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Amblyomma Tick Infestation and Occurrence of Haemogregarines in African Hinge-Back Tortoises in Ibadan, Nigeria

Adetunji, V. E. and Adeyemo, O. K.

Department of Veterinary Public Health and Preventive Medicine, University of Ibadan, Ibadan

* *Author for Correspondence: onize5@yahoo.com*

ABSTRACT

Tortoises are kept as pets in some homes and as side attraction in zoological gardens, but their usefulness could be limited by ectoparasite infestation. Ectoparasites cause harmful effects on their hosts, sometimes with high morbidity and mortality. Despite this, little attention has been paid to ectoparasite infestation in tortoises with potential extinction threat. Study was aimed at determining the presence and prevalence of tick infestation on African hinge-back tortoises (AHT) and evaluate their possible roles in the transmission of Haemogregarines a common heamoparasites in *Kinixys belliana* and *Kinixys homeana* (African bell hinge-back and home hinge-back tortoises) obtained from major wildlife markets in Ibadan, Nigeria. In total, 120 tortoises (70 *K. belliana* and 50 *K homeana*) purchased at wildlife markets over a year during rainy and dry seasons were screened for ectoparasites but only ticks were found. Ticks were detached from tortoises, using a pair of forceps. Mean prevalence for each *Kinixys belliana* and *Kinixys homeana* group was determined and ticks were identified. The effects of sex, species, and season on parasite prevalence were evaluated using the Mann–Whitney U test and Pearson's chi-square test. Data were quantified using correlation coefficient test. Level of significance was taken as 5%. Hard ticks (*Amblyomma species*) were the only ectoparasites found on 54.0% of tortoises. Higher prevalence of *Amblyomma* was observ ed in *Kinixys belliana* (55.7%) than *Kinixys homeana* (52.0%). Male tortoises recorded higher prevalence (60.7%) than females (48.4%). Overall prevalence during rainy season was 55.0 and 53.3% in dry season. A comprehensive preventive health programme aimed at controlling ectoparasites such as ticks on tortoises in zoological gardens and in private facilities is hereby recommended.

Keywords: Wildlife Ectoparasites; Haemogregarines; *Kinixys belliana*; *Kinixys homeana; Amblyomma*

INTRODUCTION

Chelonians, which include African hinge-back tortoises (*Kinixys belliana* and *Kinixys homeana*) are mostly herbivores but some species are insectivores. Distinct from other reptile's species, aside from their natural environment, they are found in homes as pets of children and adult (Kirecci *et al*., 2013). African hinge-back tortoises (AHT) are endangered species and infestation with ectoparasites is believed to be one of the factors militating against their population (Gibbon *et al*., 2000; Garces-Restrepro *et al*., 2013). Several species of land tortoises, including AHT are hosts to several haematophagus insects such as mites, ticks and larvae of dipteran flies (Jacobson, 1994, Cook *et al*., 2014). According to Jacobson, (1994) and Cook *et al*. (2014), hard ticks such as *Amblyomma tuberculatum*, *A. clypsolatum*, *A. testudinarium*; *Hyalomma aegyptium* and *H. detritum* have been reported to parasitize tortoises. Haemogregarina was first described by Danilewsky in 1885 in *Emys orbicularis* (European pond turtle) (Ozvegy *et al*., 2015). Morphologically, the parasite is elongated and found intra-erythrocytically. It stains basophilic with Giemsa stain and is surrounded by a clear zone. Merogonic differentiation occurs in the intermediate (vertebrate) host, while both gametogony and sporogony occur in the alimentary tract of the definitive hosts, which are invertebrate arthropods. Haemogregarines are parasites of cold-blooded vertebrates that commonly parasitize fishes and reptiles as intermediate hosts. In aquatic systems, blood-sucking leeches serve as definitive hosts, while in terrestrial systems, ticks and mites function as definitive hosts (Haklova-Kocikova *et al*., 2014; Ozvegy *et al*., 2015).

