Preliminary Study on Gastrointestinal Helminths in Warthogs (*Phacochoerus africanus*) at the Mole National Park, Ghana

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Abstract

Most emerging human infectious diseases originated from wildlife. To find out if warthogs in Mole National Park harbour zoonotic parasites, a total of 39 warthog faecal droppings were sampled and examined. Zinc Sulphate Floatation technique was used in processing the samples for microscopic examination and quantification of parasite eggs. Out of the 39 warthog droppings, 95% were infected with one or more parasite species. At least seven genera of helminths were identified. Nearly 72% of the warthog droppings harboured *Strongyloides* sp and trichostrongyle-type. *Enterobius* sp. was found in 64.1% of the droppings. Other parasitic helminths identified such as *Ascaris* sp., *Taenia* sp., *Monieza* sp., and *S. haematobium* occurred in less than 50% of the animals. Z-tests showed significant variations in prevalence among the various parasites ($p<0.05$). Mostly, the level of infection ranged from moderate ($100<EPG<500$) to high ($EPG \geq 500$) loads of helminth eggs. Forty-two percent of the warthogs had three or more parasites. This study reveals some helminths that are harboured by the warthogs in the Mole National Park. The presence of zoonotic parasites such as *Ascaris* sp. and *Taenia* sp. in the warthogs is an indication of potential for transmission of zoonoses in the community.

Keywords: Warthogs, gastrointestinal helminths, prevalence, wildlife, mole

Introduction

Income from wildlife tourism is one of the benefits from human-wildlife interactions. In many developing countries, tourism is a major source of foreign exchange earnings (World Tourism Organization, 2002; Manzoor et. al., 2019). National parks, game reserves and zoos promote wildlife tourism. This establishments provide a fertile ground for close interaction between humans and nonhuman animals which can engender transmission, emergence and re-emergence of zoonoses. (Kruse, Kirkemo, & Handeland, 2004; Chomel, Belotto & Meslin, 2007; Wiethoelter et al., 2015). Intestinal helminthic infections are among less studied zoonoses of wildlife. These infections pose a major threat to humans and nonhuman animals, and it has been reported that most of the emerging human infectious diseases are zoonotic and more than 70% of them originated from wildlife (Rahman et. al., 2020). At least 34 helminths are known to infect humans in which nonhuman animal play a key role (WHO, 1979). These infections can occur with or without symptoms (Kipper et al., 2011; Mathison &Pritt, 2022), however, common signs include poor development due to nutritional deprivation (Pedersen et al., 2002; Thomsen et al., 2006), higher levels of eosinophils and inflammatory reaction in the liver (Frontera et al., 2007), loss of appetite, intestinal blockage and potentiation of other infections or even death, especially in malnourished animals (Stewart & Hoyt, 2006; Nonga et al., 2014). Parasitic infections also affect survival of animals by increasing their susceptibility to predators or minimizing their competitive fitness (Nonga et al., 2014).

In the Staff Quarters of the Mole National

Park, wild animals such as warthogs and baboon interact with humans unhindered. As potential reservoir hosts of zoonoses, warthogs could shed pathogens and parasite eggs into the environment exposing humans and other nonhuman animals to infection (Nonga et al., 2014). The situation with the warthogs is unique as these animals usually heap their faecal matter along the paths and around the housing units. Thus, close association of these animals with humans and their livestock may pose serious public health threat to the park staff and their families, and fringe communities. Recent outbreaks of zoonotic diseases of wildlife origin, such as Monkeypox, Ebola virus disease, Avian influenza and COVID-19 (Chakraborty et. al., 2022), were as result of human-wildlife interaction. It is therefore imperative that the warthogs are examined for possible harbouring of infectious agents of public health importance. Moreover, warthogs are wild animals of the pig family *Suidae*. Studies have shown that animals of the family *Suidae* are very similar to humans regarding genetic, physiology, anatomy and many other functional features and can have common infections (Ibrahim, 2006; Hart, 2007; Swindle, 2012). For example, pigs have been described as excellent model for human disease (Meurens, 2012). Additionally, there is scarcity of data on parasitic infections in the warthogs in the Mole National Park. This study, therefore, aimed at determining the profile of helminthic infection in the warthogs and to find out the potential for transmission of zoonoses.

