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In Vivo and *In Vitro* Survival Rates of Protoscoleces Kept at Different Constant Temperature

Ekram Ismail Fagiree Ismail[1], Mohammed Bahaa Eldeen Ahmed Saad[2]

[1]Department of Biology, Alzaem Alazhari University, Khartoum, Sudan
[2]Alahlia University, Khartoum, Sudan
Email: ik_fagiree@hotmail.com

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Abstract

An experiment was conducted on the survival rate of protoscoleces derived from hydatid cyst brought from infected camels in Tambool area (North Eastern Sudan). The survival rate was studied *in vivo* and *in vitro* through exposure to different constant temperatures to measure their viability. The results revealed that the protoscoleces remained viable *in vitro* for 20 days at 4˚C, 7 and 5 days at 25˚C and 37˚C, respectively, while at 45˚C the protoscoleces were immediately dead. This means that the infection rate of hydatid cyst becomes high in relatively cold seasons (winter and autumn). In the *in vivo* study, each mouse (five groups, four mice in each) was inoculated intraperitoneally with 2000 protoscoleces exposed to the same different constant temperature, as previously used. 6 months later, the mice in different groups were sacrificed and necropsied to study the development of hydatid cyst inside. The results showed that the rate of development inside the mice was clear in the first and the second groups (4˚C and 25˚C), however, in the other 2 groups (37˚C and 45˚C), no sign of development of the cyst was observed, with the presence of few number of hooks compared to the control group, which showed significant difference in the development of hydatid cyst.

Keywords

Echinococcus granulosus, Hydatid Cyst, Protoscoleces, Temprature, Mice

1. Introduction

Hydatid disease caused by the tapeworm *Echinococcus granulosus* is shown to have high prevalence among camels (46.56%) in Sudan [1]. In some parts of the country, all the offal of slaughtered camels are discarded in the open except the

liver. Thus, dogs have an access to these offal.

To implement an effective control program, it is necessary to investigate survival pattern of the protoscoleces in the discarded organs.

In a previous study in Sudan, Saad and Zien Eldin [2] evaluated the survival rate of protoscoleces from hydatid cysts brought to the laboratory. They kept the protoscoleces under different constant temperatures (4˚C, 20˚C, 30˚C, 40˚C and 50˚C. The viability of the protoscoleces was determined by the complete evagination and active body movement. They examined the fluid of the cyst after 1 h, 2 h, and 4 h. The fluid was also examined after 1day, 2 days, 3 days, 4 days and 5 days. They found that the survival rate continued to its highest (83.54%) at the 5^{th} day and it was assumed to continue. However, the rate was nill on the 4^{th}, 3^{rd}, 2^{nd}, and 1^{st} day when the scoleces were kept at 20˚C, 30˚C, 40˚C and 50 ˚C respectively.

Under natural conditions, unhygienic disposal of slaughter house offals may act as a source of infection to stray dogs which are usually abundant around slaughter house in small towns in the country. As mentioned by [1], the larval stages could survive for several days during the cold seasons and this rate is reduced to several hours during the hot seasons which allow roving dogs to overwhelm the discarded parts and accordingly constitute great risk of infection to the domestic animals and man as well.

There are many studies that used various methods for detecting the fertility and assessing the viability of hydatid cysts [3]. Information relative to degree of fertility and viability taken from important intermediate hosts, have many clinical and epidemiological applications and it should be considered in many program to control and eradicate the disease [4].

The *in vitro* viability of protoscoleces was assessed on the basis of flame cell activity and staining with eosin, which were considered as criteria to determine the death or viability of protoscoleces. In addition to this, movement (flame cell activity), the other is motility like constriction-relaxation (invagination-evagination) in the protoscoleces was also noticed. Both types of movements were examined under light microscope [5].

The motility of protoscoleces examined under effect of three different temperature 25˚C, 37˚C, and 40˚C was within 15 minutes. It showed steadily increase with rising temperature. Flame cell activity increased as high as 70.01% at 40˚C, while the motility with constriction-relaxation movement increased as 100.0% at 40˚C [6].

The objectives of this study was to repeat the work of Saad and Zien Eldin (1983), however, expanding the work by adding the staining technique for the *in vitro* study. *In vivo* study was also considered using experimentally infected mice.

2. Materials and Methods

It is a case control study.

The field work was conducted in Tambool area (Central Eastern Sudan)

where slaughtered camels were checked for hydatid cysts. Laboratory work was performed at the laboratories of the Faculty of Education, Alzaem Alazhari University and the Faculty of Education, University of Khartoum.

The study included camels slaughtered at Tambool market and white swiss mice (25 - 30 gram body weight males) were used for the experimental purposes.

Hydatid cysts from infected lung of camels were brought to the laboratory and fluid of fertile cysts was aspirated with a sterile needle and kept in McCartney bottles at constant temperatures of 4˚C, 25˚C, 37˚C and 45˚C. The viability of the protoscoleces was determined by the complete evagination and active body movement with eosin stain. The fluid of the cyst was examined after one day, 2 days, and 3 days up to 20 days.

