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First report of the parasitic infection in two snail species from Burkina Faso water bodies

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Abstract

Trematodiases are important yet neglected tropical diseases, caused by trematode parasites with a multi-host life cycle, which typically involves a snail intermediate host. The many knowledge gaps regarding the trematode life cycles, pathology, and epidemiology complicate effective control. This work was initiated to inventory parasites as part of the "One Health" initiative, where human and animal trematodes are considered equally important, in order to map their distribution, detect high-risk locations and improve disease control. This paper describes the occurrence of parasitic infections in the Ouagadougou reservoirs. These reservoirs are under intensive market gardening. The infra- and component community of digenetic trematodes and other parasites in a freshwater gastropod community were examined over a 5-month period. A total of 1031 Thiaridae snails was collected. Among them, 109 belonging to 2 species were infected by larval trematodes. Seven different types of cercaria were found: xiphidiocercaria, furcocercous, megalurous cercaria, monostome cercaria, Armatae xiphidiocercaria, echinostome cercaria and gymnocephalus cercaria. In addition to trematode infections, nematode and oligochaete (*Chaetogaster limnaei limnaei*) infections have been reported during this investigation. The association of *Chaetogaster limnaei limnaei* with the snail intermediate host may be of value as a control measure against economically important parasitic diseases such as fascioliasis and schistosomiasis..

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Introduction

Freshwater snails play an important role in the ecosystems and many of them have great medical and veterinary importance. Therefore, they pose risks to human health and can lead to major socioeconomic problems in many tropical and sub-tropical countries into worldwide (Lu *et al.*, 2018). Worldwide, around 350 snail species are estimated to be possible hosts of parasites which cause human and animal diseases (Hung *et al.*, 2013). The freshwater snail fauna of Burkina Faso includes many species that transmit serious parasitic diseases such as schistosomiasis and fascioliasis. Schistosomiasis is the most studied trematodiasis and affects around 190 million people and millions of domesticated animals in Africa (Standley *et al.*, 2012; Deka *et al.*, 2020). In Burkina Faso, *Schistosoma mansoni* infection was endemic in the western and southern areas of the country with a focal distribution before the implementation of the National Schistosomiasis Control Program (NSCP) in 2004 (Poda *et al.*, 1994). The program mainly focused on mass drug administration with praziquantel in school-aged children (SAC) with a coverage rate > 90% (Gabrielli *et al.*, 2006). In 2010, the prevalence of *S. mansoni* infection among SAC ranged from 3.3 to 39.1% (Zongo *et al.*, 2013; Cisse *et al.*, 2021). Most of the studies on snails and their parasites in Burkina Faso were focused on pulmonates like Lymnaeidae and Planorbidae families (Poda *et al.*, 1996; Zongo *et al.*, 2013; Sansarricq, 1959; Sellin *et al.*, 1982; Poda, 1996; Perez-Saez *et al.*, 2016). However, some studies reported that species of the Thiaridae family serve as the first intermediate host for trematodes (Dechruksa *et al.*, 2016). Concerning this family of Thiaridae, recent studies reported the presence of *Cleopatra bulimoides*, *Melanoides tuberculata* in some reservoirs in Burkina Faso particularly in the reservoirs of Ouagadougou (Ouédraogo *et al.*, 2015). *Cleopatra bulimoides* is known to be an intermediate host of the digenean *Prohemistomum vivax* which parasitizes the Egyptian kite (Azim, 1933) and may infect humans (Nasr, 1941). It is also known to transmit the paramphistome *Gastrodiscus aegyptiacus* – an intestinal parasite of African equines (Malek, 1981). *Melanoides tuberculata* described as an invasive snail is known to be the intermediate host of at least 37 species of digeneans worldwide (Mukaratirwa *et al.*, 2005; Heneberg *et al.*, 2014; Chalkowski *et al.*, 2021). In addition to being intermediate hosts of digeneans, these two snails can harbor nematodes such as larva of *Angiostrongylus cantonensis*, a parasite with zoonotic potential (Ibrahim, 2007).

