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Some factors affecting metacercarial infections in *Oreochromis niloticus* collected from brackish water, Ismailia, Egypt

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ABSTRACT

The objective of this work is to study the environmental and biological factors affecting metacercarial infections in *O. niloticus* from brackish water, Ismailia, Egypt. Fish were collected monthly during the period from March 2009 to February 2010 from two sites in El Sayadeen pool at Ismailia. Nine different trematode metacercariae and undifferentiated cyst were recovered from *O. niloticus* fish. The identified metacercariae were *Euclinostomum* sp., *Pygidiopsis* sp., *Prohemistomum* sp., *Phagicola* sp., *Bolbophorus* sp., *Stictodora* sp., and *Haplorichis* sp. The total prevalence was higher in site 2 (75.44%) than site 1 (71.41%). The highest infection was recorded in winter (75%), and the lowest in spring (24.67%). Infection was the highest (76.86%) in the largest fishes in weight and size. Infection was higher in female fish (77.57%) compared to males (70.73%). Prevalence, intensity and abundance of each metacercaria species showed different responses to the considered factors.

Key words: *metacercariae, O. niloticus, sites, seasonal patterns, host size and sex.*

INTRODUCTION

Parasites are small players with crucial role in ecological theatres is well said by Marcogliese (2004) as he signified parasites as imperative tools for the effectual management of our natural resources by having an ecosystem based approach (Vankara *et al.*, 2011). It is an established fact that many species of parasites provoke pronounced or subtle effects on hosts affecting their behaviour, growth, fecundity and mortality (Marcogliese, 2004; Vankara *et al.*, 2011). Digenetic trematodes and their metacercariae (MC) take a great interest in most countries especially for the human care against the transmissible diseases (Taher, 2009).

The impact and significance of many factors, both biotic and abiotic, may vary according to the sampling season for each studied host species, and thus, the infection variability may change greatly during the year (Kuklin *et al.*, 2009). The study on seasonal dynamics of parasitism levels serves as a tool to understand broad aspects that determine the population biology of the host-parasite system (Chubb, 1982). Parasites may respond to increasing temperatures more strongly than their hosts. Trematodes are among the most common parasites of vertebrates and they require a molluscan intermediate host in which infective cercariae are asexually produced and released into the water in search of the next host in the life cycle (Marcogliese, 2008).

The parasite cycle may also be synchronized with the host cycle (Pampoulie *et al.*, 2000), inducing a modification of prevalence in relation with the life cycle of host. Generally, reports about the susceptible host sex to infection are varied and conflicting. Changes of the parasite fauna of a host with age have been studied by some workers, with length and weight of fish being used as indication of age (Sinha and Srivastava, 1958).

Metcercarial infections are the most common parasitic infection of the fish in Egypt. Despite the importance of MC in the life cycle of trematodes, little is known about their epidemiology and ecology in their natural hosts in Egypt. Hence, the objective of this work is to study the environmental and biological factors affecting metacercarial infections *in O. niloticus* from brackish water, Ismailia, Egypt.

MATERIAL AND METHODS

Fish were collected from El Sayyadeen pool in Ismailia city during a period extended from March 2009 to February 2010. Two sites were selected for this study, site 1 (N 30° 34' 55.02" E 32° 16' 4.25") and site 2 (N 30° 34' 28.48" E 32° 16' 24.37").

Altogether 230 specimens of the cichlid *Oreochromis niloticus* collected from El Sayyadeen pool. The fish were transported immediately to the laboratory to be examined. The total length of *O. niloticus* was determined by measuring the distance from the tip of the longest jaw or the end of the snout to the longest caudal lobe pushed together (Miller and Lea, 1972). *O. niloticus* fish were divided into three length classes: Class 1 (<11cm.), class 2 (<13 cm.) and class 3 (>13 cm.). Fish sex was determined according to Guerrero and Shelton (1974).

Body cavity was opened and the internal viscera including the muscles, liver, and kidney were examined for the possible presence of trematode metacercariae (MC). These organs were kept in saline solution for few minutes for possible recover of any parasite. Tissues were screened for the presence of metacercariae (MC) by compression method in which snipes were taken from muscles and visceral organs such as liver, kidney and gonads. Each piece was compressed between two microscopic glass slides and examined for the presence of MC (Sayasone *et al*., 2007 and Elsheikha and Elshazly, 2008).

