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Haemoparasite infections of turkeys (*Meleagris gallopavo*) reared in Maiduguri, Borno State, Nigeria: Occurrence and associated risk factors

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Abstract

This study investigated the occurrence of haemosporidian parasites in turkeys reared in Maiduguri, Borno State, Nigeria. The study adopted a cross-sectional survey design, and stratified random sampling technique. Microscopic examination of blood smears from 370 turkeys showed that 42 (11.4%) were infected with *Plasmodium* species which was the only haemosporidian parasite found. The *Plasmodium* species appeared as pigmented gametocytes within erythrocytes. Turkeys sampled at Veterinary Hospitals had the highest occurrence of *Plasmodium* parasites in blood (77.1%), followed by those from live bird markets (5.0%) and then those sampled from farms/households (4.3%). Male turkeys exhibited a significantly ($p < 0.05$) higher occurrence (17.1%), compared to females (6.5%). The occurrence of the parasite was more common during the rainy season (18.9%) than the dry season (3.8%). Clinically sick turkeys had a higher occurrence of *Plasmodium* parasites (38.0%), compared to apparently healthy ones (1.5%). Extensively reared turkeys showed a higher occurrence (16.8%) compared to those reared intensively (5.9%). These findings highlight the significant risk factors (including sex, season, health status, and husbandry practices) associated with *Plasmodium* species infection in turkeys in the study area. Increasing awareness among stakeholders about occurrence and transmission routes of the *Plasmodium* parasite, and developing targeted control strategies, emphasizing biosecurity measures, and encouraging collaborative research efforts for its effective prevention and control is advocated.

Keywords: Avian haemoparasites; *Plasmodium* species; Turkeys; Risk factors; Microscopy.

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Introduction

Haemoparasitic infections pose a significant threat to the health and productivity of all animals, including avian species, worldwide (Tembe *et al*., 2023; Grace *et al*., 2024). Among domestic birds, turkeys (*Meleagris gallopavo*) are particularly susceptible to a variety of haemoparasites; infection with such haemoparasites can result in substantial economic losses for turkey producers (Jajere *et al*., 2018; Tembe *et al*., 2023). Understanding the risk factors and seasonal variations associated with haemoparasitic infections in turkeys is crucial for implementing effective control and management strategies (Naqvi *et al*., 2017; Stuen, 2020).

The common avian haemoparasites include various genera such as *Haemoproteus*, *Leucocytozoon* and *Plasmodium* (Villalva-Pasillas *et al*., 2020; Capasso *et al*., 2023; Valkiūnas and Iezhova, 2022; 2023). Infection with these parasites can lead to a wide range of clinical manifestations, including anemia, reduced feed conversion, decreased egg production and increased mortality rates, which significantly impact on the overall health and performance of turkey flocks (Rukambile *et al*., 2020; Tembe *et al*., 2023).

The transmission dynamics of haemoparasites among turkeys is influenced by a multitude of factors, including environmental conditions, host susceptibility, vector abundance, and management practices (Lawal *et al*., 2019). Seasonal variation in these factors may further modulate the prevalence and intensity of haemoparasitic infections within turkey populations. For instance, climatic variations can directly affect vector activity and survival rates, leading to fluctuations in parasite transmission rates throughout the year (Opara *et al*., 2014; Nourani *et al*., 2020).

Moreover, several intrinsic and extrinsic risk factors can predispose turkeys to haemoparasitic infections (Pori *et al*., 2023). Host-related factors such as age, genetic susceptibility, immune status, and concurrent diseases can influence the likelihood of parasite establishment and proliferation within the host (Neyer *et al*., 2023). Concurrently, external factors like housing/husbandry system, flock density, vector control measures, and geographical location can significantly influence the exposure risk and transmission dynamics of haemoparasites in turkey farms (Yaro *et al*., 2021; Terra *et al*., 2023).