Despite the importance of Thiaridae as invasive species and the potential one health concern of some of their parasites, there are not enough reports on these snails and their infections in Burkina Faso. The present study is an attempt to establish distribution of *Melanoides tuberculata* and *Cleopatra bulimoides*, hosts of parasites and to determine the prevalence of cercariae in these molluscan species occurring in Ouagadougou. Moreover, this study constitutes the first contribution to our knowledge of the parasitic transmission risks by these molluscs in this area. The objectives were to investigate: (i) the distribution of *Melanoides tuberculata* and *Cleopatra bulimoides* in Ouagadougou reservoirs and (ii) the infection rates of trematodes in thiarid snails. The results of this study could be useful in the control and prevention of trematode infections in riverine people of these reservoirs.

Materials and methods

Study sites

The study was conducted in Ouagadougou, the capital city of Burkina Faso. Ouagadougou is crossed by four streams, which are used as drainage channels for runoff water. The city has four intra-urban dams built in 1963 on the Massili River (Fig. 1). They drain into the Nakanbe River, one of the most important river at the country level, and this, through Bangr-Weoogo, an urban park (Coulibaly *et al.*, 2020). The complex formed by the Bangr-Weoogo Urban Park and the three reservoirs (Ouaga1, 2 & 3) have been designated as a wetland of international importance (Ramsar Site No. 2367) since 2019. The waters of these reservoirs are used by the surrounding population for production activities such as urban farming, horticulture, and recreational activities such as swimming and fishing. Since 2015, special attention has been paid to these reservoirs both for their role in production activities and as an ecosystem in maintaining biodiversity.

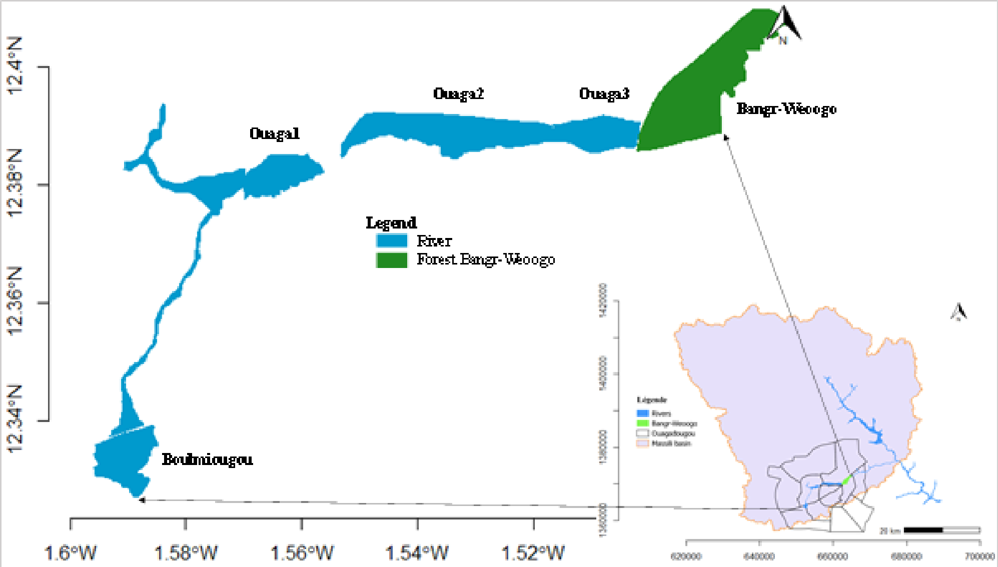


Fig. 1. Location of selected dam lakes and study sites in Ouagadougou.

