Assiut Vet. Med. J. Vol. 59 No. 139 October 2013

CRYPTOSPORIDIUM AND OTHER ZOONOTIC PARASITES IN *OREOCHROMIS NILOTICUS* IN ASSIUT GOVERNORATE

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Received at: 30/9/2013

Accepted: 25/11/2013

ABSTRACT

This study was carried out on 120 *Oreochromis niloticus* fish (60 wild and 60 cultured). The fish were randomly collected, from different areas of Assiut Governorate as well as from some fish farms. They were used to investigate the prevalence of *Cryptosporidium* and other zoonotic parasites in *O. niloticus*. Examination of gills, muscles and the contents of the gastrointestinal tract revealed highest parasitic infestation rate (80 %) in wild compared to (55 %) in cultured *O. niloticus*. The prevalence of the isolated parasites in wild and cultured *O. niloticus* was as follows: *Cryptosporidium spp*. 15.0 % and 23.3% respectively; Acanthocephela *(Acanthocentius tilapae*) 8.3 % and 9.2% respectively; microscopic encysted metacercariae 88.3% and 26.7 % respectively while macroscopic encysted metacercariae detected only in wild *O. niloticus* 40%. Killing factors (temperatures, solutions and processing methods) were studied on encysted metacercariae (EMC) in *O. niloticus* killing criteria followed the movability index (MI) from 1.000 within 24 hours. Muscle pieces containing viable EMC were incubated in different concentrations of NaCl, acetic acid or commercial vinegar at room temperature. The MI was ≤1 (killing effect) within 1, 2 hours for 30% and 20% of NaCl respectively and 1, 6 and12 hours for 5, 2.5 and 1.25%of acetic acid, respectively while commercial vinegar has MI ≥1 (no killing effect) at 5%.Storage of *O. niloticus* at (-10℃) resulted in killing the EMC after 12 hours of storage while storage at 5 ˚C for 24 hours has no killing effect. Dry salting of *O. niloticus* could kill the EMC within one hour of salt contact, while marination in 5 % acetic acid for one minute resulted in eradication of EMC after 2 hours of treatment with barely detected acetic acid odor and significant reduction in muscle pH ($P < 0.05$.).

Keywords: *Cryptosporidium, zoonotic parasites, fish, Killing factors, processing methods, encysted metacercariae (EMC).*

INTRODUCTION

Fish meat demand as human food is increasing especially in the developing countries where people income is low. World fish production has grown tremendously in the second half of the 20th century (Pillay, 1990). Egyptian per capita fish consumption is more than doubled from below 7kg in 1990 to over 14 kg in 2002, (Suloma and Ogata, 2006). Tilapia is the predominant aqua-cultured finfish in Egypt and represented 14.5% of the coounty's total fish landing and is a componant of traditional Egyptian diet (Green *et al*., 2002).

Salted, salt fulminated, smoked and fried fish are the favorite traditional fish dishes on the Egyptian people. The effect of some traditional processing methods on EMC is remained uncertain. Eating the EMC in row or under processed fish constitute a zoontic risk (Huss *et al.,* 2003).

A multitude of parasites have been reported in fish, but only a few species are capable of infecting human (Adams *et al*., 1997). Recent reports suggest that fishes act as a source of serious human parasitic infectious diseases (EFSA, 2010). Fish borne parasites infecting human results from food habits of the people by consuming raw, partially cooked, slightly salted or smoked fish (Abd El-Maksoud, 1992). Members of the genus *Cryptosporidium* are parasites of the intestinal tracts of fishes, reptiles, birds, and mammals. People may be infected by consuming contaminated water or food. Cryptosporidiosis is an emerging disease in both wild and farmed fish in numerous countries worldwide (Sitja-Bobadilla *et al*., 2005). Relatively little is known about the prevalence and geographic distribution of *Cryptosporidium* isolates that infect fish in Egypt. Heat inactivation of parasites proved effective for eliminating the risk of parasitosis but trematodes possessed a higher heat resistance (Hus *et al*., 2003). So far fish processors need alternative

easy, fast, cheap and effective measures for eradication of EMC. However the appropriate inactivation technologies in freshwater fish are not well established. Therefore the present study aimed to:

- Document the prevalence of *Cryptosporidium* and other zoonotic parasites in one of the most common edible fishes (*Oreochromis niloticus*) that might cause public health problems, if these fishes were eaten improperly cooked.
- Investigate the effect of some killing factors: temperatures, solutions and processing methods on encysted metacercariae (EMC).

