IJSRM
INTERNATIONAL JOURNAL OF SCIENCE AND RESEARCH METHODOLOGY
An Official Publication of Human Journals

Human Journals
Research Article
December 2019 Vol.:14, Issue:2

Nematode Parasites of Anurans from the Farm of the Banco National Park (South-Eastern Côte d'Ivoire)

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INTERNATIONAL JOURNAL OF SCIENCE AND RESEARCH METHODOLOGY
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Submission: 21 November 2019
Accepted: 27 November 2019
Published: 30 December 2019

HUMAN JOURNALS

www.ijsrm.humanjournals.com

Keywords: Parasitic Nematodes, Amphibians, Rainforest, Banco National Park, Côte d'Ivoire

ABSTRACT

This study proposes to know the parasitic Nematodes of the Anurans from the fish farm of the Banco National Park, south-eastern Cote d'Ivoire. Spanning a one year period (November 2016 to October 2017), the dissection of 354 anurans specimens belonging to 23 species revealed 11 parasitic nematode taxa (*Amplicaecum africanum, Anisakis simplex, Camallanus dimitrovi, Chabaudus leberrei, Cosmocerca ornata, Filaria* sp., *Oswaldocruzia* sp., *Oxysomatium brevicaudatum, Rhabdias bufonis, Rhabdias* sp1. *and Rhabdias* sp 2). The recorded global prevalence (68.36%) shows a high parasitic infestation of nematodes in anurans. This infestation is influenced by the microhabitats and parasitic specificity of the Nematodes. Most of the Nematodes harvested in the small intestine have a broad spectrum infestation. They parasitize several species of different families. They therefore have a broad or euryxene specificity. The prevalence of the Anurans is higher in the rainy season than in the dry season. The presence of water in the environments creates favorable conditions for the development of the Anurans. Thus, the increase in the number of host species causes an increase in the number of parasitic Helminth species. The knowledge of parasitic helminths of anurans and their prevalence will serve as references for further investigations of the nematofauna and the development of adequate measures for better protection of the anurans of the park.

INTRODUCTION

Anurans are known to play vital role in balancing food chains and webs in natural habitats since their larvae, eggs and adults are consumed by various predators (Rödel 1999; Channing 2001; de Armas 2001; Rödel *et al*., 2002). Likewise, they represent an important source of animal protein for humans (Channing., 2001; Angulo 2008; Gonwouo and Rödel., 2008; Mohneke *et al*., 2010). In Côted'Ivoire, the consumption of frogs, i.e., *Hoplobatrachus occipitalis*, has recently increased to a considerable extent to such a point that anurans are considered an important component of protein in the feeding habits of some local populations (Kouamé *et al*., 2015).

However, anurans are victims of various parasitic mites on the ventral side of their thighs (*e.g.* Spieler & Linsenmair., 1999; Rödel & Agyei 2003), chytrid fungus responsible of their massive decline (*e.g*. Longcore *et al*., 1999; Penner *et al*., 2013; Mesquita *et al*., 2017), and reservoir of endoparisites pathogenic to their population (*e.g.* Cunningham *et al*., 1996; Goldberg *et al*., 1998; Düşen and Oğuz., 2010; Aisien *et al*., 2011). These endoparasites are numerous, to the number of which are parasitic nematodes. The nematodes are rounded and elongated worms covered with a rigid cuticule, and able to cause many pathologies and even death to their hosts (Chanseau 1954; Durette-Desset & Batcharov 1974; Baker., 1982; Gonzalez & Hamann., 2010; Kuzmin *et al*. 2015). In most aquatic ecosystems, the high prevalence of these parasitic roundworms mostly increases the rate of infestation of the first intermediate hosts, i.e., gastropods (Johnson *et al*., 2007), before affecting vertebrates such as anurans (Sessions & Ruth, 1990). Therefore, they can transmit parasites to predators when preyed, thus affecting wildlife negatively. Hence, Imasuen *et al.* (2012) recommend the necessity to assess the parasites of amphibians, since frogs as well as wildlife are a potential source of food to humans. According to Healy (1970), anurans parasites infest humans by eating frogs badly cooked. In fact, at the young stage, the nematodes are found in ectopic sites of the host before settling permanently in their normal infestation sites.

