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Detection of Gastrointestinal Parasites of Lizards (*Agama agama*) Trapped in and Around Commercial Poultry Pens in Gombe State, Nigeria

Jallailudeen Rabana Lawal*[1], Kingsley Uwakwe Ezema[2], Abdullahi Abubakar Biu[3], Shuaibu Gidado Adamu[4]

[1]*Department of Veterinary Medicine, Faculty of Veterinary Medicine, University of Maiduguri, Borno State, Nigeria*

[2]*Veterinary Teaching Hospital, Faculty of Veterinary Medicine, University of Maiduguri, Borno State, Nigeria*

[3]*Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, University of Maiduguri, Borno State, Nigeria*

[4]*Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, University of Maiduguri, Borno State, Nigeria*

Abstract

BACKGROUND: *Agama agama* lizards are the most populous species of lizard in developing countries of African including Nigeria. These lizards are insectivores; they feed on insects and other arthropods, which may predispose them to different gastrointestinal parasitic infections, some of which are of zoonotic importance.

OBJECTIVES: This study aimed to investigate the gastrointestinal parasites in *Agama agama* lizards trapped in and around commercial poultry houses in Gombe State, Nigeria.

METHODS: The study was carried out between the months of February and December, 2017. Fresh fecal samples were collected from 200 humanly euthanized *Agama agama* lizards from six Local Government Areas of Gombe State. Samples were examined microscopically for the presence of gastrointestinal parasites eggs or adult worms.

RESULTS: Gastrointestinal parasites were detected in 154 (77.0%) *Agama agama* lizards examined, comprising of 58% gastrointestinal helminthes ova and 19% coccidian oocytes. Six species of helminthes were encountered viz: *Strongyluris* species (25.5%), *Trichuris* species (13.5%), *Oxyuris* species (8.0%), *Ascaris* species (5.5%), *Heterakis* species (4.0%) and *Capillaria* species (1.5%) all belonging to the class *Nematoda*. Two coccidian species comprised of *Eimeria* (1.5%) and *Isospora* species (1.5%) were also encountered. The prevalence of gastrointestinal helminthes was found to be significantly ($p < 0.05$) higher in male (68.5%) compared to the female (45.7%) infected lizards. Coccidiosis was also higher ($p > 0.05$) in the male (19.4%) compared to the female (18.5%) infected lizards. Prevalence of gastrointestinal parasites was found to be significantly ($p < 0.0001$) higher in the rainy season compared to the dry season of the study period.

CONCLUSIONS: This study discovered helminthosis and coccidiosis in *Agama agama* lizards roaming in and around poultry pens in Gombe State. The occurrence of six helminthes and two coccidian species were recovered from infected *Agama agama* lizards, and prevalence rates of both parasites were significantly higher during the rainy season. Similar genus of gastrointestinal parasites detected in this study have been previously documented in man and domesticated birds. This suggests the association of *Agama agama* lizards in gastrointestinal parasites transmissions between susceptible hosts. Therefore, it is recommended that further studies involving molecular characterization and comparative studies of these gastrointestinal parasites in *Agama agama* lizards and domesticated birds should be carried out. It is also recommended that strict biosecurity should be maintained in and around commercial poultry houses to curb disease transmission by lizards or wild reptiles to birds.

KEYWORDS: *Agama agama* lizards, commercial poultry pens, gastrointestinal parasites, Gombe State, Nigeria

Correspondence

Jallailudeen Rabana Lawal, Department of Veterinary Medicine, Faculty of Veterinary Medicine, University of Maiduguri, Borno State, Nigeria, Tel: +234 8032886428, Fax: +234 8032886428, Email: rabanajallailudeen@yahoo.com