Source: KML file extracted from Google Earth Pro version 1.3.36.102 and map realised by R 4.1.1

Study Design and period

This cross-sectional study involving snails collecting in different microhabitats (rocks, sediment, submerged objects of various kinds and macrophytes) and parasites examination by cercarial emission test and grinding of molluscs were done. The study was carried out monthly from May to September 2020. A total of 24 points were sampled each month with six points per reservoir.

Sampling of snails and their identification

Snails were collected every month from May to September 2020 in each reservoir using the counts per unit of time sampling method (Oliver et Schneiderman, 1956). Two researchers collected snails by handpicking and scooping every 15 minutes at each sampling site. Just living Thiaridae snails were collected and kept in dark-colored bottles with wet pieces of papers to keep them alive, and brought to the laboratory. Once in the laboratory, the collected snails were measured for length (average size) with digital calipers to the nearest 0.05mm and were then identified into species level according to their shell morphology using key identification of (Brown, 2015).

Cercarial emission test and dissection of molluscs

Snails were brought alive to the laboratory and reared in batches of 5 individuals in 25cm^3 of transparent plastic tanks containing dechlorinated tap water at room temperature with a natural photoperiod and fed with lettuce. To screen the infected individuals, the snails of each tank were examined in the morning after the sample day for cercariae emission. Individuals found to be infected were moved in separate containers in the same conditions as described above. Snails that did not shed cercariae at the first time were monitored for shedding cercariae at half-hour intervals for another 24 hours. A few drops normal saline buffer was put on the slide and covered with a cover slip and examined under microscope.

Parasites identification

Snails were examined to detect and count cercariae of trematode species and larvae of other parasite species. Snail shedding and crushing methods were used to investigate parasitic infections. Parasites were identified based on morphological features and classified to major types, using the keys of Frandsen (1984) and Gibson *et al.* (2002). Snails were checked for cercarial shedding under a binocular microscope after an hour of exposure to artificial light, and dissecting microscope was used to examine sporocysts and rediae.

Data analysis

The population structure of the parasites was described in terms of prevalence and mean intensity according to the terminology of Bush *et al.* (1997).

The prevalence and intensity of parasites were assessed in each snail. Summary of statistics were presented as mean abundance and mean intensity ± SE of infection according to Bush *et al.* (1997). Snail counts and prevalence of infections based on simple descriptive statistics. Means are presented with their standard-errors. The no-parametric test of Kruskal-wallis (with p-value = 0.05) and Wilcoxon were realised to check first, the difference between snail species abundance according to micro-habitats and secondly to compare the effect of shell size on parasitism. For interaction on shell size, *Cleopatra bulimoides* population was grouped into size classes according to the Sturge rule and *Melanoides tuberculata*, according to the rule of (Von Bertalanffy, 1938).

Results

Habitat characteristics and snails distribution

Various anthropogenic activities are carried out around water bodies such as swimming, urban farming and water sampling. The current study was swift in the rainy season, and water temperature was 25–31 °C. There were small to medium sized rocks all over the streams. The collected snails were found on the rocks, floating objects (plastic matter, used clothing, used tire), and on aquatic plants (*Nymphea lotus* L.).

A total of 1031 snails of Thiaridae belonging to two species (*Melanoides tuberculata*, and *Cleopatra bulimoides*) were collected and examined. The samples were dominated by *Melanoides tuberculata* which was counting 717 (69.5%) specimen while *Cleopatra bulimoides* was counting 314 individuals (30.5%). The shells size varied from 1.58 to 27.78 ± 0.77mm TL and 8.10 ±0.54 to 25.38mm TL respectively for *Melanoides tuberculata*, and *Cleopatra bulimoides*.

The Kruskal-Wallis test assessed for *Melanoides tuberculata* habitat occupations showed that there were statistically significant differences (p = 0.036) between the different micro-habitats.