Metacercariae were separately collected by the general feature and were tentatively identified to species level based on the morphological details, their dimensions, the shape of cysts, site of infection, presence of suckers, and shape and contents of excretory bladder under a light microscope (Amer, 1996; Elsheikha and Elshazly, 2008 and Sohn *et al.*, 2009). In addition, the characteristic occurrence of different heterophyid MC in fish either singly or in groups was considered for confirming MC identification (Elsheikha and Elshazly, 2008).

The mean prevalence, intensity and abundance of metacercarial infection in fish muscles were recorded according to Margolis *et al.* (1982). To satisfy the assumption of statistical analysis used, all the data were normalized by log (x+1) transformation to achieve homoscedasticity or linearity. For studying the differences among groups, analysis of variance was used. All data were analyzed with the software packages Microsoft SPSS version 15.0, for statistical evaluation. Values of $P < 0.05$, $P < 0.01$ and $P < 0.001$ reflected levels of significance.

RESULTS AND DISCUSSION

Nine different trematode MC and undifferentiated cyst were recovered from *O. niloticus* fish, Undifferentiated Heterophyid MC, *Euclinostomum* sp., undifferentiated MC, *Pygidiopsis* sp., *Prohemistomum* sp., *Phagicola* sp., *Bolbophorus* sp., *Stictodora* sp. and *Haplorichis* sp. The prevalence was higher in site 2 (75.44%) than site 1

(71.41%). Regarding infection with different species of MC, the highest prevalence was recorded in site 1 for *Phagicola* sp. (29.31%). No infection was recorded in the fish by *Prohemistomum* sp., *Bolbophorus* sp. and the undifferentiated cyst. The highest prevalence in site 2 was recorded also for *Phagicola* sp. (45.61%). The mean intensity of infection was higher in site 2 (98.72±6.8) than in site 1 (81.78±7.8) (Table 1). Regarding infection with different species of MC, the highest intensity of infection was recorded in site 1 (143.85±10.3) and site 2 for *Euclinostomum* sp. the highest intensity of infection was recorded also for *Euclinostomum* sp. ($P < 0.001$). The present study suggests 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 (Taher, 2009).

Table 1: Prevalence, mean abundance (±SE) and m intensity (±SE) of metacercariae infecting *O. niloticus*, from two sites of El Sayyadeen pool.

Metacercariae species	Prevalence of metacercariae			Abundance			Intensity		
	Site 1 (N=116)	Site 2 (N=114)	All sites	Site 1	Site 2	All sites	Site 1	Site 2	All sites
Undifferentiated Heterophyid MC	24.14% (n=28)	19.29% (n=22)	21.74% (n=50)	7.50 ±1.92	7.28± 2.42	7.39±1.54	31.07±6.18	37.73±5.46	34.00±5.71
Euclinostomum sp.	22.41% (n=26)	26.32% (n=30)	24.35% (n=56)	32.24± 3.53	42.41 ±4.85	37.24±3.57	143.85±10.3	153.23±11.6	151.61±9.6
Undifferentiated metacercariae	0.69% (n=8)	5.26% (n=6)	6.09% (n=14)	5.25± 2.97	0. 80± 0.33	3.07±1.52	61.00±10.49	15.00±2.24	50.00±9.91
Pygidepsis sp.	3.45% (n=4)	8.77% (n=10)	6.09% (n=14)	1.03± 0.61	4.19 ± 1.71	2.58±0.89	30.00±10.80	55.00±8.6	47.86±9.84
Prohemistomum sp.	0	1.75% (n=2)	0.87% (n=2)	0	0. 18± 0.13	0.09±0.06	0	10 (n=2)	10 (n=2)
Phagicola sp.	29.31% (n=34)	45.61% (n=52)	37.39% (n=86)	9.91±2.64	11.79 ± 2.08	10.83±1.68	32.86±7.47	26.27±3.70	28.95±3.74
Bolbophorus sp.	0	2.63% (n=3)	1.30% (n=3)	0	0.27 ± 0.15	0.13±0.07	0	10 (n=3)	10 (n=3)
Undifferentiated cyst	0	3.51% (n=4)	1.74% (n=4)	0	3.75±1.87	1.84±0.92	0	105.50±8.69	105.50±8.69
Stictodora sp.	7.76% (n=9)	7.02% (n=8)	7.39% (n=17)	1.72 ± 0.71	4.11 ± 2.27	2.89±1.17	22.22±5.96	57.50±10	38.82±8.3
Haplorichis sp.	14.66% (n=17)	6.14% (n=7)	10.43% (n=24)	1.98 ±0.49	1. 69 ± 0.94	1.8±0.53	13.53±1.47	27.14±5.3	17.50±3.77
Total prevalence	72.41% (n=84)	75.44% (n=86)	73.91% (n=170)	59.22± 3.05	74.47± 3.99	66.57±2.25	81.78±7.8	98.72±6.8	90.35±7.1

