

Assessment of enteric helminth parasites in bushmeat in Ghana

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ABSTRACT

Anthropogenic activities, such as hunting wild animals for food, increase the risk of zoonotic transmission of infective stages of parasites to humans. The handling, processing and consumption of wild animal meat, popularly known as 'bushmeat', as well as exposure to wildlife habitats, can pose a significant risk to human health through the transfer of parasitic infective stages. This study sought to assess the enteric helminth parasite burden and potentially zoonotic helminths in fresh, wild animal carcasses being processed for food. Parasitological analysis of samples of rectal and intestinal contents collected from a total of fifty (50) wild animal carcasses belonging to eight (8) different species at the Atwemonom Bushmeat Market in Kumasi showed nine (9) genera of enteric helminth parasites with an overall prevalence of 71.0%. Individual parasite species prevalence was assessed, with *Ascaris* sp. showing 25% and 87.5% by coprological and molecular assessment, respectively. Molecular analysis showed a higher parasite species prevalence in all samples analyzed. Species-specific analysis indicated the presence of two potentially zoonotic parasites, *Strongyloides stercoralis* and *Trichuris trichiura*, in wild animals, indicating the need to intensify one health approach in wild animal parasitic infections. Data from this study suggest that wild animals in addition to being natural hosts, may also serve as reservoirs for numerous parasites of medical and veterinary importance.

1. Introduction

The consumption of bushmeat, defined as the meat of wild animals hunted for food, has been an integral part of human subsistence for centuries (Kurpiers et al., 2016). In many regions around the world, particularly in rural communities with limited access to alternative food sources, bushmeat remains an important protein source (Aboagye et al., 2019; Odeniran and Ademola, 2016). The rapid expansion of human populations and increased demand for bushmeat have exerted additional pressure on wildlife populations, often resulting in unsustainable hunting practices and an increased risk of pathogen transmission (Daszak et al., 2001).

Assessing the zoonotic potential of enteric parasites, including various protozoans and helminths, is warranted, as the majority of these parasites are known to cause emerging parasitic diseases of zoonotic origin (Jones et al., 2008). In addition, understanding the prevalence and distribution of these enteric parasites in bushmeat is not only crucial for wildlife conservation but also essential for safeguarding public

health.

Significant health risks have been reported for consumers, hunters, and others involved in the wild animal meat supply chain with about 16 parasites found across various traded wildlife taxonomic groups. Notable parasites include *Sarcocystis* sp., *Trichuris* sp., and *Ancylostoma* sp. (Cantlay et al., 2017). Additionally, a study in Accra found human-infecting parasites like *Strongyloides*, *Haemonchus*, *Ascaris* and *Fasciola* in domesticated grasscutters (Rodentia: *Thryonomys swinderianus*) (Aboagye et al., 2019). Direct contact with live or dead wild animals and their habitats poses a risk of zoonotic transmission, leading to gastrointestinal illnesses and potentially life-threatening conditions (Okoye et al., 2015). Hunters, butchers, and bushmeat traders, therefore, face heightened infection risks due to their close interactions with wildlife.

This study aimed to comprehensively assess the presence of parasitic helminths in bushmeat from a major bushmeat hub, the 'Atwemonom' bushmeat market in Kumasi, Ghana. This study employed both conventional parasitological and molecular techniques to identify

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potentially zoonotic parasites in wild animals.

2. Materials and methods

2.1. Description of study area and design

Study samples were obtained from the “Atwemonom” section of the Kejetia market (6.698635°N, 1.619140°W). This market is situated in the Kumasi Metropolitan District of the Ashanti region, situated within the tropical forest zone of Ghana (Fig. 1). “Atwemonom,” which literally means “place for fresh duiker meat,” is the only accredited fresh wild animal meat market in the middle belt of Ghana and serves as an indicator of the region’s overall fresh bushmeat trade (McNamara, 2014). The market also serves as a bushmeat hub for six (6) neighboring regions, including the Ahafo, Brong Ahafo, Bono East, Western North, Savanna, and Eastern regions of Ghana.