In West Africa, whereas amphibians in Nigeria, Togo and Benin have been studied in detail with regard to parasitic infestations (*e.g.* Durette-Desset and Batcharov 1974; Aisien *et al*., 2003, 2004b; 2009; 2011; Akani *et al*., 2011; Imasuen *et al*. 2012; Aisien *et al*. 2015), comparative studies are poorly known for Côte d'Ivoire. The publications on amphibian parasitology are restricted to two papers relating an infestation of thighs of puddle frogs by larval mites (Spieler & Linsenmair 1999), and an assessment of helminths including nematodes of rocket frogs (Assemian *et al*., 2016).

From the fish farm of the Banco National Park, south-eastern Cote d'Ivoire, the Anuran studies are mainly based on stand (Assemian *et al*., 2006) and on diet and reproduction (Tohé *et al*., 2008a,b; 2015a,b). No data exists on their parasitic Helminths. Thus, this work aims to inventory the parasitic Nematode Anurans and to define their degree of infestations of the park.

MATERIALS AND METHODS

Study zone

The study was carried out from the fish farm of the Banco National Park in south-eastern Ivory Coast (Figure No. 1). Located in the midst of Abidjan, a West African mega-city, this urban forest (05°21′–05°25′N and 04°01′–04°05′W) covers an area of 3474 ha and is characterized by a diversity of micro natural and anthropogenic habitats, including a fish farm with a vast clearing of 2.5 ha (05°18′N, 4°06′W). The fish farm 16 ponds and vegetation composed of epiphytes, bamboos and important grassy field at edge of the forest. It is irrigated permanently by the Banco River and many brooks and is subject to weeding and burning on a quarterly basis. This site is known to harbour 14 species of anurans belonging to eight genera and four families (Tohé *et al*., 2008a).

Sampling of hosts and parasite collection

Data collection was conducted over a one-year period (November 2016 to October 2017). Anurans were mainly located opportunistically during visual surveys. The nocturnal (19h to21h) and diurnal (06h to 08h) surveys were conducted by three people in all available habitats. The search techniques include visual scanning of terrain and refuge examination which consisted of lifting logs and rocks and looking around water bodies. After capture, species were identified according to the published checklist from Assemian *et al*. (2006), and nomenclature follows the taxonomy of Frost (2016). In the laboratory, voucher anurans were euthanized in a 1,1,1-Trichloro-2-methyl-2-propanol hemihydrate solution and thereafter to ensure that eventual endoparasites they hosted were still intact. After thawing, an incision along the medial-ventral line (from the mouth to the anus) removed the digestive tract and its appendages. These organs are esophagus/stomach, lungs, liver/gall bladder, bladder, small intestine and large intestine/cloacae. Each organ was put into a petri dish. A longitudinal section of each organ was made and the internal cavity was rinsed with water. The water collected in each petri dish was examined with a binocular magnifying glass. After careful observation, all nematodes identified were sorted, transferred to pillboxes, and thereafter fixed and stored in 70% ethanol.

Placing and identification of nematode species

These worms were removed with a fine brush and then dropped on a slide in a drop of lactophenol. After a few minutes, the nematode species were observed under an optical microscope. The identification of nematode species was carried out according to the works of Chanseau (1954), Gagno (2006), Iyaji *et al*. (2015), Kuzmin *et al*. (2015).