Materials and Methods

Study Areas and Design

The study was conducted in Staff Quarters of the Mole National Park in the Savannah Region of Ghana. Mole National Park is situated about 20km north of Damango, the capital of the newly created Savanna region between Wa and Tamale. The area was initially established as a Game Reserve in 1958. After expansion of the Game Reserve, it was upgraded and gazetted as a National Park under Wildlife Reserves Regulation (L.I. 710) in 1971. It covers about 4480 km^2 land area and lies within latitude 9°12′–10°06′N, and longitude 1°25′–2°17′W. It is the largest park in Ghana and an open woodland savanna with patches of tree cover and forests in the middle and upslope regions of the park. The Park is a home to a wide range of animals including warthogs, elephants, baboons, monkeys, agama lizards, kobs, antelopes, bushbucks and waterbucks, just to mention a few. Other domestic animals such as goats, sheep, cats

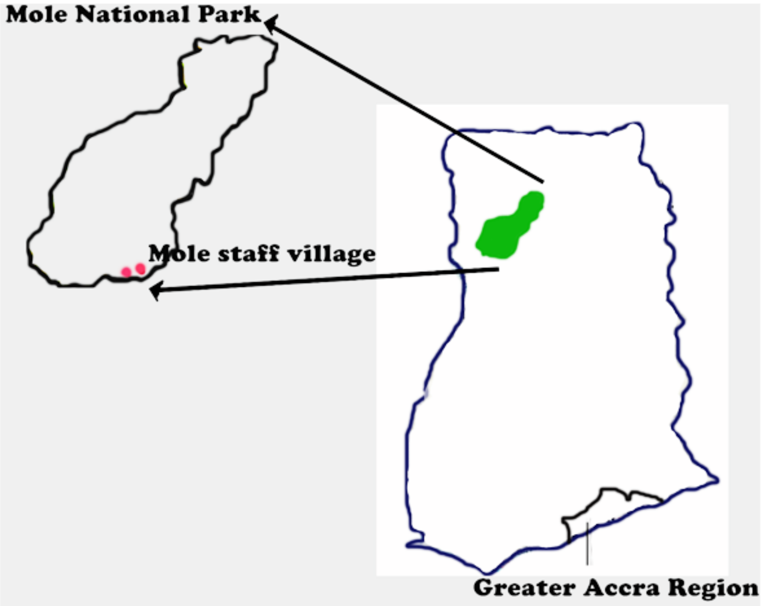

Figure 1 Map of the Mole National Park and study sites

and dogs are also raised by inhabitants of the Mole Staff Quarters. It received about 18,000 visitors in 2015 (Acquah, Dearden & Rollins, 2016, Mole National Park, 2023).

Thirty-nine (39) warthogs faecal droppings were sampled at Mole National Park on August 21, 2020. Thirty-three (33) of the samples were collected from warthogs that were interacting with humans in the settlement areas and six (6) from those far away from human settlements. In the Staff Quarters, every dropping that was fresh was collected. In this way, most of the animals that were interacting with humans were sampled. Sampling of animal far away from human settlements was to examine the possible impact of human-wildlife interactions on helminthic infections in the warthogs.

Sample Collection

Interior parts of the faecal matter were carefully collected with spatula into labelled sterile plastic stool containers. Each container was appropriately labelled with recognizable numbers. Samples were collected early in the morning at the various locations in the Staff Quarters to ensure that they were neither dry nor contaminated. Sampling of only fresh droppings and the characteristic single riniform (kidney-shaped) faeces of warthogs was helpful in the identification and ensuring that the chances of sampling the same animal twice was avoided (Chame, 2003). The samples were transported on ice to the Parasitology Laboratory of the Department of Animal Biology and Conservation Science for analysis.

Analysis of Samples

Zinc Sulphate Floatation technique was employed in this study. Two grams of faeces were weighed and pushed through a strainer into a dish of saline (0.85%). The saline-faecal mixture was sieved into a centrifuge tube with the help of a funnel, capped and centrifuged at 2000 rpm for two minutes. The supernatant was gently discarded and Zinc Sulphate floatation solution (1.18 specific gravity) was added. The tube was shaken gently to resuspend sediments in floatation fluid and again centrifuged at 2000 rpm for two minutes. Thirty millilitres of the solution were removed from the surface into a tube with Pasteur pipette, and after mixing, a drop was placed on each glass slide, stained with iodine solution and covered with a coverslip for examination. The slides were examined with the 10× and 40× objective lenses of the microscope for the presence of and identification of parasite eggs. Identification was done using standard practical manuals and based on morphological features (Ballweber, 2001). For estimation of helminth eggs, the two chambers of the McMaster slide were filled with the solution, examined and eggs counted. The eggs count from the two chambers was multiplied by 50 to obtain number of eggs per gram (EPG) as specified by the protocol.