The Wilcoxon pairwise test between these micro-habitats showed that only the difference between the banks and floating object was significant (Wilcoxon test, p = 0.048). However, they were no statistically significant differences (p = 0.294) between the number of *Cleopatra bulimoides* specimen recorded in the different micro-habitats. To conclude, the spatial occupation of *M. tuberculata* seems to be influenced by the type of micro-habitats while those of *C. bulimoides* seems to be no influenced by type of micro-habitats (Fig. 2).

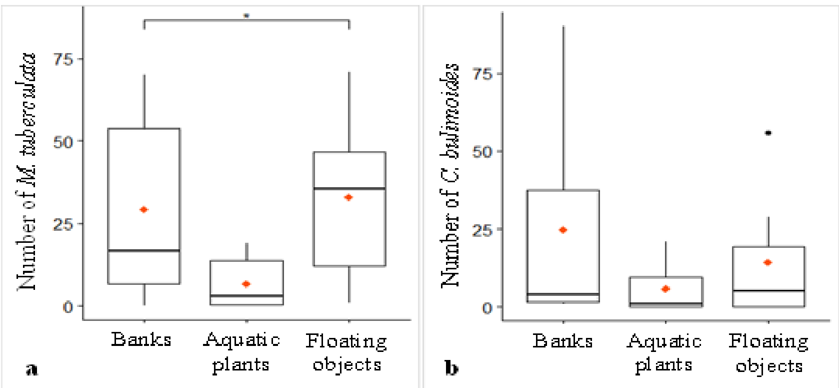


Fig. 2. Distribution of Thiaridae species according to micro-habitats.

Parasitic infections

From the total number of snails examined for trematode infections, 109 specimens (10.57%) were infected by trematode cercariae. The most prevalent snail overall was *Cleopatra bulimoides* with 17.83% (56/314) while *Melanoides tuberculata* was occurring for 7.39% (53/717). However, statistical analysis of prevalence shows no significant difference (p = 0.354) between *Melanoides tuberculata* and *Cleopatra bulimoides*. Thus, in our context, both species present the same risk of infestation.

Seven types of cercariae were categorized: (1) xiphidiocercaria, (2) furcocercous, (3) megalurous cercaria, (4) monostome cercaria, (5) Armatae xiphidiocercaria, (6) echinostome cercaria and (7) gymnocephalus cercaria. Parasitological data (prevalence, mean intensity, abundance) are summarised in Table 1. The size of *Melanoides tuberculata*, was classified in thirteen class size according to their total length from 0mm to 27mm. This allowed to group them in two classes: immature and mature molluscs which contain respectively 10 immature snail infected and 43 mature snail infected. However, there were no statistically significant differences ($p > 0.05$) between the number of immature infected and those of mature (Fig. 3).

Table 1. Occurrence, average intensities and abundance of parasites found in *Melanoides tuberculata* and *Cleopatra bulimoides*.

Snails species	Snails size classes (mm)	Snails Number	Prevalence% (n/N)	Mean intensity	Mean abundances
M. tuberculata	S_1= 1.58 ± 0	1	100	2	1
	S_2= 5.26 ± 0.42	16	6.25	1	0.06
	S_3 = 8.34 ± 0.81	27	7.40	2	0.07
	S_4= 10.92 ±0.76	47	12.77	2	0.12
	S_5=13.36 ±0.81	94	3.19	1	0.03
	S_6 =16.01 ±0.71	117	7.69	1	0.08
	S_7 = 18.7 ± 0.77	119	5.88	2	0.06
	S_8 = 21.23±0.73	135	9.62	4	0.1
	S_9= 23.55±0.75	158	6.96	2	0.07
C. bulimoides	S_1= 8.1 ± 0.5	2	50	21	0.50
	S_2=10.04± 0.49	10	10	16	0.40
	S_3 = 11.95±0.54	114	8.77	12.76	0.22
	S_4= 13.92 ±0.44	164	10.98	7.50	0.16