Despite the growing recognition of haemoparasitic infections as a significant threat to poultry health and production, comprehensive studies investigating the epidemiology and risk factors for these infections in turkey populations remain relatively scarce in Nigeria. The present study evaluated the occurrence and risk factors associated with haemoparasite infections in turkeys reared in Maiduguri, Borno State, Nigeria.

Materials and Methods

Study Area: The study was conducted in Maiduguri, Borno State, Nigeria. Maiduguri is the capital and largest city of Borno State, and is located in northeastern Nigeria. The city is situated at approximately 11.8451° N latitude and 13.1600° E longitude. Maiduguri occupies a strategic position within the Lake Chad Basin region, and it is 300 metres elevation above sea level. It occupies an area of 69,436 square kilometres. Maiduguri has a projected population of l,146,397 million persons with annual growth rate of 2.8% (NPC, 2006). Maiduguri is composed of two local government areas (LGAs) namely: Maiduguri metropolitan council (MMC) and Jere LGA, with some sources including Konduga and Mafa LGAs into 'greater Maiduguri'. As of 2022, Maiduguri is estimated to have a population of approximately two million in the

metropolitan area (NPC, 2006). The majority of the populace of Maiduguri Metropolitan Council are farmers, civil servants and traders. Major crops cultivated in the study area are millet, sorghum, groundnut, wheat and cowpea, and the major livestock reared are cattle, sheep, goats and poultry (Bulama *et al*., 2019).

Study Design and Study Population: This study adopted a cross-sectional design. The study targeted a representative sample of domesticated turkeys sourced from different turkey breeder households located at GRA, Mairi area, University of Maiduguri (UniMaid) staff quarters, live birds' markets (Monday and Custom), and Veterinary Hospitals (UniMaid Veterinary Teaching Hospital, and Senator Ali Sheriff veterinary Hospital). The inclusion criteria involved apparently healthy and clinically sick turkeys of varying ages and sexes. Turkeys lacking complete records were excluded.

Sampling Strategy: A stratified random sampling technique was used to ensure representation from different geographical regions, live birds' markets and farms. Sample size calculation was based on 40% prevalence of haemoparasites among turkeys from previous study by Opara *et al*. (2014) following standard epidemiological formula (Fisher's formula for cross-sectional descriptive study) for sample size determination for scientific research. A sample size of 368.79 was calculated and 370 samples were collected.

Sampling Period: Sampling was carried out between January and September, 2023 within two seasonal periods, that included, the dry season (January – May) and rainy season (May – September).

Informed consent: Informed consent was obtained from turkey owners prior to data collection.

Data Collection using a Questionnaire Survey: A structured questionnaire was administered to turkey farmers or owners to collect data on potential risk factors such as: housing conditions, management practices, feeding methods, presence of other birds, previous disease history, environmental factors (temperature, humidity).

Biological Sample Collection from the Turkeys: Before collecting blood samples, each turkey that was sampled underwent gentle restraint to minimize stress. Blood collection was done following the procedures outlined by Cheesbrough (2000) and Rukhsana (2005); blood was drawn from the wing vein or jugular vein of turkeys using a sterile 23-gauge needle attached to a 5ml syringe. Approximately 1 – 2 ml of blood per turkey was collected. The blood samples that were collected were then transferred into labeled tubes containing an anticoagulant (EDTA). To effectively prevent clotting, the tubes were gently rocked and rolled to ensure thorough mixing of the blood with the anticoagulant. Proper labeling was done to ensure accurate identification of each sample. All sampled turkeys were handled humanely following standard animal welfare guidelines. Measures were taken to minimize stress and discomfort during sample collection, and all procedures were done humanely.