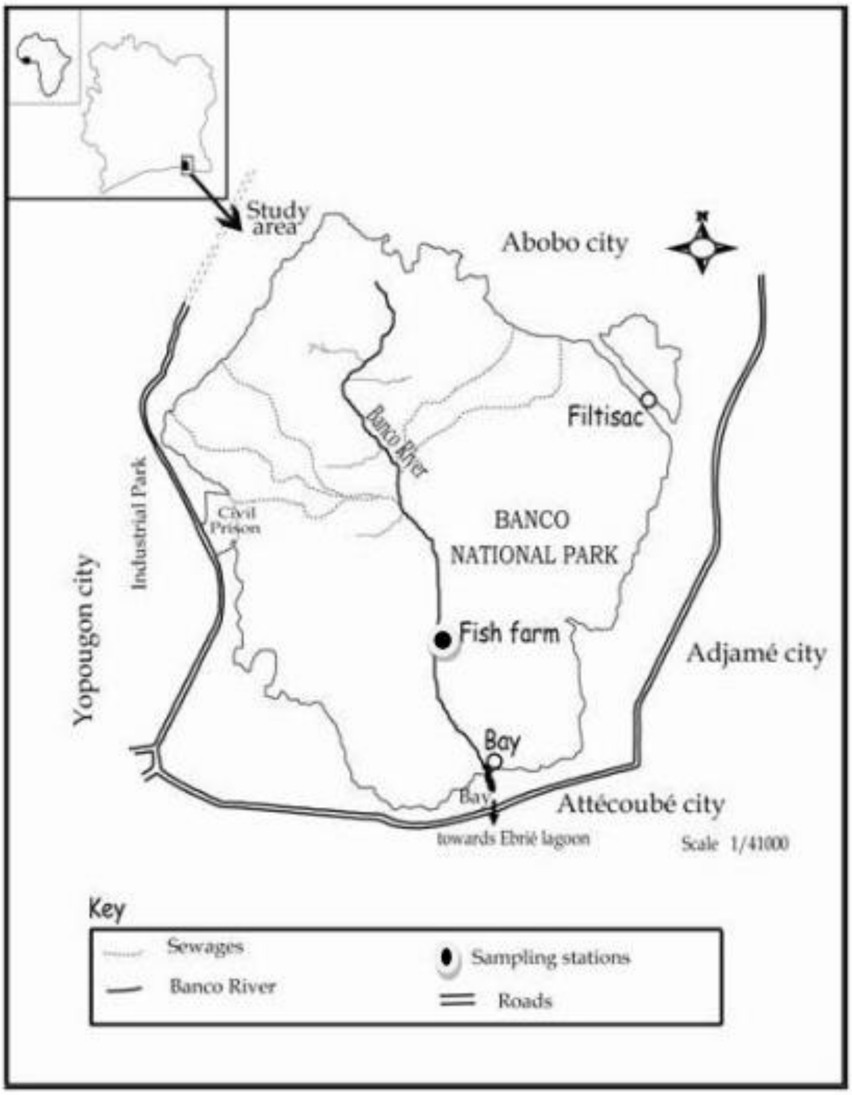

Figure No. 1: Overview of the Banco National Park and the location of the fish farm. From Tohé *et al*. (2014).

Prevalence rate and mean intensity of infection

The prevalence rate and mean intensity of infection were calculated according to Anderson (1993).

Prevalence (P) is the ratio (expressed as a percentage) between the number of hosts infested (n) by a species of parasite and the number of hosts (h) examined.

$$P = \frac{n}{h} \times 100$$

Parasite mean intensity (MI) is the ratio of the number of individuals (p) of parasite species in a sample to the number of infested host individuals (n) in the same sample. It therefore represents the average number of individuals of a parasitic species per parasitized host in the sample.

$$MI = \frac{p}{n}$$

Statistical analysis

One Chi-square test (χ^2) are were used to compare parasite prevalence according to the season. The differences were considered significant at the 5% level. These tests were performed using the statistica 7.1 software.

