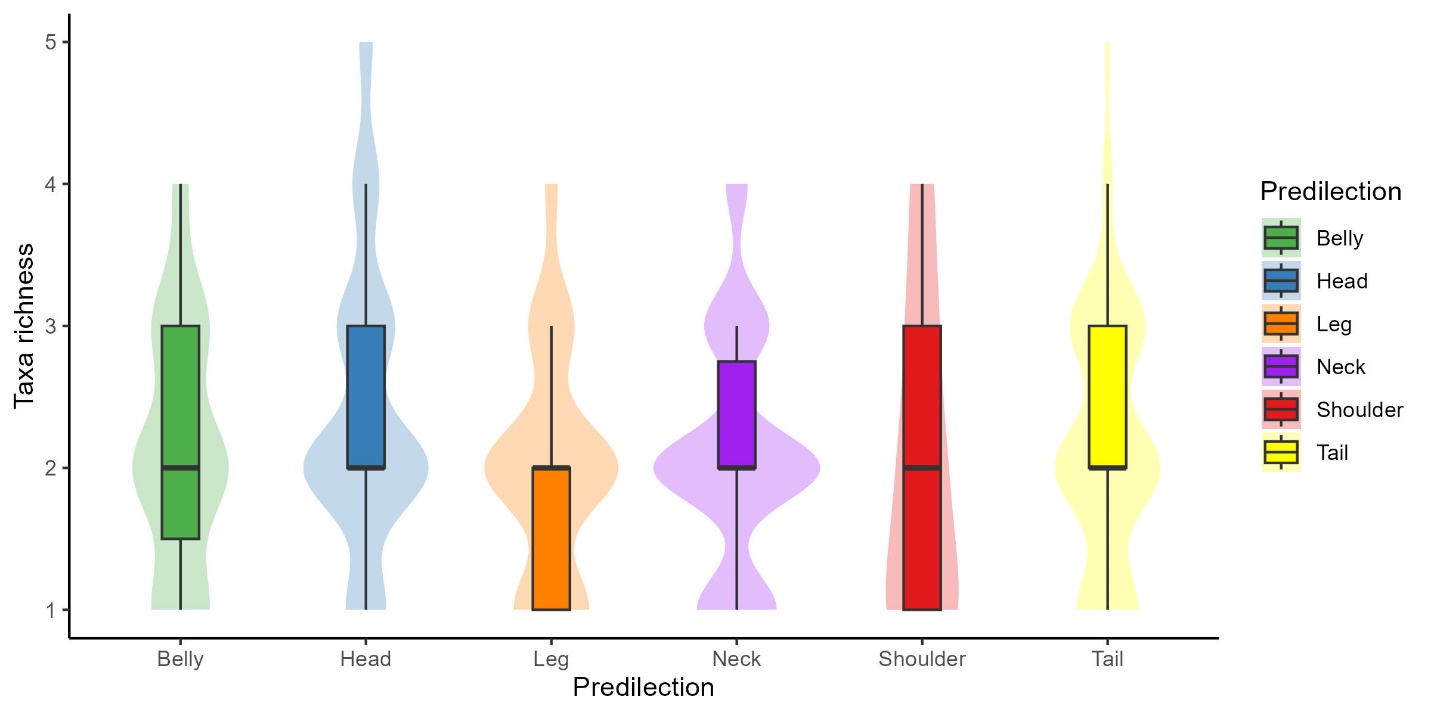


Figure 5: Species accumulation curve (SAC) of ticks sampled from 95 cattle in the ranch.