MATERIAL and METHODS

1- Samples:
A total number of 120 specimens of *O. niloticus* (60 farmed fish & 60 wild fish) were randomly collected from different localities of the River Nile and from some fish farms at Assiut Governorate.

2- Parasitological examination:

(A) Macroscopic examination:
Macroscopic examination was carried out either by naked eye or by the magnifying hand lens (2 X and 4X) for detection of encysted metacercariae (EMC) in musculature and gills (Mahdy *et al.*, 1995).

(B) Microscopic examination:
Muscles were screened for the presence of EMC by compression method in which snips were taken from muscles of each part of fish body (head, trunk and tail). Each piece was compressed between two microscopic glass slides and examined microscopically for the presence of EMC (Sayasone *et al.,* 2007).

Metacercariae were identified to genus level based on the morphological details, their dimensions, shape of cysts, site of infection, and shape and contents of excretory bladder under light microscopy (Amer, 1996; Sohn *et al.,* 2005 and Elsheikha and Elshazly, 2008).

The stomachs and intestines were dissected and placed in separate Petri dishes with physiological saline solution, and washed by saline solution. Mucosal scrapings were collected and centrifuged for concentration of oocysts (Waldman *et al*., 1986). Smears were done from the sediment and stained by a modified Zeil- Neolsen acid-fast (Henriksen and Pohlenz, 1981).

The collected *Acanthocephela* were first washed in saline solution, refrigerated in cold water for 12 hours to protrude its proboscis, were relaxed and fixed in AFA (Alcohol formalin acetic acid), dehydrated and mounted (Pritchard and Kruse, 1982).

3- Studying the effect of sodium chloride (NaCl), acetic acid and vinegar on encysted metacercariae in *O. niloticus* muscle pieces:

Ten pieces (about 1 cm^3 each) of heavily infected fish muscles containing active encysted metacercariae were soaked in a 20ml solution of (40,20,10,5,2.5, 1.25 and 0.3 %) concentrations of acetic acid, (40,20,10,5,2.5, 1.25 and 0.3 %) Na Cl or (5, 2.5, 1.25 and 0.3 %) vinegar, individually in a plastic plate. Infected fish muscle pieces containing active encysted metacercariae were soaked in 20ml distilled water as a control. For each concentration and control the muscle pieces were examined at 0,1,2, 4,6, 12, 18 and 24h of soaking and transferred to another plate contains 20ml of 0.89% Na Cl and examined for metacercariae movement using light microscope. The assessment of the effect of treatment was done using the movability index (MI) recommended by (Prawang, 2001) and the killing criterion described by (Wongsawad *et al*., 2005). Since MI value $\leq$ 1.0 within 24h refers to the death while MI > 1.0 refers to the survival of the metacercariae.

4- Studying the effect of storage temperature or different processing methods on EMC:
Freshly caught *O. niloticus* (50 -70gm each) were examined for presence of active EMC. A sufficient number of highly infected fish were eviscerated, washed and divided into the following groups:

GA were put in tightly closed plastic bags and subdivided into GA1 (were refrigerated at 5˚C) and GA2 (were frozen at -10 ˚C). GB were soaked in acetic acid 5% for one minute at room temerature. GC were dry salted at room temperature using NaCl (NaCl/fish =3/10gm). GD was control group. The assessment of the effect of different factors on EMC was done by reporting the MI at 0, 1, 2, 4, 6, 12, 18 and 24h according to (Prawang, 2001) and (Wongswad, 2005).

5- Evaluation of the effect processing fish with acetic acid on the chemical and sensory quality of processed fish:(Sallam, 2007)

(A)- pH measurement: :(Sallam, 2007**)**

Ten grams of each sample were blended with 20 ml distilled water in a blender for 30 s and pH value of fish homogenate was measured by a digital pH-meter (Gallenhamp No.101284) standardized at pH 4 and 7.

(B)- Sensory assessment: (Sallam, 2007)
Fish samples soaked in acetic acid for one minute as well as control samples were separately wrapped with aluminum foil, cooked in steaming pot until the core temperature of each sample reached 70 ċ. stick water was drained and allowed to cool to room temperature (25-28 ċ).The acceptability of cooked samples was

evaluated by 5 semi-trained panelists.