This was a cross-sectional study that involved sampling from fresh game for sale at the study site. Sampling was done for three (3) consecutive days in March 2022. All wild mammal carcasses received at the study site were assessed for enteric helminths.

2.2. Sample collection

Two sets of fresh samples were collected from the rectal contents of individual wild animals’ carcasses and placed in sterile, labeled disposable containers with fasteners. One set of samples used for parasitological analysis was preserved in 10% formalin, while those for molecular analysis were placed in cool boxes and later transported to the parasitology laboratory at the Department of Animal Biology and Conservation Science, University of Ghana, for storage and processing. Samples for molecular analysis were stored at -20 °C, while those for parasitology analysis were kept at room temperature.

2.3. Parasitological analysis of samples of rectal content from wild animals

Rectal content samples stored in formalin were examined for the

presence of helminth eggs/cysts using conventional parasitological techniques, including zinc sulphate flotation and formalin-ethyl acetate sedimentation techniques.

Zinc Sulphate Flotation Technique: Formalin-preserved faecal samples were strained into a 15 mL centrifuge tube and centrifuged at 500 g for 10 min. The sediment was diluted with 0.85% saline, and it was centrifuged for 5 min at 500 g. Again, the supernatant was decanted, and the sediment was thoroughly mixed with zinc sulphate solution and centrifuged at 500 g for 5 min. A few drops were aliquoted from the surface of the supernatant with the aid of a disposable pipette and observed under the microscope for parasites (CDC, 2016).

Formalin-Ethyl Acetate Sedimentation technique: The sedimentation technique as described by the CDC (2016) was employed to examine for the presence of parasite eggs and/or oocyst. Briefly, formalin-preserved faecal samples were strained through a gauze into a 15 mL centrifuge tube. Additional 10% formalin was added through the debris on the gauze to the 15 mL mark on the centrifuge tube and centrifuged at 500 g for 10 min. The supernatant was decanted, and 10 ml and 4 ml of 10% formalin and ethyl acetate were added, respectively, to the sediment and mixed thoroughly. The mixture was centrifuged at 500 g for 10 min. The top layers of the supernatant were poured off, and drops of the sediment were examined under the microscope (CDC, 2016).

With the aid of standard keys, parasitic helminth eggs were identified (Genchi et al., 2019; Sohn and Chai, 2024; WHO, 2019). These procedures were performed at the Parasitology Lab of the Department of Animal Biology and Conservation Science.

2.4. Molecular analysis of samples of rectal content of wild animals

All samples were assessed molecularly by conventional polymerase chain reaction (PCR) using selected genus and species-specific primers.

2.4.1. DNA Extraction

Genomic DNA was extracted from about 150 mg of rectal content samples using Zymo Research’s Quick-DNA Fecal/Soil Microbe Kit (catalog #. D6010) and following the manufacturer’s instructions with