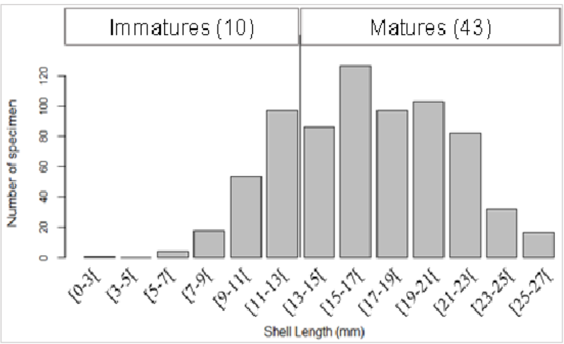


Fig. 3. Size frequency distribution for *Melanoides tuberculata*.

The most common type of cercariae recovered from the study area was xiphidiocercaria cercaria, which accounted for 48% of all infections by trematodes in snails. In total, 12 of snails were infected by xiphidiocercariae. During the survey, 119 xiphidiocercariae metacercaria type were recorded with a mean intensity of 10 cercariae per infected snail. This was followed by gymnocephalus cercaria (19.23% of all infections) with 64 metacercariae recovered, mean intensity of 12.8 per snail infected and megalurous cercaria (15.38% of all infections) (Fig. 5).

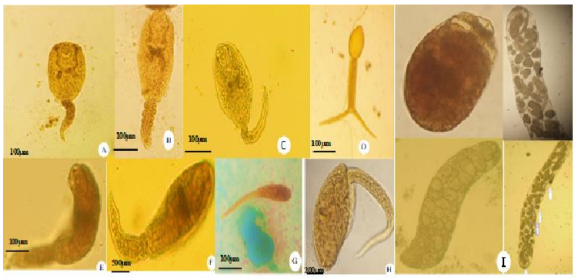
Trematode infections

The overall Trematode prevalence was 5.14% (53/1031). Of these trematode-bearing snails, nearly half were found to harbour mainly immature forms (rediae). *Melanoides tuberculata* were found to be infected with only one types of cercariae, monostome cercariae. Whereas, six (06) types of cercariae (Fig 4), xiphidiocercaria, armatae xiphidiocercaria, gymnocephalus, echinostome, megalurous cercaria and furcocercous were recorded in *Cleopatra bulimoides*. Co-infections (with two different types of cercariae) were observed in only four specimens of *C. bulimoides*.

Nematode and annelide infections

The overall prevalence of these infections was 5.53% of infected snails. *Melanoides tuberculata*, which seemed less susceptible to trematode infection was more prone to nematodes and annelids infections. In fact, 44.95% (49/109) of the infected *Melanoides tuberculata* individuals were carriers of at least one specimen of annelid and nematode belonging respectively to the gender *Chaetogaster* and *Angiostrongylus*. A total of 54 *Angiostrongylus* sp. fixed in the digestive tracts were recorded with a mean infection intensity of 3 per infected snail.

The morphometric characteristics of these larvae would appear to correspond to those of *Angiotrongylus cantonensis* (Fig. **6**). Larvae belonging to the genus *Chaetogaster* were collected from both *C. bulimoides* and *M. tuberculata* with an average infection intensity of 2.61 per infected snail.



Legend: A-C. types of xiphidiocercariae; D. furcocercous; E. echinostome cercaria; F. megalurous cercaria; G. monostome cercaria; H. gymnocephalus cercaria; I. rediae

Fig. 4. Morphotypes of cercariae and rediae recorded from *Thiaridae snail* in Ouagadougou freshwater bodies.