Panelists were asked to evaluate the overall acceptability with regard to appearance, odour intensity, flavour and aftertaste, tenderness, juiciness, off-odour, and off-flavour according eight-point hedonic scoring scale. Samples receiving overall scores of more than 4 were considered acceptable, while a score between 3 and 4 was considered the borderline of acceptability. The packaged SPSS program for windows version 12.0.1 was used for statistical analysis according to (SPSS 2007). Data were expressed as mean ± standard error (SE). Differences between groups were determined by means of a Student "t"-test. Significance level was set at $P < 0.05$.

RESULTS

Table 1: Prevalence of different zoonotic parasites in examined fish.

Source of fish	Ex. fish	Inf. fish	%	*Cryptosporidium*		*Acanthocephalus*		Encysted metacercariae: Macroscopic m. (Gills)		Encysted metacercariae: Microscopic m. (Muscles)		Total infection	
				Inf. No	%	Inf. No	%	Inf. No	%	Inf. No	%	Inf. No.	%
Farmed Fish	60	33	55.0	14	23.3	6	10.0	0	0	16	26.7	16	26.7
Wild fish	60	48	80.0	9	15.0	5	8.3	24	40.0	53	88.3	59	98.3
Total	120	81	67.5	23	19.2	11	9.2	24	20.0	69	57.5	75	62.5

Table 2: Prevalence of different metacercariae detected in examined fish.

Source of fish	Infected Fishes: Total Macroscopic meta.		Total Microscopic meta.		Mixed infection		Single Macroscopic meta .		Single Microscopic meta.		Total infection	
	infected No.	%	infected No.	%	infected No.	%	infected No.	%	infected No.	%	infected No.	%
Farmed Fish	0	0	16	26.7	0	0	0	0	16	26.7	16	26.7
Wild fish	24	40.0	53	88.3	18	30.0	6	10.0	35	58.3	59	98.3
Total	24	20.0	69	57.5	18	15.0	6	5.0	51	42.5	75	62.5

Table 3: Prevalence of different species of metacercariae in examined fish.

Types of metacercariae	Farmed fish		Wild fish		Total	
	N0. of Inf. fish	%	N0. of Inf. fish	%	N0. of Inf. fish	%
Microscopic m:	16	26.7	53	88.3	69	57.5
1-*Prohemistomum spp.m*	9	15.0	33	55.0	42	35.0
2- *Haplorchis spp.m*	0	0	3	5.0	3	2.5
3- *Diplostomum spp*.m	7	11.7	17	28.3	24	20.0
Macroscopic m:	0	0	24	40.0	24	20.0
1-*Clinostomum*	0	0	23	38.3	23	19.2
phalacrocoracis	0	0	1	1.7	1	0.8
2-*Clinostomum tilapiae*						

Table 4: Effect of chemicals on movability index (MI) of ECM.

Parameter	Acetic acid		Sodium chloride		Vinegar	
	Conc	Duration in hours	Conc.	Duration in hours	Conc	Duration in hours
MI≤1	5%	1	30%	1	-	-
	2.5%	6	20%	2	-	-
	1.25%	12	-	-		
MI≥1	0.6%	24	10%	24	5%	24
	0.3%	24	5%	24	2.5%	24

Table 5: Effect of processing or storage temperature on movability index (MI) of EMC.

MI≤1		MI≥1	
Processing	Duration in hours	processing	Duration in hours
Soaking in acetic acid 5% for 30s	2 hours after treatment	Soaking in acetic acid 2% for 60s	24 hour after treatment
Dry salting	1	Storage at 5ć	24
Storage at -10ć	12		

Table 6: Effect of processing *O.nioticus* with acetic acid on pH and sensory quality.

Parameters.	pH	Sensory evacuations					
		Odour intensity	Juicen-ess	Tenderness	Appearance	Unpleasant odour	Overall acceptability
Mean Treat.	5.07±0.22	5.33±0.33.	7.67±0.33	7.33±0.33	7.00±0.00	5.00±0.00	31.67±0.22
		Slight intense	Extreme juicy	Very tender	Moderate	Barely detected	Acceptable
Mean. Cont.	6.03±0.12	6.67±0.33	7.33±0.33	5.67±0.33	7.33±0.33	6.00±0.00	32.67±0.12
		Very intense	Very juicy	Moderate tender	Moderate	Not detected	Acceptable
P-value	0.027	0.092	0.211	0.065	0.211	0.0005	0.113

DISCUSSION

Parasitic fauna of fishes respond strongly to alterations in the physical and chemical characteristics of aquatic environment and modifications in the physiological and biological conditions of hosts (Ferrari- Hoeinghaus *et al*., 2006).