Statistical Analysis

The data was entered into Microsoft excel sheets and imported to GraphPad Prism (GraphPad Prism, GraphPad Software, San Diego, CA, USA) for analysis. The prevalence of helminth eggs in the droppings and multiple infections were calculated. Mean intensity of infection was estimated as the mean of number of eggs per gram of faecal matter. Chi-square test was used to compare variations in frequencies of multiple infections between the sites and z-test of proportions was employed to evaluate differences in prevalence among the parasites.

Results

Diversity and Prevalence of Parasite in Warthogs

At least seven genera of parasites were identified, comprising four nematodes, two cestodes and one trematode (Table 2). In all, there were three contributing classes and six families. Two classes and five families were found in warthogs that were interacting with humans, and three classes and six families were identified in the warthogs that were far away from human settlements.

Out of the 39 warthog droppings examined, 95% (37/39) were infected with one or more parasite species. At least seven helminths' genera were identified. These include *Strongyloides* sp., trichostrongyle-type species, *Enterobius* sp., *Ascaris* sp., *Taenia* sp., *Monieza* sp. and *Schistosoma* sp.

In general, *Strongyloides* sp and trichostrongyle-type species had the highest prevalence of 71.8% (28/39) in the warthog droppings. These were followed by *Enterobius* sp. *Ascaris* sp., *Taenia* sp., *Monieza* sp., and *S. haematobium*. with prevalent rates of 64.1% (25/39), 48.7% (19/39), 30.8% (12/39), 23.1% (9/39) and 2.6% (1/39), repectively. This pattern of prevalence was also observed in warthog droppings at the two sites with *Strongyloides* sp. and trichostrongyle-type species accounting for the most prevalent helminths (Table 1). The prevalence differed significantly among the various parasites identified in the warthog droppings at the two sites (p<0.05).

Mean Intensity of Helminthic Infections in Warthogs

Based on number of eggs per gram of faecal matter (EPG) as described by Nwafor (2019), most warthog droppings in this study had moderate (100<EPG<500) to high infection (EPG ≥ 500) levels of helminth eggs in both sites. However, higher intensities of infection (EPG ≥ 500) with trichostrongyle-type helminths and *Ascaris* sp. were observed in warthogs that were interacting with humans whereas high intensity of infection with *Strongyloides* sp. was observed in warthogs that were far away from human settlements (Table 2).

Multiple Infections

Out of the 39 samples examined, 37 (95%) had at least one parasite genus and 18 (46.2%) had three or more (Figure 1). Multiple infections did not differ significantly between warthogs that were interacting with humans (42.4%)

TABLE 1

Classification and Prevalence of Parasites identified in Warthogs

Classification		Prevalence (%)	
		WF	WFB
Nematodes			
Trichostrongyle-type#	Families: Trichostrongylidae, Strongylidae	66.7	100
Strongyloides sp.	Class: Chromadorea Family: Strongylidae	69.7	83.3
Enterobius sp.	Class: Chromadorea Family:Oxyuridae	64.1	50.0
Ascaris sp.	Class: Chromadorea Family: Ascarididae	48.5	50.0
Cestodes			
Taenia sp.	Class: Cestoda Family: Taeniidae	30.3	33.3
Monieza sp.	Class: Cestoda Family: Anoplocephalidae	30.3	33.3
Trematodes			
S. haematobium	Class: Trematoda Family: Schistosomatidae	0	16.6

WF: Warthogs interacting with humans; WFB: Warthogs far away from human settlements. #Trichostrongyle-type includes *Trichostrongylus*, *Ostertagia*, *Teladorsagia*, *Haemonchus*, *Cooperia*, *Oesophagostomum*, *Chabertia* and hookworms which are not easily distinguishable by eggs (Ballweber, 2001).

TABLE 2

Mean eggs per gram of faeces of warthogs

Parasites	WF	WFB
	EPG (Mean ± SEM)	
Trichostrongyle-type#	1452±850***	333±138**
Strongyloides sp	391±124**	2800±1707***
Enterobius sp.	98±27*	ND
Ascaris sp	2394±1733***	83±40*
Taenia sp	103±49**	233±114**
Monieza sp	188±115**	150±115**
S. haematobium	0	17±8*

WF: Warthogs interacting with humans; WFB: Warthogs far away from human settlements. EPG: Eggs per gram; SEM: standard error of the mean; *low infection (EPG ≤ 100); **moderate infection (100<EPG<500); ***high infection (EPG ≥ 500) as described by Nwafor (2019). #Trichostrongyle-type includes *Trichostrongylus*, *Ostertagia*, *Teladorsagia*, *Haemonchus*, *Cooperia*, *Oesophagostomum*, *Chabertia* and hookworms which are not easily distinguishable by eggs (Ballweber, 2001). ND: Not determined.