RESULTS

Species that are infested

A total of 354 anuran specimens belonging to 22 species were examined (Table No. 1). However, 10 species of anuran were infested by parasitic nematodes *Amnirana albolabris* (Hallowell, 1856), *Aubria occidentalis* Perret, 1995, *Hoplobatrachus occipitalis* (Günther, 1859), *Hyperolius guttulatus* (Günther, 1859), *Ptychadena aequiplicata* (Werner, 1898), *P. longirotris* (Peters, 1870), *P. mascareniensis* (Dumeril & Bibron, 1841), *P. pumilio* (Boulenger, 1920), *Sclerophrys maculata* (Hallowell, 1855) and *S. regularis* (Reuss, 1834), while 13 species of anurans were non-infested with a prevalence of 0% (*Afixalus dorsalis* (Peters, 1875), *A. fulvovittatus* (Cope, 1860), *Arthroleptis* sp., *Hyperolius concolor* Rapp, 1842, *H. Fusciventris* Schiøtz, 1967, *Leptopelis spiritusnoctis* Rödel, 2007, *Morerella cyanophthalma* Rödel, Assemian, Kouamé & Tohé, 2009, *Phrynobatrachus latifrons* Ahl, 1924, *Phrynobatrachus liberiensis* (Barbour & Loveridge, 1927), *Phrynobatrachus* sp. and *Ptychadena* sp1., *P* sp2. and *Xenopus mulleri*). The overall prevalence of Nematode infestation of anurans from the fish farm was 68.36%.

Inventory and parasitic specificity of the Nematodes

After dissection, 11 species of parasitic nematodes were identified (*e.g. Amplicaecum africanum* Taylor, 1924, *Anisakis simplex* (Rudolphi, 1809), *Camallanus dimitrovi* Durette-Desset and Batchvarov, 1974, *Chabaudus leberrei* Bain & Phillipon, 1969, *Cosmocerca ornata* Dujardin, 1845, *Filaria* sp., *Oswaldocruzia* sp., *Oxysomatium brevicaudatum* (Zeder, 1800), *Rhabdias bufonis* Schrank, 1788, *Rhabdias* sp.1. and *Rhabdias* sp.2) (Figure No. 2). The infestation sites of nematode species in the host are the esophagus/stomach, lungs, small intestine and large intestine/rectum. However, the intestine remains the preferred site of Nematode infestation. The species (*Amplicaecum africanum*, *Anisakis simplex*, *Camallanus dimitrovi*, *Cosmocerca ornata*, *Filaria* sp, *Oxysomatium brevicaudatum*) parasitize several hosts of different families (euryxenes) and the species (Chabaudus leberrei, Camallanus silurana, Rhabdias bufonis, Rhabdias sp1. and Rhabdias sp2.) are specific to a host (oioxenes). (Table No. 2).

Point of infestation

- *Prevalence and mean intensity of parasitic infections in anurans specimens*

The prevalence and mean intensity of parasitic infection by nematode species are presented in table No. 3. The prevalence of parasites varied from 2.17 to 100%, while the mean intensity was comprised between 1 and 72.2 parasites / infested host. The highest prevalence and mean intensity was recorded in *Sclerophrys maculata*. This species was more parasitized by *Cosmocerca ornata* Dujardin, 1845 with a prevalence of 100% and an infection mean intensity of 89.69. These parasites are also found mostly in eight hosts with high prevalence. These were *Amnirana albolabris* (94.23%), *Aubria occidentalis* (50%), *Ptychadena aequiplicata* (33.33%), *P. longirotris* (34.21%), *P. mascareniensis* (50%), *P pumilio* (44.44%), *Sclerophrys maculata* (100%) and *S. regulalis* (55%). Two-host parasites such as *Rhabdias bufonis* Schrank, 1788 and *Rhabdias* sp.1 had also a high prevalence of *Sclerophrys maculata* (83.33%) and *Amnirana albolabris* (86.54) respectively.

- *Anurans infestation according to the seasons*

The infestation rate is higher in the rainy season than in the dry season. However, the χ^2 comparison test ($p > 0.05$) did not show any significant differences with the seasons. The study of the influence of the season on the degree of Anuran infestation by parasitic Helminths is recorded in Table No. 4.