N = number of examined fish, n = number of infected fish.

The epidemiological picture of food borne trematodiasis has changed in recent years, in some setting, the prevalence of food borne trematode infections increases while in other, it decreases significantly, which can be explained by factors such as social, economic development, urbanization, adequate food inspections, health, education campaigns, use of chemical fertilizers and water pollution (Keiser and Utzinger, 2004).

The effect of seasonal variation on the prevalence, abundance and intensity of infection in *O. niloticus* in El Sayyadeen pool is shown in table (2). The highest prevalence was recorded in winter (75%), and the lowest in spring (24.67%). In spring, the highest prevalence was recorded for undifferentiated heterophyid MC (37.93%). No infection was recorded for *Stictodora* sp. and *Haplorichis* sp. In summer, the highest prevalence was recorded for *Phagicola* sp. (58.33%). No infection was recorded for undifferentiated MC, *Prohemistomum* sp., *Bolbophorus* sp. and *Haplorichis* sp. In autumn, the highest prevalence was recorded for *Euclinostomum* sp. (39.29%). In winter, the highest prevalence was recorded for *Phagicola* sp. (58.93%), while no infection was recorded for *Pygidiopsis* sp., *Prohemistomum* sp., *Bolbophorus* sp., undifferentiated cyst, and *Stictodora* sp. The mean intensity of infection was higher in autumn (133.07±14.65) ($P < 0.001$) (Table

2). Regarding infection with different species of MC, the highest intensity of infection was recorded in spring for the undifferentiated cyst (106.67±6.02) (P < 0.001). In summer, the highest intensity of infection was recorded for *Euclinostomum* sp. (135.00±12.35) (P < 0.001). In autumn and winter, the highest intensity was represented by *Euclinostomum* sp. (150.45±13.49; 197.06±16.53) respectively (P < 0.001).

Table 2: Prevalence, mean abundance (±SE) and m intensity (±SE) of metacercariae infecting *O. niloticus,* of El Sayyadeen pool during different seasons.