Parasites have global distribution and are an integral part of the ecosystem. However, they can impair growth, reproduction and exert negative effects on biochemical and hematological parameters of their hosts, thereby altering their genetic traits, fitness and survival (Knapp *et al*., 2019). Ectoparasites harm their hosts by feeding or defecating on them, which can lead to skin irritation, inflammation, and secondary infections (Yalew, 2022). When infestations are severe, they can cause debilitating conditions such as anemia due to blood loss, hypersensitivity reactions, and even anaphylaxis (Yalew, 2022). In addition, ectoparasites often act as vectors for various pathogens, exacerbating the risk of diseases in infested animals (Yalew, 2022). Health assessments in veterinary practices rely greatly on haematological parameters and plasma biochemistry evaluations, among others (Stacy *et al*., 2011; Norte *et al*., 2013; Knapp *et al*., 2019).

Ectoparasites can be categorized broadly into haematophagus arthropods, such as ticks, mosquitoes, fleas, lice, that suck blood, and burrowing ectoparasites such as mites that lodge underneath the skin (Visser *et al*., 2001). They are not only capable of causing diseases but could also act as vectors of pathogens with high morbidity and mortality (Visser *et al*., 2001).

Ticks are ectoparasites of veterinary importance considering their roles as vectors of many disease agents (Basu *et al*., 2012). They are blood-sucking obligatory parasites of mammals, reptiles, birds and amphibians and cause clinical conditions such as anaemia, loss of condition, reduced milk production, tick paralysis, irritations and injury due to their bites (Basu *et al*., 2012). Ticks rank next to mosquitoes in the transmission of human infectious diseases globally. Haemogregarines parasitizing South African hinge-back tortoise, *Kinixys zobensis* are believed to be spread by hard ticks; *Amblyomma* species (Cook *et al.,* 2015).

Despite the growing popularity of tortoises as pets, coupled with the associated health hazards posed by ectoparasites especially ticks, information on the presence and abundance of ectoparasites in *Kinixys belliana* and *Kinixys homeana* sourced from the wild and being sold in open market in Oyo State is limited. This study was designed to determine the presence and abundance of ectoparasites (ticks, mite and other arthropods) as well as haemogregarine infection in *Kinixys belliana* and *Kinixys homeana* sourced in Wildlife market at Bode, Ibadan, Oyo state, Nigeria.

MATERIALS AND METHODS

Study Area

This cross-sectional study was carried out in Ibadan Oyo State. Ibadan is a major hub for traders and marketers of traditional medicine and wild animals from across the states in western Nigeria. Bode Wildlife Market is a major wildlife market in Oyo State known for trading in wildlife, including tortoises and accommodates an average of 300,000 people who are either selling or buying wildlife animals (Oduntan *et al*. 2012). Ethics and ethical statement

The ethical approval for this study was obtained from the University of Ibadan Animal Care and Use Research Ethics Committee (UI-ACUREC), with approval number: UI-ACUREC/App/10/2016/01

Study Design and Sample Collection

This was a cross-sectional study conducted on *K. belliana* and *K. homeana* available at the central herb/wil dlife market at Bode, Molete in Ibadan, Oyo State. One hundred and twenty AHT {*Kinixys belliana* (70) and *Kinixys homeana* (50)} were purposively sourced from the market for the study. The sampling of the tortoises was done over a period of one year during the rainy seasons between April 2016 and December 2017, spanning both rainy and dry seasons. All sampled tortoises were housed singly in a wooden vivarium and examined for the distribution and abundance of ticks. The tortoises were not treated with any acaricide throughout the period of study. The tortoises were observed systematically from the anterior body to the posterior for the presence of ectoparasites. A pair of forceps was used to carefully detach ticks from infested tortoises into labeled bijou bottles containing preservative solution to maintain the integrity of the integument of the ticks and prevent them from dehydration. The solution was prepared by adding 80ml of 90% ethanol with 5ml of 5% glycerol and 15 mL distil water (ethanol-glycerol preservative). Ticks were counted and recorded for each tortoise and later kept over ice careful to prevent freezing.