Table No. 1: Total number of Anuran dissected and parasitic by nematodes from the Banco fish farm

Anuran species	Total number of dissected individuals	Total number of parasite by nematodes
Afrixalus dorsalis	5	0
Afrixalus fulvovittatus	1	0
Amnirana albolabris	104	102
Arthroleptis sp.	1	0
Aubria occidentalis	6	4
Hoplobatrachus occipitalis	18	17
Hyperolius concolor	11	0
Hyperolius fusciventris	1	0
Hyperolius guttulatus	46	10
Leptopelis spiritusnoctis	1	0
Morerella cyanophthalma	1	0
Phrynobatrachus latifrons	2	0
Phrynobatrachus liberiensis	1	0
Phrynobatrachus sp.	1	0
Ptychadena aequiplicata	5	2
Ptychadena longirotris	38	20
Ptychadena mascareniensis	40	34
Ptychadena sp.1	1	0
Ptychadena pumilio	18	13
Ptychadena sp.2	1	0
Sclerophrys masculatus	30	30
Sclerophrys regularis	20	10
Xenopus mulleri	2	0
Totaux 23	**354**	**242**

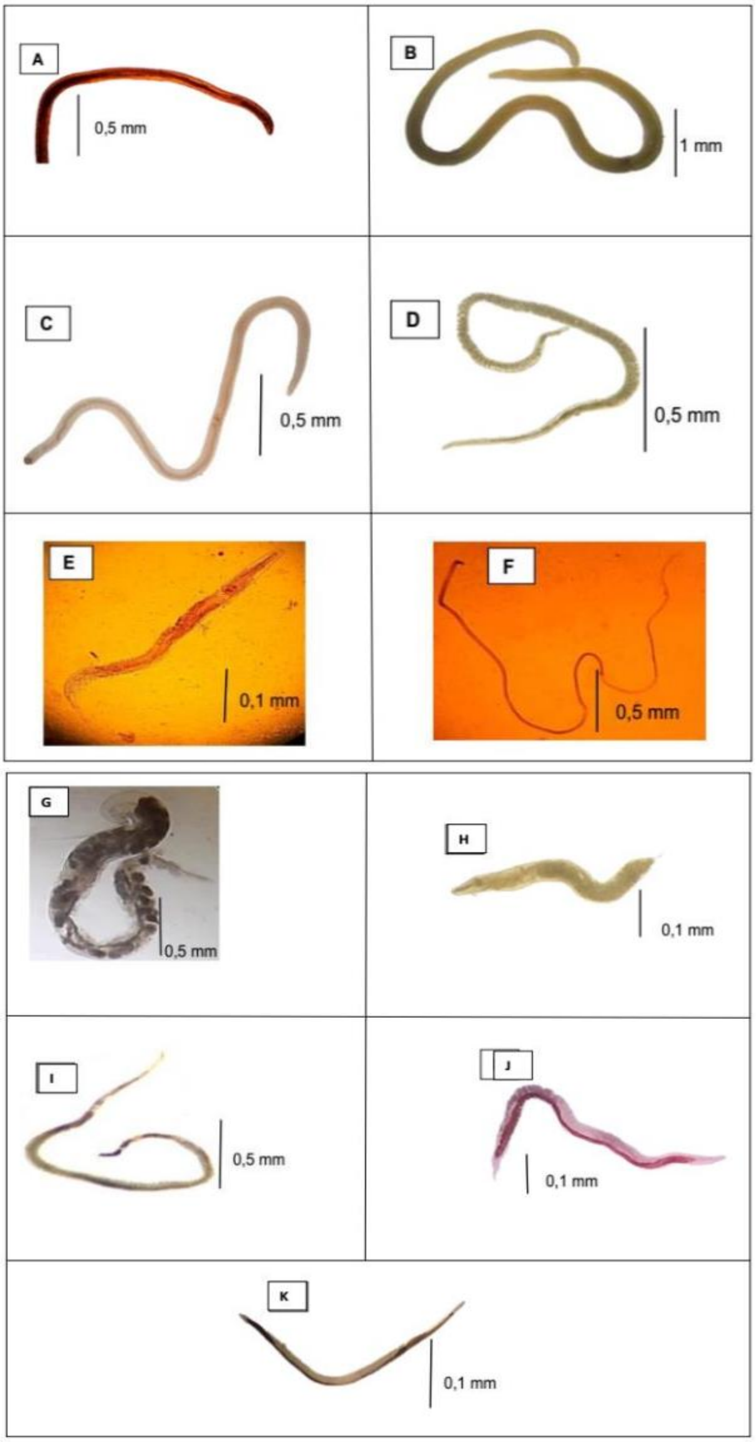

Figure No. 2: Nematodes parasites of Anuran in the fish farm of the Banco National Park

A: *Amplicaecum africanum* Taylor, 1924, **B**: *Anisakis simplex* (Rudolphi, 1809), **C**: *Camallanus dimitrovi* Durette-Desset and Batchvarov, 1974, **D**: *Chabaudus leberrei* Bain and Phillipon, 1969, **E**: *Cosmocerca ornata* Dujardin, 1845, **F**: *Filaria* sp, **G**: *Oswaldocruzia sp,* **H:** *Oxysomatium brevicaudatum* (Zeder, 1800), **I**: *Rhabdias bufonis* Schrank, 1788, **J**: *Rhabdias* sp1. *And* **K**: *Rhabdias sp2.*

Table No. 2: Parasitic nematodes, their host anurans and target organs

Parasitic nematodes	Host anurans	Target organs
Amplicaecum africanum	*Hoplobatrachus occipitalis*	Esophagus / stomach
	Sclerophrys maculata	Esophagus / stomach