Blood Sample Analysis: Blood sample analysis involved microscopic examination of blood smears for the identification of haemoparasites. Thin blood and buffy coat smears were made from each blood sample on two different clean dry slides and were left for few minutes to air dry and then labeled appropriately (Mello *et al*., 2014). The slides were then fixed with methanol for five minutes, allowed to air dry, packaged and then transported to the Department of Veterinary Parasitology and Entomology Research Laboratory, University of Maiduguri for staining with diluted 10% Giemsa stain, according to the standard procedures described by Zajac and Conboy (2012). The slides were later viewed under low magnification (×40) and under high

magnification (×100; oil immersion), using the light Olympus® (Japan) microscope, for the presence of intracellular or extracellular blood parasites and their gametocytes as previously described by Valkiûnas (2005) and Valkiûnas *et al*. (2008).

Data Analysis: Descriptive statistical analysis and Chi-square or Fisher's exact tests were performed on the data obtained as appropriate, using GraphPad Prism software (GraphPad Inc., San Diego, CA). Percentage occurrences were calculated and presented in tabular form. Differences were considered statistically significant at a threshold of $p < 0.05$.

Results

Out of the 370 blood smears examined, 42 (11.4%) were found to be infected with *Plasmodium* species (Table 1), which were seen under the microscope as pigmented gametocytes within the cytoplasm of mature erythrocytes (Figure 1).

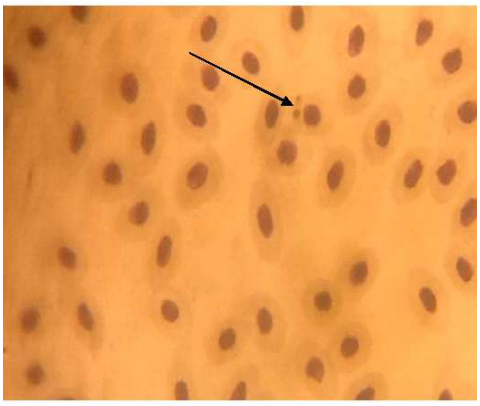

Figure 1. *Plasmodium* species (black arrow), viewed as pigmented gametocytes within the cytoplasm of mature erythrocytes. (×100).

Among the 80 turkeys sampled at live bird markets, 4 (5.0%) were found to be infected with avian *Plasmodium*. But, out of the 255 turkeys sampled from farms/households, 11 (4.3%) were infected with avian *Plasmodium.* Among the 35 turkeys sampled from veterinary hospitals, 27 (77.1%) tested positive for avian *Plasmodium*. The occurrence of *Plasmodium* species in blood of turkeys sampled at Veterinary Hospitals was significantly higher ($p < 0.05$) than those sampled at live bird markets and farms/households.

Twenty nine out of the 170 male turkeys sampled (17.1%) were infected with avian *Plasmodium*, but among the 200 female turkeys sampled, 13 (6.5%) were infected with avian *Plasmodium* (Table 2) The difference in occurrence of avian *Plasmodium* between male and female turkeys was statistically ($p < 0.05$) significant. (Table 2).

Out of the 150 juvenile turkeys sampled, 17 (11.3%) were infected with avian *Plasmodium*, but among the 220 adults sampled, 25 (11.4%) were infected. The difference in prevalence rates between juvenile and adult turkeys was not statistically ($p > 0.05$) significant (Table 2).

Seven out of the 185 turkeys sampled during the dry season (3.8%) were infected with avian *Plasmodium* species, whereas, among the 185 turkeys sampled during the rainy season, 35 (18.9%) were infected (Table 2). The difference in occurrence between seasons was statistically significant ($p < 0.05$).

Four turkeys out of the 270 apparently healthy ones sampled (1.5%) were infected with avian *Plasmodium*, while among the 100 clinically sick turkeys sampled, 38 (38.0%) were infected (Table 2). The occurrence of *Plasmodium* species was significantly higher ($p < 0.05$) in the clinically sick turkeys when compared to the apparently healthy ones.

When the occurrence under the two different husbandry systems was compared, out of the 185 intensively reared turkeys sampled, 11 (5.9%) were infected with avian *Plasmodium*, while among the 185 extensively reared turkeys sampled, 31 (16.8%) were infected. The difference in occurrence between turkeys raised under husbandry systems was statistically significant ($p < 0.05$).

