Assiut University web-site: www.aun.edu.eg

EFFECT OF FREEZING AND CHILLING ON THE VIABILITY AND INFECTIVITY OF THE METACERCARIAE OF *HAPLORCHIS PUMILIO* AND *PROHEMISTOMUM VIVAX*

YOUSSEF, T.H.[1]; HEFNAWY, Y.A.[1]; KHALIFA, R.[2] and MAHMOUD, A.E.[2]
[1] Department of Food Hygiene, Faculty of Veterinary Medicine, Assiut University, Egypt
[2] Department of Parasitology, Faculty of Medicine, Assiut University, Egypt

Received: 31 December 2015; **Accepted:** 30 January 2016

ABSTRACT

Effect of freezing and chilling on the metacercariae of *Haplorchis pumilio* and *Prohemistomum vivax* infecting the fresh water fish (*Tilapia nilotica* and *Clarias lazera*) in Assuit province is studied. Test of the viability of the metacercaiae was done not only by microscopic examination but also by experimental infection in albino rats. Chilling of *Tilapia nilotica* and *Clarias lazera* at (4 ^{o}C) for 24 hr and 48 hr, respectively has ended the viability of metacercariae of *Prohemistomum vivax* and *Haplorchis pumilio*. Exposure of infected fishes of both species to freezing at (-10 ^{o}C) for 24 hr has proved to be lethal to the metacercariae of both *Prohemistomum vivax* and *Haplorchis pumilio.*

Key words: *freezing , Chilling , Metacercariae , Tilapia nilotica , Clarias lazera*

INTRODUCTION

Fishes are considered as one of the most valuable nutritive, tasty, palatable and easily digested protein for human. They can compensate the shortage in the animal protein where there is need to food as a result of rapid increase of world population particularly in developing countries and also due to the dramatic changes that occurred in animal and poultry production from emerging zoontic diseases such as bovine spongiform encephalopathy and avian influenza which affect the protein resources. However, fish may harbor many pathogens which constitute great problems either in cultured or wild fishes as they limit the fish production especially in subtopic countries like Egypt (Elamei, 2001).

Under natural conditions 50 – 90 % of freshwater fishes harbor at least one species of parasites (Sineszko, 1979). Parasitic diseases are considered to be serious problem rather than bacterial diseases in warm water fishes (Eissa *et al.*, 1996).

Fresh water fishes are considered as one of the important sources of parasitic infection to man and fish eating mammals particularly after the increased pollution of rivers and lakes in Egypt (Mohamed, 1996). The public health importance of some internal parasitic diseases affecting *Tilapia nilotica* such as yellow grub which can be transmitted to human as a result of ingesting raw or improperly cooked fish and causing Halzoun like disease leading to laryngopharyngitis (Williams and Jones, 1976) while *Prohemistomum vivax* was rarely recorded to infect human and may cause death (Nasr, 1941).

Corresponding author: Dr. Youssef, T.H.
E-mail address: dr_toko@yahoo.com
Present address: Department of Food Hygiene, Faculty of Veterinary Medicine, Assiut University, Egypt

MATERIALS AND METHODS

Fresh *Tilapia nilotica* and *Clarias lazera* fishes were examined for the presence of different metacercariae. Snips of muscles obtained from the head region (anterior part) of the fish, trunk region (middle part) and tail region (posterior part) were compressed between two slides and examined microscopically. Samples of the discovered metacercariae were fixed in 5% formalin, stained in acetic acid alum carmine and mounted in Canada balsm. Heavily infected fish were divided into three groups. The first group was used as positive control, the second group was exposed to chilling at temperature of (4 °C) and the third group was exposed to freezing at temperature (-10 °C). The viability of the three groups was observed after interval 24, 48 and 72 hours microscopically. Then the time required for death of the encysted metacercariae was determined. Moreover, confirmation test was done by feeding parasites free laboratory albino rats on the three groups of encysted metacercariae and sacrification of rats from the 7th day post infection to detect and count the adult flukes in their intestine (Wells and Randall, 1956, Syme, 1966).

Daily infection was done from the the three groups. In each experimental trial, confirmed viable 50 *Haplorchis pumilio* and 20 *Prohemistomum vivax* encysted metacercariae were given to each albino rat.