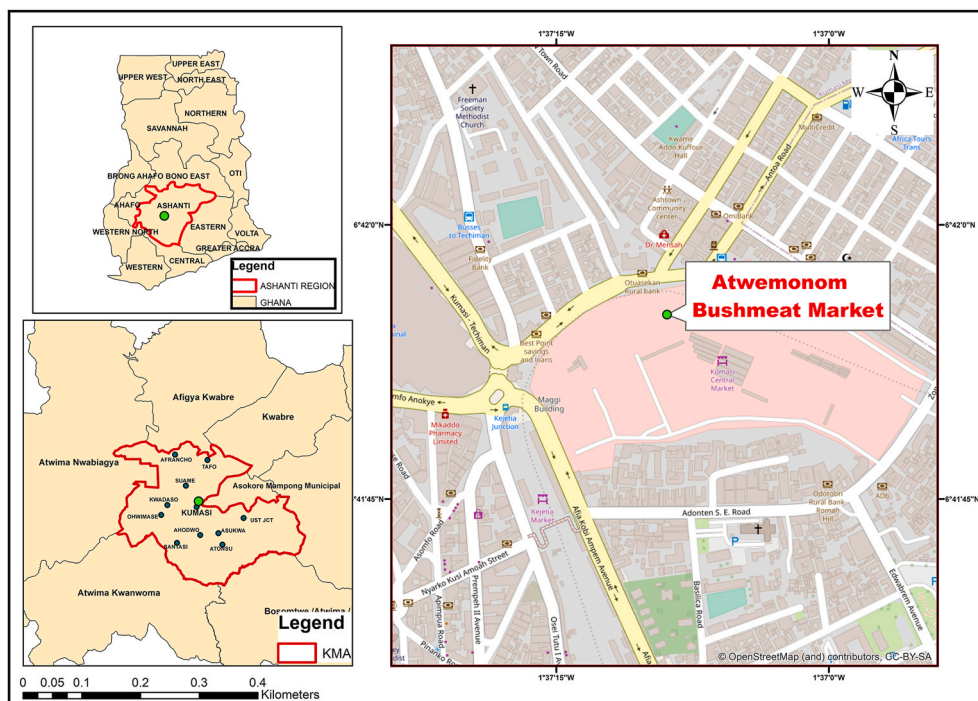


Fig. 1. Map of Atwemonom bushmeat market, Kumasi.

some modifications. Briefly, rectal content samples were lysed and homogenized by continuous bead beating for 35 min on a shaker. The manufacturer's protocol was then followed to extract total DNA from each sample.

2.4.2. PCR analysis

Following morphological identification to genus, four (4) genus-specific primers (for *Ascaris* sp., *Strongyloides* sp., *Fasciola* sp., and *Ancylostoma* sp.), and three (3) species-specific primers (*Strongyloides stercoralis*, *Trichuris trichiura*, and *Trichuris suis*) were selected for PCR analysis. Hookworm could not amplify due to issues with primer sequences. Primers for pinworms and cestodes were not available for confirmation of *Enterobius* sp., *Taenia* sp., and *Hymenolepis* sp. PCR reactions were performed in a total volume of 12 μ L containing 6 μ L of Phusion® High-Fidelity PCR Master Mix with HF Buffer using the manufacturer's protocol. Amplification of *Strongyloides stercoralis* was performed using OneTaq® Quick-Load® 2X Master Mix. Primer sequences and cycling conditions are indicated in [Supplementary Table 1](#).

PCR products were visualized on a 2.0% agarose gel under UV using a Gel Doc system. The molecular analysis was performed at the Noguchi Memorial Institute of Biomedical Research.

2.5. Statistical analysis

GraphPad Prism (version 10.2.3) was used to perform all statistical analysis. The non-parametric Kruskal-Wallis test was used to assess statistically significant differences between the three wild animal groups, grasscutters (*Thryonomys swinderianus*), African civet (Viverrinae: *Civettictis civetta*) and antelopes (Cephalophinae). A post hoc test (Dunn's multiple test) was done to determine significant differences between paired groups. Parasite prevalence was determined by dividing the number of infected wild animals by the total number of sampled wild animals, and this ratio was then expressed as a percentage ([Ebert, 2005](#))

3. Results

A total of 50 rectal content samples were obtained from individual animal carcasses from the Atwemonom market in Kumasi. The carcasses consisted of eight (8) different species of wild animals, with the majority (74%) being grasscutter (*Thryonomys swinderianus*). Other animal groups included the African civet (*Civettictis civetta*) (6%) and antelopes (20%). Antelopes included bushbucks (*Tragelaphus sylvaticus*), red-flanked duikers (*Cephalophus rufilatus*), Maxwell's duikers (*Philantomba maxwellii*), and black duikers (*Cephalophus niger*).

3.1. Prevalence of enteric helminth parasites by coprological assessment

Using both ethyl acetate sedimentation and zinc sulphate floatation techniques, the majority (71%) of the animals were found to be infested with helminth eggs. Eggs of some of the observed parasites are shown in [Supplementary Fig. 1](#). Overall, nine (9) genera of enteric helminth parasites were identified in the wild animals, with *Ascaris* sp. being the most prevalent (25%). *Enterobius* sp. and *Hymenolepis* sp. had the least prevalence (1.9%) ([Fig. 2](#)).