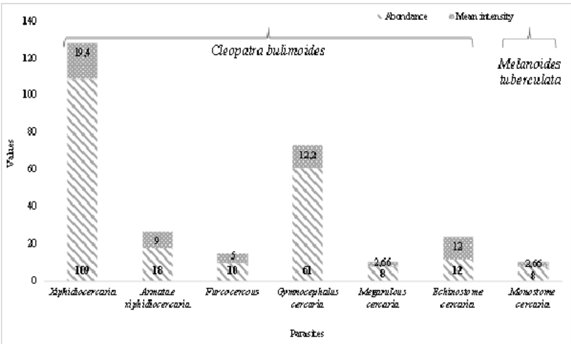
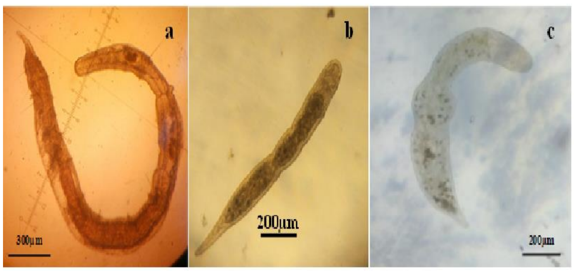


Fig. 5. Average Abundance and intensities of parasites found in *Cleopatra bulimoides* and *Melanoides tuberculata*.



Legend: a. *Angiostrongylus* sp. b. *Chaetogaster* sp. c. *Chaetogaster limneai limneai*

Fig. 6. Morphotypes of nematode and annelide recorded from *Thiaridae snail* in Ouagadougou freshwater bodies.

Discussion

Most freshwater snails can become intermediate hosts for parasitic infections. This study is the first known report on parasitic infection in the Burkina Faso watersheds concerning Thiaridea family snails. The present study identified 2 species of snails belonging to this family commonly found in West African ecoregions in Ouagadougou watershed: *Melanoides tuberculata* and *Cleopatra bulimoides* (Tchakonté *et al.*, 2014; Koudenoukpo *et al.*, 2020). The distribution of these snail species was in function of the type of microhabitat. In our case, the species were most abundant in banks and floating object. Many studies have also reported the abundance of Thiaridae particularly the species *M. tuberculata* in the standing water in general (Dudgeon, 1989) and particular on the fine particles as banks (Tina Liu et Resh, 1997). Moreover, other authors argued that abiotic and biotic factors can infuence the occurrence of snails and parasites (Duan *et al.*, 2021).

The overall Trematode prevalence of 5.14% recorded in the present study corroborates the studies of (Opisa *et al.*, 2011; Mohammed *et al.*, 2016; Chontananarth *et al.*, 2017; Mereta *et al.*, 2019) that reported prevalence within the range of 1.8% to 14.1% in various freshwater snail hosts. However, the species richness of the trematode collection in the whole gastropod community (7 types which suggest a minimum of 7 species of cercariae) was relatively high compared with other studies, e.g. two types and five cercarial species for fourteen gastropod species in the Khek River in Thailand (Dechruksa *et al.*, 2007), seven morphological types for six gastropod species in Kaduna state (Nigeria) (Abdulkadi *et al.*, 2018). This could be also explained by the host sample size which is argued to influence the trematode diversity (Duan *et al.*, 2021).

Double infections were rare during the study, in agreement with the report that fewer multispecies infections are observed than those expected by chance (Kuris et Lafferty, 1994). Sousa (1993) and Keeney *et al.* (2008) suggested that a parasitized snail loses its chemical attraction to other searching parasites, or that the infection alters its physiology to hinder or prevent the development of a subsequent infection, or during their exposure in the field, some initial infections were invaded by another parasite species that often excluded the first parasite. The rare double infections would probably be due to roughly simultaneous infection with two species of trematodes.