RESULTS

Table 1: Effect of chilling and freezing on the viability of encysted metacercariae in muscles of Tilapia nilotica.

Time / h	Chilling		Freezing	
	Temperature	Viability	Temperature	Viability
0 hr	25°C	+	25°C	+
24 hr	4°C	+	-10°C	-
48 hr	4°C	-	-10°C	-
72 hr	4°C	-	-10°C	-

+: viable encysted metacercariae
-: not viable encysted melacercariae

Table 2: Effect of chilling and freezing on the viability of encysted metacercariae in muscles of Clarias lazera.

Time / h	Chilling		Freezing	
	Temperature	Viability	Temperature	Viability
0 hr	25°C	+	25°C	+
24 hr	4°C	+	-10°C	-
48 hr	4°C	+	-10°C	-
72 hr	4°C	-	-10°C	-

+: viable encysted metacercariae
-: not viable encysted melacercariae

Table 3: Effect of freezing and chilling on the infectivity of metacercariae of Haplorchis pumilio and Prohemistomum vivax in Tilapia nilotica.

		Haplorachis pumilio		*Prohemistomum vivax*	
		No. given	No. produced	No. given	No. produced
	Fresh fishes	50	30-35*	20	8-12*
Freezing	After 12 hrs	50	9-11	20	2-4
	After 24 hrs	50	0	20	0
	After 48 hrs	50	0	20	0
	After 72 hrs	50	0	20	0
Chilling	After 12 hrs	50	16-20	20	5-6
	After 24 hrs	50	4-9	20	1-2
	After 48 hrs	50	0	20	0
	After 72 hrs	50	0	20	0

* The number of adults was within the same range throughout the three days of the experiment.
Freezing = -10 ^{O}C
Chilling = 4 ^{O}C

Table 4: Effect of freezing and chilling on the infectivity of metacercariae of Haplorchis pumilio and Prohemistomum vivax in Clarias lazera.

		Haplorachis pumilio		*Prohemistomum vivax*	
		No. given	**No. produced**	**No. given**	**No. produced**
	Fresh fishes	50	32-37*	20	10-14*
Freezing	After 12 hrs	50	10-12	20	3-5
	After 24 hrs	50	0	20	0
	After 48 hrs	50	0	20	0
	After 72 hrs	50	0	20	0
Chilling	After 12 hrs	50	18-22	20	8-10
	After 24 hrs	50	9-12	20	5-6
	After 48 hrs	50	3-5	20	1-2
	After 72 hrs	50	0	20	0

* The number of adults was within the same range throughout the three days of the experiment.
Freezing = -10 ^{O}C
Chilling = 4 ^{O}C

DISCUSSION

Effect of chilling of *Tilapia nilotioca and Clarias lazera* on viability and infectivity of encysted metacercariae:

1- Effect of chilling of *Tilapia nilotioca*:
Investigation of infested muscular tissues of *T. nilotioca* with encysted metacercariae at interval periods (0, 24, 48 and 72 hours) at chilling temp. (4°C) revealed that the encysted metacercariae were viable for 24 hours only and complete destruction occurred after 24 hours of chilling storage.

2- Effect of chilling of *Clarias lazera*:
Examintion of infested *C. lazera* at interval period of chilling (0, 24, 48 and 72 hours) at (4°C) revealed that the encysted metacercariae were viable for 48 hours and complete destruction occurred after 48 hours of chilling storage. The variation between the viability of encysted metacercariae infested the muscular tissues of *T. nilotioca* & *C. lazera* may be attributed to the variation in the host species.

3-Effect of freezing of *Tilapia nilotioca* & *Clarias lazera* on viability of encysted metacercaria:
Investigation of infested muscular tissues of *T. nilotioca* & *C. lazera* with encysted metacercariae at interval periods (0, 24, 48 and 72 hours) at of freezing temp. (-10°C) revealed that the encysted metacercariae were destroyed within 24 hours only. Experimental infection of laboratory rats with freezed and chilled 50 EMC of *Haplorchis pumilio* and 20 EMC of *Prohemistomum vivax* confirmed the data obtained for their viability.

CONCLUSIONS

Freezing of fishes for one to two days before consumption remains to be the best method for avoiding human transmission of fish borne parasites.

