د راسة على تأثير التمليح والتجميد على حيوية ميتا سركاريا

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محفوظ فهمى ، رفعت خليفة ، حسين يوسف

د رس الباحثون تأثير التمليح (١٠٪ ، ٢٠٪) والتجميد تحت د رجة - ٤°م لفترات مختلفة على ميتاسركاريا الهابلوركس بوميليو والبروهيستوم فيفاكس الموجود ة بين عضلات سمك الأماية (الستس نيرس) ، فى محافظة أسيوط . وقد تـــم التأكد من كون الميتا سركاريا حية أو ميته بفحصها ميكروسكوبيا وبعد وى الجرذان البيضاء واتضح من الد راسة أن تأثير التجميد اشد وطأة من تأثير التمليـــح وقورنت النتائج المختلفة للتمليح والتجميد . وقد أثبت البحث شد ة حساسية هذ ه الأنواع من الميتا سركاريا لتأثير التمليح والتجميد ، واتضح أن مقاومتهـــا أقل بكثير من مقاومة ميتا سركاريا الهتروفس هتروفس الذى يصيب الأنسان بكثرة فى الوجه البحـــرى .

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EFFECT OF SALTING AND FREEZING ON THE VIABILITY AND INFECTIVITY OF THE METACERCARIAE OF HAPLORCHIS PUMILIO (LOOS, 1896) AND PROHEMISTOMUM VIVAX (SONSINO, 1893)

(With One Table and One Figure)

By

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SUMMARY

Effect of salting (10% and 20%) and that of freezing on the metacercariae of Haplorchis pumilio and Prohemistomum vivax infecting the fresh water fish Alestes nurse in Assiut Province is studied. Test of the viability of the metacercariae was done not only by microscopic examination but also by experimental infection in albino rats. Effect of freezing was also studied on the same matacercariae and was found to be more drasting than the effect of salting. Both metacercariae were found to be less resistant than the metacercariae of Heterophyes heterophyes which is more commonly encountered as a human parasite of Lower Egypt.

INTRODUCTION

Some Egyptians like to consume "Sweet Fesikh" which is a sort of raw fishes salted for about three days. This mild salting was reported by KHALIL (1937) to be insufficient to kill the encysted metacercariae of Heterophyes heterophyes which is a common parasite of natives living near the Egyptian Lakes; BOROLLOS and MANZALAH. KHALIL (op. cit.) concluded on experimental basis that 7-10 days salting is the minimum peroid necessary to render these fishes non-infective. ELIAS (1968) studied the effect of salting on the viability of the metacercariae of Heterophyes

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heterophyes in the mullet fish (Mugil sp.). Nevertheless,some other intestinal trematodes are now believed to be transmissible to man through the agency of fishes. NASR (1941) showed that Prohemistomum vivax was the cause of death in a human case. KHALIL (1932) demonestrated that Haplorchis pumilio is transmissible to man and he was able to infect himself with this parasite. KHALIFA et al. (in press) found the eggs of Haplorchis pumilio in the faeces of a young boy from Assiut city. Other haplorchid parasites were also recorded to infect man (WATSON 1960). Salted fish in Upper Egypt is usually done by salting of the fish Alestes nurse. During the course of this study. The present authors found that Alestes nurse is parasitized by the metacercariae of Haplorchis pumilio and Prohemistomum vivax. Therefore, the present authors studied the effect of salting and freezing on these fishes aiming to find their effect on the encysted metacercariae of the mentioned parasites.

MATERIAL AND METHODS

Fresh Alestes nurse fishes were examined for the presence of different metacercariae. Snips of muscles obtained from the area near the dorsal fins and the tail were pressed 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 . These specimens were used for description of the metacercariae as well as for camera lucida drawings. Heavily infected fishes were divided into four groups. First group was left fresh while the second and third groups were subjected to 10% and 20% salt respectively. The fourth group was submitted for the effect of freezing (-4 C°). The first group was used as a control which were fed with fresh unsalted metacercariae and to detect the exact identity of the parasites included. Experimental final host used was the albino rat. Daily infection was also done from the second and third groups. From the freezed group, experimental infection was tried every 12 hours. In each experimental trial, 300

Haohorchis pumilio and 100 Prohemistomum vivax metacercariae were giben to each albino rat. Three rats were infected daily from each group. Meanwhile,a sample was microscopically examined daily to detect any sign of viability of treated metacercariae. The experiment was repeated for three days after freezing. Experimentally infected rats were sacrified for searching and counting the raised adults.

RESULTS

Metacercariae encountered naturally in the muscles of Alestes nurse were of two types:

a) Metacercaria of H. pumilio.

