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Molluscs Intermediate Hosts of Distomes in Some Natural Cattle Water Points in Ngaoundere

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Authors' contributions

This work was carried out in collaboration among all authors. Authors CGA, MA and MM designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors CGA, YC, NACN, MA, AS and ND managed the analyses of the study. Authors CGA and YC managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Background: Freshwater gastropod molluscs act as indispensable intermediate hosts in the life cycle of many species of distomes of medical and veterinary interest. The aim of this study was to carry out an inventory of molluscs in natural cattle water points and show their contamination and therefore their role as intermediate host.

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Material and Methods: Between October to December 2018, a total of 1631 molluscs were collected in five natural cattle water points: Four lakes (Dang, Bini, Djalingo, Calmet) and one river (Mardock). The cattle water points were chosen based on their accessibility and the presence of vegetation around. Molluscs were collected manually by hand from shallow areas or using a rigid fine mesh net for deep areas. Molluscs were then placed in plastic jar and transported to the laboratory where they were identified.

Results: Four species of molluscs belonging to the subclass Pulmonata were identified. These species were *Bulinus forskalii* (Morelet, 1866), *Bulinus globosus* (Morelet, 1866), *Biomphalaria pfeifferi* (Krauss, 1848) and *Lymnaea natalensis* (Krauss, 1848) with respective numbers of 244 (18%), 796 (58.4%), 293 (21.5%) and 298 (21.9%). Molluscs were not encountered in all the water points. All the four species of molluscs were collected in lake Dang, Bini and Djalingo. Lake Dang showed great specific richness with the presence of four identified molluscs. In lake Bini two species of molluscs were encountered with a high density of *B. pfeifferi*. The frequency of infestations with *Fasciola gigantica* and *Paramphistomum daubneyi* varied in certain localities according to the species of molluscs present in the cattle water points. The majority of molluscs that were subjected to the cercariae emission test showed their infectious nature by emitting cercariae which were larval forms of the distomes.

Conclusion: It is very important to set up a mechanism for controlling molluscs in farming areas because these animals are the main sources parasites dissemination.

Keywords: Lymnaea natalensis; Biomphalaria pfeifferi; Bulinus globosus; Bulinus forskalii; distomes; cattle; Ngaoundere.

1. INTRODUCTION

Freshwater gastropod molluscs act as indispensable intermediate hosts in the life cycle of many species of distomes of medical and veterinary interest [1]. Several studies have shown high prevalence of fasciolosis, schistomatosis, paramphistomatosis and dicrocoeliosis which are the main distomatoses in areas where animals are in constant contact with water [2]. Distomatoses are infections caused by distomes belonging to the subclass trematoda. These diseases are related to poor hygienic conditions in tropical and subtropical countries, where the biotope (temperature, pools, creeks, rivers, natural lakes and artificial water catchments, etc.) promotes the development of infestation of molluscs intermediate hosts [3]. Animals are infected by ingesting encysted metacercariae on aquatic plants, by drinking contaminated water or by transcutaneous penetration of cercariae [1]. Despite all the measures taken by the breeders and state institutions for the control of distomatoses, they remain endemic in large breeding areas of our country [4]. They are at the origin of a drop in the demand for animal protein [5]. To better control these distomatoses, the knowledge of malacological fauna of the main rivers located closed to the high breeding areas is necessary. The main objective of this study was to carry out an inventory of freshwater molluscs intermediate hosts of the main bovine distomes in Ngaoundere and to highlight the role played by molluscs in the transmission of distomes in natural water points.

2. MATERIALS AND METHODS

2.1 Study Area

The study was carried out in Ngaoundere (Adamawa-Cameroon). Ngaoundere town is located between Latitude 7°19N and Longitude 13°35E. There are 2 seasons a dry season (November – May) and a rainy season (May – November). The dry season is marked by a dry and warm wind coming from the North while the rainy season is sometimes prominent with violent and continuous rains. The water points surveyed in the present study (Lake Dang, Lake Bini, Lake Calmet, Lake Djalingo and River Mardock) were located in two localities in Vina subdivisions: Lake Dang and Lake Bini in Ngaoundere 3, Lake Djalingo, Lake Calmet and River Mardock in Ngaoundere 2 as presented in Fig. 1.