Metacercariae species	**Prevalence of metacercariae**				**Abundance**				**Intensity**			
	Spring 1 (N=58)	**Summer 2 (N=60)**	**Autumn (N=56)**	**Winter (N=56)**	**Spring**	**Summer**	**Autumn**	**Winter**	**Spring**	**Summer**	**Autumn**	**Winter**
Undifferentiated Heterophyid MC	37.93% (n=22)	28.33% (n=17)	16.07% (n=9)	3.57% (n=2)	10.70±3.05	9.66±2.87	6.79±4.36	0.57±0.42	31.36±3.75	35.29±3.76	42.22±12.99	15.00±2.50
Euclinostomum sp.	18.97% (n=11)	10% (n=6)	39.29% (n=22)	30.36% (n=17)	17.89± 3.81	13.97±5.24	59.11±6.89	60.91±5.60	92.73±7.89	135.00±12.35	150.45±13.49	197.06±16.53
Undifferentiated metacercariae	10.34% (n=6)	0%	5.36% (n=3)	8.93% (n=5)	7.02±5.47	0	3.75± 2.66	1.69± 0.75	66.67±12.83	0	70.00±11.42	18.00±1.00
Pygidepsis sp.	12.07% (n=7)	11.67% (n=7)	0%	0%	3.68 ± 2.07	6.03±2.79	0	0	41.43±7.55	54.29±7.97	0	0
Prohemistomum sp.	3.45% (n=2)	0%	0%	0%	0.34 (n=2)	0	0	0	10 (n=2)	0	0	0
Phagicola sp.	6.89% (n=4)	58.33% (n=35)	25% (n=14)	58.93% (n=33)	2.46±1.53	17.93 ±3.85	10.36±4.36	12.83 ±2.97	35.00±7.55	29.72±3.39	41.43±7.93	21.88±2.27
Bolbophorus sp.	5.17% (n=3)	0%	0%	0%	2.98± 0.52	0	0	0	10 (n=3)	0	0	0
Undifferentiated cyst	5.17% (n=3)	1.67% (n=1)	0%	0%	5.61±3.23	1.67 (n=1)	0	0	106.67±6.02	100 (n=1)	0	0
Stictodora sp.	0%	20% (n=12)	8.93% (n=5)	0%	0	4.48±1.48	7.14±4.47	0	0	21.67±2.58	80.00±9.25	0
Haplorichis sp.	0%	0%	28.57% (n=16)	14.29% (n=8)	0	0	5.71±1.97	1.81±0.56	0	0	20.00±3.55	12.50±0.75
Total prevalence	24.67% (n=39)	33.83 (n=50)	64.69 (n=39)	75% (n=42)	46.67±7.85	51.38±5.94	92.68±6.85	77.27±5.77	72.31±6.12	61.60±6.99	133.07±14.65	101.67±10.47

N = number of examined fish, n = number of infected fish.

The effect of the seasonal variations on the parasitic infections in fish is a debate issue (Soliman and Ibrahim, 2012). Touch *et al.* (2009), and Thuy *et al.* (2010) reported that the prevalence of infection is higher during the rainy season compared to the dry season. In contrast, El-Naffar and El-Shahawi (1986), and Raef (1994) reported that the highest percentage of infection in *Tilapia* sp. is found during hot months and the lowest prevalence is during winter, and Abou-Zakham *et al.* (1990) found that the intensity of *Stictodora tridactyla* MC in *Mugil* and *Tilapia* are higher in summer. However, the effects of climate change will be superimposed on a multitude of other anthropogenic environmental changes (Marcogliese, 2008). The consistent seasonal pattern, with variable amplitudes of fluctuation in different fish species in different seasons also suggests the presence of a seasonal infection-modifying factor (Elsheikha and Elshazly, 2008). Both prevalence and intensity may be locality-dependent because the snails and fish live at different places in the lake at different seasons. Trematode infection of snails is known to be affected by physical conditions, size, location and fauna diversity of the habitat (Taraschewski and Paperna, 1981). Thus, seasonal variations of the encysted MC prevalence and intensity in the fish host may probably be dictated by many environmental conditions such as dry, rainfall periods, since the snail vector availability was a limiting factor in determining seasonality of the parasites as reported by (Elsheikha and El Shazly, 2008). In addition, the combination of these factors in addition to the abundance and availability of the fish host may also have influenced the seasonal dynamics of parasite prevalence and intensity in this aquatic ecosystem (Elsheikha and El Shazly, 2008).

The effect of fish length on the prevalence, abundance and intensity of infection in *O. niloticus* of El Sayyadeen pool is shown in table (3). Results showed that prevalence was the highest (76.86%) in fish within length class 3 (>13), and it was the lowest (55%) in fish within length class 1 (<11). Regarding infection with different species of MC, the highest prevalence was recorded in class 1 for *Phagicola* sp. (50%). While no infection was recorded for undifferentiated heterophyid MC, *Pygidiopsis* sp., *Prohemistomum* sp., *Bolbophorus* sp., undifferentiated cyst, and *Stictodora* sp.

Table 3: Prevalence, mean abundance (±SE) and m intensity (±SE) of metacercariae infecting *O. niloticus,* of El Sayyadeen pool according to host size.