The results of the present study showed that, parasite community of wild and farmed *O. niloticus* consisted of, one protozoon (*Cryptosporidium spp*. 19.2%), one Acanthocephalan (*Acanthocephalus spp* 9.2%) and five trematode metacercariae (*Prohemistomum spp.* 35.0%, *Diplostomum spp.* 20.0%, *Haplorchis spp.* 2.5%, *Clinostomum phalacrocoracis* 19.2% and *C. tilapiae* 0.8%).

The overall prevalence of examined fish was 67.5 %, highest infestation rate was detected in wild *O. niloticus* 80 % compared to 55 % in farmed one (table 1). Contrary to these results were recorded by (Eissa, *et al.*, 1996) who reported that the infection rate in farmed and wild fish in Sharkia Governorate

was 67.33% and 42.33% respectively. Also Ibrahim (2012) recorded that the infection rate in farmed fish was 63.51% and in wild fish was 44.37% in Ismailia Governorate.

The difference of percentage may be attributed to the sanitary condition of the place, the location of the river from living place, number and class of people visiting the river and their purpose, biological pollution and snails propagation which act as intermediate hosts to complete the life cycle of some trematodes. In addition to good environmental conditions carried out by fish farmers in the study area.

Skinner (1982) mentioned that chronic exposure to pollutants or any environmental stress which lead to immuno-suppression through the release of corticosteroids make the fish more susceptible to any pathogenic organisms. This is considered the main factor responsible for the high rates of parasitic diseases in fish.

Examination of MZN stained intestinal contents smears in the present study revealed that 23 fish (19.2%) were positive for *Cryptosporidium sp*. The infection rate in farmed fish was 23.3% while in wild fish it was 15.0 % (table1). Gastric and intestinal cryptosporidiosis has been previously identified in 14 species of marine and freshwater fish (Alvarez-Pellitero *et al*., 2004 and Xiao *et al*., 2004). Hefnawy (1989) detected *Cryptosporidium* in 30 % of Tilapia in Assiut. Al- Taee (2008) detected it in 28.97 % in examined fish from Mosul. Abd-Allah (2009) detected *C. parvum* oocysts with infection rate of 13.75% in crayfish at Zagazig. Also Mahmood (2012) detected it in 16.9 % of carp fish in Iraq. The variation in the rate of infections may be due to the genus and numbers of fish, time of samples collection and examination, the rate of contaminated water, stress exposed in addition to the methods used for the diagnosis.

On the other hand, highest infection rate in farmed fish may be related to food supply in addition to over crowding which leads to the high and quick spread of infectious diseases in farmed fish. Additionally, fish farming in rural areas is always integrated with poultry production and this might have contributed to the parasitic infection which some species may cross infect between fish and domestic poultry (Aiello and Mays 1998).

The appearance of detected *Cryptosporidium* oocysts after staining with modified Zeihl- Neelsen was bright red spherical oocysts with a diameter of 5.2 x 4.4 µm, and that agree with the description by (Xiao *et al*., 2004 and O' Donghue, 2005).

Cryptosporidium lacks species host specificity (Xiao *et al*., 2004). Therefore, domestic and wild species of animals may be reservoirs of infection for susceptible human individuals, whether they are immunodeficient or immunologically competent. Accordingly, the infected fish play a role as reservoirs and can shed massive amount of infective oocysts to the aquatic environments, so this pathogen has been isolated worldwide from rivers, lakes and other sources of water (Baruš *et al*., 2002 and O' Donghue, 2005).

On the other hand adult worms of Acanthocephela were detected in 9.2% of examined fish, their infection rate in farmed and wild fish was 10.0 % and 8.3 % respectively (table 1). This result some what agrees with that of Eissa *et al*. (1996) and Abdel-Mawla and El-Ekiaby (2012) in both farmed and wild *Tilapia*, but high prevalence was detected in *Tilapia* by El- Naffar *et al*. (1983) 43.2% and Eissa *et al*. (2010) 46.9 %. Although, they disagreed with the prevalence, all agreed that *O. niloticus* is the only species of fish infested with *Acanthocephala spp*. through feeding on fresh water crustacean (Duijn, 1973). Ramadan (1991), mentioned that the kind of food is a fairly factor in determining the type of helminth parasites which can be found in a fish. Martin *et al*. (2009) reported that, high stocking densities favors increased parasite populations.