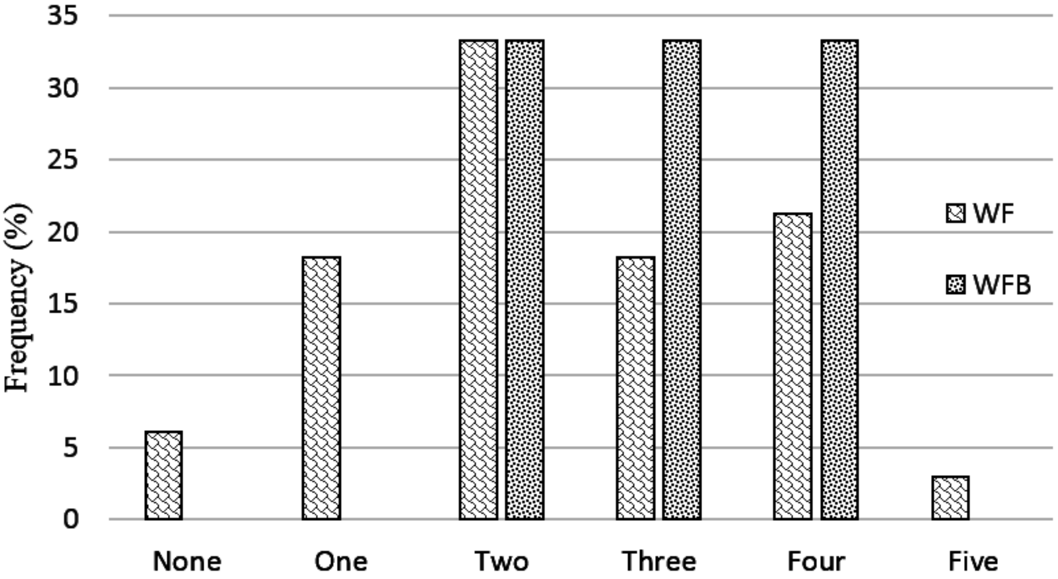

Figure 2 Frequency of multiple infections. WF: warthogs interacting with humans; WFB: warthogs far away from human settlements

and warthogs that were far away from human settlements (66.7.%) ($\chi2$ (5) =1.175, p=0.278).

Discussions

The spread of infectious pathogens, particularly soil-transmitted intestinal parasites, depends on environmental characteristics, reservoirs of parasites, host populations and complex interactions of these factors. Human-nonhuman animal interactions are known to lead to development and transmissions of zoonoses (Cunningham, Daszak & Wood, 2017). Warthogs and baboons in the Staff Quarters of the Mole National Park were sighted interacting with the residents as well as visitors.

Almost all the warthog droppings examined (95%) were infected with at least one parasite and this is not surprising because the risk factors for infection were present. Our observations over the years showed that warthogs in the community have a habit of heaping their faeces along paths and few metres away from the buildings, and in portions of grasses they feed on. They were also sighted around toilet facilities in the community and could be exposed to parasite species that usually infect humans.

Seven genera and six families of parasites were identified. Out of seven genera, three, *Strongyloides*, *Ascaris* and *Taenia*, were found in a previous study in the park (Larbi et. al., 2020). However, all the parasitic helminths identified in a study in Burkina Faso (Belem, 2012) differed from those found in this study. This is not surprising because geographical variations in the distribution of parasites have been well documented (Medlock et. al., 2013; Camacho-Alvarez et. al., 2021).