Twenty mice were selected for the study. They were divided into 5 groups of 4 each.

Group 1: received 2000 protoscoleces kept at 4˚C

Group 2: received 2000 protoscoleces kept at 25˚C

Group 3: received 2000 protoscoleces kept at 37˚C

Group 4: received 2000 protoscoleces kept at 45˚C

Group 5: received 2000 protoscoleces directly from camels as the untreated control. The mice were sacrified 6 months post inoculation and the survival rate was based on the number of cysts developed compared to the control group.

3. Results

The *in vivo* results revealed that the establishment rates in different groups compared to the control group were 28.57%, 20%, 0% and 0% for those kept at 4˚C, 25˚C, 37˚C and 45˚C respectively (Tables 1-3, Figure 1, Figure 2(a) & Figure 2(b), and 100% with the control groups (Table 1).

The *in vitro* results showed that the maximum survival rate was observed with

Table 1. *In vivo* survival rates of protoscoleces kept at different constant temperatures.

Groups	Temperatures	Number of cyst encountered	Establishment rate	Probability
1	4˚C	10	28.57%	0.06
2	25˚C	7	20.00%	0.04
3	37˚C	0	00.00%	0.00
4	45˚C	0	00.00%	0.00
5	Control	35	100%	0.22

Table 2. Survival rate of protoscoleces *in vivo* at 4˚C.

No of Mice	No. of cyst	Size	Site	Case
1	4	small	Liver	sterile
2	2	small	liver	fertile
3	4	small	Liver	fertile
Total	10	-	-	-

Establishment rate = 10 ÷ 35 × 100 = 28.57%.

Table 3. Survival rate of protoscoleces *in vivo* at 25˚C.

No of Mice	No. of cyst	Size	Site	Case
1	2	small	Liver & spleen	fertile
2	3	small	liver	fertile
3	2	small	Liver	fertile
Total	7	-	-	-

Establishment rate = 7 ÷ 35 × 100 = 20.0%.

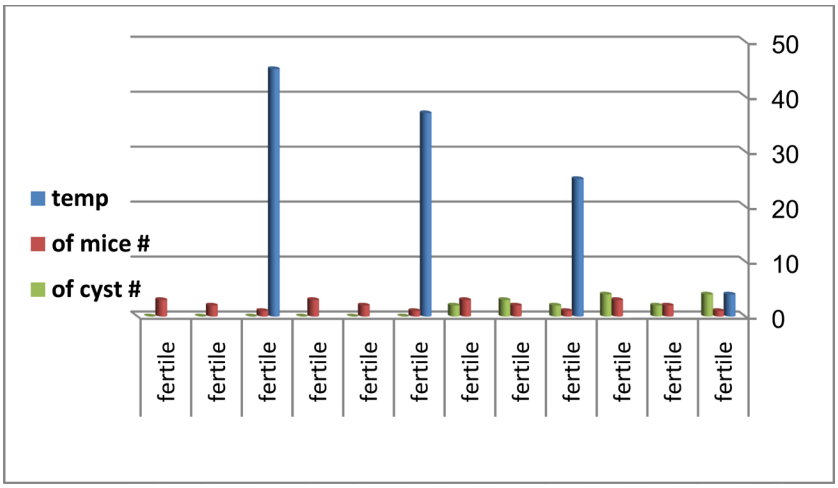

Figure 1. *In vivo* survival rates of protoscoleces kept at different constant temperature.

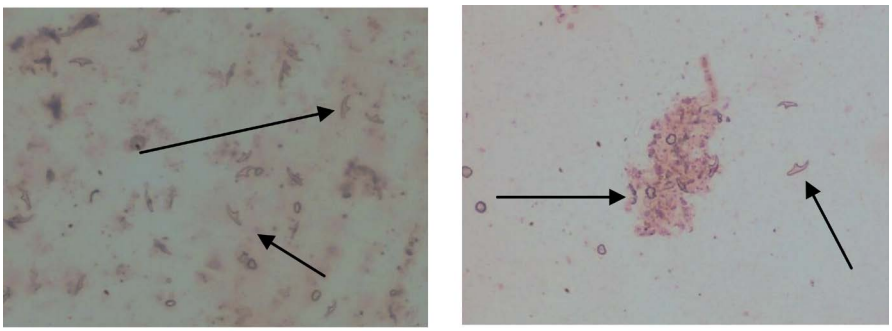

Figure 2. (a) *In vivo* survival of protoscoleces at 4˚C (hooks); (b) *In vivo* survival of protoscoleces at 25˚C (hooks).

Table 4. The *in vitro* survival rates of protoscoleces kept at different constant temperatures.