2.2 Collection and Identification of Mollusks

Freshwater molluscs were collected between October (end of rainy season) and December (start of dry season) manually by hands from shallow areas or using a rigid fine mesh net for deep areas [6]. The molluscs were collected in an area of about 20 m^2 in 20 minutes by two

people moving in opposite directions [7]. They were then placed in plastic jar containing water from their natural environment and transported to the Laboratory of Veterinary and Medical Entomology of Special Mission of tse-tse fly Eradication. Once in the laboratory, molluscs were carefully washed with tap water, then counted and classified according to morphological differences such as shell shape, size and type of opening (dextral or sinistral). They were identified on the basis of the work of Brown [8]. The physicochemical parameters of the water points were measured using the HANNA multi parametric device according to the techniques recommended by Rodier et al., [9]. These parameters were temperature, pH, solid contents and salinity as seen on Table 1. Site density was expressed as the number of molluscs harvested by an individual within an hour and the abundance was represented as the number of molluscs harvested in one site [6].

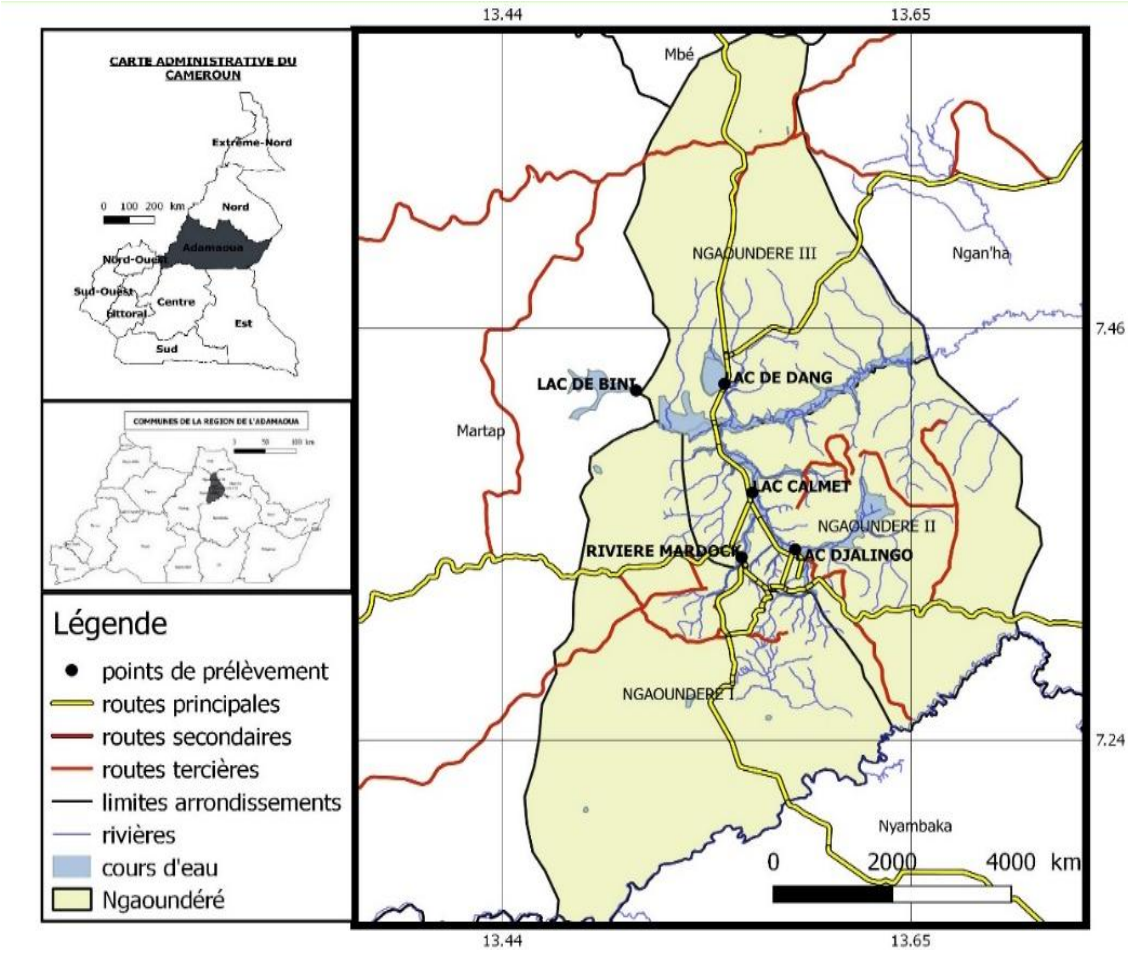

Fig. 1. Prospected water points [5]

Table 1. Physicochemical parameters of water points

Sites	T / °C	PH	TDS / ppm	Conductivity / siemens	Salinity /ppm
Bini lake	22.4	7.7	15	36	27
Calmet lake	28.9	7.7	17.5	33.5	25.1
Dang lake	28.5	7.2	38.3	76.7	57.5
Djalingo lake	24.1	7.1	303	537.5	403.1
Mardock river	20	8.2	26	43	32.3

2.3 Cercariae Emission Test

In order to confirm the contamination of molluscs and therefore their role as intermediate host in the life cycle of the main distomes encountered during the study, thirty-two (32) molluscs (08 individuals of each of the 04 species) were randomly selected and each placed in a 35 mm diameter petri dish containing a 2 cm thick layer of water and a piece of lettuce. All the containers were placed in a widely open room, but not receiving direct sunlight. For 10 days, the containers were inspected twice a day (in the morning at 8 a.m. and in the evening at 4 p.m.) for cercariae. When there was a cercariae emission, the cercariae were counted using a binocular magnifier and the molluscs was immediately returned to another petri dish with its lettuce fragment, and the water changed. If there were no cercariae emission, the water in each container was replaced during the evening inspection [10].

