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First report of helminths parasites in frog (*Hoplobatrachus occipitalis,* Günther 1858) from Daloa, Issia and Sinfra (Côte d'Ivoire)

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Abstract
Frog are common inhabitants of water and are exposed to infection by many types of helminths. This study constitutes the first report of helminth in *Hoplobatrachus occipitalis* from Middle West of Côte d'Ivoire. Samples of three localities including Daloa, Issia Sinfra were investigated. A total of 210 specimens of edible frog *H. occipitalis* were examined for parasites research in intestine and lung. Results showed that 82.0% of frog were parasitized by at least one species of helminth. A total of 756 helminths comprising: 408 nematodes and 348 trematodes were recorded from these frogs. The nematodes included: *Camallamus dimitrovi* (38.7%) and *Chabaudus leberrei* (12.7%). The trematodes were represented by *Haematoloechus exoterorchis* (32.5%) and *Diplodiscus fischtalicus* (13.5%). The prevalence and mean intensity of infection were higher in females than males but generally no significant. The highest value of parasitic load of *H. exoterorchis* and *C. dimitrovi* were 4.9±2.4 and 3.9±1.2 respectively. Thus to minimize the probability of contamination of frog, their breeding will be requisite.

Keywords: *Hoplobatrachus occipitalis,* helminths, prevalence, Côte d'Ivoire

1. Introduction
Frogs are amphibians belonging to the order Anuran and they are spread in the wild on every continent [1]. They gradually disappear over time because of diseases [2]. Studies have shown that there is a possible link between parasitic infections and amphibian population decline [3, 4]. In general, the diseases are linked to a large number of trematodes encysted in the host tissues [5]. Other helminths including Cestoda and Acanthocephala were isolated from different species of frog. Cestoda cause gastrointestinal lesions, obstructions and death of frog in cases of heavy infection [6]. Metacercariae of trematode Ribeiroia induce polydactyly and other limb deformities in frogs and are responsible for high mortalities in populations of wild frogs [7]. The information on parasitic infections of frog have been reported in several studies in Africa [8, 9, 10, 11, 12]. In spite of the numerous records and wide distribution of these parasites, the data on parasitic infection of frog in Côte d'Ivoire are poorly studied, old for over 30 years and not updated [13, 14, 15]. The only recent study on the helminth parasite is that of Assemian *et al*. [16] on *P. mascareniensis* in Daloa city wetlands. Furthermore, no data on parasites of *Hoplobatrachus occipitalis* is not yet available from localities such us Daloa, Issia and Sinfra in the Middle West of Côte d'Ivoire where frog meat are used as an important source of animal protein. Herein, we present the data on helminths infection of the edible frog *H. occipitalis* in this area of Côte d'Ivoire.

2. Materials and Methods
2.1 Sample collection and procedures
This study was done in Middle West of Côte d'Ivoire. These part of area were selected based on the importance of frog consumption and the supply of frog market. In total, 210 adult fresh frog *H. occipitalis* (Figure 1) were brought in three markets including Daloa (66 frogs), Issia (84 frogs) and Sinfra (60 frogs). Due to nocturne activities, the adult frogs were harvested in shallow, swamp and pond by collectors between 8 h and 11 PM and sold soon morning on market.

Frog were transported to the laboratory where they were sexed, and dissected using standard procedures. Males were identified by the presence of a vocal sac at super position. Our study was performed on 115 females and 95 males. After dissection, the lung and the intestine were removed separately and opened longitudinally using scissors and dissecting pins for examination. The contents were washed separately with water and the larger parasites visible were directly harvested and the small parasite with a magnifying glass. Then rinsing water was also poured into a Petri dish and the parasites were searched with a magnifying glass. The helminth were observed on a microscope slide at X 10 and X 40 magnification. The identification was performed based on morphological description from literature [17, 18, 19]. All parasites were counted and thereafter preserved in fresh 70% alcohol [9]. Voucher specimens of helminthes has been deposited in the Laboratory Central Veterinary of Bingerville.

Fig 1: Specimen of *Hoplobatrachus occipitalis* (Günther 1858)

2.2 Data analysis

The prevalence and intensity of infections were calculated in accordance with Bush *et al*. (1997) [20]. Prevalence rate was calculated as a percentage of the number of a particular host species infected with a specific helminth parasite divided by the total number of host examined. The mean intensity of infection refers to the number of parasites per host (Calculated for the total host population uninfected individuals). The effect of host sex on intensity was tested using a Mann-Whitney *U* test and Kruskal Wallis test were used to compare the prevalence of infection based on locality. The assumption of normality was checked with Shapiro-Wilk's test. All statistical analyses were carried out using STATISTICA 7.0.

3. Results

3.1 Prevalence of frog helminths

The results showed that 82.0% of frogs were parasitized by at least one species of helminth. In total 756 helminths, comprised 54.0% of nematodes and 46.0% of trematodes were extracted from all of the 210 frogs analyzed. In addition, 29.0%; 7.6% and 0.5% of frogs harbored two, three and four parasites respectively. The nematodes isolated from the intestine were *Chabaudus leberrei* and *Camallamus dimitrovi*. While, *Haematoloechus exoterorchis* and *Diplodiscus fischthalicus* isolated respectively in lung and intestine were identified as trematodes. Among the helminths isolated, *C. dimitrovi* (38.7%) was the most dominant and *Chabaudus leberrei* with 12.7% was the least (Figure 2).