Metacercariae species	Prevalence of metcercariae			Abundance			Intensity		
	Class 1 (N=20)	Class 2 (N=89)	Class 3 (N=121)	Class 1	Class 2	Class 3	Class 1	Class 2	Class 3
Undifferentiated Heterophyid MC	0%	14.61% (n=13)	30.58% (n=37)	0	5.00 ±2.79	9.83 ±2.01	0	33.84±8.82	34.05±4.97
Euclinostomum sp.	15% (n=3)	33.71% (n=30)	19.01% (n=23)	46 ±6.35	42.95±3.06	31.58 ±3.55	306.67±27.28	126.00±11.69	146.78±18.63
Undifferentiated metacercariae	5% (n=1)	8.99% (n=8)	4.13% (n=5)	1.00 (n=1)	6.98± 3.99	0.68± 0.33	20 (n=1)	75.00±6.69	16.00±4.00
Pygidepsis sp.	0%	0%	11.57% (n=14)	0	0	4.92± 1.68	0	0	47.85±9.8
Prohemistomum sp.	0%	0%	1.65% (n=2)	0	0	0.17 (n=2)	0	0	10 (n=2)
Phagicola sp.	50% (n=10)	35.96% (n=32)	36.36% (n=44)	10± 3.24	10.93± 3.12	11.02± 2.27	20.00±4.71	30.00±7.32	30.22±7.32
Bolbophorus sp.	0%	0%	2.48% (n=3)	0	0	0.25± 0.15	0	0	10±0.00
Undifferentiated cyst	0%	0%	3.31% (n=4)	0	0	3.50± 1.57	0	0	105.00±8.66
Stictodora sp.	0%	6.74% (n=6)	9.09% (n=11)	0	4.77± 2.93	2.12± 0.75	0	68.33±14.87	22.73±4.87
Haplorichis sp.	15% (n=3)	15.73% (n=14)	5.79% (n=7)	2±1.17	3.18± 1.24	0.84± 0.35	13.33±3.33	20.00±6.29	14.28±2.97
Total prevalence	55% (n=11)	74.16% (n=66)	76.86% (n=93)	59.00±4.29	73.18±3.09	63.00 ±3.48	107±22.22	97.88±8.82	83.01±12.35

N = number of examined fish, n = number of infected fish.

In class 2, the highest prevalence was recorded for *Phagicola* sp. (35.96%) while no infection was recorded for *Pygidiopsis* sp., *Prohemistomum* sp., *Bolbophorus* sp. and undifferentiated cyst. In class 3, the highest prevalence was recorded for *Phagicola* sp. (5.97%). The highest total mean intensity of infection (107±22.22) was recorded in fish within length class 1 (<11) ($P < 0.01$) (Table 3). Regarding infection with different species of MC, the highest intensity of infection was recorded in class 1 for *Euclinostomum* sp. (306.67±27.28) ($P < 0.001$). In class 2, the highest intensity of infection was recorded for *Euclinostomum* sp. (126.00±11.69) ($P < 0.001$). In class 3, the highest intensity of infection was recorded for *Euclinostomum* sp. (146.78±18.63) ($P < 0.001$). This agrees with Takemoto and Pavanelli (1994) who found that increased size and age of fish result in significant increase in the levels of parasitism. On the other hand, Wang *et al.* (2002) recorded that there is no positive correlation between infection rates of *Haplorchis* MC in fish and sizes of infected fish. Poulin (1995) stated that helminth diversity and parasite communities' richness are more consistently correlated with host size. The mean intensity of infection is inversely proportional to *O. niloticus* length and weight. Chappell (1969) also revealed that the infestation level of parasites attained a negative relationship with the length of host fish. On the other hand, Corrêa and Brasil-Sato (2008) stated that the total length of the hosts does not influence the parasite prevalence.

Results show that the prevalence was higher in female fish (77.57%) compared to male one (70.73%) (Table 4). Regarding infection with different species of MC, the highest prevalence in males was recorded for *Phagicola* sp. (33.33%). The highest prevalence in females was recorded also for *Phagicola* sp. (42.06%), No infection was recorded for *Prohemistomum* sp. The mean intensity of infection was higher in males (93.79±12.22) than in females (86.75±10.09) (Table 4). Regarding infection with different species of MC, the highest intensity of infection was recorded in males and females for *Euclinostomum* sp. (161.38±14.47; 141.11±12.95 respectively) (P < 0.001).

Table 4: Prevalence, mean abundance (±SE) and m intensity (±SE) of metacercariae infecting *O. niloticus*, of El Sayyadeen pool according to host sex.