2.4 Statistical Analysis

The data collected were recorded in the Microsoft Excel software 2016 version for plotting of graphs and calculating means, then imported into the stat graphic software for variance analysis. The relative abundance (A) of the species, which is the ratio between the total number of each species and the total number of molluscs harvested, was calculated as well as the similarity indices of Sorensen (B) and that of Shannon (H') diversity.

3. RESULTS

3.1 Molluscs Identified

The various surveys in the selected water points (Lake Dang, Lake Bini, Lake Calmet, Lake Djalingo and River Mardock) during the study led to a total collection of 1631 molluscs all belonging to the Subclass of pulmonata. These were *Bulinus forskalii* (Morelet, 1866), *Bulinus globosus* (Morelet, 1866), *Biomphalaria pfeifferi* (Krauss, 1848) and *Lymnaea natalensis* (Krauss, 1848), with respective numbers of 244 (18%), 796 (58.4%), 293 (21.5%) and 298 (21.9%).

3.2 Distribution and Abundance of Molluscs

It appears from Fig. 2 that molluscs were not found in all water points. Indeed, in Lake Dang, Lake Bini and Lake Djalingo all four species of molluscs were seen. Lake Dang shows great specific richness with the presence of four species of molluscs identified. At lake Bini, 02 species of molluscs (*Biomphalaria pfeifferi* and *Lymnaea natalensis*) were encountered with a high density of *B. pfeifferi*. At Djalingo lake, only *L. natalensis* was present during the collection period. This species (*L. natalensis*) was present at all sites while *Bulinus forskalii* and *Bulinus globosus* were found exclusively in Lake Dang.

3.3 Specific Diversity of Mollusks

The Shannon index (H´) measures the specific diversity of species present in their natural range. According to the various calculations carried out, it appears that Lake Dang presents a high specific diversity of molluscs (H´ = 1) compared to all the other sites where collections were made. At the level of Lake Djalingo, the presence of a single species of molluscs was noted (H´ = 0). As for Bini Lake, two species were present (H´ = 0.45).