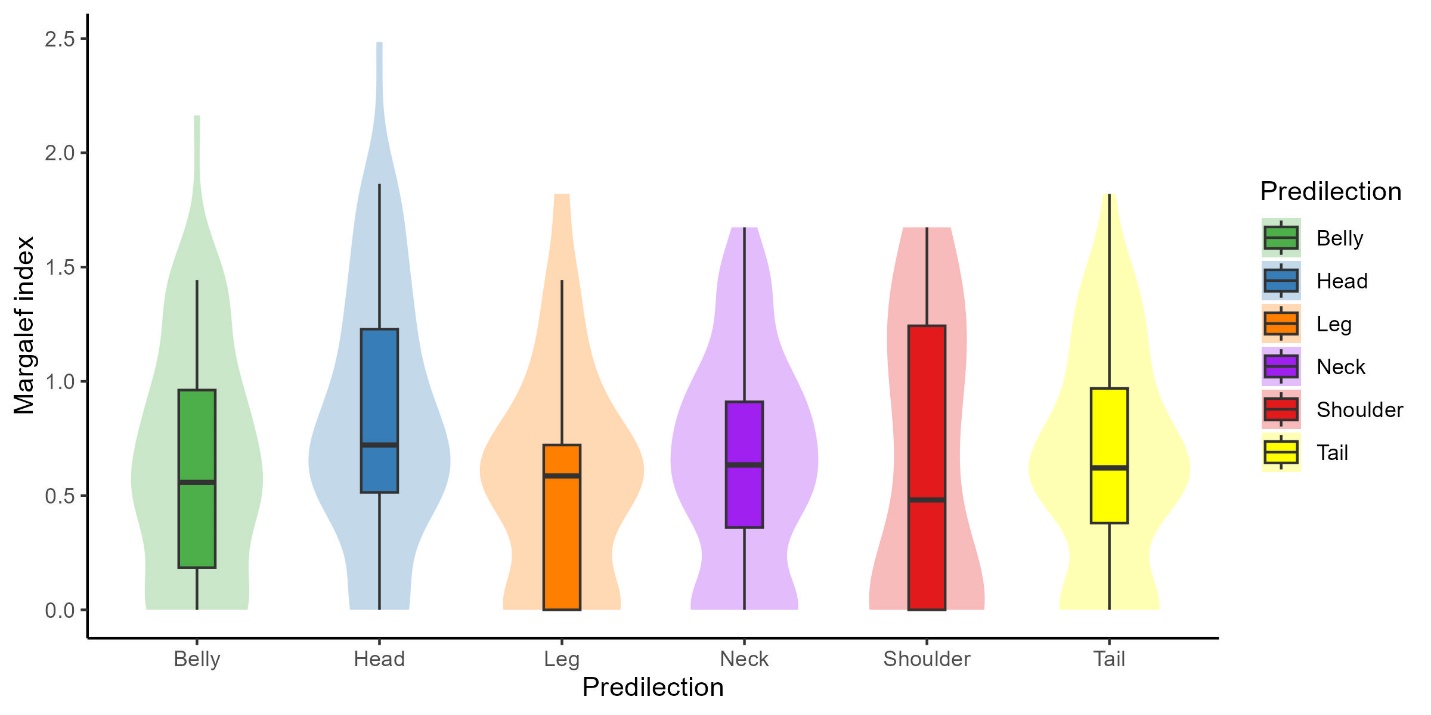
Figure\_: Abundance of ticks at the predilection areas in cattle sampled. different letters show significant differences between predilections.

The tick species abundance is represented in the figure\_. The tick community in the belly had the highest mean abundance (7.32±0.62), followed by the tail, leg, neck and head, which had a mean abundance of 5.49+0.77, 6.51+0.68, 6.13+0.64 and 6.03+0.55. Particularly, the Shoulder had the lowest number of ticks with mean abundance of 4.30+0.55. Negative binomial model showed that the abundance of ticks at the shoulders were significantly (P<0.05) lower than those in the belly.



Figure\_: Species richness of ticks at the predilection areas in cattle sampled.