Significant variations in prevalence were observed among the parasites identified with nematode genera such as, *Strongyloides*, *Enterobius*, *Ascaris* and trichostrongyle-type species being the most prevalent. The high prevalence of helminths in the warthog droppings, for that matter in the warthogs, particularly those far away from human settlements, could be due to the conducive environment for their transmission. Moisture is essential for development and emergence of parasites from the faecal matter (O'Connor et al., 2007; Akinbo et al., 2011). In the thickets, far away from human settlements, moisture may be preserved better than in the open and mainly unshaded spaces in the residential enclave. However, warthogs that are interacting with humans are more likely to encounter and contaminate themselves with parasite laden faeces due to the abundance of their faeces in the community. These might account for the high prevalence at both sites. The high prevalence of *Strongyloides* sp., also known as threadworm could be due to autoinfection. Auto-infection is known in the complex life cycle of *Strongyloides* sp. (CDC, 2019). Similar high prevalence of *Strongyloides* sp. were observed in warthogs in the Mole National Park and Saadani National Park in Tanzania (Nonga et al., 2014; Larbi et. al., 2020) and in the grasscutter (Aboagye et. al., 2019) in prior studies. But the prevalence of some of the parasites in the current study were much higher than previously reported (Larbi et. al., 2020). The variations in prevalence could be due to differences in sampling season (Akinbo et al., 2011; Khajuria et. al., 2013), the techniques employed and/or the sample size.

The intensities of infections were also high and varied significant among the parasite species. Most of the parasites with high prevalence such as *Strongyloides* sp. and *Ascaris* sp., also had high intensity of infection, indicating their dominance in the warthog population. Droppings from warthogs that were interacting with humans had high intensity for trichostrongyle-type species and *Ascaris* sp. (2394±1733 EPG) whereas those from their counterparts that far away from human settlements had high parasite loads for *Strongyloides* sp. (2800±1707 EPG). These differences might be due to variations in the environmental conditions at the two sites as alluded to above. In addition to the high intensities of infection, frequency of multiple infections also known as multiparasitism was high. About forty-six percent of the warthog droppings had three or more different parasite species, indicating the high parasite burden of the animals. The prevalence of trichostrongyle-type helminths was high, but it is a composite group that includes the genera, *Trichostrongylus*, *Ostertagia*, *Teladorsagia*, *Haemonchus*, *Cooperia*, *Oesophagostomum*, *Chabertia* and hookworms are not reliably distinguishable by eggs (Ballweber, 2001; Roeber, Jex, & Gasser, 2013). Trichostrongyle-type species are known to infect wildlife, domesticated and companion animals. *Trichostrongylus*, *Haemonchus*, *Oesophagostomum*, and hookworms are prevalent and highly pathogenic blood sucking parasites that exact a heavy toll on the health and wellbeing of their hosts (Stewart & Hoyt, 2006; Nonga et al., 2014). Further analysis is required to identify the genera of helminths constituting this trichostrongyle-type group.

Some of the species of *Strongyloides*, trichostrongyle-type, *Taenia* and *Ascaris*, and *S. haematobium* are parasites of public health importance. Though further analysis is required to identify the species of zoonotic potential, some are easily recognizable. For instance, the possible species of *Ascaris*, *A. suum* and *A. lumbricoides* which are found in pigs and humans, respectively, are both zoonotic (Monteiro et.al., 2019). The high prevalence and intensities of the zoonotic species reveal suitable conditions for their transmission. The transmission could easily spill over into humans, especially in the prevailing improper hygienic setting where warthogs' faeces were heaped in the community along paths and close to the residences. Also, the warthogs in the community could be infected with pathogens from humans as they interact with the inhabitants and roam about unrestrained.

S. haematobium was found in only one warthog dropping collected from warthogs that were far away from human settlements, but this could be the tip of the iceberg as the eggs of this parasite is usually found in the urine rather than the faecal matter. *Schistosoma* sp. were first identified in warthogs in South Africa in 1988 and many studies have reported the presence of *S. haematobium* in other nonhuman animals, either in the wild or domesticated (Boomker et. al., 1991; Futagbi et. al., 2010; Squire et. al., 2018). Aboagye et. al., 2019; Savassi et. al., 2020). These are indications that *S. haematobium* may have many more nonhuman animals as reservoirs than identified so far. Molecular analysis is underway to identify other parasites that were missed in the microscopic analysis. Most of the helminth genera identified are of species that pose nutritional and/or pathological problems for the host. But *Moniezia* sp. do not pose such problems (Elliott, 1986; Dever, Kahn, & Doyle, 2015) but just like other cestodes, heavy infections can have impact on the nutritional physiology of the host.

The results reveal that warthogs in the Mole National Park have a considerable helminth load that may affect their wellbeing. The data also indicate that some helminths harboured by the warthogs are of zoonotic relevance. Some measures may be required to mitigate possible transmission of zoonoses, especially in the Staff Quarters of the park.

Ethical Consideration

Before samples were collected, permission was sought from the Ghana Wildlife Division of the Forestry Commission and the Management of the Mole National Park.

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