Tick Identification

Ticks were identified using field guide of Estrada-Pena *et al*. (2017) and Lartha and Azhahianambi (2013). The ticks were first identified with the naked eyes, then using a simple hand lens of X10 magnification. Identification to the genus level was achieved by analyzing morphological features such as mouth part, presence of the eyes, presence of scutum in male, coscutum in females, presence of festoon, presence or absence or vestigial adanal plate and banded legs. They were removed from the preservative solution, blotted with Whatman filter paper and cleaned with a very fine artist brush, sorted into a petri dish placed on a white surface. Ticks were then viewed dorsally from various angles under a dissecting microscope (Optika® St-40-2L SN 20611, Ponteranica, Italy) at X10 and X20 magnifications. Image was recorded with a digital camera

Blood Collection

In addition, 2 mL of blood was withdrawn through the sub-carapacial sinuses of each tortoise, using a sterile 25G needle and syringe according to McArthur *et al*. (2004) to determine the prevalence of haemogregarines. Thin blood smears were prepared, air-dried, fixed for 10 minutes in absolute methanol and stained with Giemsa-stain for 30 minutes (Houwen, 2000). Four slides per tortoise were prepared, rinsed under running tap, drained, and arranged vertically on a slide rack to dry naturally. They were screened using the 100x oil immersion on an Olympus BX41 microscope. Images were captured using a digital camera. Parasite's prevalence and intensity for each tortoise were calculated as numbers of parasitised red blood cells (RBC) per 100 RBCs. Altogether, 25 fields of 100 were observed per slide to equate for 10,000 RBC on the four slides. Tortoises were donated to the University of Ibadan zoological garden after sample collection to promote chelonian conservation as returning them to wild will lead to recapture.

Data Analyses

Ectoparasite prevalence per sex, species and seasons were determined with Mann U Whitney Test. Sex, seasons and species prevalence were evaluated using Pearson's chi-square test. The level of association of tick infestation and abundance as well as level of parasitaemia of haemogregarine parasites on tick infested tortoises were evaluated with Pearson's Correlation Coefficient and the Frequency polygon was used to depict the level of association. The level of significance was taken as 5%.

RESULTS

Hard ticks belonging to the genus *Amblyomma* were the only ectoparasites identified on 65 out of the 120 AHT examined, resulting in an overall prevalence of 54.2% (65/120) (Figure 1). The ticks were attached to various body parts, including the head, neck, shoulders, legs, tail region, and occasionally in the fissures of the carapace and plastron. Male ticks were smaller and ornately patterned compared to females. (Figures 2A and 2B). The overall prevalence of *Amblyomma* tick infestation was 54.2% (Table 1). The prevalence was slightly higher in *Kinixys belliana* (55.7%) than *Kinixys homeana* (52.0%). Male tortoises showed a higher infestation rate of 60.7% (34/56) compared to females at 48.4% (31/64). (Figure 3). Seasonal variations indicated similar infestation rates, with 55.0% (33/60) during the rainy season and 53.3% (32/60) during the dry season. Within species, 58.3% (21/36) of *K. belliana* and 50.0% (12/24) of *K. homeana* were infested during the rainy season, while 52.9% (18/34) of *K. belliana* and 53.9% (14/26) of *K. homeana* were infested in the dry season. (Figure 4, Table 2). Giemsa-stained blood smears revealed haemogregarine gametocytes as sausage- or banana-shaped organisms within the cytoplasm of infected erythrocytes (Figure 5). Parasites were observed at ×100 magnification (Panel A) and ×1000 magnification (Panel B), confirming haemogregarine infection in *Amblyomma*-infested tortoises. The overall prevalence of haemogregarine was 53.3% (64/120) in the African hinge-back tortoises. The prevalence level was higher in *Kinixys belliana* (57.1%) than *Kinixys homeana* (48.0%). Haemogregarine parasite intensity was also higher in *K. belliana* (0.23±0.23) than *K. homeana* (0.21±0.23). A weak positive correlation (r = 0.3758, p = 0.002) was observed between tick infestation and the level of haemogregarine parasitaemia (Figure 6).