These are samll cysts measuring about 150-170 by 120-150 microns . They are usually not surrounded by any tissue reaction. Some of them were till immature as indicated by the presence of the eye spots (Fig. 1:a); others were mature, bigger in size and lacking the ocelli (Fig. 1:b).Experimental infection of the albino rats proved that they belong to Haplorchis pumilio (LOOSS, 1896). Adults produced were noticed to differ in maturity as evidenced by differene in size as well as the development of the genital organs and eggs.

b) Metacercariae of P. vivax.

This is a moderate sized cyst, ranging in size between 300-320 by 310-350 microns. Cercarial body could be easily seen through the cyst wall. It was usually samller than the cyst capacity and the space around it was filled by darkly pigmented structures as well as refractile ones (Fig. 1:C). Cysts were usually surrounded by a thick layer of infilterated cells; the layer differed in thickness at different levels. Younger cysts were proportionally sammler (Fig. 1:d). Experimental infection into albino rats produced mature and immature adults of Prohemistomum vivax (SONSINO, 1893). Effect of salting (Table 1) .

a) 10% concentration:

After one day, most of the metacercaria of both parasites were noticed to be still rolling inside their cysts indicating viability.

Nevertheless, experimental infection into albino rats produced less than albino rats produced less than half the number of adults in controls. Drastic fall in the number of living metacercariae occurred after the second day. On the third day, Prohemistomum vivax metacercariae signs of death and experimental infection confirmed the microscopic findings. In case of Haplorchis pumilio metacercariae, microscopic examination indicated inviability of cysts, but experimental infection produced 0-4 parasites per host (Table 1). On the fourth to the seventh day, both microscopical and experimental infection proved that metacercariae present had already lost their infectivity.

b) 20% concentration:

Such high concentration lead to drastic fall in the number of adults produced by experimental infection, particularly in cases of P.vivax (Table 1). One day after infection, about one eighth of the number of adults produced in controls was obtained in case of H.pumilio while only 0-2 adults per host were found in case of P. vivax. The second day shoed that all P. vivax metacercariae became non-infective, while only 0-5 adults of H. pumilio per host were obtained. The latter were found to be completely non-infective on the third day.

Effect of freezing at - 4 C°

Exposure of infected fishes to - 4 C° has proved to be quickly lethal to the metacercariae of both H. pumilio and P. vivax. After 12 hrs. exposure, only one sixth to one fourth of the metacercariae of H.pumilio were found to be still viable while one tenth to one sixth of those of P. vivax survived. Twenty four hours exposure was enough to kill most of the metacercariae of both parasites (Table 1). After 36 hours exposure, all metacercariae of P. vivax were completely non viable while only 0-1 worm of H.pumilio could be raised in albino rats given 300metacercariae. After 48 hours,all the metacercariae of both trematedes were found dead.

DISCUSSION

According to the data shown in table 1, the present authros could conclude that the effect of freezing was the most drastic to both metacercariae of H. pumilio and P. viviax although the former was slightly more resistant than the latter. One day freezing was enough to kill nearly all the metacercariae of P. vivax while two days were needed for H. pumilio.

Metacercariae of H. pumilio were also characteristically more resistant to the effect of salting. Fishes which had been 10% salted were free from any danger of infection with P. vivax after two days, while at least three days were needed for killing the metacercariae of H.pumilio. On the other hand heavily salted fishes (20%) were found free from P. vivax after one day and free from H. pumilio after two days. Therefore , it is advisable to consume highly salted fishes although lightly salted ones in Assiut are also free from any danger, as it is usually salted for at least four to five days.

In Egypt, the effect of salting was only studied in relation to the metacercariae of Heterophyes heterophyes by KHALIL (1937) & ELIAS (1968). The present study is therefore done for the first time on other metacercariae, H. pumilio and P. vivax which are known to be transmissible to man. It shows that these two metacercariae are less resistant to the effect of salting and freezing particularly that of P. vivax. This may explain why Heterophyes heterophyes is more prevalent parasite than H. pumilio and P. vivax.

Effect of freezing on different metacercariae was not studied before in Egypt. This study shows that its effect is very lethal to the metacercariae of H. pumilio and P. vivax. They could not survive for more than two days in the former and one day in the latter. This excludes any danger of infection from imported frozen fishes.

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Table (1)

Effect of Salting and Freezing on Metacercariae of Haplorchis pumilio and Prohemistomum vivax.