As for the Sorensen index (S), which is a statistical indicator making it possible to measure the degree of similarity of two samples from different environments, it appears that the samples of molluscs collected at the level of Lake Bini and Lake Dang were similar because two identical species of molluscs (*Biomphalaria pfeifferi* and *Lymnaea natalensis*) were noted at the two sites (S = 0.86), while in the Bini and Calmet sites (S = 0.4), Calmet and Dang (S = 0.33) and Dang and Djalingo (S = 0.4), the samples were different (Marcon, 2018).

3.4 Prevalence of Cattle Infections and Abundance of Molluscs Species

The prevalence of cattles infected with *Fasciola gigantica* and *Paramphistomum daubneyi* varied from one locality to another depending on the species of molluscs present in the environment as well as their abundance during the collection period of the Intermediate Hosts (September - December). In fact, the prevalence of *P. daubneyi* infestation was higher (19.6%) in cattle breeding in Ngaoundere 3 subdivision who drank along the lakes of Dang and Bini where they had a high abundance of the molluscs *Bulinus globosus*, *Bulinus forskalii* and *Biomphalaria pfeifferi* compared to cattle raised in Ngaoundere 2 Subdivision (9.3%) where *B. globosus*, *B. forskalii* and *B. pfeifferi* were not present or were in very small numbers. Unlike the water points located at Ngaoundere 3, those in the second

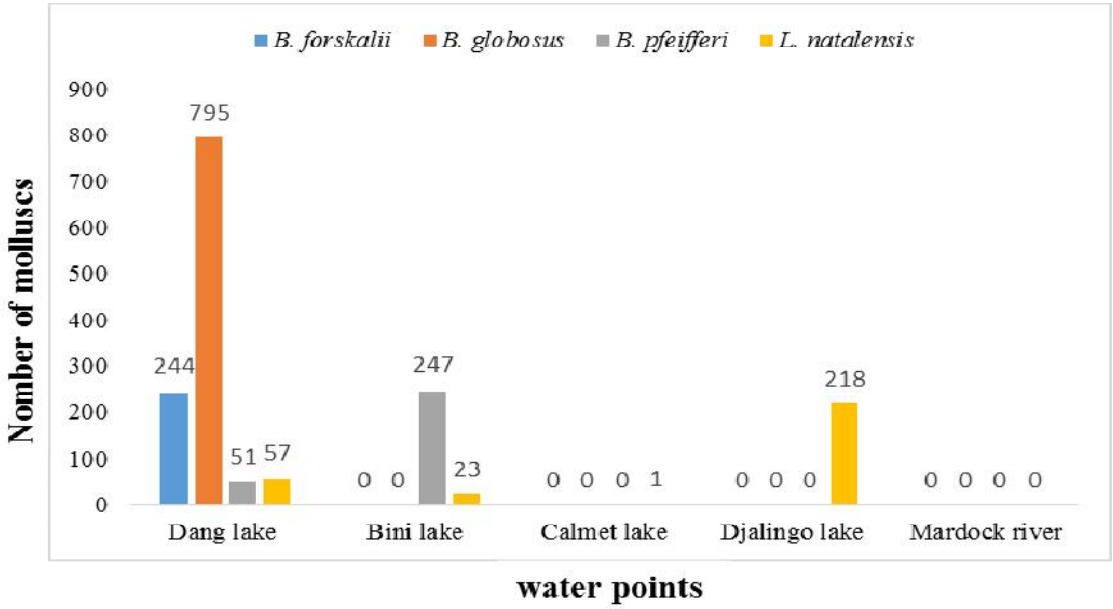

Fig. 2. Distribution and abondance of species in function of site

subdivision of Ngaoundere showed a greater abundance of *Lymnaea natalensis* molluscs. The prevalence of *F. gigantica* infestation in cattle raised in this locality was higher (22.4%) than that of Ngaoundere 3 (8.2%) where just a few individuals of *Lymnaea* were found [11].

3.5 Evaluation of Cercarian Emissions

After ten (10) days of exposure of the 32 molluscs, a total of 1420 cercariae were counted. The cercariae were emitted by 20 molluscs, an average of 71 cercariae per molluscs. During the first phase (first 5 days), about 450 cercariae were removed from the molluscs. This number increased considerably in the second phase (last 5 days) during which 970 cercariae observed were of various shapes.

