NIGERIAN VETERINARY JOURNAL
ISSN 0331-3026
Nig. Vet. J., September 2018 Vol 39 (3): 209 -216.
https://dx.doi.org/10.4314/nvj.v39i3.4 ORIGINAL ARTICLE

An Evaluation of Intestinal Parasites in Edible Frogs (*Hoplobatracus* spp) Sold for Consumption in Zaria, Kaduna State, Nigeria

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SUMMARY

To determine the presence of eggs, oocysts and larvae of intestinal parasites in edible frogs (*Hoplobatrachus* species) sold for human consumption in Hanwa Zaria, Kaduna State, Northwestern Nigeria. Gastrointestinal tract (GIT) samples of edible frogs (n = 225) were collected from the frog market (FM) at Hanwa, Zaria. The samples were analysed for parasites eggs, oocysts and larvae using zinc sulphate-sucrose floatation method of specific gravity 1.21, sedimentation tests followed by microscopy. Parasitic eggs, larvae, and oocyst were found in 54.7% of the total gastrointestinal tract samples using floatation technique while, eggs were found in 40 (17.8%) of the samples using sedimentation technique. The overall prevalence of the parasitic oocyst, eggs or larva using the simple floatation and sedimentation techniques techniques in the examination of the frog was 63.1% (142/225). The presence of parasites in both the male and female frogs were 92 (75.4%) and 50 (48.5%), respectively, there was statistically significant association between gender and parasite prevalence, P = 0.0001. There was no significant association between original location of frogs and prevalence of parasites, but there was statistically significant association between gender and parasite prevalence, p = 0.525. Coccidia oocysts mean counts were highest (8.11 ± 0.423). People should be educated on how to prevent possible zoonotic transmission to humans during capture, slaughter, processing and/or consumption of raw or improperly cooked frogs.

Key words: Frog, *Hoplobatrachus* spp, eggs, oocysts, larvae, Zaria.

INTRODUCTION

Frogs inhabits all types of water bodies and belong to a major group or class of vertebrates called amphibians. Frogs commonly consumed by some people in Nigeria belong to the genus Hoplobatrachus. The Hoplobatrachus spp of frogs are in great abundance and widely distributed in most West African countries (Doris, 1991). A study to determine the proximate Composition and Social Acceptability of Sun-Dried Edible Frog in south west Nigeria reveals that Edible frog (Hoplobatrachus spp) which man has not substantially exploited for food in Nigeria can serve as a competent source of animal protein and

other vital nutrients in human diets (Odutan, 2012)

There is a fast changing trend in eating habits of people towards "unusual foods", resulting in frogs becoming an important food item in many parts of the world. Frog consumption has gained recognition worldwide with many countries engaging in the export and import of frogs (Fletcher and Frahman, 1989, Akinyemi, 2015, Kia et al., 2017). In the absence of frog farming edible frogs may become extinct. The marketing of frogs should be monitored and regulated in Nigeria and Ibadan city in particular as the increase in frog trade puts a lot of constrains on wild frog species (Akinyemi, 2015)

Frogs may harbour or serve as reservoirs for important parasitic diseases some of which may be zoonotic parasites which may be infectious to humans that consume raw or improperly cooked frogs. Frogs are widespread and together with numerous other aquatic vertebrates represent a very important link in the food chain. Amphibians especially frogs are victims of many parasites ranging from protozoans to helminths (Abdulkareem, 1989). Conversely, infections by parasites with a direct life cycle may be magnified in a closed environment (Whitaker, 2016**).** Researchers in other parts of the world have begun documenting parasites of edible frogs, but few of such have been done in Nigeria, mostly in the South western part of the country (Ugbeda, 1995, Penner et al., 2013).

Meat-borne parasitic nematodes and cestodes are prevalent in several regions of the world, and can be sourced from livestocks, wildlife meat, fish, snail and amphibians such as frogs. Frogs may harbour several parasites that may be zoonotic to man (Urquhart, 1999). There is paucity of information on the parasites of edible frogs processed and sold in Nigeria, hence the need for this study to evaluate the presence of helminth parasites in those frogs sold in the area.