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Introduction

Lizards are one of the most common reptiles that apparently exist in many tropical parts of the world because of their adaptive and poikilothermic nature. The African lizard family *Agamidae* consists of several species of lizards usually found in Africa and Eurasia (Brown et al., 2002; Brito et al., 2008). Studies have revealed that the *Agama agama* lizards are the most popular species amongst the *Agamidae* identified (Spawls et al., 2002), and are usually considered to be native to western African countries especially the sub-Saharan (Matthee and Flemming, 2002; Enge et al., 2004; Mediannikov et al., 2012). They live in social groups including a lead male, about half a dozen females, and subordinate males (Wagner et al., 2009a; Wagner et al., 2009b; Wagner et al., 2009c). The *Agama agama* lizards are primarily insectivores; they play a significant role as biological control of insects and other arthropod pests around households as well as in agriculture involving crop production (Vasconcelos et al., 2009; Lazić et al., 2013). Unfortunately, *Agama agama* lizards have been reported to serve as transport and reservoir host to several protozoan and helminthes parasites, some of which are of zoonotic importance (Wekhe and Olayinka, 1999; Adeoye and Ogunbanwo, 2007). Human beings can get infection during handling of objects contaminated with infected faeces or saliva, and accidentally ingesting the parasite eggs (Nash, 2005). The prevalence of several species of gastrointestinal parasites in *Agama agama* lizards has been reported from some parts of Nigeria (Adeoye and Ogunbanwo, 2007; Omonona et al., 2011; Mbaya et al., 2013; Nwadike and Ilozumba, 2015; Sowemimo and Oluwafemi, 2017). *Ascaris* spp. *Capillaria spp.* and *Raillietiella* spp. from *Agama agama* lizard origin have been documented in human beings. Despite the various studies, there is still a paucity of information on the prevalence of gastrointestinal parasites in *Agama agama* lizard from Gombe State, and the risk of transmission to human and commercial poultry flocks. Therefore, the present study aimed at providing information on the occurrence of gastrointestinal parasites of free-living *Agama agama* lizard in close proximity with commercial poultry farms and to determine those that harbor parasites of zoonotic importance so as to provide control measures where necessary.

Materials and Methods

Study Area

This study was conducted in Gombe State, Northeastern Nigeria (Fig. 1) which shares boundaries with Bauchi, Taraba, Adamawa, Yobe and Borno states. Gombe State is located between latitude 9◦ 30' and 12 3' N and longitude 8◦ 45' and 11◦ 45' E (Anonymous, 2017). The state has Eleven Local Government Areas *viz*: Gombe, Akko, Funakaye, Kwami, Dukku, Billiri, Shongom, Nafada, Yamaltu-Deba, Kaltungo and Balanga Local Government Areas. The state has a mean annual rainfall of 818.5mm, with a mean maximum temperature of 37 ◦C and a mean minimum temperature of 12 ◦C. The major economic activities of the people of Gombe State include crop and livestock production as well as trading.

Study Design

Cross-sectional study involving Non-probability convenience sampling method was adopted for the selection of 6 out of the 11

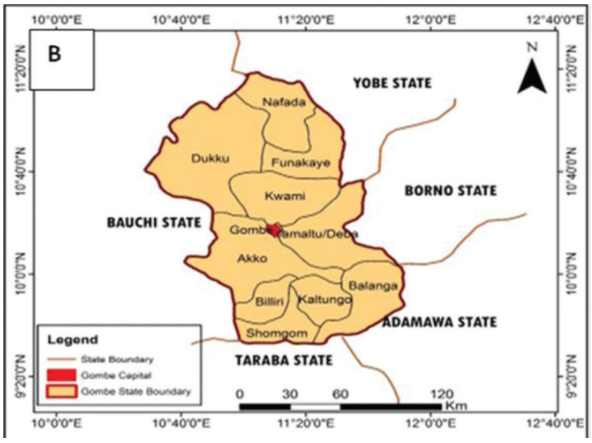

Figure 1. Map of Gombe State showing the study areas

Figure 2. Male and female *Agama agama* Lizards in commercial poultry pen in the study area (Black arrows)

Figure 3. Female *Agama agama* Lizard outside commercial poultry pen in the study area