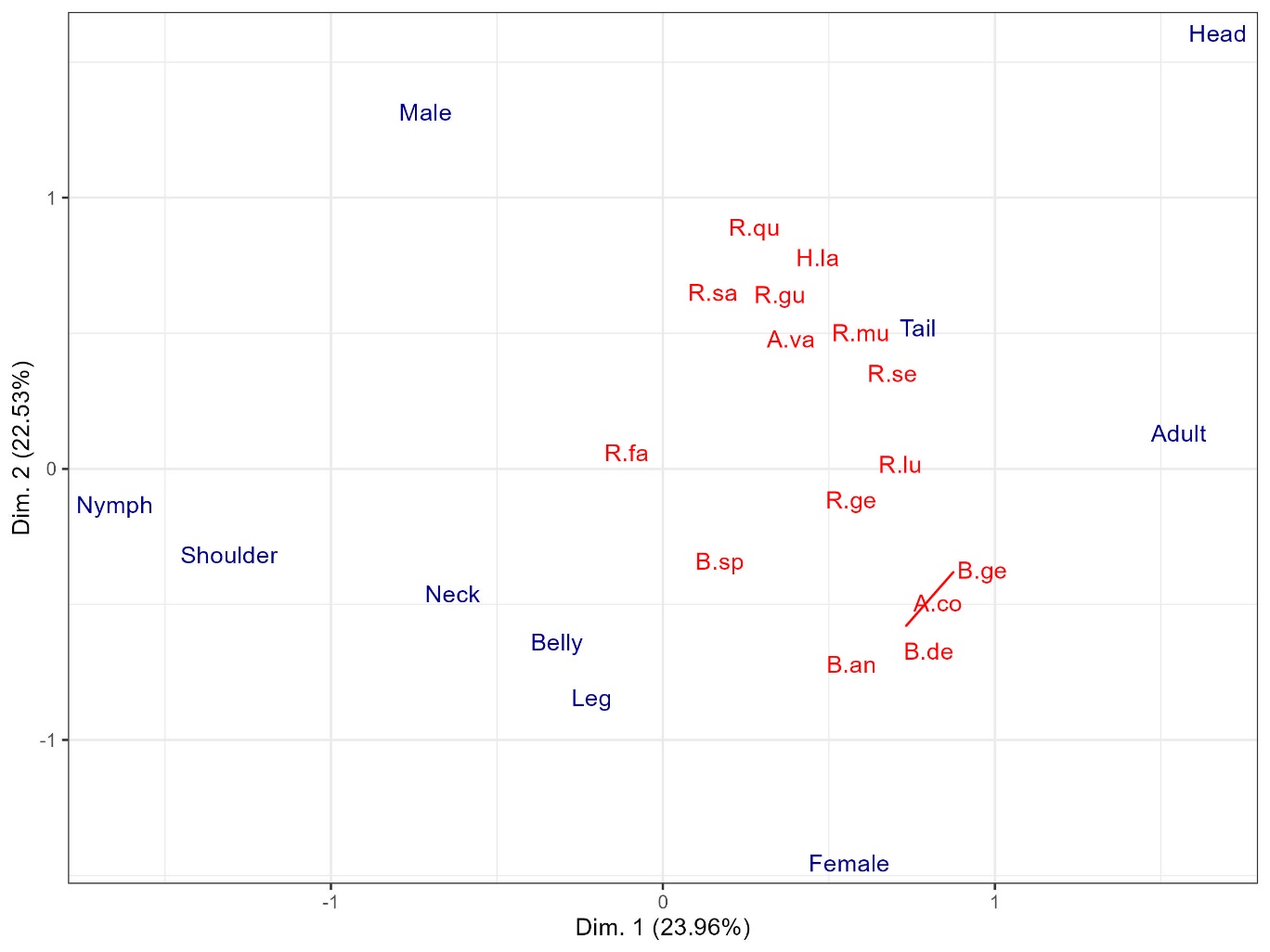
Multiple species of ticks were found in most predilection sites, as shown in Figure\_. The median species richness was 2 across all predilection sites. However, non-significant variation in tick richness persisted. For example, the cattle’s head had the highest mean tick-species richness of 2.42+0.55, followed by tail, belly and neck which had a richness of 2.19+0.77, 2.13+0.62 and 2.09+0.64, respectively. Furthermore, the mean species richest was lowest in the leg (1.98+0.68). Additionally, a few cattle had up to 5 distinct tick species infestations in the head and tail.



Figure\_: Margalef index of ticks at the predilection areas in cattle sampled.

Margalef diversity estimates of ticks at the predilection sites are shown in Figure\_. While this index statistically did not vary significantly (P>0.05), slight differences were observed. The Tick head had the highest mean Margalef index (0.83+0.07), followed by the tail (0.65+0.07), neck (0.64+0.07), belly (0.63+0.07) and shoulder (0.62+0.13). The legs had the lowest Margalef index (0.57+0.06) amongst all predilection sites.

The FAMD explains 46.5% of the variance in the tick data using the two dimensions only, with the first and second explaining 23.96% and 22.53%, respectively. The FAMD has revealed notable differences in the qualitative variables (Tick life stages, Predilection area and Sex) of the ticks. The FAMD plot shows that the ticks community show no notable dissimilarity in their preference for certain predilection area. However, the adult and Nymph stages of ticks would contain different tick species compositions as shown in their high disparity on the FAMD plot. The neck, belly and leg of the cattle are qualitatively more similar. Overall, the Nymph had greater affinity for the shoulder and least for the head.



***Figure \_: Factorial design of predilection site, life stage and species of ticks in the cattle ranch. A.va, A. variegatum; A.co, A. coharenses; B.an, B. annulatus; B.de, B. decoloratus; B. ge, B. geigyi; H.la, H. laechi; R.gu, R. gulhoni; R.lu, R. lunulatus; R.mu, R. muhsame; R.sa, R. sanguineus; R.se, R. senegalensis; B.sp, Boophilus species; R.qu, R. quilhoni; R.ge, R. gemma; R.fa, R. fanguineus.***

# DISCUSSION

In this study, we were able to investigate –at a community level—the prevalence of ticks at predilection sites of cattle, after sampling ticks from 95 cattle in a trade market in Edo State, Nigeria. Generally, our survey has shown that 78.95 % of cattle were infected with at least one tick species. This was far significantly higher than in Adane *et al*. (2019)’s study, from which a prevalence of \_% was reported.

To the best of our knowledge, this study has shown the highest diversity of ticks from a single cattle ranch in Nigeria. Overall, the observed species richness and the Chao 2 and Jacknife2 estimates suggest that while a substantial number of tick species were recorded, further sampling is likely to reveal additional species in the cattle ranch.