Table 1. The occurrence of *Plasmodium* species infection in turkeys sampled in Maiduguri, Borno State, Nigeria, and the distribution of the occurrence based on sampling location (n = 370).

Sample origin/ Study location	Number of turkeys sampled	Number of turkey infected with Plasmodium species, with % in brackets
Live bird markets	80	4 (5.0%)
Farms/Households	255	11 (4.3%)
Veterinary Hospitals	35	27 (77.1%)
Total	370	42 (11.4%)

Table 2. Distribution of the occurrence of *Plasmodium* species infection in turkeys sampled in Maiduguri, Borno State, Nigeria, based on sex, age, season, health status and husbandry system.

Parameter	Categories	Number of turkeys sampled.	Number infected with *Plasmodium* species, with % in brackets
Sex*	Males	170	29 (17.1%)
	Females	200	13 (6.5%)
Age (months)	Juveniles (4 – 5)	150	17 (11.3%)
	Adults (> 5)	220	25 (11.4%)
Season*	Dry season	185	7 (3.8%)
	Rainy season	185	35 (18.9%)
Health status*	Apparently healthy	270	4 (1.5%)
	Clinically sick	100	38 (38.0%)
Husbandry* system	Intensive	185	11 (5.9%)
	Extensive	185	31 (16.8%)

* Asterisk on a parameter indicates significant difference ($p < 0.05$) in occurrence between the different categories.

Discussion

The overall 11.4% occurrence of *Plasmodium* species in the turkeys surveyed implies the presence of mosquitoes which are suitable arthropod vectors capable of transmitting these haemoparasites to turkeys in the study area. These findings align with a previous study by Mohammed *et al*. (2023), which documented various species of mosquitoes in both residential and non-residential areas within the current study location. Moreover, the abundance of vectors has been linked to the transmission of blood protozoans by various blood-sucking arthropods, found in both tropical and non-tropical regions, as long as there are favorable breeding environments available (Ogbaje *et al*., 2019; Marzal *et al*., 2022; Tembe *et al*., 2023). The present study is

the first to investigate and document haemoparasites in domesticated turkeys in the study area; turkey rearing is becoming increasingly popular among poultry farmers in the area. Several recent studies in Nigeria have reported the prevalence of haemoparasites in domesticated avian species of which infections in chickens are the most documented (Lawal *et al*., 2021 a & b; Buhari *et al*., 2022). However, Tembe *et al*. (2023) in a review, reported several species of avian haemoparasites from a wide range of poultry species distributed across Nigeria, Kenya, South Africa, Tanzania, Uganda, Botswana, Zimbabwe, Ghana, Cameroon, and Zambia, with the presence of mixed infections observed in quails, pigeons, chickens, ducks, turkeys, and guinea fowls, but predominantly in chickens.

The findings in the present study that avian malaria infection is most prevalent in turkeys sampled from Veterinary Hospitals, followed by those from farms and households, with the lowest occurrence recorded among turkeys from live bird markets, is believed to be due to differences in overall health of the turkeys in these locations, management practices, and exposure to vectors. Turkeys brought to Veterinary hospitals were initially presented at such hospital as a result of suspected disease conditions. The detection of avian malaria parasites in these turkeys suggests that the haemoparasites may be part of their health problems, or the malarial parasites exercised immunosuppressive effects, potentially making the turkeys more susceptible to the other diseases for which they were brought to the hospital. These findings concurs with similar study by Lawal *et al*. (2024) on domesticated pigeons, which found a higher occurrence of avian haemoparasites in pigeons from Veterinary Health Centres compared to other locations. This was also linked to the possible immunosuppressive capabilities of haemosporidian parasites, which may increase the host's susceptibility to additional infections by secondary pathogens, as emphasized by Sol *et al*. (2003). Moreover, the secondary infections could have prompted the birds to seek medical care at hospitals, where they were subsequently sampled. The detection of avian haemoparasites in turkeys sampled from live bird markets is not surprising. This may largely be due to the common practice of vendors mixing different species of birds in close proximity. Such conditions are ideal for the spread of blood protozoans carried by arthropods, especially in the presence of abundant blood-sucking arthropod vectors (Lawal *et al*., 2021a & b; Rodrigues *et al*., 2021). These vectors transmit parasites from infected birds to healthy ones through their bites during blood meals. Maiduguri, known for prevalent *Plasmodium* vectors, according to Balogun *et al*. (2019) and Ibrahim *et al*. (2021), offers conducive conditions for the mosquito-transmitted haemosporidian parasites. Furthermore, management practices such as biosecurity measures and vector control can influence infection prevalence in turkey populations (Dhaka *et al*., 2023; Grace *et al*., 2024)