Figure 4. Male *Agama agama* Lizard outside commercial poultry pen in the study area

Local Government Areas of the state *viz*: Dukku, Yamaltu-Deba, Kaltungo, Shongom, Akko and Kwami Local Government Areas. By the consent of commercial poultry farmers, selected poultry houses within these LGAs were visited for *agama* lizard capture. Birds in any selected pen were first relocated to other different pens within the poultry farm, and then capture of *Agama* lizard was done using traps with cockroaches as bait on alternate days throughout the sampling periods. Criteria considered for the selection of commercial poultry pens for lizard capture include: availability of *Agama agama* lizard of both sexes and willingness of the poultry farmers to allow researchers into the pens during the period of study for lizard capture.

Sample collection

A total of 200 *Agama agama* lizards of both sexes were captured between the months of February and December, 2017. The captured lizards were humanly euthanized using halothane, and then taken to the Parasitology Laboratory of Department of Biological Sciences, Gombe State University. All procedures employed for the examination of the lizards for the presence or absence of gastrointestinal parasite were in accordance with standard parasitological procedures. Following evisceration, the entire gastrointestinal tract for each *Agama* lizard was examined for the presence of adult worm. Adult worms

recovered were placed in petri dish containing saline and were later identified according to reference parasitology pictures.

Coprology

In the laboratory, each lizard was placed in dorsal recumbence on a dissection board, then using thumb forceps, scalpel blade and scissors, the abdomen was incised to expose the gastrointestinal tracts, removed and placed in a Petri dish containing physiological saline. Each organ was cut open longitudinally and fresh faecal samples were collected, expressed in a Petri-dish containing physiological saline. The contents were then examined closely on a dark background under a dissecting microscope. Adult worms observed were removed and sorted using reference atlas. Faecal materials were washed in saline to remove adhering debris; concentrated salt solution was used to increase the possibility of finding ova, cyst, oocyst, or larva in the sample where they are too insufficient to be seen by direct microscopy. Standard coproscopic methods comprising direct smear, flotation technique followed by sedimentation technique, direct saline and iodine smear observations were used to detect parasite infections as described by Kandasamy et al. (2013). Protozoans were identified by sodium-acetate acetic acid formaldehyde (SAF) method and modified Ziehl-Neelsen staining. Parasites eggs were identified using standard parasitological techniques as described by Garcia et al. (2017).

Data analysis: Data were analyzed using the Graphpad prism software package (version 17). Prevalence was expressed as percentages, differences in prevalence between lizards' sexes and season of sample collection were assessed using Chi-squared (χ^2) test.

Results

Table 1 summarizes the result of the prevalence of gastrointestinal parasites in *Agama agama* lizards captured in and around commercial poultry pens in Gombe State Nigeria. Out of the 200 lizards examined, 154 (77.0%) were found to be infected with gastrointestinal parasites. Out of the gastrointestinal parasites infected lizards, 116 (58.0) were found to harbor gastrointestinal helminthes while 38 (19.0%) were found to harbor coccidian oocyts.

Table 2 summarizes the prevalence of the species of the gastrointestinal parasites encountered in the infected *Agama agama* lizards in Gombe State. *Strongyluris spp.* (25.5%) was found to be the more prevalent helminthes, followed by *Trichuris spp.* (13.5%), *Oxyuris spp.* (8.0%), *Ascaris spp.* (5.5%), *Heterakis spp.* (4.0%), and the least prevalent helminthes encountered were *Capillaria spp.* (1.5%). However, *Eimeria spp* (17.5%) was found to be the most prevalent coccidian parasites followed by *Isospora spp.* (1.5%).