3.2. Prevalence of enteric helminth parasites in wild animal groups

The prevalence of enteric helminth parasites varied in the various animal groups assessed. *Ascaris* sp. were more prevalent ($p < 0.05$) in antelopes (80%) compared to grasscutters (10.8%). All African civets (100%) were infected with hookworm, with 13.5% and 10% of grasscutters and antelopes being infected, respectively. Like *Ascaris* sp., *Strongyloides* sp. and *Fasciola* sp. were more prevalent in antelopes ($p < 0.05$) compared to grasscutter ([Table 1](#)). Lower prevalences of other helminths (*Trichuris* sp., *Dicrocoelium* sp., *Enterobius* sp., *Taenia* sp., and *Hymenolepis* sp.) were recorded in grasscutter and antelopes, while none

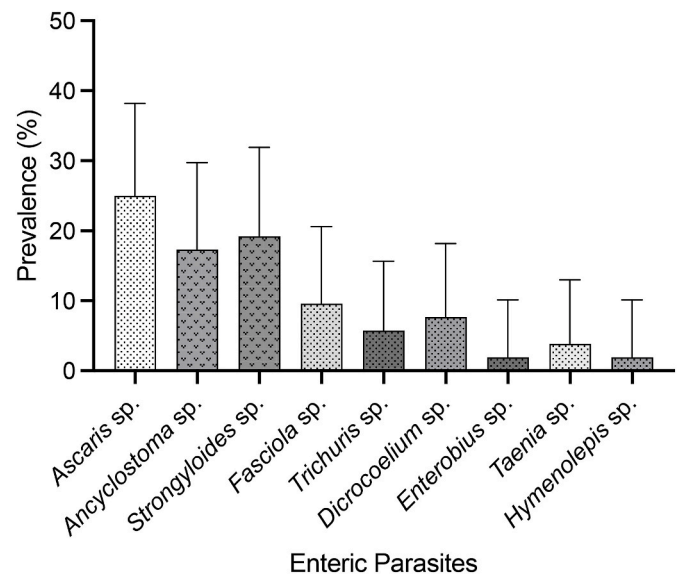


Fig. 2. Prevalence of enteric helminth parasites in wild animals by morphological assessment.

of these parasites were recorded in African civets.

3.3. Molecular identification of selected potentially zoonotic parasites

Using three genus-specific and three species-specific primers for selected parasite genera and species, 38 rectal content samples were analyzed. Molecular analysis demonstrated higher prevalence rates compared to traditional methods ([Supplementary Table 2](#)). Except for *Strongyloides stercoralis*, the prevalence rates of all parasitic helminths assessed by PCR were above 65%, with *Ascaris* sp. and *Strongyloides* sp. recording the highest (86.8%). All African civets (100%) were infected with *Ascaris* sp. About 92% and 83% of grasscutters were infected with *Strongyloides* sp. and *Fasciola* sp., respectively ([Table 2](#)). Antelopes exhibited the highest burden of *Trichuris* infections, with *T. trichiura* and *T. suis* prevalence rates reaching 81.8% and 72.7%, respectively. The prevalence of *S. stercoralis* was significantly higher in antelopes (18%) compared to African civets, which yielded no positive records of the parasite.

3.4. Rates of multiple helminth infections in wild animal hosts

A PCR-based analysis of 38 rectal content samples revealed widespread multiple infections with four enteric helminth parasite genera. All samples harboured at least two genera, and over 50% were infected with all four ([Fig. 3](#)). In contrast, traditional morphological assessment of 37 of the 38 samples detected lower multiple infection rates, with over 50% showing no parasites.

4. Discussion

The transmission of pathogens from animals (predominantly domestic or companion animals) to humans and vice versa has been documented. This study, however, assessed the presence of potentially zoonotic enteric parasites in wild animals using both traditional parasitological and molecular methods.

