Gut digeneaiasis in African catfish *Clarias gariepinus* with estimating the efficacy of some anthelmintics

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Abstract: A total number of 200 fish (50 fish in each season) were collected randomly and examined for presence of digenea. Two species were recovered and identified as *Eumasenia aegypticus* and *Orientocreadium bactrachoid* with infestation rate of 13 % (26 out of 200) and 19.5 % (39 out of 200) respectively. Seasonally, the highest prevalence of digenea occured in autumn, spring, winter and the lowest prevalence in summer. The histopathological alterations were manifested bypresence of degenerative changes, sloughing and atrophy of gastric villi of glandular stomach and presence of degenerative changes in the lining intestinal mucosa with goblet cell proliferation. Besides, our results indicated the in vitro efficacyof both praziquantel and triclabendazole against *O.bactrachoid*.

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1.Introduction:

Fish is one of the most important sources of proteins available for humans and animals. In Egypt, parasitic diseases represent about 80% of fish diseases **(Eissa, 2002)**. Parasites reduce fish production by affecting the normal physiology of fish **(Imam and Dewu,2010)** and can result in mass mortalities of fish, or in some cases infection of manand other invertebrates that consume them **(Woo, 2006,Noga, 2010 ; Lima dos Santos and Howgate, 2011)**. However, there are few reports of parasites causing mortality or serious damage toferal fish populations, but this may be largely because such effects go unnoticed. Parasites in wild fish are usually onlyremarked upon when they are so obvious as to lead to rejection of fish by fishermen or consumers **(Roberts, 2012)**.

The present study was designed to investigate the prevalent diseases caused by digenea in wild African catfish *Clarias gariepinus*. Besides, clinical picture, determination of total and seasonal prevalence, histopathological alterations and estimating the invitro efficacy of praziquantel and triclabendazole against *Orientocreadium bactrachoid* were tried.

2.Materials and Methods:

Fish:

A total number of 200 of alive African Catfish *Clarias gariepinus* ranged between 45 to 315 g (body weight) and from 18 to 39 cm (total length) were collected randomly from river Nile at Kafr El-Sheikh Governorate during 2011 as 50 fish seasonally.Fish were kept in fully prepared glass aquaria, supplied with chlorine free tap water , continuous aeration and filtration according to **Innes (1966)**.

Clinical picture:

Alive fish were examined for clinical signsand postmortem lesions using the methods described by **Lucky (1977)**.

Parasitological examination:

The stomach wasseparated from the intestine and each examined separately.Stomach was opened andintestinal mucosa was stripped off by scalpel and washed with normal saline. Flukes were collected and preserved inalcohol formalin acetic acid and stained with Semichon'sacetocarmine stain . The whole mount of collected trematodes was done according to **Woodland (2006)**. They were identified according to the identification key of **Yamaguti (1958)**.

Histopathological examination:

The histopathological examinations of affected tissue (intestines and stomach) were performed as described by **Drury and Wallington (1980)**.

Drugs:

Distocide® tablet each one contains, praziquantel 600 mg, manufactured by Egyptian International Pharmaceutical Industries CO.Egypt. under license of SHIN POONG Pharm. Co., LTD. Seoul, Korea.

Fasinex®10% triclabendazole suspension. Novartis Animal Health CO.

In vitro efficacy of Praziquantel and Triclabendazole against *Orientocreadium bactrachoid*:

100 ml of RPMI mediumwas supplied with 2 mg ceftriaxone to achieve a final concentration of 20 µg / 1 ml, then 25 µg amphotericin B were

added to achieve a final concentration of 0.25 µg/1 ml media as described by **Kotzen*et al*. (2004)**.