MATERIAL AND METHODS

Study area

The research study was carried out in Zaria, Kaduna state Nigeria. Zaria lies in the Northern Guinea Savannah zone, within $11^0$4N, $7^0$42E, a region that has a tropical savannah climate with distinct wet (April-September) and dry (October-March) seasons, with an annual mean rainfall of about 1047.08mm. The dusty, dry, cold hamattan wind is observed between November and January. Zaria is characterized by mainly open woodland vegetation (Kaduna State Government, 2000).

Sample collection

Nine batches, each containing live frogs (n=25), totalling two hundred and twenty-five frogs (*Hoplobatrachus spp*) of both sexes and weighing between 40-170g were collected and used for the survey. Samples were collected between the months of February and March, 2013. The frogs were collected in batches, each representing a location and were labelled accordingly.

Most of the frogs were brought in from neighbouring states i.e Katsina (locations: Maraban danja, Funtua, Mairuwa dam), Kano (location: Kwanar dangora), Kaduna (locations: Birnin gwari, Ikara, Zuntu, River Kaduna) and within Zaria (locations: Napri dam, Shika) to the frog market at Hanwa, Zaria which serves as the processing and distribution centre; it also served as our collection point.

Sample processing and analysis

The frogs were euthanized by means of ice-cold bathing after which the body cavity of the frogs was slit open from the throat to the anus to expose the internal organs (Plate I). The gastrointestinal contents were isolated and an incision was made throughout the entire length of the gastrointestinal tract using scissors and thump forceps. The sexes of *R. spp* were determined after dissection by examination of their reproductive organs. The males have conspicuous testes, while

the females were differentiated by the absence of testes, but the presence of ovaries and/or the presence of eggs in most cases.

The intestinal contents were scraped out with a spatula into a centrifuge tube; the already prepared floatation medium (consisting of zinc sulphate-sucrose) about 4ml was added to it and mixed thoroughly with a spatula. The mixture was filtered into another test tube through a guaze using a funnel. The filtrate in the test tube was topped to the brim with the floatation medium to form a convex meniscus. A clean coverslip was gently placed on the preparation and left for 4 minutes. The coverslip was then placed on a clean glass slide and examined under the microscope for eggs, oocysts and cysts(Urquhart,1999); using a magnification of x10 objective.

Afterwards, the supernatant was decanted leaving the sediments in the test tube, to which water was added and decanted again then the sediment was pipetted and smeared on the glass slide to examine for heavier eggs, cysts or oocysts like those of trematodes and some cestodes.

The bench aid for the diagnosis of intestinal parasites as described by WHO (WHO, 1994) was used for preliminary identification of eggs, cysts and oocysts.

Statistical analysis

The data obtained were summarized into percentages and tables. Prevalence was calculated using numbers with parasite divided by total number of frogs sampled multiplied by 100. Chi-square was used to test for association between sex and location of frogs sampled and, the presence of parasites using Graphpad Prism version 4.0 for Windows. Values of $P < 0.05$ were considered significant.

RESULTS

Parasitic eggs, larvae, and oocyst were found in 54.7% of the total GIT samples using floatation technique while, 40 (17.8%) parasitic eggs were recovered by sedimentation technique. The overall prevalence of the parasitic oocyst, ova or larva using the simple floatation and sedimentation techniques in the examination of the frog was 63.1% (142/225). The parasites isolated/identified had an individual prevalence of the following; Coccidia oocyst (32.4%), Ancylostoma eggs (8.9%), nematode larvae (7.1%), Taenia eggs (3.6%), Hymenolepis eggs (2.7%), Dicrocelium eggs (9.8%) and Fasciola eggs (8.0%). The parasites and the mean parasitic eggs, oocyst or larva obtained from the 225 frogs examined using both techniques were; Coccidia (8.11 ± 0.423), Ancylostoma (2.22 ± 0.49), Nematode larvae (1.778 ± 0.40), Taenia (0.89 ± 0.20), Hymenolepis (0.75 ± 0.25), Dicrocelium (2.44 ± 0.56) and Fasciola (2.00 ± 0.83). (TABLES 1 and 2).