The study identified a statistically significant difference in the occurrence of avian *Plasmodium* between male and female turkeys, with males showing a higher occurrence. This difference may be attributed to physiological disparities between sexes, which could have implications for breeding and management strategies, as both male and female turkeys have large body areas accessible to vectors for blood meals, which facilitates parasite transmission. In contrast, male chickens exhibit a more pronounced difference in comb and wattle sizes, providing distinct sites for blood-sucking arthropod vectors, particularly in roosters compared to hens. The findings in the present study align with the report by Lawal *et al*. (2021b), which observed a higher prevalence of *Plasmodium* in male chickens and attributed this to the

variations in comb and wattle sizes. Other studies have also noted differences in the prevalence rates of avian haemoparasites based on host plumage color, with darker-feathered birds showing higher prevalence compared to those with brighter feathers (Gangoso *et al*., 2016). It has been reported that most arthropod vectors are nocturnal and are typically attracted to darker-colored hosts even during the day, and darker colors are generally more attractive to mosquitoes, potentially increasing host-vector contact rates (Aouissi *et al*., 2021). The specific reasons for these variations are not entirely clear. However, the feeding preferences of these vectors concerning color attractiveness remain unknown and should be explored in future research, as recommended by Che-Ajuyo *et al*. (2023). Other studies have attributed the significant difference in haemoparasite prevalence between male and female birds to variations in body mass, noting that male birds generally have larger body masses compared to females, thus providing more extensive sites for blood-sucking arthropods during their blood meals (Che-Ajuyo *et al*., 2023).

Endocrinological differences between sexes may also significantly contribute to differences occurrence between males and females. For instance, testosterone is known to have immunosuppressive effects, which can interact with behavioral and environmental factors (Foo *et al*., 2017). Additionally, the social status of male vertebrates within their communities is a significant predictor of parasite risk compared to females (Habig *et al*., 2018).

The lack of significant difference in occurrence of *Plasmodium* parasite in the blood of juvenile and adult turkeys in the present study indicates that the two age groups share equal chance of getting infections in the presence and abundance of suitable arthropod vectors, which is consistent with reports by Tembe *et al*. (2023).

The finding in the present study that *Plasmodium* infections were significantly more prevalent during the rainy season compared to the dry season may be attributed to the higher level of vector activity and favorable environmental conditions for parasite development and transmission during the rainy season. This finding is consistent with the reports of Ranford-Cartwright (2023) and Tamayo-Quintero *et al*. (2023), who have attributed the rise in avian malaria cases to the role of humid habitats in enhancing vector breeding.