600 mg praziquantel tablet was finely ground in clean dry mortar and dissolved in 187.5 ml DMSO to achieve final concentration of 3.2 µg / 1 µl of the solution. Standard solution of praziquantel was made by addition of 10 µl of praziquantel in DMSO to 9990 µl of prepared RPMI media to achieve final concentration of(3.2 µg/ 1 ml) of the standard solution. Double fold serial dilutions were made to achieve the following concentrations of praziquantel (3.2, 1.6, 0.8, 0.4, 0.2, 0.1, 0.05, 0.025, 0.0125, and 0.00625 µg / ml) as described by **Smout*et al.* (2010).**

Triclabendazole 10 % suspensionswere well shaked and 320 µl were dissolved in 680 µl DMSO to achieve final concentration of 32 µg / 1 µl of the solution. Standard solution of triclabendazole was made by addition of 10 µl of triclabendazolein DMSO to 9990 µl of prepared RPMI media to achieve final concentration of (32) µg triclabendazole/ 1 ml of the standard solution. Double fold serial dilution was made to achieve the following concentrations of triclabendazole (32, 16, 8, 4, 2, 1, 0.5, 0.25, 0.125, and 0.0625 µg / ml) as described by **Smout*et al.* (2010).**

Orientocreadium bactrachoid were collected and washed using prepared RPMI media, and in 3 clean dry Petri dishes were divided into 4 quarters. In each quarter, five actively motile flukes were added. 100 µl of the first concentration of praziquantel was added to first quarter and 100 µl of the second concentration was added to second quarter and so on for the remaining concentrations, the last 2 quarters remainedas control (with media only). The same was performed in other three clean dry petri dishes except it was inoculated with the different dilutions of triclabendazole. All petri dishes were observed microscopically for any changes occur in the fluke shape or motility with the time.

3.Results and Discussion:

In the present study, the clinical signs of the infested catfish were manifested as weakness, severe emaciation, imbalanced swimming, some fish showed sluggish movement, loss of condition with paler coloration. These clinical signs were nearly agree with that described by **Eissa (2002)**, **Nadia Ali (2007)** and **Dalia Sabri*et al.* (2010)**.

*Eumasenia aegypticus*was 1.5 - 2 mm in body length and 0.7 mm in body width. The widest region of body present in the middle part of the worm. Oral sucker is large and funnel shaped, wide, surrounded by double row of alternating spines interrupted dorsally. The ventral sucker is rounded and larger than oral sucker and situated at the end of the anterior third of the worm. Cirrus pouch is S shaped, more wide in its middle part and extending to level of posterior margin of ventral sucker. Eggs are small, oval golden yellow and numerous, occupy from the last third to the last half of the worm (plate,1). These descriptions were identical to that given by **Burton (1962)** and **Olfat Mahdy *et al.* (1994)**. *E aegypticus* was isolated from glandular stomach of infested *C gariepinus*. The dominant PM lesions of infested fish were presence of congestion in the stomach (plate,1a) that may be caused by the parasite's large oral sucker which surrounded by double row of alternating spines interrupted dorsally leading to destruction of gastric mucosa. The same results were given by **Eissa *et al.* (2011)** who recorded presence of slight bulging of the stomach, congestion and haemorrhage on the mucous membrane with watery food especially in heavily infested cases.

Orientocreadium bactrachoid were extremely mobile and capable of great elongation and contraction. Its dimensions were 2.5 - 3 mm in body length and 0.5 - 0.7 mm in body width. The body was elongate, rounded anteriorly, slightly tapering posteriorly. Ventral sucker, almost equal in size to oral sucker and situated at one third of body length. Cirrus pouch is elongate and round present beside the ventral sucker. Eggs are small, yellow filling the posterior part of the worm (plate,1 d).These findings were similar to the descriptions given by **Burton (1962)**, **Olfat Mahdy *et al.* (1994)**, and **Eissa *et al.* (2011)**.It was isolated from the intestine of infested fish. The dominant post mortem lesion of heavily infested fish was presence of congestion (plate, 1b). Nearly similar results described by **Amal Atwa (2006)** and **Eissa *et al.* (2011)** who recorded presence of enteritis, haemorrhage and ulceration of intestinal mucous membrane. This congestion is mainly attributed to the effect of the parasite attachment by its oral and ventral sucker to the intestinal mucosa.