An overall enteric helminth parasite prevalence of 71% was recorded by coprological assessment of the rectal contents of eight (8) wild animal species being processed for food at the Atwemonom bushmeat market in Kumasi, Ghana. This is consistent with similar studies, which reported prevalences of 78.8%, 71.2%, and 77.4% in different parts of Nigeria ([Olayemi, 2011](#)). The study identified a total of nine enteric helminth

Table 1
Prevalence of enteric helminth parasites in three animal groups by coprological assessment.

Parasites	Grasscutter (n = 37)		African civet (n = 3)		Antelopes (n = 10)		p-value
	Prevalence	95% CI	Prevalence	95% CI	Prevalence	95% CI	
<i>Ascaris</i> sp.	10.8 ^a	4.3–24.7	33.3 ^{a,b}	1.7–88.2	80 ^b	49.0–96.4	0.006
Hookworm (<i>Ancylostoma</i> sp.)	13.5 ^a	5.9–28.0	100 ^b	43.9–100	10 ^a	0.5–40.4	0.004
<i>Strongyloides</i> sp.	10.8 ^a	4.3–24.7	33.3 ^{a,b}	1.7–88.2	40 ^b	16.8–68.7	0.03
<i>Fasciola</i> sp.	2.7 ^a	0.1–13.8	33.3 ^{a,b}	1.7–88.2	30 ^b	10.8–60.3	0.005
<i>Trichuris</i> sp.	2.7	0.1–13.8	0	0–56.2	10	0.5–40.4	0.15
<i>Dicrocoelium</i> sp.	8.1	2.8–21.3	0	0–56.2	10	0.5–40.4	0.87
<i>Enterobius</i> sp.	2.7	0.1–13.8	0	0–56.2	0	0–27.8	0.83
<i>Taenia</i> sp.	2.7	0.1–13.8	0	0–56.2	10	0.5–40.4	0.60
<i>Hymenolepis</i> sp.	2.7	0.1–13.8	0	0–56.2	0	0–27.8	0.83

Data are represented as prevalences (%) and confidence intervals. In each case, there is no significant difference for values with the same alphabet (a, b). Significant values are all $p < 0.05$.

Table 2
Prevalence of enteric helminth parasites among the three wild animal groups assessed by molecular analysis.

Parasite species	Overall prevalence (%)	Grasscutter (n = 24)		African civet (n = 3)		Antelopes (n = 11)	
		Prevalence	95% CI	Prevalence	95% CI	Prevalence	95% CI
<i>Ascaris</i> sp.	86.8	91.7	74.2–98.5	100	43.9–100	72.7	43.4–90.3
<i>Strongyloides</i> sp.	86.8	91.7	74.2–98.5	66.7	11.8–98.3	81.8	52.3–96.8
<i>Fasciola</i> sp.	78.9	83.3	64.1–93.3	66.7	11.8–98.3	72.7	43.4–90.3
<i>Trichuris trichiura</i>	78.9	79.2	59.5–90.8	66.7	11.8–98.3	81.8	52.3–96.8
<i>Trichuris suis</i>	68.4	70.8	50.8–85.1	33.3	1.7–88.2	72.7	43.4–90.3
<i>Strongyloides stercoralis</i>	10.5	8.3	1.5–25.8	0	0–56.2	18.2	3.2–47.7

Data are represented as prevalences (%) and confidence intervals.

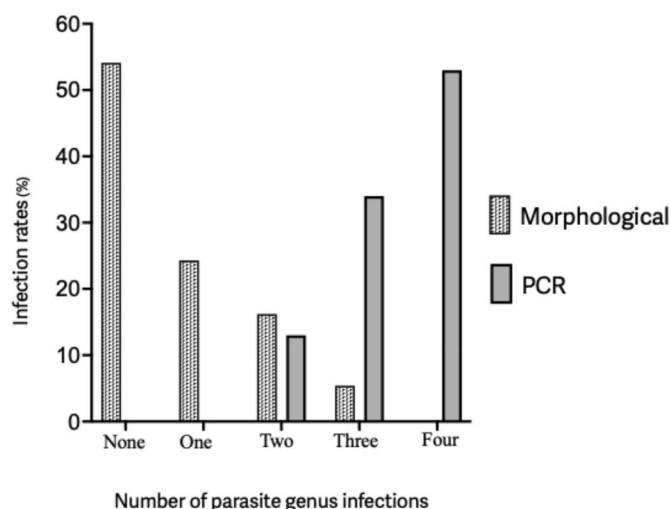


Fig. 3. Rate of multiple helminth parasite infections in wild animal hosts.