Furthermore, we see that at median, tick-infested cattle have a median of two distinct species of ticks in every predilection site of the cattle. Overall, each cattle have a median of \_ distinct species of tick. This much way higher than in Adane et al. (2019)’s report of 3 species of ticks (*A. variegatum*, *Rhipicephalus* species, *R. microplus*) in 209 out of 258 tick-infested cattle in the ranch.

Based on the community dynamics since Adene et al. (2019)’s study, we had seen signicant changes in the species of ticks found. In this study, we find that *B. annulatus, B. decoloratus and B. geigyi were the most abundant ticks in the cattles sampled, constituting* 42.38%, 29.38% and 20.93% of total ticks sampled from the cattles. However, Adene et al. (2019) had only *A. variegatum*, *R. microplus* and a *Rhiphicephalus* species. In this study *R. microplus* was among the rare species (with relative abundance of 2.44%), but had a significantly high prevalence (21.5%) in Adene et al. (2019)’s survey. A study in Maiduguri, Northern Nigeria, by (Musa et al., 2014) showed *Boophilus microplus*, *Amblyomma variegatum*, *Hyalomma* spp., *Rhipicephalus* sanguineous and Ornithodorus spp. as the only ticks present in the area.

The high level of tick diversity and prevalence in the cattle trade area of Edo state, Nigeria calls for quick public animal health intervention. Most of the species of ticks that are highly prevalent in our survey are known to transmit multiple tick-borne diseases (TBDs). For example, *Boophilus* *decoloratus*, a common tick species in Africa, is an important vector of several tick-borne diseases affecting livestock. It transmits *Babesia bigemina* and *Anaplasma marginale* to cattle, causing babesiosis and anaplasmosis respectively (Akinboade et al., 1981). Additionally, *B. annulatus*, the, is capable of transmitting *Anaplasma marginale* through transstadial transmission, leading to anaplasmosis (Samish et al., 1993). Finally, *B. geigyi* has been seen to transmit\_\_\_\_\_\_\_ (REFERENCE).

It was clear that the female ticks in this study showed greater homogeneity in the overall community structure on all tick-infested cattle. This means that the community of tick species that were females were less diverse in structure compared to males with higher diversity. However, there were more female than male ticks in our survey. Female ticks are typically the main carriers of TBD, as they feed for longer periods and consume more blood than males, making them more likely to acquire and transmit pathogens. A larger female tick population increases the risk of disease spread to both cattle and humans. Additionally, since female ticks can lay thousands of eggs, a higher number of females could lead to a larger overall tick population, heightening the chances of future infestations and the spread of TBD. While male ticks are less likely to transmit diseases due to shorter feeding times, their diverse population, as shown in this study, may still pose indirect risks by contributing to the ecological dynamics that support female ticks' survival and reproduction.

Adult ticks are often more involved in disease transmission due to their longer feeding duration and greater blood meal sizes compared to nymphs. The higher prevalence of adult ticks on cattle could elevate the risk of transmitting tick-borne diseases to both cattle and potentially humans.

*Amblyomma variegatum* have been collected in cattles from multiple regions in Nigeria such as in Plateau (reference#), Borno (James-Rugu & Jidayi, 2004), Kano (Unsworth, 1952), Nigeria.

This study revealed that, at median, each tick-infested cattle was infested by approximately three distinct tick species. The presence of multiple tick species on a single host raises concerns about the potential for co-infections with tick-borne diseases (TBDs), as different species may serve as vectors for various pathogens.

The diversity of tick species, as measured by the Margalef index, did not show significant variation across the different predilection sites on the cattle. This suggests that the diversity of tick infestation is relatively uniform across the body (at least for the body parts we investigated), with no specific predilection site harbouring a significantly more diverse tick population. The non-significant difference in tick diversity between body regions also points to the fact that ticks may not exhibit strong site preferences when it comes to attaching to their hosts. Thus, each predilection site—whether head, tail, belly, or leg—had similar levels of tick diversity.

The absence of significant differences in tick diversity between predilection sites also implies that factors such as cattle movement, environmental exposure, and host immune responses are likely to play a more substantial role in determining tick attachment and diversity than the physical characteristics of the predilection sites themselves. For this cattle ranch study, we have shown that at a community and population level, there is no obvious attachment preference at the predilection sites. However, this, in many ways, contrasts with previous studies that suggested certain body areas, could be more favourable for tick attachment due to easier access or proximity to blood vessels (REFERENCES).

While we have observed 15 species, some are however rare, we do not doubt that further sampling would have revealed more tick infestation in the cattle at the ranch. The SAC, Jackknife 2 and Chao 2 estimates also attest to this claim. We are limited on how many species of ticks are there. The cattle trade market is known to receive cattle from every part of Nigeria. This is of public health importance. This is a resounding call for country-wide monitoring of ticks.

Cattle in Nigeria are mostly raised by the Hausa herdsmen, and these people are largely not formally educated.

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