REFERENCES

Eissa, I.A.M.; Diab, A.S. and Badran, A.F. (1996): Studies on some internal parasitic diseases among wild and cultured Oreochromis niloticus fish. 7th Sci. Cong., 17-19 Nov. 1996, Fac. Vet. Med., Assiut, Egypt.

Elamie, M.M.M.T. (2001): Studies on the diseases resulting from encysted metacercariae in some freshwater fishes. M.V.Sc. Thesis (Fish diseases and management), Fac. Vet. Med. Suez Canal Univ.

Mohamed, I.B. (1996): Pathological changes in some fish eating mammals as a result of consumption of fish infested with encysted metacercariae. PhD Thesis, (Pathology), Fac. Vet. Med., Cairo Univ.

Nasr, M. (1941): The occurance of *Prohemistomum vivax* infection in man, with redescription of the parasite. Lab. Med. Prog., 2: 135-149.

Sineszko, S.F. (1979): Effects of environmental stress on outbreak of infectiou diseases of fishes. J. Fish Biol., 6: 157-208.

Syme, J.D. (1966): Fish and fish inspection. 2nd Ed. 52-64. Univ. of Toronto.

Wells, H.H. and Randall, B.H. (1956): New hosts for trematodes of the genus Heterophyes in Egypt. J. Parasitol., 42: 287-292.

Williams, H.H. and Jones, A. (1976): Marine helminthes and human health. Commonu. Inst. Helminthol. Misc. Publ.3, 47.

تأثير التبريد والتجميد علي حيويه وعدوي يرقات الهابلوركيس باميليو والبروهيموسيتموم فايفكس

طارق حسين يوسف ، يحيي عبد البديع حفناوي ، رفعت خليفه ، عبير السيد محمود

E-mail: dr_toko@yahoo.com Assiut University web-site: www.aun.edu.eg

تم فى هذا البحث دراسة تأثير التبريد (٤°م) والتجميد (-١٠°م) لفترات مختلفه علي الميتاسركاريا الموجودة بين عضلات سمك البلطي والقراميط في محافظة أسيوط. وقد تم التأكد من كون الميتاسركاريا حية أو ميتة بفحصها ميكروسكوبيا وبعدوى جرذان المعامل البيضاء وأتضح ما يلى: (١) تأثير درجة حرارة التبريد على أسماك البلطى: باجراء تجربة تأثير درجة حرارة التبريد (٤ °م) على حيوية الأطوار اليرقية المتحوصلة فى أنسجة أسماك البلطى على فترات مختلفة (صفر، ٢٤ ساعة، ٤٨ ساعة و ٧٢ ساعة) أتضح حيوية هذه الأطوار اليرقية لمدة ٢٤ ساعة فقط، وبعد ٢٤ ساعة فقدت هذه الأطوار اليرقية حيويتها نتيجة لتحطمها. (٢) تأثير درجة حرارة التبريد على أسماك القراميط: باجراء تجربة تأثير درجة حرارة التبريد (٤ °م) على حيوية الأطوار اليرقية المتحوصلة فى أنسجة أسماك القراميط على فترات مختلفة (صفر، ٢٤ ساعة، ٤٨ ساعة و ٧٢ ساعة) أتضح حيوية هذه الأطوار اليرقية لمدة ٤٨ ساعة وبعد ٤٨ ساعة فقدت هذه الأطوار اليرقية حيويتها نتيجة لتحطمها. (٣) تأثير درجة حرارة التجميد على أسماك البلطى والقراميط: باجراء تجربة تأثير درجة حرارة التجميد (-١٠ °م) على حيوية الأطوار اليرقية المتحوصلة فى أنسجة أسماك البلطى والقراميط على فترات مختلفة (صفر، ٢٤ ساعة، ٤٨ ساعة و ٧٢ ساعة) أتضح تحطم هذه الأطوار اليرقية خلال ٢٤ ساعة فقط. هذا وقد أكدت نتائج قدرة ميتاسركاريا الهابلوركس بوميليو والبروهيميستومم فايفاكس المتحوصلة المعرضة للتبريد والتجميد على عدوى الجرذان بالديدان البالغة نفس النتائج المذكورة سابقا بالنسبة لحيوية هذه اليرقات.