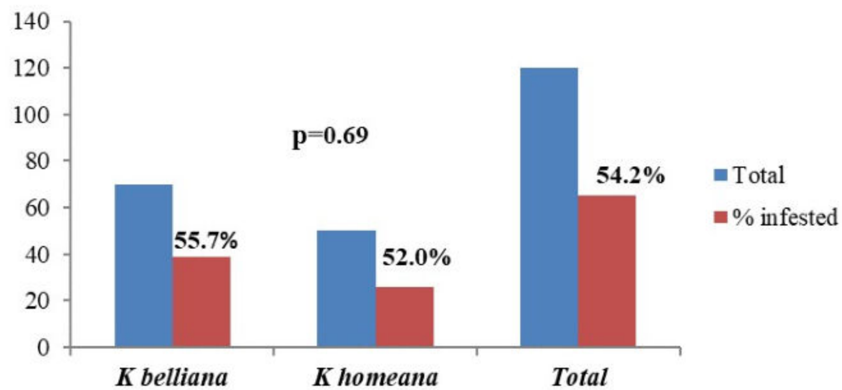

Figure 1: Prevalence of ectoparasites in *K. belliana* and *K. homeana*

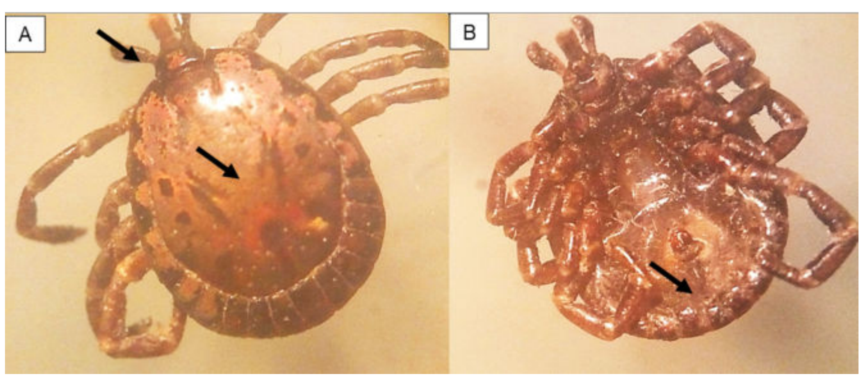

Figure 2: (A) Dorsal view of a male *Amblyomma* tick Note palp article 2 longer than 1 and 3 and the elaborate ornamentation on the dorsum (orange enamel) arrowed (B) Ventral view of a male Amblyomma tick Note the arrowed anal and reduce adanal plate.

DISCUSSION

Ticks pose significant health risks to both animals and humans, serving as vectors for numerous diseases including haemogregarinosis, hepatozoonosis, babesiosis, borreliosis, and rickettsial infections. The overall tick prevalence of 54.2% observed in African hinge-back tortoises (AHT) in this study aligns with previous reports from other chelonian species (Tavassoli *et al*., 2007; Kirecci *et al*., 2013). Segura *et al*. (2023) reported a prevalence of 100 % infestation rates in spur-thighed tortoises (*Testudo graeca*) with an intensity of 4 ticks per tortoise, while our findings represent a moderate infestation level that nonetheless warrants attention given the zoonotic potential of tick-borne pathogens.

Recent molecular studies have confirmed the presence of zoonotic pathogens in tortoise-associated *Amblyomma* ticks. Notably, reptile-associated ticks in South Africa have been found to harbor spotted fever group *Rickettsia* species, *Coxiella*-like endosymbionts, and *Borrelia* species, many of which have zoonotic importance (Mofokeng *et al*., 2022). The report of *Rickettsia africae* in *Amblyomma* species from African tortoises is particularly concerning, as this pathogen causes African tick-bite fever in humans, frequently affecting travelers and individuals handling reptiles (Zhang *et al*., 2024). These findings underscore serious public health implications for handlers of AHT in Nigeria and emphasize the need for appropriate biosafety measures when managing these chelonians.