		Haplorchis pumilio		Prohemistomum viax	
		No. given	No. produced	No. given	No. produced
	Fresh fishes	300	200-250	100	50-80
SALTING	1st day				
	10%	300	77-85	100	30-45
	20%	300	25-40	100	0-2
	2nd day				
	10%	300	20-40	100	0-8
	20%	300	0-5	100	0
	3rd day				
	10%	300	0-4	100	0
	20%	300	0	100	0
	4th to 7th day				
	10%	300	0	100	0
	20%	300	0	100	0
FREEZING	After 12 hours	300	55-70	100	10-14
	After 24 hours	300	1-5	100	1-2
	After 36 hours	300	0-1	100	0
	After 48 hours	300		100	0
	After 60 hours	300	0	100	0
	After 72 hours	300	0	100	0

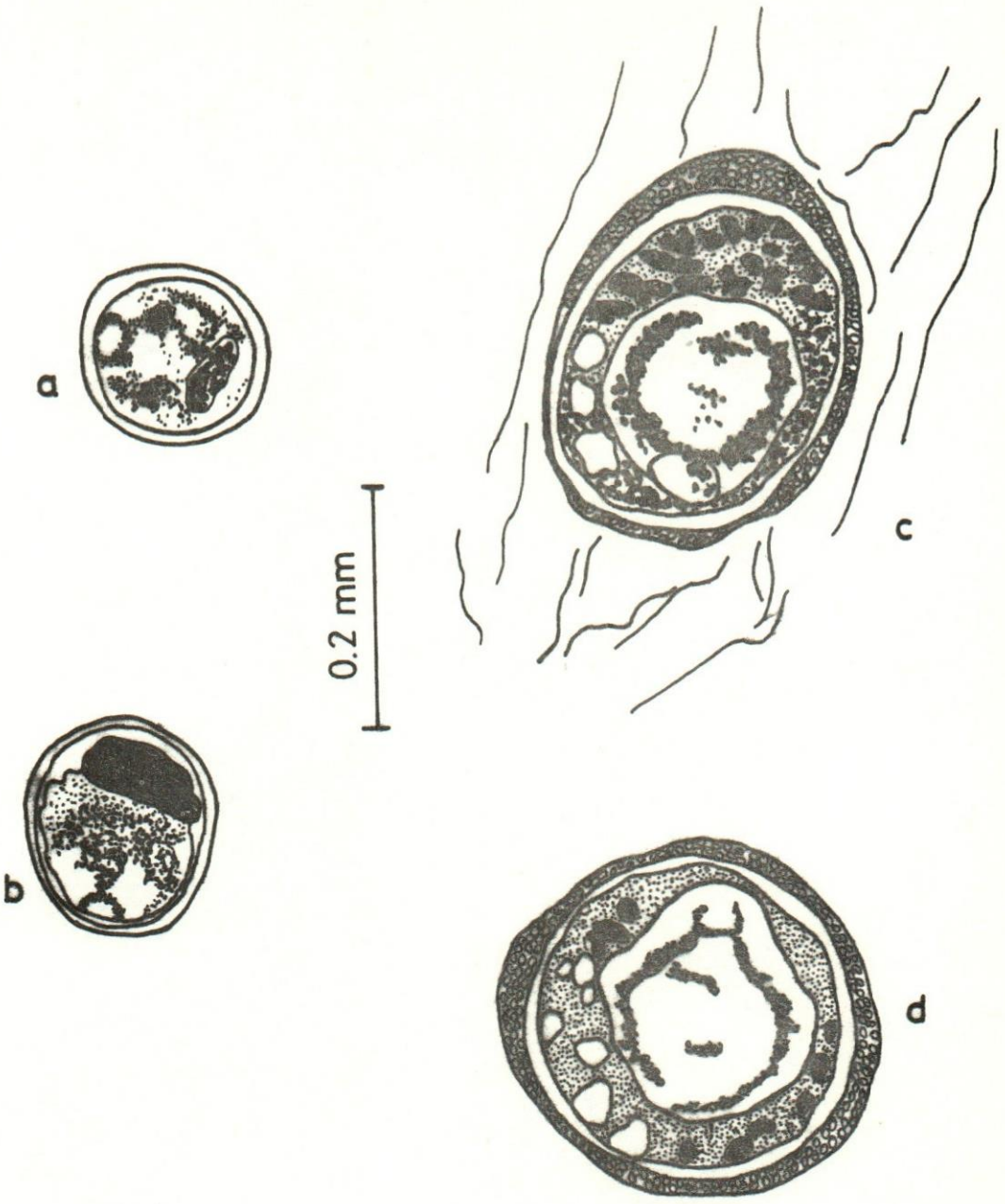

Fig. 1: a & b: Camera lucida drawings of different developmental stages of the metacercariae of Haplorchis pumilio.

Fig. 2: c & d: Camera lucida drawings of different developmental stages of the metacercariae of Prohemistomum vivax.