Natural infestation of man with Acanthocephala is rare, but possible where eating habits are unclean. In some cases they can cause great pain to the host if the proboscis completely perforates the gut wall. Subsequent natural infections with Acanthocephala have since been reported, (Tada *et al*., 1983). Eight species have been isolated from humans to date (Haustein *et al.,* 2009).

Concerning to the prevalence of encysted metacercariae in the present work, total infection rate

was 63.3%. They were differentiated into microscopic encysted metacercariae 57.5% and macroscopic 20.0 % (table 1). These findings are nearly similar to those given by Arafa *et al*. (2005) 42.86% Abd-El- Rahman (2005) 45% and Abd-Allah (2009) 32.2 %. However, higher prevalence war recorded by Khattab (1990) 87.06 % and Taher (2009) 84.75%. Taher, (2009) suggested that such variation in prevalence may be related to the difference in the habitat, food supply, abundance of both aquatic snails (the intermediate host), and the aquatic piscivorous birds, which play the main role to complete the life cycle of some digenetic trematodes.

The highest infection rate with microscopic metacercariae was detected in wild fish (88.3%), while macroscopic metacercariae were not detected in farmed fish (table1). The present authors suggest that such results may be related to the wide area of surface water of River Nile and their richness with aquatic snails (the intermediate host) and presence of aquatic birds around it is considered the most important points which lead to increase the infestation rate in wild fish.

Microscopic metacercariae detected in the present work were differentiated into: *Prohemistomum sp.* (35.0%), *Haplorchis sp.* and (2.5%) *Diplostomum sp.* (20.0%), while macroscopic metacercariae detected in the present work were differentiated into*:* *Clinostomum phalacrocoracis* (19.2%) and *Clinostomum tilapiae* (0.8%).

Most of these metacercariae were detected previously by El- Shahawi (1983), El-Naffar, and El-Shahawi (1986), Hussein (2007) and Taher, (2009).

Trematode metacercariae are considered as one of the most common parasites infesting fish; some of these parasites may have zoonotic importance (Hernandez *et al*., 1998). Yellow grub (*Clinostomum* metacercariae) causing Halzoun like disease, while *Prohemistomum vivax* may causing death for human being (Nasr, 1941 and Williams and Jones, 1976).

The majority of human infections with trematode result from the consumption of raw or lightly cooked freshwater fish or shellfish containing viable metacercariae. Depending on the parasite species, these infections may occur in the liver, lungs, small intestine and occasionally brain or other tissues producing symptoms ranging in severity from mild to debilitating and life-threaten (Amin, 2005). In surveys a true picture of the prevalence in humans with these parasites is difficult to attain because of the similarity of their eggs of many species (Healy, 1970).

Encysted metacercaria (EMC) develop in *O. niloticus* as intermediate host. The effects of aberrant physico-chemical microenvironment (e.g. salt, vinegar and acetic acid) concentrations for different exposure times on EMC were studied. The *in vitro* investigation showed that the tested EMC were killed within 1, 6 and 12 hour of incubation in 5, 2.5, and1.25% acetic acid, respectively (Table 4). Acetic acid is generally recognized as safe by The United States Food and Drug Administration (FDA). Most research on individual or combined effect of acetic acid has focused on surface contamination of fish and fresh meat surface (Ahmed 1999). However, few studies directed toward the wormcidal effect of acetic acid. With The Far East *Stellantchasmus falcatus* EMC, Wongsawad *et al*. (2005) found that the metacercariae were killed in acetic acid at concentration of 5 or 10% within 12 and 6 hours, respectively. Sukontason *et al*. (1998) stated that 5% acetic acid has no killing effect on trematode EMC within 3hours.

Vinegar is one of the oldest fermented food items enjoyed by man and widely used in food industry. In our study, commercial vinegar (5% Conc.) has no killing effect on ECM (MI≥1 within 24hour), (Table 4). Murrell, (2002) reported that commercial vinegar (4% Conc.) has no killing effect on *O. viverrini* EMC within one hour.