Table 1. Prevalence (%) of Gastrointestinal Parasites of Lizard (*Agama agama*) in Gombe State, Nigeria

Number of Lizards Examined	Number of Lizards Infected (%) N= 200		Prevalence (%)
	Helminth (%)	Coccidia (%)	
200	116 (58.0)	38 (19.0)	154 (77.0)

N= Total Number of Lizards Examined

Table 2. Species-specific Prevalence (%) of Gastrointestinal Parasites of Lizard (*Agama agama*) in Gombe State, Nigeria

Parasites	Species	Number of lizard infected N=200	(%) Prevalence	Confidence Interval 95%	
				Lower limit	Upper limit
Helminthes	*Strongyluris* spp.	51	25.5	0.1996	0.3196
	Trichuris spp.	27	13.5	0.0945	0.1893
	Oxyuris spp.	16	8.0	0.0498	0.1260
	Ascaris spp.	11	5.5	0.0310	0.0958
	Heterakis spp.	8	4.0	0.0204	0.0769
	Capillaria spp.	3	1.5	0.0051	0.0432
Coccidia	*Eimeria* spp.	35	17.5	0.1286	0.2336
	Isospora spp.	3	1.5	0.0051	0.0432

N = Total number of Agama Lizards examined

Table 3. Sex-specific Prevalence (%) of Gastrointestinal Parasites of Lizard (*Agama agama*) in Gombe State, Nigeria

Parasites	Sex	Number of Lizards Examined	Number of Lizard infected (%)	(Confidence Interval) 95%		p-value	Relative risk
				Lower limit	Upper limit		
Helminthes	Male	108	74 (68.5)[a]	0.2289	0.4118	0.0018	0.5793
	Female	92	42 (45.7)[b]	0.4360	0.6473		
Coccidia	Male	108	21 (19.4)[a]	0.7183	0.8753	0.8622	0.9881
	Female	92	17 (18.5)[a]	0.7209	0.8887		

[ab]Different superscripts within columns indicates statistically significant difference (P<0.05)

Table 3 reveals the sex – specific prevalence of gastrointestinal parasites in *Agama agama* lizards in Gombe State, helminthes infection was found to be significantly (p <0.05 at 95% confidence Interval) higher in male (68.5%) lizards compared to the female (45.7%). Moreover, the prevalence of coccidian oocyts was found to be higher in the male lizards (19.4%) compared to the female (18.5), although the difference was not statistically significant (p> 0.05 at 95% confidence Interval).

Table 4 summarizes the results of the season – specific prevalence of gastrointestinal parasites in *Agama agama* lizards in Gombe State. Helminthes infection was found to be significantly (p <0.0001 at 95% confidence Interval) higher during the rainy (86.4%) season compared to the dry (23.3%). Moreover, the prevalence of coccidian oocyts was also found to be higher during the rainy (29.1%) compared to the dry (6.7%) season, the difference in the prevalence rates was also statistically significant (p> 0.0001 at 95% confidence Interval).

Table 4. Season-specific Prevalence (%) of Gastrointestinal Parasites of Lizard (*Agama agama*) in Gombe State, Nigeria

Parasites	Season	Number of Lizards Examined	Number of Lizard infected (%)	(Confidence Interval) 95%		p-value	Relative risk
				Lower limit	Upper limit		
Helminthes	Dry	90	21 (23.3)[a]	0.6658	0.8494	< 0.0001	5.622
	Rainy	110	95 (86.4)[b]	0.0783	0.2153		
Coccidia	Dry	90	6 (6.7)[a]	0.8607	0.9751	< 0.0001	1.316
	Rainy	110	32 (29.1)[b]	0.6149	0.7921		

[ab]Different superscripts within columns indicates statistically significant difference (P<0.05)