	Sclerophrys regularis	Esophagus / stomach
Anisakis simplex	*Hoplobatrachus occipitalis*	Small intestine
	Sclerophrys maculata	Small intestine
Camallanus dimitrovi	*Amnirana albolabris*	Small intestine
	Aubria occidentalis	Small intestine
	Hoplobatrachus occipitalis	Small intestine
	Ptychadena mascareniensis	Small intestine
	Sclerophrys maculata	Small intestine
Chabaudus leberrei	*Hoplobatrachus occipitalis*	Small intestine
Cosmocerca ornate	*Amnirana albolabris*	Small intestine; large intestine / cloacae
	Aubria occidentalis	Small intestine; large intestine / cloacae
	Ptychadena aequiplicata	Small intestine; large intestine / cloacae
	Ptychadena longirotris	Small intestine; large intestine / cloacae
	Ptychadena mascareniensis	Small intestine; large intestine / cloacae
	Ptychadena pumilio	Small intestine; large intestine / cloacae
	Sclerophrys maculata	Small intestine; large intestine / cloacae
	Sclerophrys regularis	Small intestine; large intestine / cloacae
Filaria sp.	*Hoplobatrachus occipitalis*	Small intestine
	Ptychadena mascareniensis	Small intestine
	Ptychadena pumilio	Small intestine
	Sclerophrys regularis	Small intestine
Oswaldocruzia sp.	*Ptychadena longirotris*	Small intestine
Oxysomatium brevicaudatum	*Amnirana albolabris*	Large Intestine / cloacae
	Aubria occidentalis	Large Intestine / cloacae
	Hyperolius guttulatus	Large Intestine / cloacae
	Ptychadena longirotris	Large Intestine / cloacae
	Ptychadena mascareniensis	Large Intestine / cloacae
	Ptychadena pumilio	Large Intestine / cloacae
Rhabdias bufonis	*Sclerophrys maculata*	Lungs
Rhabdias sp1.	*Amnirana albolabris*	Lungs
Rhabdias sp2.	*Ptychadena pumilio*	Lungs