Metacercariae species	Prevalence		Abundance		Intensity	
	Male (N=123)	Female (N=107)	Male	Female	Male	Female
Undifferentiated Heterophyid MC	21.95% (n=27)	21.49% (n=23)	6.22± 2.24	8.00±2.12	31.85±8.84	36.52±7.02
Euclinostomum sp.	23.58% (n=29)	22.43% (n=27)	39.33±5.01	33.24±4.43	161.38±14.47	141.11±12.95
Undifferentiated metacercariae	6.50% (n=8)	5.61% (n=6)	4.12±2.66	2.00±1.36	61.25±15.78	35.00±11.09
Pygidepsis sp.	4.07% (n=5)	8.41% (n=9)	0.84±0.68	4.38±1.76	42.00±15.94	51.11±13.06
Prohemistomum sp.	1.63% (n=2)	0%	0.16 (n=2)	0	10 (n=2)	0
Phagicola sp.	33.33% (n=41)	42.06% (n=45)	9.08±2.08	12.95±2.79	27.56±4.97	30.22±5.57
Bolbophorus sp.	0.81% (n=1)	1.87% (n=2)	0.08 (n=1)	0.19±0.13	10 (n=1)	10 (n=2)
Undifferentiated cyst	0.81% (n=1)	2.80% (n=3)	0.84 (n=1)	2.19±1.50	100 (n=1)	106.67±12.02
Stictodora sp.	9.76% (n=12)	4.67% (n=5)	3.95±2.09	1.81±0.94	39.17±8.36	38.00±11.58
Haplorichis sp.	8.94% (n=11)	12.15% (n=13)	2.07±0.42	1.43±0.42	7.99±2.73	13.08±1.33
Total prevalence	70.73% (n=87)	77.57% (n=83)	65.95±5.56	67.29±4.70	93.79±12.22	86.75±10.09

N = number of examined fish, n = number of infected fish.

The parasite cycle may also be synchronised with the host cycle (Pampoulie *et al.*, 2000), inducing a modification of prevalence in relation with the life cycle of host to indicate that the female host is more susceptible to the infection. El-Naggar and Khidr (1986), Khidr (1990), Hagras *et al.* (1995), and Hagras *et al.* (2001) found similar results, where there is significant preference for one sex over the other. Generally, reports about the susceptible host sex to infection are varied and conflicting. Harrod and Griffiths (2005) reported that the female hosts are more heavily infested than male ones. However, different levels of parasitism in females and males may result from ecological divergence between genders that could lead to different exposure to parasites (Reimchen and Nosil, 2001).

In conclusion, the present study reflects the different responses of MC infecting *O. niloticus* to the environmental and biological factors which were varied according to genus of MC.

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ARABIC SUMMARY

بعض العوامل المؤثرة على الإصابة بالميتاسركاريا فى أسماك البلطى النيلى من مياه قليلة الملوحة بالإسماعيلية

إحسان حسن ، مها سليمان ، عبد الفتاح على غباشى
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هذا العمل يهدف إلى دراسة تأثير بعض العوامل البيئية و البيولوجية على انتشار الميتاسركاريا فى أسماك البلطى النيلي من موقعين من بحيرة التمساح فى الفترة من مارس 2009 إلى فبراير 2010. تم تجميع الأسماك شهريا وتم قياس أطوالها وتحديد جنسها. أوضحت النتائج وجود عشرة أنواع من الميتاسركاريا وكانت نسبة الإصابة فى المنطقة الثانية (75.44%) أعلى من المنطقة الأولى (71.41%). وكانت نسبة الإصابة الأعلى خلال فصل الشتاء (75%) والأدنى فى الربيع (24.76%). وقد تم دراسة تأثير أطوال الأسماك على نسبة انتشار الميتاسركاريا وأظهرت النتائج ان نسبة الإصابة كانت الأعلى في الأسماك الأكثر طولاً (76.86%). و تم أيضاً دراسة تأثير الجنس على وجود الميتاسركاريا وكانت نسبة الإصابة الأعلى فى الإناث (77.57%) مقارنة بالذكور (70.73%). تعكس هذه الدراسة استجابات الميتاسركاريا المختلفة للعوامل المختبرة.