The present study revealed that 65 out of 200 examined *Clarias gariepinus* were infested with flukes in a total prevalence of 32.5 % as shown in table (1), The number of infested fish was 25, 4, 26 and 10 with a seasonal prevalence of 50, 8, 52 and 20% in spring, summer, autumn and winter respectively. Regarding the highest seasonal prevalence of trematodes, it was shown in autumn, spring, winter and the lowest occurred in summer. There was a great variation between the results describing the seasonal prevalence of trematodes infestation as **Mona Khattab (1990)** who found that the peak was during summer and the lowest in spring, **Amal Atwa (2006)**who found that the peak was in winter and the lowest in spring, **Eissa *et al.* (2010 b)**whofound that the peak in spring and the lowest in winter. These variations may be attributed to the difference in study areas. Nearly similar results were met by **El-Seify *et al.* (1997)** who found the highest seasonal prevalence of trematodes occurred in autumn and the lowest in winter.

The seasonal prevalences of *E aegypticus*were 30, 16, 4 and 2 % in spring, autumn, summer and winter respectively.While, in *O bactrachoid* were 36, 20, 18 and 4 % in autumn, spring, winter and summer respectively as shown in Table (2).

Our results indicated that use of praziquantel and triclabendazole against *Obactrachoid* in concentration of 0.2 and 8 µg / ml respectively were effective. *O bactrachoid* showed body deformity with swelling of oral and ventral suckers after treatment with both drugs. The fluke died after 75 and 60 minutes respectively. **Kim and Cho (2000)**, **Kim *et al.* (2001)** and **Williams (2009)** indicated the efficacy of praziquantel in treatment of monogenean infestation in fish. Also, **Sharp *et al.* (2004)** recorded that praziquantel is a parasiticide which is effective against tapeworms, monogeneans, and trematodes. Moreover, the present findings were in harmony with those described by **Tojo and Santamarina (1998 a)** who recorded the efficacy of triclabendazole in treatment of infested rainbow trout *Oncorhynchus mykiss* with gyrodactylosis and **Tojo *et al.* (1992)** who recorded the invitro efficacy of triclabendazole against gyrodactylosis. In addition, **Athanassopoulou*et al.* (2009)** recorded that triclabendazole is effective in treatment of monogenean infections in fish. From the economical view,praziquantel is more preferable to be used against such infestation not only due to the low dose required but also for its low price in comparison with triclabendazole.

Histopathologically, *O bactrachoid* in glandular stomach of infested fish revealed presence of degenerative changes, sloughing and atrophy of gastric villi with absence of gastric gland plate 2(a) and presence of lymphocytic infiltration (plate 2b). These alterations may be due to presence of large oral sucker which surrounded by double row of alternating spines that interrupted dorsally as that described by **Burton (1962)** which leading to destruction of gastric mucosa. In case of *E aegypticus*, the infested fish intestine revealed presence of degenerative changes of the lining intestinal mucosa with goblet cell proliferation associated with massive submucosal lymphocytic infiltration (plate 2c). These descriptions were similar to that given by **Eissa *et al.* (2010 b)**

Table (1): Showing the total prevalence of trematodiasis in *Clariasgariepinus*.

Parasite	No. of examined fish	Total number of infested fish	%
Eumasenia aegypticus	200	26	13
Orientocreadm bactrachoid		39	19.5
Total	200	65	32.5