The results summarized in Table 4 revealed that common salt (NaCl) could kill the EMC (MI≤1) at Conc. 30, 20% within 1and 2 hours, respectively. Lower Conc. (10 and 5%) have MI≥1 within 24 hour (no killing effect). The sodium chloride micro environment effect on ECM was investigated by other researchers. Wiwanitkit *et al*. (2002) stated that degeneration of all Haplorchinae spp. metacercariae took approximately 3 hours by marination in 5% sodium chloride. With The Far East indigenous *Stellantchasmus flacatus* metacercariae, Wongswad *et al*. (2005) found that the worms were killed in NaCl 20, 30, 40 % solutions within 12, 6 and 2 hours, respectively.

Fish processors are in need of methods to eliminate parasites in fish rather than cooking. In our study, different storage and processing methods were evaluated as control measures of the ECM in *O. niloticus* within 24 hour. Chilling and freezing are the normally employed methods for fish preservation. Cold storage of *O. niloticus* at 5℃ for 24 hours dose not affect the viability of ECM (MI ≥1within 24h), (Table5).

Youssef *et al*. (1993) found that chilling muscle pieces of fish at 4ċfor 24hour has no killing effect on trematode EMC. Abdalah *et al*. (2009) stated that it took 11-15 days of storage at 5˚ċ to achieve complete loss of viability of four different trematode ECM while Wiwanitkit *et al*. (2002) found that refrigeration of freshwater fish for approximately 5hours resulted in degeneration of all Haploirchinae spp.

By freezing, killing of parasites depends on several factors. The temperature of freezing process, length of time that fish is held frozen and type of parasite appear to be the most important, FDA (2001). In the present study storage of *O. niloticus* at -10℃ for 12 hours resulted in complete loss of ECM viability (Table5).

With the same fish species, Abdallah *et al*. (2009) mentioned that it took longer time (16h of storage at -10℃) to gain complete loss of *Dilpostomatidae* ECM viability. A shorter time (5h) was enough to degenerate all Haplorchinae spp. ECM in freshwater fish, Wiwanitkit *et al*. (2002).

Marinating is the process of soaking foods in a seasoned, often acidic, liquids with or without cooking. The aim of marinating is not only to inhibit the action of bacteria but also tenderize the connective tissue, EFSA (2010). In this study the present authors investigated the effect of soaking of *O. niloticus* in acetic acid on viability of EMC. The data summarized in (Table5), revealed that it took two hours after soaking of fish in acetic acid for one minute to gain MI≤1 (killing effect). In general, little was known about the effect of acid on fish trematodes, mainly ECM., Zviagina and Beer (1997) found that 3% acetic acid was minimally effective in suppressing the viability of *O. felineus* ECM while 6% concentration was about 6 times higher. Wiwanitkit *et al.* (2002) reported that marinating freshwater fish for approximately 3 hours was enough to degenerate Haplorchinae spp. EMC.

Dry salting was the most effective processing mean in our study. It could kill the ECM in *O. niloticus* muscles within one hour, (Table, 5). Parasites vary in their response to common salt, research studies indicated that parasites can be eliminated at high salt concentrations for a short period, some of them can also be killed at low salt content. That will depend on fat content, temperature, amount of salt, salt composition, et. (Mol and Ozden 2004).

Fan (1998) stated that keeping the freshwater fish in heavy salt (fish/salt=10gm/3gm) may not be effective in the prevention of clonorchiasis. Sukontasoy *et al*. (1998) found that 10 % NaCl has no killing effect on trematode ECM in fish within 3 hours. Murrell (2002) reported that dry salting was lethal for anisakids within ten minutes of direct contact.

Knowledge about the pH of fish flesh may give valuable information about its condition. The pH value of seafood varies from 5.8-7.2 depending on struggling at time of harvesting but normal variation is of pH 5.8-6.5 Ali (2011). The present data summarized in Table 6 verify that acetic acid soaked fish had a significant lower pH ($p<0.05$) values compared to control. Lowering the pH by acid application is the chief application in foods, (Ahmed, 1999).

Sensory assessment has always played a key role in quality and freshness evaluation in fish industry. In our study, the five attributes tested by panelists are given in Table 6. Except for barely detected unpleasnt odour (barely vinegar like odour) scores, sensory attribute of cooked Tilapia treated with acetic acid did not differ significantly compared with control ($p<0.05$).In this respect, Marshall and Kim (1996) stated that catfish fillets treated with 2% acetic acid for 5-60seconds were liked less by sensory panels than control due to acidic odour. The detected vinegar-like odour in treated fish in our study did not significantly affects the overall acceptability compared with control, and judged as acceptable Table 6. Ahmed (1999) concluded that acetic acid should not be used in concentration higher than 2% because consumer may reject such acid treated fish as they alter sensor quality especially odour and color of such fish. our experiment revealed that with 5 % acetic acid fish still acceptable by panelists and that may lead to the fact that intact fish less affected by acid than filleted fish.