Although among *Melanoides tuberculata*, we could distinguish between mature and immature molluscs. We found that there was no significant likelihood of attachment based on snail size category. Therefore, it seems that infection intensity did not depend significantly of snail size, which is likely driven by the lack of observed infections in many of the small snails. These results corroborate with those of Brachtel *et al.* (2018) in Upper Mississipi River. Indeed, all the *Melanoides* collected would have an age lower than 1.5 years. According to (Von Bertalanffy, 1938) for a *Melanoides* shell length of about 25mm, the age of the mollusc would be about 1.3 years. However, the length size of *Melanoides tuberculata*, in addition to being affected by ecological conditions as highlighted by (Oliveira et Oliveira, 2018), other studies have shown that, snail of Thiarid need more than 30 months to reach size of 20mm (Pointier *et al.*, 1993). In addition, the same study showed that the rainy season (June-November) is the period of reproduction of the Molluscs. This could explain the relative abundance of juveniles of *Melanoides tuberculata*.

Like several other studies, our results suggest that larger snails show higher levels of infection than smaller snails (Sousa, 1993; Guralnick *et al.*, 2004). This is probably related to tissue changes, particularly the development of gonadal tissue. Trematode feeding of gonadal tissues (and resulting castration of snails) occurs frequently in *M. tuberculata* and may be necessarily for some trematodes to complete their larval development (Hechinger *et al.*, 2008; Papkou *et al.*, 2016). Despite the lack of statistical significance, there was an overall positive relationship between snail body size and percentage infection. In practical terms, a known snail size range that is vulnerable to infection could improve trematode detection in invaded systems by allowing managers to develop targeted survey methods. Our results support this assertion for water bodies in the city of Ouagadougou. However, it is clear that further work is necessary to refine the approach for application in invaded aquatic systems.

In addition to trematode infections, nematode and oligochaete infections have been reported during this investigation. Indeed, *Chaetogaster limnaei limnaei*, an oligochaete, was always found infesting many freshwater snails, living in the mantle cavity, pulmonary cavity or embedded in the mucus of the foot under natural conditions (Ibrahim, 2007; Wajdi, 1963). An intimate association between the *C. limnaei* and many aquatic snail species has long been recognized as commensal or parasitic in several freshwater ecosystems (Callisto *et al.*, 2005; Mitchell et Leung, 2016). The presence of *Chaetogaster* sp. in snail vectors hinders and even stops infection by their trematode larvae (Ibrahim, 2007). In our study, a relative large number of snail individuals, especially of the *Melanoides tuberculata* species, harboured this oligochete. This could explain on the one hand, the low prevalence of trematode infections and on the other hand, the low diversity of trematode (only monostome cercariae) isolated in this group. The association of *C. limnaei* with the snail intermediate host may be of value as a control measure against economically important parasitic diseases such as fascioliasis and schistosomiasis.

In this investigation, the Thiaridae snails found in the Ouagadougou basin served as the intermediate host of a zoonotic nematode (which seemed to be *Angiostrongylus cantonensis*) and various species of zoonotic trematodes. Regarding the prevalence of the cercarial types, in this investigation, the Megalurous and echinostome cercaria was observed to occur in *Cleopatra bulimoides* in this study. This type of cercaria developed in many important trematodes of the family Echinostomatidae which infected the gastrointestinal tract of animals and humans'. Although echinostomiasis occurs worldwide and most human infections are reported from Asia. Despite being relatively rare, at least 19 species of echinostomes from 8 genera have been reported in humans from China, India, Indonesia, Japan, Korea, Malaysia, Philippines, Russia, Taiwan, and Thailand (Jung *et al*., 2014; Sah *et al*., 2018). This study listed and classified the parasites into major groups. It would be interesting to investigate further in order to isolate the zoonotic parasite species. Finally, during this study, the largest number of infected molluscs were harvested from the shoreline. This implies that users of the water bodies of the city of Ouagadougou are at risk of being infected by these parasites.

Conclusion

In conclusion, freshwater snails have played an important role with regard to the health of the public, as well as within the veterinary field. This study has contributed in the way of new information on the trematode fauna that is present in Ouagadougou, Burkina Faso. The findings could serve as an initial step for understanding the epidemiological situation and establishment of control program of trematode infections in humans and animals. This study also provided important information regarding which cercarial type is developed into specific Thiaridae snail's family or genus.

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