Report of our study confirm *Amblyomma* species were the predominant ixodid ticks infesting chelonians, this is consistent with widespread patterns across Africa (Kirecci *et al*., 2013). This study corroborates reports from southern Africa where *Amblyomma marmoreum* and *A. hebraeum* are the most frequently encountered tick species on tortoises (Horak *et al*., 2006). The overall prevalence of 54.2% recorded in our study is lower than the 71.4% in *T. graeca* from Turkey (Kirecci *et al*., 2013), 77.8% in *Stigmochelys pardalis*, and 100% in *Kinixys zombensis* from South Africa, but higher than the 43.8% reported in *T. graeca* from Iran (Tavasoli *et al*., 2007). These variations may reflect differences in environmental conditions, host population densities, sampling methodologies, and regional tick fauna composition.

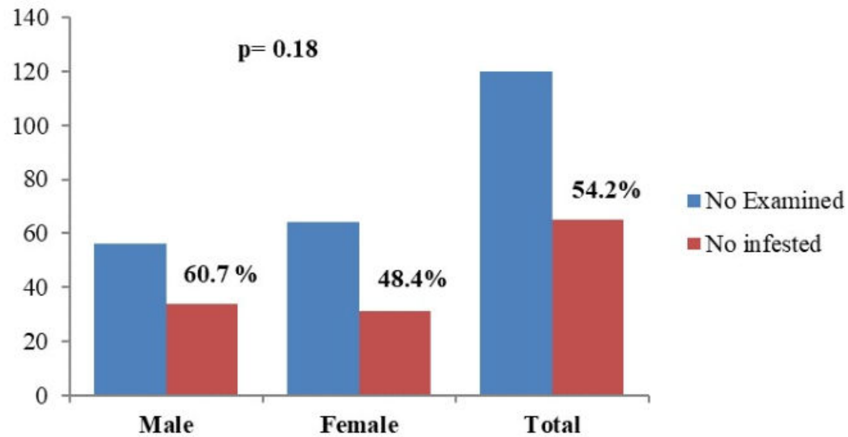

Figure 3: Prevalence of ectoparasites in male and female *Kinixys* tortoises

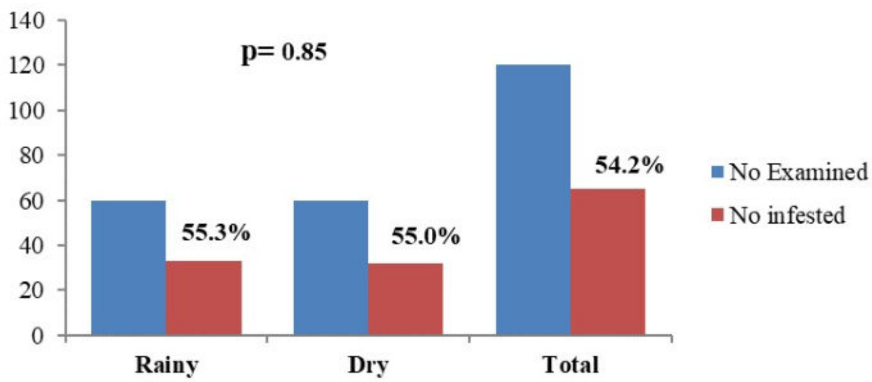

Figure 4: Prevalence of ectoparasites during rainy and dry seasons

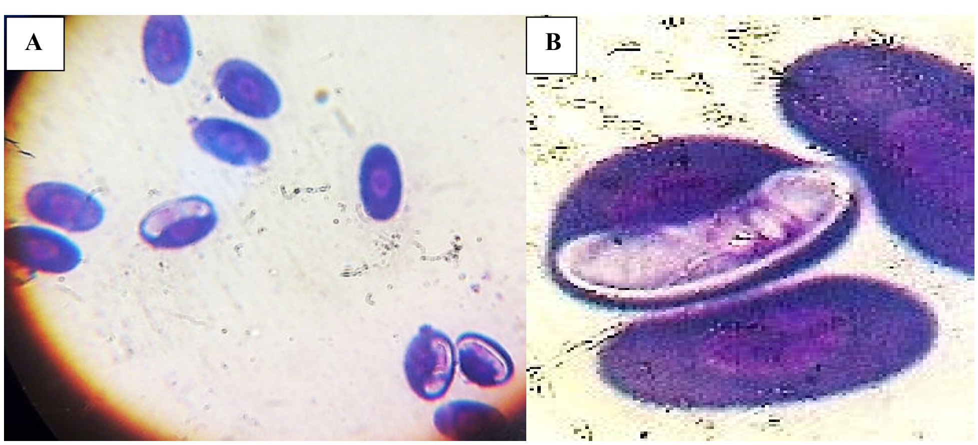

Figure 5: Giemsa-stained gametocytes of parasites observed as sausage or banana-shaped organism lying within the cytoplasm of infected erythrocytes. Magnification: A = x 100; B = x 1000

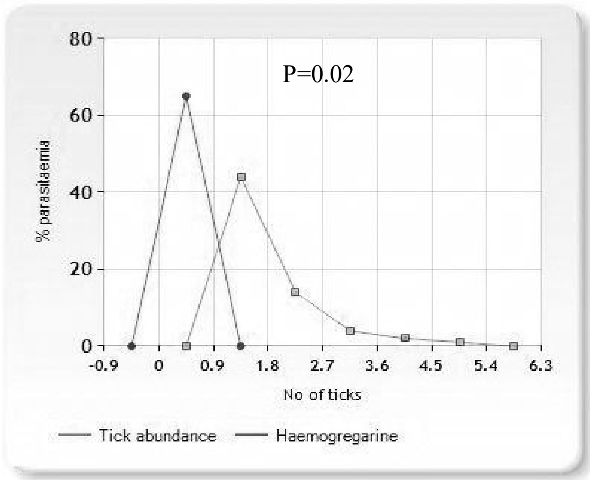

Figure 6: Tick infestation and Haemogregarine parasitaemia

The significantly higher tick burden in male tortoises (60.7%) compared to females (48.4%) (p = 0.18) observed in this study is consistent with findings from Mediterranean and North African tortoise populations. This sex-skewed parasitism has been attributed to multiple factors including behavioral ecology and endocrinological differences. Laghzaoui *et al*. (2022) and Segura *et al*. (2023) reported similar patterns, suggesting that male-biased tick infestation relates to home range differences between sexes, with males typically covering larger territories during breeding seasons.