Discussion

This result of the present study revealed that all the helminthes detected in the gastrointestinal parasites infected *Agama agama* lizards belong to the class *Nematoda.* This finding of the present study suggests that the nematodes species are the most prevalent helminthes in Agama lizards in Gombe State, Nigeria. Our finding coincides with several reports from previous studies which revealed nematodes as the most prevalent gastrointestinal parasites of lizards of the Agama species in Nigeria (Adeoye and Ogunbanwo, 2007; Omonona et al., 2011; Mbaya et al., 2013; Nwadike and Ilozumba, 2015; Sowemimo and Oluwafemi, 2017). Even though other species of helminthes belonging to the classes *Trematoda*, *Cestoda, Pentastomes* and *Acanthocephalan* have been previously reported from Agama lizards and other lizards species from some developing countries of Africa and other parts of the world (Goldberg and Bursey, 2005; McAllister et al., 2011, Norval et al., 2013; Yang et al., 2014; Norval et al., 2014), most of these studies have also implicated nematodes as the most dominant of amongst the helminthes species that infect *Agama* lizards. The predominance in the incidence of nematodes species in *Agama* lizards as reported in the present study concurs with the findings of other previous similar studies in Nigeria. The high prevalence of nematode as reported could be attributed to the feeding habits of lizards and the direct life cycles of nematodes which do not usually require intermediate host in the transmission of infective stage of the parasite to host (Sanchis et al., 2000; Martin and Rocha, 2004). This may result in their widespread infective stages. Infections by nematode parasites in the vast majority of the land-dwelling vertebrates are bound to happen during the terrestrial stages of the life cycle of these parasites (Al-barwari and Saeed, 2007). The microscopic detection of gastrointestinal parasites of *Agama* lizard in the present study did not reveal the presence of cestodes, trematodes, pentastomes or acanthocephalan from the infected lizards examined. This might be attributed to

the complex life cycle of these parasites and their requirement for an intermediated host for the transmission of the infective stage of the parasite (Roberts and Janovy, 2005; Kennedy, 2006). There is the possibility that the *Agama* lizards are less susceptible to these classes of gastrointestinal parasites due to some barriers of phylogenetic incompetency and host specificity nature (Norval et al., 2013). Moreover, there were no previous reports of these parasites from the *Agama* lizards examined in previous similar studies in Nigeria (Adeoye and Ogunbanwo, 2007; Omonona et al., 2011; Mbaya et al., 2013; Nwadike and Ilozumba, 2015; Sowemimo and Oluwafemi, 2017).

The result of the present study has also encountered the occurrence of *Eimeria* and *Isospora* species in infected *Agama* Lizards. This finding concurs with the reports of Amo et al. (2005) and Adeoye and Ogunbanwo (2007) who have also reported the *Eimeria* species as one of the most prevalent protozoans in reptiles. These ubiquitous protozoans are considered as one of the most important gastrointestinal organisms that cause the disease of coccidiosis in many vertebrates including the *Agama* lizards (Sharma et al., 2013; Opara et al., 2014). The occurrence of coccidian parasites in *Agama* lizards is not surprising due to the feeding habit of the lizards. They might possibly ingest oocyst of the organisms from contaminated soil or by ingestion of other accidental hosts such as arthropods during feeding activities. The results of the present study showed that the overall prevalence of gastrointestinal parasites infection in *Agama* lizards in Gombe State was 77.0%. This finding is lower than the 95.5% reported by Adeoye and Ogunbanwo (2007) in Lagos and 100% reported from Maiduguri and Ibadan by Mbaya et al. (2007) and Sowemimo and Oluwafemi (2017) respectively. However, the prevalence rate reported from this present study is higher than prevalence rate of 76.1% that was reported by Rataj et al. (2011) in Slovenia. These variations in the prevalence rates from various studies might be attributed to difference in method of diagnosis, scope of the research, study areas, sample sizes, type of lizard and organs examined, geographical regions and season of samples collections which might influence the detection and occurrence of the parasites. To the best of our knowledge, the present study is the first of its kind to report the presence of *Isospora* species in *Agama* lizards in Nigeria.