4. DISCUSSION

4.1 Molluscs Identified

Knowledge of malacological fauna has been the subject of several studies around the world. In Cameroon, Njiokou et al., [12] revealed the presence of *Bulinus forskalii; Bulinus globosus*; *Biomphalaria pfeifferi* and *Lymnaea natalensis* in Yaoundé. Diaw et al., [6] mentioned in addition to the species mentioned, the presence of several other species of molluscs at Richard Tall in Senegal. The most common of which were *Bulinus truncatus* and *Bulinus senegalensis*. Thiam and Diallo [13] also noted the presence of many species of freshwater molluscs in the Gambia basin. In Tanzania, Nzalawahe et al., [14] showed a high prevalence of *Fasciola gigantica* and *Paramphistomum* sp in certain villages which were due to the presence of molluscs raised in the irrigated areas. Few species encountered in this study could be justified by the fact that the number of sites explored were less and that the period of study was also short.

4.2 Distribution and Abundance of Mollusks

The lakes of Dang and Bini presented the highest specific richness. This is justified by the fact that these lakes are covered with abundant vegetation. In fact, a direct relationship has been shown between aquatic macrophytes and the specific richness of pulmonata in Africa [15]. The low presence of molluscs in Lake Calmet could be attributed to the low vegetation observed at this site. The absence of molluscs in River Mardock can be justified by the fact that the water isn't stagnant there [16]. The particular frequency of *Lymnaea natalensis* in four (4) sites out of the five (5) surveyed, is justified by the very wide spectrum of tolerance vis-à-vis the salinity manifested by *Lymnaea* [17]. The same authors also reported that *Lymnaea* can live in environments where the temperature is between

10 and 28°C; and the temperature of our sites fell within this range (20 to 28°C). According to Tchakonté et al., [18], the variation in the distribution of molluscs in the sites is due to variations in the physicochemical parameters (temperature, pH, TDS conductivity and salinity). Indeed, Thiam and Diallo [13] think that the presence, distribution and abundance of freshwater molluscs depends on the characteristics of the environment, in particular the salt content, the vegetation and the nature of the sea bed.

4.3 Cattle Infection and Abundance of Mollusks

In the present study, a relationship was observed between *Fasciola gigantica* and *Paramphistomum daubneyi* infections and the abundance of intermediate host molluscs in Vina Division. *Lymnaea natalensis* is said to be the intermediate host of *Fasciola gigantica*. According to Nzalawahe et al., [14], the freshwater molluscs L. *natalensis* is involved in the transmission of *F. gigantica*. *Biomphalaria pfeifferi* and *Bulinus forskalii* are believed to be potential intermediate hosts for *Paramphistomum daubneyi* in the Adamaoua Region of Cameroon. Moser et al., [19] stated that *B. forskalii* has the capacity to transmit several parasites including three species of paramphistomes but also several species of schistosomes [20]. Nzalawahe et al., [14] made the same observation. These authors indicated that the previously mentioned molluscs also served as intermediate hosts in the transmission of Paramphistomes to cattle in Tanzania. The same observation was made for *B. pfeifferi* [21]. This is the reason why Thiam and Diallo, [13] stipulated that the presence of freshwater molluscs in farming areas poses a risk of multiplication and spread of the distomes.

4.4 Cercarian Emissions

The number of cercariae emitted by these freshwater gastropods increased over time and depended on certain characteristics. Cercarian emissions have made it possible to highlight the infectious nature of molluscs and to show the role played by these gastropods in the perpetuity of distomes in a breeding environment. Bouix-busson et al. [10], Njiokou et al. [12], Ibikounlé et al. [21] and Nzalawahe et al. [14] also made the same observation. Indeed, theses authors have all shown the involvement of the fresh water gastropods in the transmission of larval forms of distomes.

5. CONCLUSION

It is very important to set up a mechanism for controlling molluscs in farming areas because these gastropods are the main sources of parasites dissemination. However, it will be necessary in perspective to analyze more samples and sampling should be spread over the whole year.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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