TABLE 1: Mean parasites in frogs from Hanwa frog market in Zaria, based on simple floatation technique

Variable	Coccidia	Ancylostoma	Nematode larvae	Taenia	Hymenolepis
Mean	8.11	2.22	1.778	0.89	0.75
S.E.M*	0.423	0.49	0.40	0.200	0.25

S.E.M* = Standard error of the mean

TABLE 2: Mean parasite ova in frogs from Hanwa frog market in Zaria based on Sedimentation technique

Variable	Dicrocelium	Fasciola
Mean	2.44	2.00
S.E.M*	0.56	0.83

S.E.M* = Standard error of the mean

With the aid of the simple floatation technique, 123 (54.7%) of the frogs sampled were positive for one or more parasitic eggs, oocyst or larva. NAPRI dam had the highest amount of parasites isolated from the frogs sourced from it (72.0%), followed closely by Zuntu (68.0%). There was no significant association ($p > 0.05$) between the detection of parasitic eggs, oocyst or larva and the locations where the frogs were sourced using simple floatation technique (TABLE 3).

On examination of the frogs using sedimentation technique, 40 (17.8%) of the frogs were positive for parasitic eggs of either Dicrocelium or Fasciola or both. Frogs from Napri dam again had the highest infection rate (32.0%) followed closely by frogs obtained from Ikara (28.0%). There were no parasitic eggs seen in frogs sourced from Kwanar dangora using the sedimentation technique. However, there was also no significant association ($p > 0.05$) between the detection of the ova using sedimentation technique and the location where the frogs were sourced (TABLE 3.).

The male frog had an overall prevalence of 75.4%, while the female frog had a prevalence of 48.5%. However, there was a significant association ($p < 0.05$) between the presence or detection of parasitic eggs, oocyst or larva and the sex of frog examined. (TABLE 4).

Furthermore 91.5% of the frogs had a single infection, while 8. 2% had co-infection with two or more parasites. (TABLE 4). All the locations sampled had frogs co-infected with two or more parasites. Plates II, III, IV, V, VI, VII and VIII shows the phtotomicrographs of Coccidia oocyst, Ancyclostoma ova, Nematode larva and the ova od Taenia, Fasciola, Hymenolopis and Dicrocelium ova, respectively.

TABLE 3: Locational distribution of frogs from Hanwa frog market infected with parasites using simple floatation test

Location	Total number Sampled	No Positive (%)
Kwanar Dangora	25	13 (52)
Maraban Danja	25	12(48)
Birnin Gwari	25	13 (52)
Ikara	25	12(48)
Zunta	25	17(68)
River Kaduna	25	14(56)
Mairuwa Dam	25	11(44)
Funtua Dam	25	13 (52)
NAPRI Dam	25	18(72)
Total	225	125(54.7)
$\chi^2 = 7.102$	df = 8	p = 0.525

TABLE 4: Locational distribution of frogs infected by parasites using Sedimentation technique

Location	Total number Sampled	No Positive (%)
Kwanar Dangora	25	0 (0)
Maraban Danja	25	3(12)
Birnin Gwari	25	5 (20)
Ikara	25	7(28)
Zunta	25	5(20)
River Kaduna	25	3(12)
Mairuwa Dam	25	4(16)
Funtua Dam	25	5(20)
NAPRI Dam	25	8 (32)
Total	225	40(17.8)
$\chi^2 = 12.10$	df =8	p = 0.1467

TABLE 5: Association between parasite detection in frogs and the sex of the frog in Hanwa Market

Sex	No Sampled	No positive (%)	Chi-square (χ^2)	df	OR	95% CI lower upper	P value
Male	122	92(75.4)	17.32	1	3.251	1.847 - 5. 720	0.0001
Female	103	50(48.5)					
Total	225	142(63.1)					

TABLE 6: Co-infection of intestinal parasites in frogs sold for consumption in Zaria

S/N	Sample identification	Parasitic co-infection Genera	Source
1	011/2013	*Ancylostoma* ova & *Coccidia* oocyst	Kwanar dangora
2	049/2013	*Coccidian oocyst & Taenia ova*	Maraban danja
3	065/2013	*Taenia ova & nematode larvae*	Birnin gwari
4	080/2013	*Coccidia* oocyst & *nematode larvae*	Ikara
5	108/2013	*Ancylostoma* ova & *nematode larvae*	Zuntu
6	117/2013	*Coccidia* oocyst & *nematode larvae*	Zuntu
7	122/2013	*Ancylostoma* ova & *Coccidia* oocyst	Zuntu
8	182/2013	*Coccidia* oocyst & *nematode larvae*	Funtua dam
9	198/2013	*Ancylostoma* ova & *Coccidia* oocyst	Funtua dam
1	202/2013	*Ancylostoma* ova & *Coccidia* oocyst	NAPRI dam
1	206/2013	*Coccidia* oocyst & *Nematode larva*	NAPRI dam
1	221/2013	*Coccidia* oocyst, & *Hymenolepis ova*	NAPRI dam