Amongst the six nematodes species detected in the infected *Agama* lizards, *Strongyluris* species is the most predominant with a prevalence of 25.5%. This finding is in agreement with that of Adeoye and Ogunbanwo (2007), Nwadike and Ilozumba (2015) as well as Sowemimo and Oluwafemi (2017) who have also reported this parasite as the most frequently encountered gastrointestinal parasite in *Agama* lizard in Nigeria, although Mbaya et al. (2013) in a similar study did not recover the parasite in their study area. However, the finding of the present study is lower than 82.3%, 85.6% and 92.5% reported from Lagos, Anambra and Ibadan by Adeoye and Ogunbanwo (2007) and Nwadike and Ilozumba (2015), Sowemimo and Oluwafemi (2017) respectively.

The result of this present study also revealed the 13.5% prevalence rate of *Trichuris* species. This finding is lower than 70.0% reported from Maiduguri by Mbaya et al. (2013). The variation in the prevalence rates of these two studies may be associated with difference in method of detection, geographical location, study areas, study sites, period

of sample collection, and the species of *Agama* lizard examined (wild, captive reared or pet). It has been well established that some lizard species are more susceptible to gastrointestinal parasites infection compared to others (Rataj et al., 2011). The finding of the present study encountered respectively 8.0% and 1.5% prevalence rates of *Oxyuris* and *Capillaria* species from the gastrointestinal parasites infected *Agama* lizards. This report is in contrast to previous findings by Adeoye and Ogunbanwo (2007) who in a similar study revealed 1.6% and 8.4% prevalence rates of *Oxyuris* and *Capillaria* species from Lagos respectively. The prevalence of *Ascaris* species as reported from the present study was 5.5% which is lower than 30% reported by Mbaya et al. (2013). The present study represents the first to discover *Heterakis* species in free range *Agama* lizards trapped in pens of intensively reared exotic breed of chickens in Nigeria. However, *Ascaris* and *Capillaria* species have been implicated to pose zoonotic risk to man (Nash, 2005) while *Heterakis* species have been reported in chickens (Kaingu et al., 2010; Lawal et al., 2015).

The result of the present study revealed more frequency of multiple gastrointestinal parasites infections while single infections were less commonly detected in the infected Agama lizards. This finding is consistent with the results previously reported by Nwadike and Ilozumba (2015) and Sowemimo and Oluwafemi (2017) who have also detected more frequency of multiple infections of gastrointestinal parasites compared to single infections.

The result of the present study revealed that the male *Agama* lizards are more susceptible to helminthes and coccidian infections compared to the females. The difference in the prevalence rates of helminthes infection between the two sexes was found to be statistically significant, but the difference in the prevalence rates of coccidian infection between the two sexes was not statistically significant. This finding might be attributed to certain genetic factors which might make the male more susceptible to gastrointestinal parasites than females. The findings of the present study tallies with reports of Adeoye and Ogunbanwo (2007) who have also reported high prevalence of gastrointestinal parasites in males compared to the Agama lizards and there was significant difference in the intensity of infection. However, Fadiel et al. (2005) in a similar study has reported an insignificant difference between prevalence of gastrointestinal parasites among sexes of lizards in Libya. Moreover, Amo et al. (2005), Nwadike and Ilozumba (2015) as well as Mbaya et al. (2013) have reported that the sex of lizards had no influence on the overall prevalence of helminthes infections in the *Agama agama*, as both sexes have the same prevalence of infection. This finding was attributed to the fact that both sexes of lizards share equal chance of exposure to similar infection. Although recent studies in lizards have revealed that males are more susceptible to parasites infection probably due to the immune suppressive effects of testosterone at least during the reproductive period (Uller and Olsson, 2003; Roberts et al., 2004). It has been reported that some parasites species could be transmitted from one host to another during copulation; therefore, they are usually only recorded from sexually mature lizards (Norval et al., 2011; Langford et al., 2013).

This present study was unable to detect or categorize the occurrence of gastrointestinal parasites in *Agama* lizards in Gombe State

based on age of lizards. This is because the study limited its scope to sample collection from adult lizards trapped in the poultry pens as well as considering some factors related to welfare issues.