Therefore, the higher prevalence of ticks on male tortoises in this study could be attributed to elevated levels of circulating testosterone in sexually mature and active males coupled with their sexual activities. According to Isaac, (2005) and Kao *et al.,* (2014), mating multiple females could also promote exposure of males to more ectoparasites.

The slightly higher prevalence in *K. belliana* (55.7%) versus *K. homeana* (52.0%) (p = 0.69), though not statistically significant, may be attributed to morphological and behavioral differences between these species. *K. homeana* possesses a unique anatomical feature among hinge-back tortoises: the ability to fully retract all appendages into their shell, creating a complete seal. This characteristic, documented by Luiselli and Diagne (2013), provides enhanced protection against both predators and ectoparasites by minimizing exposed body surface area available for tick attachment. In contrast, *K. belliana* exhibits incomplete retraction, leaving portions of the limbs, head, and tail exposed, thereby providing more attachment sites for questing ticks.

The marginally higher seasonal tick infestation during the rainy season (55.3%) compared to the dry season (55.0%) (p = 0.85) in both *K. belliana* and *K. homeana* aligns with established patterns of hard tick ecology in tropical and subtropical regions. Segura *et al*. (2023) reported similar seasonal patterns in spur-thighed tortoises (*T. graeca*) from Maamora Forest, Morocco, associating higher infestation rates with decreased temperatures and increased humidity during wet periods.