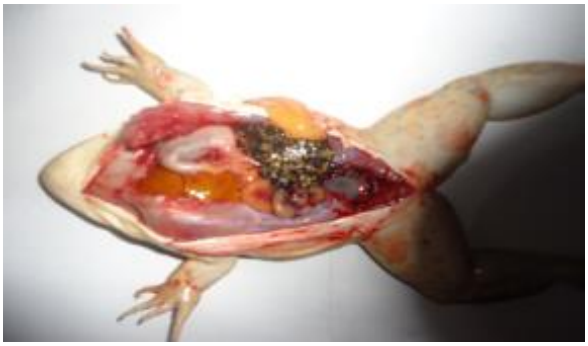

PLATE I: Dissected frog sampled from Hanwa frog Market in Zaria, Kaduna State

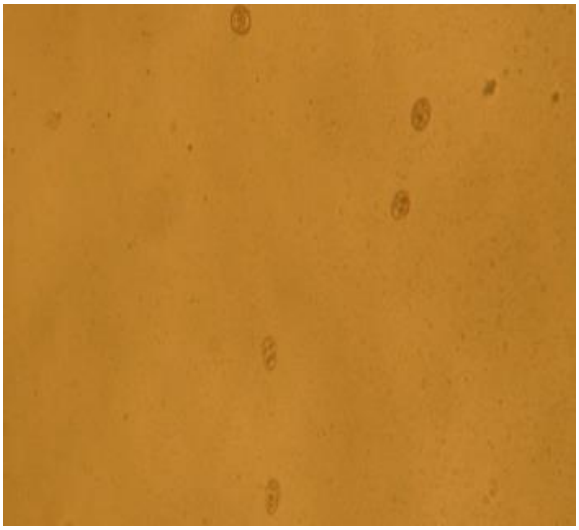

PLATE II: Photomicrograph of *Coccidia Oocysts* from an infected Frog (X 10)

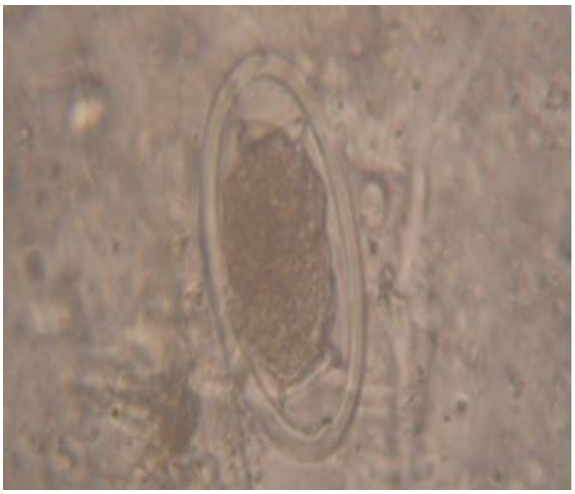

PLATE III: Photomicrograph of *Ancylostoma Ova* from an infected Frog (X 10)

PLATE IV: Photomicrograph of *Nematode Larva* from an infected Frog (X10)

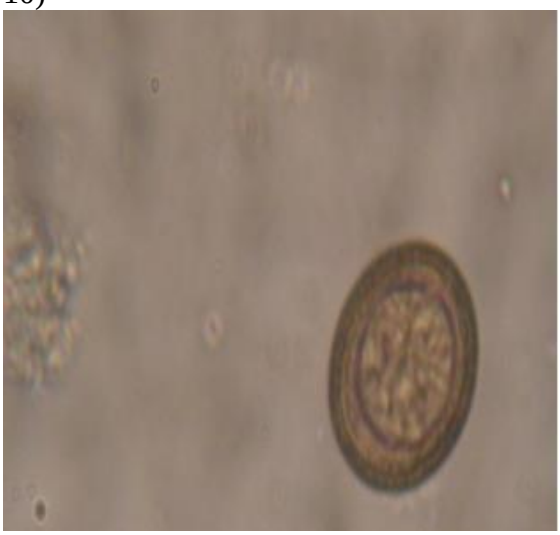

PLATE V: Photomicrograph of *Taenia ova* from an infected Frog (x 10)