CONCLUSION

From the present study it could be concluded that the fresh water fishes (*O. niloticus*) are subjected to infection with *Cryptosporidium*, *Acanthocephalus* and encysted metacercariae of different trematode worms, which could be transmitted to the consumers causing dangerous disease. Therefore such parasites must be well controlled in order to avoid their transmission to man. This study also revealed that some fish processing methods or treatments are adequate to kill ECM in *O. niloticus*. Storage of fish at -10 ˚C for 12 hour, marination of fish in 5% acetic acid for one minute and waiting for 2 hours or dry salting for one hour using table salt (sodium chloride /fish=3gram/10gram).

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دراسات على الكريبتوسبوريديم وبعض الطفيليات الأخرى التي تصيب الإنسان في اسماك البلطي النيلي في محافظه أسيوط

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تم إجراء هذه الدراسة علي ١٢٠ عينة من أسماك البلطي الطازج (٦٠ بلطي مزارع و٦٠ بلطي من نهر النيل) والتي تم جمعها من أماكن متفرقة من محافظة أسيوط وذلك بهدف دراسة مدي تواجد طفيل الكربتوسبوريديم وبعض الطفيليات الأخرى التي تصيب الإنسان وكذلك دراسة التأثيرات المختلفة لملح الطعام ، حمض الخليك ، الخل والتبريد علي الطور اليرقي المتحوصل. بفحص عينات الخياشيم ، العضلات والقناة الهضمية لتلك الأسماك تبين تواجد الطفيليات في الأسماك النيلية المصدر بمعدل أعلي (٨٠%) مقارنة بأسماك المزارع (٥٥%) كما تواجدت الطفيليات المختلفة في اسماك البلطي النيلي واسماك المزارع علي النحو التالي: الكربتوسبوريديم ١٥% ، ٢٣.٣ % ، الاكانثوسفلا ٨.٣ ، ٩.٢ % الطور اليرقي المتحوصل الميكروسكوبي ٨٨.٣ ، ٢٦.٧ % علي التوالي. بينما تواجد الطور المتحوصل العيني في البلطي النيلي المصدر فقط وبنسبة ٤٠%. بدراسة التأثيرات المختلفة علي الطور اليرقي المتحوصل (الميتاسركاريا) في العضلات للديدان المفلطحة تبين الآتي : بغمر قطعة من عضلات الأسماك المحتوية علي الطور اليرقي المتحوصل النشط بتركيزات مختلفة من محلول ملح الطعام ، حمض الخليك والخل التجاري نتج فقد لحيوية الطور اليرقي المتحوصل بعد ١ ، ٢ ساعة من الغمر في تركيز ٣٠ ، ٢٠ % من ملح الطعام علي التوالي ، ١ ، ٦ ، ١٢ ساعة من الغمر في محلول حمض الخليك تركيز ٥.٠٠ ، ٢.٥٠ ، ١.٢٥ % علي التوالي ، بينما لم تتأثر حيوية الطور اليرقي المتحوصل بالغمر في محلول الخل التجاري تركيز ٥% لمدة ٢٤ ساعة. والتمليح الجاف باستخدام ملح الطعام نتج عنة فقد لحيوية الطور اليرقي المتحوصل بعد ساعة واحدة من التمليح. بحفظ الأسماك عند ٥ درجه مئوية لمدة ٢٤ ساعة لم تتأثر حيوية الطور اليرقي المتحوصل بينما حدث فقد للحيوية عند حفظ الأسماك عند (-١٠) درجه مئوية لمدة ١٢ ساعة. وبغمر الاسماك في محلول حمض الخليك بتركيز ٥% لمدة دقيقة ، فقد الطور المتحوصل حيويته بعد ساعتين من المعاملة وتبع ذلك انخفاض معنوي ($p<0.05$) في الاس الهيدروجيني (pH) مع اكتساب رائحة الخل عند الفحص الحسي للأسماك المعاملة بحمض الخليك مقارنة بالأسماك الضابطة للتجربة ولكن ظلت مقبولة لدي مجموعة التقدير الحسي (Sensory evaluation panelist).