Ours findings revealed that the female frog were more infected by nematodes and trematodes than the males (Figure 3). About nematode *C. dimitrovi*, 41.7% of the females frogs were infected by this helminth against 37.9% for males. *Chabaudus leberrei* was isolated from 14.8% of female frog and 14.7% of male. Concerning the trematode *H. exoterorchis*, the results showed 30.4% and 27.4% us prevalence rate respectively for female and male. The trematode *D. fischthaliscus* was isolated from 23.5% of female frogs and 17.9% of male.

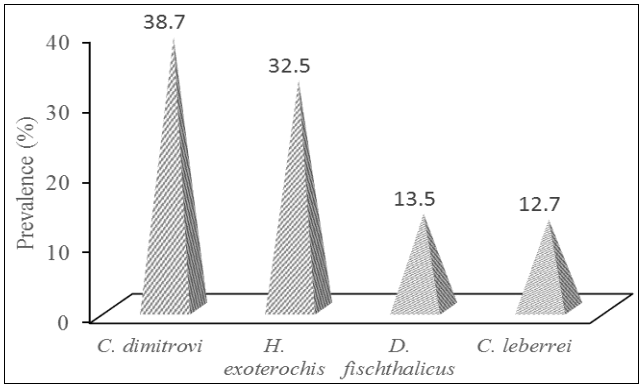

Fig 2: Prevalence of helminth parasite isolated from *Hoplobatrachus occipitalis*

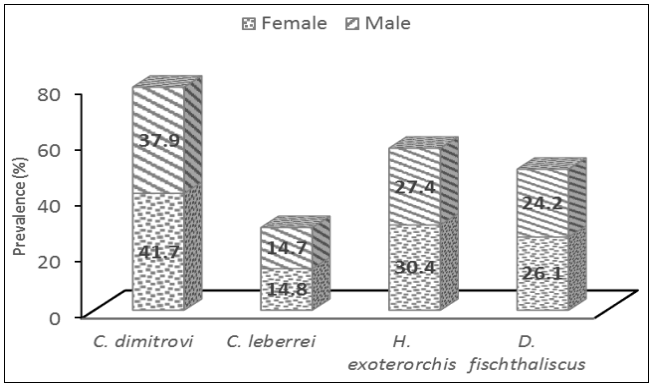

Fig 3: Prevalence of helminth parasite isolated from *H. occipitalis* with respect to frog sex

3.2 Prevalence of helminth according to sample sites

The four helminths identified in this study have been reported on all the sampling sites with different prevalence (Table 1). The prevalence of *C. dimitrovi* was between 34.8 and 43.3% obtained in Daloa and Sinfra markets respectively. Relatively to *C. leberrei*, the results showed high prevalence in Daloa market with value of 22.7% against 13.3% and 9.5% for Sinfra and Issia market respectively.

Regarding trematodes, *H. exoterorchis* and *D. fischthaliscus* were more isolated in *H. occipitalis* from Issia market with prevalence of 34.5% and 31% respectively. The determination of parasite load showed that 3.62 ± 1.7 *C. dimitrovi* were collected per frog on all sites. The number of *C. dimitrovi* ranged from 1 to 8. The highest mean intensity were recorded in Sinfra (3.9 *C. dimitrovi* per frog) followed Issia (3.6) and Daloa (3.4). The mean intensity (MI) of *H. exoterorchis* ranged from 2 to 9 and the highest MI was found to Issia with 4.9 parasites per frog. Only parasitic intensity of *H. exoterorchis* was related to sampling sites (P = 0.031).

Table 1: Prevalence and mean intensity of infection according to sampling sites (P: prevalence; MI: Mean intensity; SD: Standard deviation)

	Locality surveyed (Number of frog analyzed)								
	Issia (84)			Daloa (66)			Sinfra (60)		
Parasite isolated	**Frog infected**	**P (%)**	**MI±SD (range)**	**Frog infected**	**P (%)**	**MI ±SD (range)**	**Frog infected**	**P (%)**	**MI±SD (range)**
Nematode									
C. dimitrovi	35	41.7	3.6±2.1 (1-8)	23	34.8	3.4±1.3 (1-7)	26	43.3	3.9±1.2 (1-6)
C. leberrei	8	9.5	2.1±1.1 (1-4)	15	22.7	3.2± 1.0 (1-5)	7	11.7	3.4 ±1.4 (1-5)
Trematode									
H. exoterorchis	29	34.5	4.9±2.4 (2-9)	18	27.3	3.1±1.4 (1-6)	14	23.3	3.8±1.4 (2-6)
D.fischthaliscus	17	20.2	2.4±1,3 (1-5)	11	19.7	2.2±0.9 (1-4)	16	26.4	2.3±0.8 (1-4)

3.3 Mean intensity of helminths according to sex

The parasite load or mean intensity was determined by gender and are reflected in figure 4. In analyzing the results, it appears that with the exception of *D. fischthaliscus*, the mean infection was higher in females than in males. The parasite load of *C. dimitrovi* was 3.7 ± 1.9 and 3.4 ± 1.6 respectively in female and male. Mann-Whitney *U* test showed that mean intensity not significantly different between sex (U = 5266, P = 0.572). It was 3.37 in male and 3.47 species/hots in female.