Table No. 3: Prevalence and mean intensity of parasitic infections in anurans specimens

Anurans *(number examined)*	**Parasites** *(number of infested hosts)*	**Prevalence (%)**	**Total number of parasites collected**	**Mean Intensity**
Amnirana albolabris (104)	*Camallanus dimitrovi (18)*	17.3	22	1.22
	Oxysomatium brevicaudatum (3)	2.88	10	3.33
	Cosmocerca ornata (98)	94.23	624	6.37
	Rhabdias sp.1 *(90)*	86.54	287	3.18
Aubria occidentalis (6)	*Camallanus dimitrovi (2)*	33.33	3	1.5
	Oxysomatium brevicaudatum (1)	16.66	8	8
	Cosmocerca ornata (3)	50	16	5.33
Hoplobatrachus occipitalis (18)	*Camallanus dimitrovi (3)*	16.66	10	3.33
	Chabaudus leberrei (3)	16.66	7	2.33
	Filaria sp. *(3)*	16,66	4	1.33
	Anisakis simplex (6)	33.33	176	29.33
	Amplicaecum africanum (5)	27.77	13	2.6
Hyperolius guttulatus (46)	*Oxysomatium brevicaudatum (2)*	4.35	2	1
Ptychadena aequiplicata (6)	*Cosmocerca ornata (2)*	33.33	22	11
Ptychadena longirotris (38)	*Oswaldocruzia* sp. *(5)*	13,15	8	1.6
	Oxysomatium brevicaudatum (2)	5.26	3	1.5
	Cosmocerca ornata (13)	34.21	24	1.85
Ptychadena mascareniensis (40)	*Oxysomatium brevicaudatum (11)*	27.5	228	20.72
	Cosmocerca ornata (20)	50	108	5.4
	Filaria sp. *(3)*	7.5	6	2
	Camallanus dimitrovi (7)	17.5	7	1
Ptychadena pumilio (18)	*Oxysomatium brevicaudatum (7)*	38.89	93	13.28
	Cosmocerca ornata (8)	44.44	25	3.12
	Filaria sp. *(2)*	11.11	12	6
	Rhabdias sp2. *(3)*	16.67	12	4
Sclerophrys maculata (30)	*Cosmocerca ornata (30)*	100	2166	72.2
	Amplicaecum africanum (10)	33.33	220	22
	Anisakis simplex (6)	20	243	40.5
	Camallanus dimitrovi (8)	26.67	24	3
	Rhabdias bufonis (25)	83.33	244	9.76
Sclerophrys regularis (20)	*Cosmocerca ornata (11)*	55	60	5.45
	Amplicaecum africanum (5)	25	18	3.6
	Filaria sp. *(2)*	10	6	3

Table No. 4: Seasonal variation of Anuran infestation rate from November 2017 to December 2018

National Park	Seasons	Number Anurans examined	Number Anurans infested	Infestation rate (%)
Banco	Rainy	181	129	71,2
	Dried	173	113	65,3

DISCUSSION

Compared to other West African areas such as the Pendjari Reserve in Benin (Aisien *et al*., 2011: eight species of anuran parasitic nematodes) and the Delta region in Nigeria (Aisien *et al.*, 2017b: 13 species of anuran parasitic nematodes), the number of nematode species recorded in the Banco National Park is considerable. In Côte d'Ivoire, this is the first time that these species have been reported. However, with the exception of *Rhabdias* sp.1 and *Rhabdias* sp.2, 8 species found in this study have already been identified in other African countries, including Nigeria (*Aisien et al.*, 2001, 2003, 2004, 2009, 2015, 2017a, 2017b, Imasuen *et al.,* 2012).