The findings of the present study have also revealed season-specific prevalence of gastrointestinal parasites in infected *Agama* lizards in the study area. Both helminthosis and coccidiosis were more frequently encountered in samples collected and examined during the rainy season compared to samples collected during the dry season. This finding suggested that *Agama* lizards are more predisposed to gastrointestinal parasites infection during the raining season compared to the dry season in the present study area. This might be attributed to the abundance of optimum temperature and humidity experienced during the rainy season which may favor sporulation of oocyst and helminthes eggs that facilitates direct infections.

There was significant statistical difference ($P < 0.0001$ at 95% confidence interval) between the prevalent rates of the gastrointestinal parasites in the infected lizards during the two seasons. This finding of high frequency of gastrointestinal parasites in *Agama* lizards captured from poultry farms during the rainy compared to dry season is consistent with several reports of high prevalence of gastrointestinal parasites species in free range poultry species in developing countries including Nigeria (Alam et al., 2014; Opara et al., 2014; Udoh et al., 2014; Lawal et al., 2015; Biu et al., 2016). The dry season is usually considered as the period that is unfavorable for the development and persistence of parasitic phases of parasites (Mbaya et al., 2006). This could have been partly responsible for the comparative low prevalence of gastrointestinal parasites in the present study.

Reports from similar studies have suggested the role of reptiles and rodents in the transmission of the pathogenic parasites to intensively reared poultry species (Mbaya et al., 2013; Gulani et al., 2016). It was observed and noted in the present study locations, that *Agama* lizards frequently visit the poultry pens to feed on poultry feeds and other arthropods. This indicates that during these times they may accidentally contaminate the pen, litter and feeds with the parasites they may have harbored from contaminated environment. However, birds can become infected by the ingestion of contaminated material or feed. Therefore, it reasonable to keep all species of reptiles including the *Agama* lizards away from the poultry pens to prevent the incidence of gastrointestinal parasites.

Conclusion: The findings of the present study unleashed evidence of likelihood which may initiate the sharing of gastrointestinal parasites between the reptile fauna and intensively reared poultry. In situations where free range *Agama* lizards frequently have access into poultry pens and possibly contaminate poultry feed and water with gastrointestinal parasites infected faeces will inevitably aid in the sharing of vectors of diseases faster and more efficiently, thereby making disease transmission faster, effective and efficient between the lizards and birds. The fact that some similar species of the gastrointestinal parasites detected in Agama lizard in Gombe State have previously been reported in man and domesticated birds elsewhere; these parasites might utilize Agama lizards as host or reservoirs to enhance zoonotic diseases transmission. The 77.0% prevalence rate of helminthosis and coccidiosis is considered high in *Agama* lizards captured in commercial poultry houses in

Gombe State, which may suggest the lizards play a vital role in the epidemiology of the parasites in the study area. A total of five helminthes which belong to the nematodes species and two coccidian species were encountered in the infected lizards. Some of which have been previously reported in wild and pet reptiles including the *Agama* lizards in Nigeria and other parts of the world. The prevalence of gastrointestinal parasites was higher among the male lizards compared to the females; the prevalence was also high during the rainy compared to the dry season. It was speculated that the high level of parasitic infections in *Agama* lizards may have played a vital role in the maintenance of some related species of these parasites in intensively reared commercial poultry flocks in the study area.

Recommendations: It is recommended that further studies should be carried out using more sensitive diagnostic tool such as molecular detection and characterization to justify the possibility of the *Agama* lizards' susceptibility to trematodes, cestodes, pentastomes and acanthocephalan including other possibilities like the absence or presence of some appropriate intermediate hosts and vectors to convey these parasites to the reptile species. The control of *Agama* lizards as vectors and reservoirs of poultry diseases depends largely on the construction of poultry houses equipped with reptile proof facilities. Removal and destruction of refuse which would provide feeding material and place for bedding. To prevent spread of disease, new technology for routine deworming of free range lizards visiting poultry houses where the destruction of lizards using poisoning is against the ethics of the animal welfare needs to be established.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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