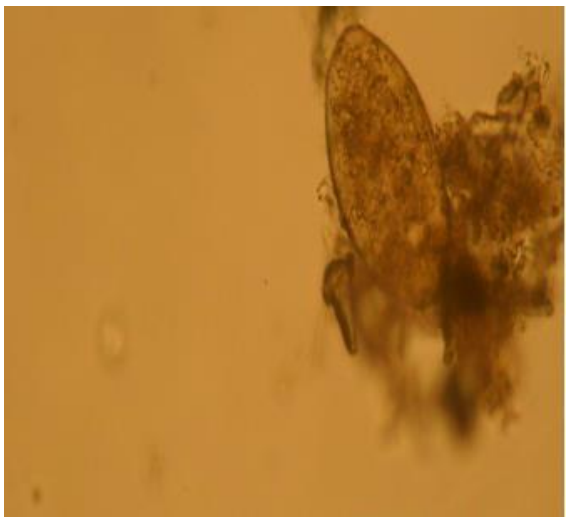

PLATE VI: Photomicrograph of *Fasciola Ova* from an infected Frog (X 10)

PLATE VII: Photomicrograph of *Hymenolepis* Ova from an infected Frog (X 10)

PLATE VIII: Photomicrograph of *Dicrocelium Ova* from an infected Frog (X10)**.**

DISCUSSION

Hanwa located within Sabon gari Local Government Area in Zaria northern Nigeria, frogs are processed, sold to interested community buyers but majority of the frogs are packed and transported to states in the eastern, western and southern part of the Nigeria, where there is insufficiency of frog meat due to high demand by consumers. This is the first study to demonstrate the presence of parasitic eggs, oocysts and larvae in frogs sold for human consumption in northern Nigeria

The study revealed a high prevalence (64.1%) of parasites in the frogs sampled in Zaria. This finding is similar to that reported by Aboluwarin (2012) in Abeokuta, Ogun State (southern Nigeria), who reported a high prevalence. This indicates high level of contamination of these frogs, as they may serve as a means of transmission of zoonotic parasites to individuals consuming raw or improperly cooked frogs.

The intestinal parasites found in this study include: Ancylostoma, Nematode larvae, Taenia, Hymenolepis, Fasciola and Coccidia. Similar parasites were observed by Yakubu (2013) and Aboluwarin (2012) in Nigeria. There is the possibility that coccidia detected from a frog cannot infect humans since coccidia are species specific (Aboluwarin,2012). However, Guzman *et al.* (2007) in Mexico reported different intestinal parasites in frogs. This may be due to differences in geographic location and climatic conditions that may favor intestinal parasite infection.

Frogs may serve as host of many parasites ranging from protozoan parasites to parasitic helminths like flatworm, tapeworm, roundworm, and *Acanthocephalan*. The size or weight of the eggs, oocyst or larva determines the technique for their detection. A higher prevalence of intestinal parasites was observed in male frogs than female frogs. This report agrees with that of Aboluwarin (2012) who also reported a higher prevalence of intestinal parasites in male frogs. This high prevalence in males may be due to their increased activity compared to the females. It may also be due to the fact that they are more malnourished in the dry season as a result of their increased activity, thus making them more susceptible to heavy or higher parasitic burden (Tyler, 1976).

There was no significant association between the detection of the intestinal parasites and the various locations where the frogs were sourced. This indicates that the various sources were contaminated with parasites which may infect frogs in those locations, thus making the frogs' sources of food–borne products contaminated with parasites to man and other animals that consume them. The result from the study has established that frogs processed and/or sold of which some may be zoonotic and, may serve as source of infection to humans that consume raw or improperly cooked frogs due to changing eating habits of man. Since these frogs are found on the tables in countries of developing world as well as developed, as consequence of the shifting of food habits towards 'unusual foods' or to increase animal protein intake.

Results from this study suggest the need for proper protection and strict hygienic practices when handling and processing frogs. It is also important that meat from frogs should be properly cooked before consumption to prevent the transmission of meat-borne diseases. There may be need for large-scale frog farming and formalization of the industry so that their activities may be known and monitored by stake-holders.

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