Days	No. of cyst% 4˚C	25˚C	37˚C	45˚C
1	98	85	89%	Hooks
2	97	77	70%	Hooks
3	96	50	60%	Hooks
4	80	37	51%	
5	77	27	Hooks	
6	70	18		
7	68	Hooks		
8	65			

Continued

9	60
10	51
11	43
12	40
13	37
14	31
15	27
16	23
17	16
18	12
19	8
20	3

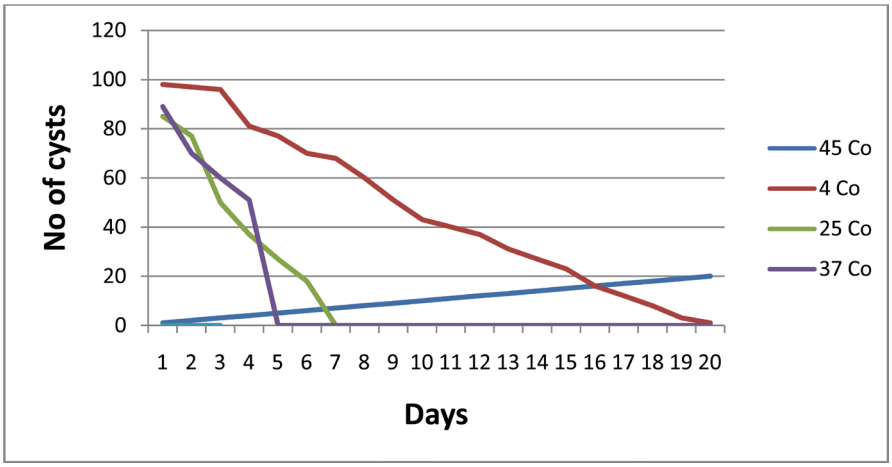

Figure 3. The *in vitro* survival rates of protoscoleces kept at different constant temperatures.

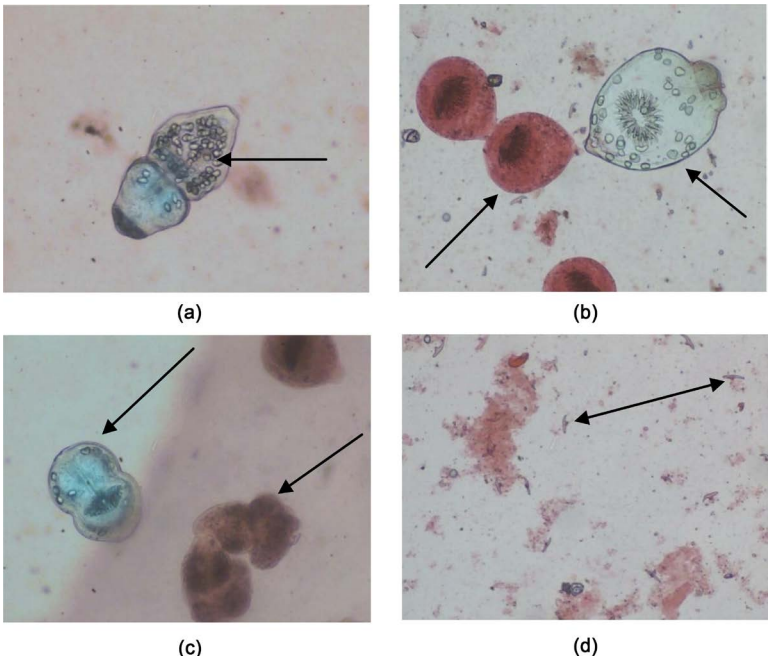

Figure 4. *In vitro* survival of protoscoleces. (a) 4˚C (1st day); (b) 25˚C; (c) 37˚C; (d) 45˚C.

protoscoleces kept for one day at 4˚C, 25˚C and 37˚C (98%, 85% and 89% respectively). Survival rates was in its highest with the 4˚C till the third day and started to decline gradually till it reached 3% in day 20. The 25˚C and 37˚C showed the same pattern but the development ceased in day 7 and 6 respectively. Survival rate in the 45˚C was nill since the beginning of the trails. The rate sharply decreased in the 4th day, for the 25˚C and 37˚C (37% and 51% respectively), however it was reasonably high (80%) in those kept at 4˚C (**Table 4**, **Figure 3**, **Figures 4(a)-(d)**).

For those kept at 4˚C, the viability decreased gradually till it reached its minimum (1%) in the 20th day.

4. Discussion

The results of the survival rate of scoleces *in vitro*, showed that it was in its highest with the 4˚C. This indicates that 4˚C is optimum temperature. Our finding agrees with the work of [2] who reported that scoleces were alive after 5 days at 4˚C. Also, our result was in line with [7] who recognized that hydatid protoscolices remain viable for a long period in different medias at different temperatures. In the absence of nutritive factors, the protoscolices of *E. granulosus* do not survive in for more than 4 days at 37˚C.

In addition to survival of protoscoleces in the preservative solutions, there was evagination of some protoscolices. This could be explained as a step of starting their development in the preservative solution. [7] reported similar morphological changes in culture media. Also, our results of the *in vivo* survival rate of protoscoleces in mice reported that, only scoleces kept at 4˚C and 25˚C succeeded to develop inside the mice while no development of cyst was observed in those kept at 37˚C and 45˚C. Also, our finding showed that the number of cysts found in the 4˚C and 25˚C in the experimented mice were very few when compared with those of the control group.

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