The overall high prevalence (69.46%) revealed a high parasitic infestation of nematodes of anurans in the fish farm. This important parasitic infestation may be attributable to amphibians microhabitats and the parasitic spécificity. In fact, the 10 infested hosts (e.g. *Amnirana albolabris, Aubria occidentalis, Hoplobatrachus occipitalis, Hyperolius guttulatus, Ptychadena aequiplicata, P. longirotris, P. mascareniensis, P. pumilio, Sclerophrys maculatus* and *S. regularis*) were mostly ground dwelling amphibians and usually need humid habitats (ponds and puddles) to feed and reproduce. They were consequently more exposed to parasites. In contrast, 13 other species of non-infested frogs (e.g. *Afixalus dorsalis, A. fulvovittatus, Arthroleptis* sp. *Hyperolius concolor, H. fusciventris, Leptopelis spiritusnoctis, Morerella cyanophthalma, Phrynobatrachus latifrons, Phrynobatrachus liberiensis, Phrynobatrachus sp., Ptychadena* sp.1 and *P.* sp.2) were arboreal or semi arboreal and most often live on trees, shrubs and grass above water. They were less exposed to nematodes. It is therefore obvious that the habitat has an impact on the parasitic infestation of these anuran species. According to Chabrier (2008), nematodes are generally found in majorities on the mainland and in the mud. In addition, these nematodes parasitize several species of different families "euryxene".

Our results also show that nematodes were is located the small intestine and large intestine/cloacae. Similar findings were already highlighted by several authors (*e.g.* Aisien *et al.,* 2001, 2017; Imasuen *et al.,* 2012). Among the multi-host species, *Cosmocerca ornata* Dujardin, 1845 infested the majority of hosts (eight species of anurans) with high prevalence. Our results corroborate those of Kiran Bala (2016) who noted that *Cosmocerca ornata* specie is now recognized as the common parasite of amphibians. It should also be noted that one host parasites from the genus *Rhabdias* had also a high prevalence. According to Paillot *et al*. (2000), this genus lives as a protandrous hermaphrodite in the lungs and feeds on blood. During the larva release process, it proliferates and migrates into the excrements. On the ground, the filariform grows, some can penetrate the skin of the anurans, migrate to the lungs or they reach their maturity again, others can enter the mollusks or they accumulate and infest the host after consumption, the reason for its high prevalence.

Although the statistical test found no significant difference, the rate of Anuran infestation in the rainy season is relatively higher than in the dry season. This difference would be related to the favorable development conditions of Anoures in rainy seasons. Indeed, in the rainy season, the presence of water in the environments creates favorable conditions for the development of the Anurans (Oungbe *et al.,* 2018). Thus, the increase in the number of host species causes an increase in the number of parasitic Helminth species. This analogy is confirmed by the works of Aisien *et al.* (2011) which records 18 species of Helminths for 14 species of Anurans and Aisien *et al.* (2017b) which records 16 species of Helminths for 17 species of Anurans. Our observations are in agreement with that of Sinsch (1991) and Vallan (2000) who affirm that the permanent presence of water points in a medium is essential for reproduction and conditions their spatial distribution.

CONCLUSION

At the end of the various dissections of the 354 specimens of Anurans on the fish farm of Banco National Park, 11 taxa of parasitic nematodes were inventoried. The overall prevalence (68.36%) shows a large parasitic infestation of Anurans. In fact, the nematodes found for the most part in the small intestine have a broad spectrum of infestation. They parasitize several species of different families "euryxene" and are mostly ground dwelling amphibians and usually need humid habitats (ponds and puddles) to feed and reproduce. The present work has also shown that the rate of Anuran infestation in the rainy season is relatively higher than in the dry season. The presence of water in the environments creates favorable conditions for the development of the Anurans. Thus, the increase in the number of host species causes an increase in the number of parasitic Helminth species.

ACKNOWLEDGEMENTS

The Authors are grateful the Office Ivorian of Parks an Reseves (OIPR) for issuance of collecting and export permits the specimens Anurans (N° 742/MINEDD/OIPR/DG/ora) from the fish farm of the Banco National Park, south-eastern Cote d'Ivoire.

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