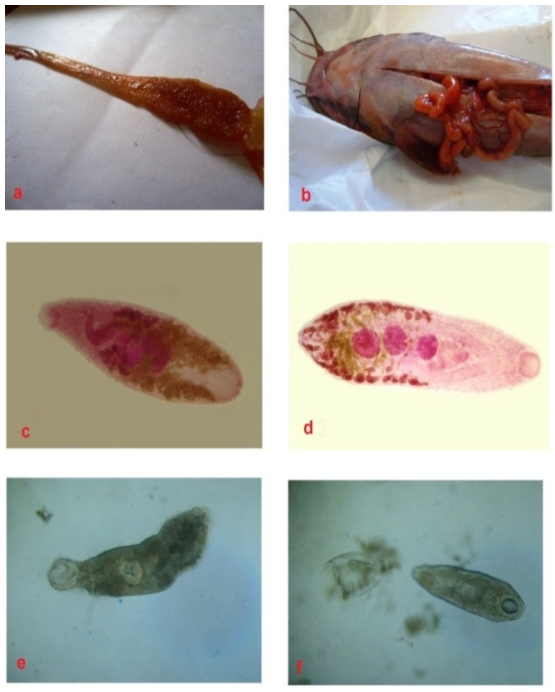

Plate 1

a. Congested stomach of infested *C. gariepinus* with *Eumasenia aegypticus.*
b. Congested intestine of infested *C. gariepinus* with *Orientocreadium bactrachoid*.
c. *Eumasenia aegypticus* isolated from glandular stomach of *C. gariepinus*. (Semichon's acid carmine stain, X = 160).
d. *Orientocreadium bactrachoid* isolated from intestine of *C. gariepinus*. (Semichon's acid carmine stain, X = 110).
e. *Orientocreadium bactrachoid* showing body deformity with swilling of oral and ventral sucker after treatment with praziquantel 3.2 µg / ml. (X = 200)
f. *Orientocreadium bactrachoid* showing swilling of oral sucker after treatment with triclabendazole 32 µg / ml. (X = 200).

Table (2): Showing the seasonal prevalence of trematodes in *Clariasgariepinus*.

Parasite / Season	No.	*Eumasenia aegypticus*		*Orientocreadium bactrachoid*		Total	%
		No. of infested fish	%	No. of infested fish	%		
Spring	50	15	30	10	20	25	50
Summer	50	2	4	2	4	4	8
Autumn	50	8	16	18	36	26	52
Winter	50	1	2	9	18	10	20
Total	200	26	13	39	19.5	65	32.5

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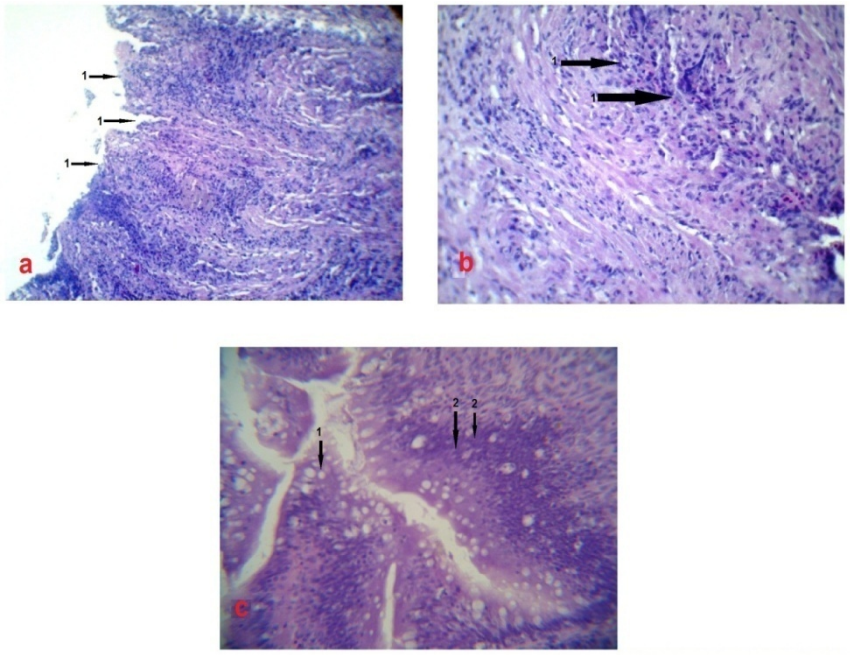

Plate 2

a. Glandular stomach of *Clarias gariepinus* infested with *Eumasenia aegypticus*showing sloughing, atrophy of gastric villi with absence of gastric gland (1). (H & E, X = 400).

b. Glandular stomach of *Clarias gariepinus* infested with *Eumasenia aegypticus*showing obliteration of gastric gland with presence of lymphocytic infiltration (1). (H & E, X = 800).

c. Intestine of *Clarias gariepinus* infested with, *Orientocreadium bactrachoid* showing degenerative changes with goblet cell (1) proliferation of the lining mucosa associated with massive sub mucosal lymphocytic infiltration (2). (H & E, X = 800).

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