parasites, with *Ascaris* sp. being the most prevalent. All grasscutters assessed recorded at least one of the nine parasites, indicating a 100% prevalence of enteric helminth. Previous studies on grasscutters in Ghana reported an enteric parasite prevalence of 100%, which is consistent with the findings in this study (Futagbi et al., 2010). A lower prevalence (38.3%) of parasites has, however, been reported in grasscutters trapped around human habitats in Nigeria (Ajayi et al., 2007).

Most of the wild animals assessed in the study were hunted within the middle-forested belt of Ghana. Other studies conducted within the southern (coastal) belt of Ghana identified a similar number of helminth species (Aboagye et al., 2019). However, parasites such as *Ancylostoma* sp., *Enterobius* sp., and cestodes (*Taenia* sp. and *Hymenolepis* sp.) observed in this study were not identified in the study by Aboagye et al. (2019). The two studies indicate differences in enteric helminth species prevalence in the different ecological zones. Some studies have

attributed differences in parasite prevalence not only to disparities in ecological types (Opara and Fagbemi, 2008), but also to cultural practices, hygiene, infrastructure, and the level of education of human inhabitants (Abara et al., 2021). Some studies have, however, reported no significant differences in parasite prevalence across different ecological zones (Olayemi, 2011). Limitations relating to difficulties in obtaining fresh faecal samples from live wild animals have also been implicated in the observed differences in parasite prevalence (Hewavithana et al., 2022; Opara and Fagbemi, 2008).

Most parasitological studies have always recorded a higher prevalence of nematodes compared to other helminths, such as trematodes and cestodes. This was reiterated in this study, as nematode prevalence in the wild animals was higher compared to the other helminths. Nematodes have a direct life cycle that does not require an intermediate host during transmission. This gives the nematodes an upper edge over the other helminths, which require an intermediate host.

Ascaris sp. was the most prevalent (25%) among the wild animals in this study. This is in agreement with previous findings by Adeniyi et al. (2015) and Okoye et al. (2015), which also recorded *Ascaris* sp. prevalence of 48.8% and 22.6%, respectively. This result, however, contradicts findings by Aboagye et al. (2019) and Futagbi et al. (2010), where *Ascaris* sp. were the least prevalent enteric parasites in the wild animals examined. In the current study, *Enterobius* sp. and *Hymenolepis* sp. were the parasites with the least prevalence.

Fewer studies in Ghana have reported on the prevalence of enteric parasites using molecular techniques. In this study, higher prevalences were observed in the molecular compared to the coprological assessment. For instance, prevalences of *Ascaris* sp. in grasscutter and African civets were 10.8% and 33.3%, respectively. However, PCR showed 91.7% and 100% in grasscutters and African civets respectively. Additionally, parasites such as *Strongyloides* sp., *Fasciola* sp., and *Trichuris* sp. were more prevalent in all animal groups when assessed by molecular techniques compared to coprological assessment. This suggests that the previous assessment of the prevalence of most enteric parasites may have been underestimated. Molecular methods, particularly polymerase chain reaction (PCR), have demonstrated greater sensitivity than microscopy. PCR can amplify even minute quantities of parasite DNA from

samples, including degraded ones, thereby enhancing the likelihood of detecting parasites. (Villamizar et al., 2019). Effective traditional morphological identification of parasite eggs in stool samples is hindered by multiple challenges, specifically uneven egg distribution within rectal samples, insufficient egg quantities for detection, inadequate sampling volumes, and compromised sample transport and storage protocols (Anderson and Schad, 1985; Easton et al., 2017). This may lead to lower prevalence rates compared to those obtained through PCR, as observed in this study.