Nigeria's tropical climate, characterized by warm temperatures and high humidity during the rainy season, creates optimal conditions for hard tick breeding, development, and host-seeking behavior. These environmental factors accelerate tick developmental rates and enhance survival of off-host stages. Salkeld and Schwarzkopf (2005) demonstrated that seasonality significantly influences parasite prevalence, abundance, and intensity across multiple host-parasite systems, with peak infestation typically occurring during periods of optimal temperature-humidity combinations. However, the minimal difference observed in our study suggests that the relatively stable warm conditions throughout the year in Ibadan may support year-round tick activity, a pattern characteristic of equatorial and near-equatorial regions.

Table 1: Prevalence of ectoparasites infestation in Africa hinge-back tortoises

Species	No of tortoise examined	No. infested ectoparasites	% infested
K. belliana	70	39	55.7
K. homeana	50	26	52.0
Total	120	65	54.2
$X^2 = 0.16$ P = 0.69			

Table 2: Seasonal variation in ectoparasites infestation in *Kinixys belliana* and *Kinixys homeana*

Species	***Kinixys belliana***			***Kinixys homeana***		
	No tested	**No. infested**	**% infestation**	**No tested**	**No. infested**	**% infestation**
Rainy season	36	21	58.3	24	12	50.0
Dry season	34	18	52.9	26	14	53.9
Total	70	39		50	26	
	$X^2 = 0.21$; p = 0.65			$X^2 = 0.07$; p = 0.79		

The weak but statistically significant weak positive association (r = 0.3758; p = 0.02) between tick infestation and haemogregarine parasitaemia represents a crucial finding that advances our understanding of haemoparasite transmission in African chelonians. This correlation supports the hypothesis that *Amblyomma* ticks serve as vectors for haemogregarine parasites in AHT populations.

In addition, the recent groundbreaking research by Mofokeng *et al*. (2024) has provided definitive molecular evidence confirming *Amblyomma* species as definitive hosts and vectors for *Hepatozoon fitzsimonsi*, a haemogregarine commonly found in southern African tortoises. Their study documented complete sporogonic development in three *Amblyomma* species (*A. marmoreum*, *A. sylvaticum*, and *A. hebraeum*), with molecular confirmation linking sporocysts and sporozoites in unengorged ticks directly to *H. fitzsimonsi* in infected tortoises. This represents the first molecular confirmation of ticks as definitive hosts for a chelonian *Hepatozoon* species.

The high prevalence of haemogregarines in K. *belliana* and K. *homeana* confirms their presence in Nigerian chelonians. Infected erythrocytes displayed hypertrophy, with atrophied and marginalised nuclei, consistent with findings in other tortoise and turtle studies (Cook *et al*. 2015; Molla *et al*. 2015). The intraerythrocytic parasites demonstrate their capacity to destroy red blood cells in Kinixys tortoises. This finding has important implications for understanding parasite maintenance in tick populations and the potential for vertical transmission.

Limitations

While our study provides valuable epidemiological data on tick-haemogregarine associations in Nigerian AHT, several limitations should be acknowledged. Molecular isolation, identification and characterization of haemogregarine parasites from tick vectors could not be carried out

Conclusion

This study provides important baseline data on *Amblyomma* tick infestation and haemogregarine parasitism in African hinge-back tortoises from Nigeria. The observed association between tick burden and haemogregarine intensity, combined with recent molecular confirmation of *Amblyomma* species as definitive hosts for chelonian haemogregarines, strongly supports the vectorial role of these ticks. The findings underscore the need for integrated approaches to understanding tick-borne pathogen ecology in African chelonians, with implications for wildlife health, conservation, and zoonotic disease prevention. Therefore, an all-inclusive preventive health programme aimed at controlling ectoparasites such as ticks on pet tortoises, in zoological gardens and other facilities is highly recommended.

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Conflict of Interests

The authors have no conflict of interest to declare.

Authors' Contributions

VEA and OKA conceived, designed and conducted the research, and drafting of the manuscript. VEA did the data collection. VEA and OKA designed and supervised the drafting of the manuscript. OKA supervised the research. All authors have read and approved the final manuscript.

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