Clinically sick turkeys showed a substantially higher prevalence of avian *Plasmodium* compared to relatively healthy ones. This suggests an association between haemoparasite infections and the overall health status of turkeys. Sick birds may have compromised immune systems due to underlying health issues or stress, making them more susceptible to various infections, including avian haemoparasites, as reported by Nebel *et al*. (2020) and Salem *et al*. (2022a). Mirzaei *et al*. (2020) also documented that illness-related stress can further suppress the immune response, facilitating the proliferation and infection of haemoparasites. Additionally, sick birds might engage in behaviors that increase their exposure to haemoparasite vectors or sources, such as becoming less active and spending more time in areas where parasites or vectors are abundant (Schumm *et al*., 2021; Salem *et al*., 2022b; Martín-Maldonado *et al*., 2023). Moreover, their weakened condition might attract vectors, increasing the likelihood of transmission. It is also possible that some turkeys in the sick group had latent haemoparasite infections that became active or intensified due to the stress of illness, contributing to the higher incidence observed.

Turkeys raised in extensive husbandry system showed a significantly higher prevalence of avian *Plasmodium* compared to those raised in intensive systems. This difference can be

attributed to varying levels of exposure to vectors and the stress associated with different husbandry practices. Turkeys in extensive systems, being more exposed to outdoor environments, are more likely to come into contact with potential carriers of haemoparasites, such as insects or other birds. This higher level of exposure ultimately leads to a higher risk of parasite transmission and infection. This finding is consistent with those of Alkharigy *et al*. (2018), who reported that birds in free-range systems, having unrestricted outdoor access, are more likely to encounter infected vectors. Conversely, intensive husbandry systems, where birds are caged, provide more controlled environments that limit exposure to external factors and reduce contact with potential haemoparasite-infected vectors, as also noted by Alkharigy *et al*. (2018).

One limitation of this present study was the lack of data on vector populations in the study area. Vector distribution and feeding preferences play crucial roles in the transmission and host-specificity of avian haemosporidians (Hellgren *et al*., 2009). Without this information, it is challenging to explain whether the absence of a particular genus of haemosporidians was due to sample size or the lack of necessary vectors. This study did not find any infections from the *Haemoproteus* and *Leucocytozoon* subgenera among the avian haemoparasite-infected turkeys, despite these haemoparasites being previously reported in other domesticated birds in the study area by Lawal *et al*. (2024) and elsewhere by Tembe *et al*. (2023). *Haemoproteus* is transmitted by louse flies (family Hippoboscidae) and primarily infects members of the family Columbiformes. On the other hand, *Leucocytozoon* parasites are transmitted by biting midges (family Ceratopogonidae) and black flies (family Simuliidae), which require high-quality running water for their early development (Buchheit *et al*., 2021; Kleinschmidt *et al*., 2022; Valkiūnas and Iezhova, 2022 & 2023). Previous studies have documented the occurrence of *Haemoproteus* and *Leucocytozoon* in other domesticated birds, such as pigeons and chickens, indicating the presence of suitable arthropod vectors capable of transmitting these parasites to susceptible birds (Mirzaei *et al*., 2020; Tembe *et al*., 2023; Valkiūnas and Iezhova, 2023; Lawal *et al*., 2024). However, the absence of these haemoparasites in turkeys suggests the need for further investigation to uncover the intrinsic or extrinsic factors that may have made turkeys more resistant to these genera of haemoparasites in the study area.

Conclusion: Microscopy of stained blood smears of 370 turkeys surveyed at Maiduguri, Nigeria showed an overall 11.4% occurrence of *Plasmodium* species. Turkeys sampled at Veterinary Hospitals had significantly higher occurrence of *Plasmodium* species, when compared with those sampled at farms/households and live bird markets. Male turkeys showed a significantly higher occurrence than females, while higher occurrence was recorded during the rainy season when compared to the dry season. Also, clinically sick turkeys had a significantly higher occurrence than the apparently healthy ones, while turkeys under extensive husbandry system had significantly higher occurrence than those reared under intensive husbandry systems.

Recommendations: Increasing the awareness among stakeholders about the occurrence of the parasite and its transmission routes, development of targeted control strategies, emphases on biosecurity measures, and collaborative research efforts for effective prevention and control of the parasite are being recommended. Further investigations are also recommended.

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Conflict of interest

The authors declare that they have no competing interests.

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