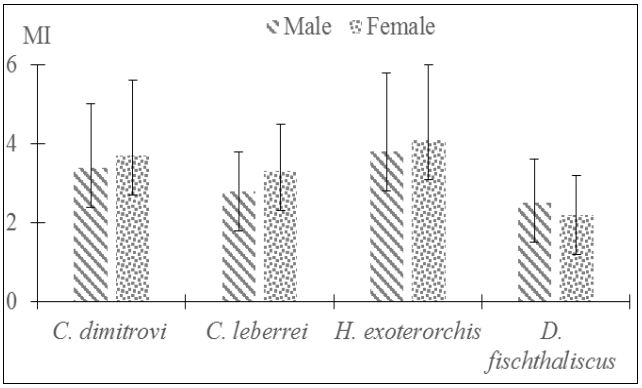

Fig 4: Mean intensity of infection of *Hoplobatrachus occipitalis* according to sex

4. Discussion

The present work examines for the helminth in a species of frogs that are most consumed in Côte d'Ivoire. In this study, four helminths were found to infect *H. occipitalis*: two species of nematodes (*C. dimitrovi* and *C. leberrei*) and two species of trematodes (*H. exoterorchis* and *D. fischthaliscus*). These helminths were present on all sites with a higher prevalence for *C. dimitrovi*. The presence of these helminths could be explained by the fact that amphibians meat many aquatic organisms that serve as intermediate hosts of the parasites (primarily trematodes) of vertebrates living in the same habitat. All these helminths isolated from *H. occipitalis* have already been identified in various species of amphibians [21, 22, 9, 23]. The prevalence obtained in this study are higher than those reported by Aisien *et al*. (2009) [9]. Indeed, the analysis of 39 frog species *H. occipitalis* by these authors found that 0.6% of *Haematoloechus* sp ; 10.8% *C. dimitrovi* and 3.0% *Chabaudus* sp. Otherwise higher prevalence 66.7% for *C. dimitrovi* have been reported by Aisien *et al*. (2011) [24]. The helminths most isolated were nematodes (54.0%) against 46.0% for trematodes. Our results are similar to those of Bhutia and Rizvi (2010) [23] which proved also in their study that the contamination is more observed in nematodes (53%) than in trematodes (46%). Moreover, Nguiffo *et al*. (2015) [12] found 90.5% and 9.4% of nematodes and trematodes. But the opposite results have been reported by Assemian *et al*. (2016) [16] which isolated more trematodes (58.04%) from *P. mascareniensis* collected in Daloa site (Côte d'Ivoire). The infection rates of frog were high in this study. This could be due to the fact that our analysis were performed on adult frogs sold on the markets. It was revealed that usually adults harbor a large number of parasites that smaller because of a change in feeding habits [25]. In addition, prolonged exposure to the accumulation of parasites in adults is the most important factor explaining the high rate of infection in adult population [26]. In this study, the prevalence of helminths was higher among females of *H. occipitalis* than males. These results are in agreement with those of several authors [26, 27]. However, these results are contrary to data of Sulieman *et al*. (2015) [28] and Assemian *et al*. (2016) [16] who found a higher prevalence in males respectively in *Amietophrynus* (bufo) *xeros* and *Ptychadenae mascareniensis*. The results showed that there was no significant difference between the prevalence of helminths in sex despite a high rate observed in females. This difference, although it was not significant could be justified by the feeding behavior between sexes. Indeed, Tohé (2009) [29] has reported that the diet of *H. occipitalis* differs between sexes.

The results also showed that the parasite intensity was higher in females than in males. Our results are consistent with those of Assemian *et al*. (2016) [16] who found that parasite load of nematodes and intensities higher acanthocephalans females *P. mascareniensis*. These results are justified by the fact that female frogs are generally larger in size than males and feed more food and therefore ingest more parasites than males [23]. Besides that, other environmental factors may influence the composition of the parasitic host. The relationship between the prevalence and sampling site was not found to be statistically significant. The presence of helminths on all sampling sites could be due to the fact that frogs are majority harvested in unsanitary areas such as swamp, shallow and pond favorable to the development of parasites. As reported by Rahman and Shakinah (2014) [30], such type of habitat contributes to the parasite infection and promote heavy infestation and can result to frog losses.

5. Conclusion

The edible frog *H. occipitalis* were infected by four different helminth species. *C. dimitrovi* was the most helminth isolated. The prevalence of contamination of the frog by helminth depend to sex. Females frog harbor more parasite than males. This preliminary work is the first step for future studies on the helminth parasites of *H. occipitalis* to understand their diversity and provide more information on their sanitary impact on frog body. However the breeding of frog will be necessary to continuous monitoring of the quality of water which is essential to reduce the contamination of frog.

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