Identification of parasites such as *Trichuris trichiura*, *Enterobius* sp., and *Strongyloides stercoralis* raises concern since these parasites are known to infect humans. Even though a lower prevalence of *Trichuris trichiura* has been reported in humans in Ghana (Ahiadorme and Morhe, 2020), 79% and 100% in grasscutter and African civets, respectively, in this study suggest the zoonotic potential of these parasites, with the animal groups being their reservoirs. *Trichuris trichiura* is known to be the third most common roundworm in humans, with infections being more frequent in tropical conditions where sanitation is very poor (CDC, 2016). A prevalence close to 80% in wild animals poses a significant challenge in the control of the whipworm. *Strongyloides stercoralis*, also a known roundworm that infects humans (CDC, 2016), with a 10.5% overall prevalence in wild animals, must be of major concern.

Considering the life cycles of these nematodes, with the routes of transmission being either through soil-contaminated hands or direct penetration of the intact skin of humans, bushmeat traders, including hunters, butchers, and vendors, are at a greater risk of infection due to exposure to the infective stages of these parasites. The occurrence of these parasites has been reported in studies in Africa, and it is most prevalent in rural communities (Adejinmi and Emikpe, 2011). Despite their significant impact on health, these parasites have received less attention, and data from this study demonstrate that wild animals may serve as reservoirs, suggesting continuous zoonotic transmission if not addressed. Zoonotic transmission of enteric helminths can occur through various routes. Hunters may come into direct contact with animal carcasses or consume food and water contaminated with helminth eggs in wild environments. Additionally, butchers processing wild animal carcasses are at risk of exposure to infested surfaces and internal organs. Maintaining good hygiene practices during the handling and processing of wild game is essential to reduce the risk of contamination and transmission of these zoonotic parasites. While the infective stages of parasitic helminths may not be present in wild meat, other pathogens, such as *E. coli*, can still be found. Therefore, it is essential to cook meat to safe temperatures to eliminate any potential parasites and pathogens (Rabatsky-Ehr et al., 2002).

5. Conclusion

The overall prevalence of parasitic helminth infections by microscopic examination in wild animals was 71.0%. Parasites such as *Ascaris* sp., *Strongyloides* sp., *Fasciola* sp., *Taenia* sp., Hookworm, *Enterobius* sp., and *Trichuris* sp. were prevalent in wild animal groups such as grasscutters and antelopes which are common bushmeat consumed in Ghana. Assessment of enteric parasites by molecular techniques used in this study shows a higher prevalence than previously reported. Potentially zoonotic enteric parasites such as *Strongyloides stercoralis* and *Trichuris trichiura* are prevalent in wild animals. The data from this study suggest that wild animals may serve as reservoirs of parasites of medical and veterinary importance. Therefore, there is a high risk of transmission of these parasites to hunters, butchers, bushmeat vendors, and consumers of wild animal meat. This study has provided comprehensive data on enteric parasites in wild animals used as bushmeat, which may provide the basis for interventions and further studies.

Further study using molecular techniques is recommended to assess the prevalence of these parasites among hunters, butchers, bushmeat vendors, and consumers to ascertain their zoonotic transmission.

CRedit authorship contribution statement

Joanita Asirifi Yeboah: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Winnifred Offih-Kyei:** Investigation, Data curation. **Caleb Kobina Danso-Coffie:** Methodology, Investigation, Data curation. **Emmanuel Bofo:** Investigation, Data curation. **Philip Banahene:** Investigation, Data curation. **Rhoda Yeboah:** Investigation, Data curation. **Godfred Futagbi:** Writing – review & editing, Formal analysis. **Langbong Bimi:** Writing – review & editing, Investigation. **Daniel Oduro:** Writing – review & editing, Supervision, Resources, Project administration, Investigation, Funding acquisition, Formal analysis.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ijppaw.2024.101005>.

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