THE EFFECT OF VARIOUS CHEMICALS AND TEMPERATURE IN DESTRUCTION OF THE EGGS OF ASCARIS LUMBRICOIDES: A PROGRESS REPORT

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Abstract

Infestation of soil and night-soil with the eggs or larvae of soil-transmitted helminths is very important factor in the transmission of these infections.

The effect of various temperatures and different chemicals on the development of larva inside the eggs of Ascaris, which is the most resistant eggs or larvae of helminths, or destruction of developed larvae inside the eggs has been evaluated by infecting white mice or by direct observations.

In eggs with developed larva, temperature of 60°C for one hour kills all larvae while in lower temperature, 40% or more larvae are still alive. In a temperature of 60°C for 15 and 20 minutes no larvae is found in the liver of mice.

Of levamisole, thiabendazole, mebendazole, sodium-nitrite and calcium superphophate, thiabendazole and mebendazole have higher effect in destruction of eggs.

In eggs with undeveloped larva, it was shown that in a temperature of 60°C for 15 minutes or more, development of larva does not take place, while in lower temperature development of larva in some eggs takes place. In a temperature of 70°C for 10 minutes no development of larva has been observed.

Evalaution of the effect of levamisole , thiabendazole, mebendazole, urea, iodine and potassium dichromate has shown that mebendazole with a concentration of 1/100,000 or more inhibit the development of larvae. Other chemicals have some effects on the development of larvae. Results of previous investigations have shown that although many chemicals are effective in the laboratory conditions, they are not very effective when used in the field. Therefore, more studies on this

subject in the field are necessary, in order to find application of this control method.

INTRODUCTION

Access to more effective anthelminthics in recent years created much hope in the control of soil-transmitted helminths which are very prevalent in many parts of the world. (1)

However, because of the infestation of environment with the eggs and larvae of these parasites, success in the control can only be achieved by repeated mass-treatment at least once every 3 months.(2) .Proper disposition of human excreta which can be exercised by the provision of sanitation facilities in rural areas, where the high infections with these parasites prevails, can not be achieved because the low socio-economic and cultural conditions of the inhabitants as well as the high cost needed in this type of approach.

Another method for reducing the risk of re-infection with soil-transmitted helminths is destruction of eggs or larvae of these parasites in the environment by use of ovicidal substrances.

This type of approach has been suggested and tried by several workers as early as 1930 and so far the effect of hundreds of various chemicals in the form of solutions or gases, food additives and other physical means have been evaluated. (3)

Of various soil-transmitted helminths the egg of Ascaris lumbricoides is the most resistant to different compounds and therefore any means effective in its destruction can be used for the control of other types of soil-transmitted helminth infections. The present study has been started since September 1975.

MATERIAL AND METHODS

The effect of few chemicals and different temperatures are evaluated on the types of eggs, i.e. Ascaris eggs with fully developed larvae collected in late July from the inhabitants of Isfahan, Central Iran, and brought by the author to New Orleans, and immature eggs collected using glycerine sedimentation method from patients referring to Charity Hospital or brought from infested area in Mississippi and Louisiana. Eggs were cultured in distilled water in room temperature (22-26°C) during the experiments.

The effect of temperature was evaluated by incubating

eggs with fully developed larvae in various temperatures for different times, 40°C for 3 hours; 50°C for 20 and 120 minutes; and 60°C for 10, 15, 20 and 25 minutes; and 65°C for 10 minutes, and the mortality of larvae either by hatching them under the coverslip pressure or by infecting white mice and recovery of larvae, 4-6 days after infection from the liver, and 10-12 days after infection from the lungs have been assessed.

To assess the effect of various temperatures on the development off eggs, eggs collected from patients were exposed to various temperatures for different times, 50°C for 15, 20 and 25 minutes; 60°C for 10, 15, 20 and 25 minutes; 65°C for 5 and 10 minutes; and 70°C for 3, 5 and 10 minutes and were examined 20-25 days after collection and percentage motile larvae were determined by direct examination or recovery of Ascaria larvae from the liver and lung of infected mice. Few animals were also infected with eggs of Ascaris as the controls.

To stimulate the natural environment infested with Ascaris ova, the eggs were incubated in a neutral dust (Decalite) in a depth of one centimeter and then exposed to 50°C for 15, 20 and 25 minutes and examined 25 days after collection.

The effects of various concentrations of chemicals, including Levamisole, Thiabendazole, Mebendazole, Iodine, Urea, Sodium Nitrite and Sodium Nitrite + Calcium Superphosphate on both immature and eggs with fully developed larvae was evalauted by methods previously described.

RESULTS

1. Eggs with fully developed larvae:

1.1. Temperature:

More than 40% of eggs had motile larvae inside after exposing them to 40°C and 50°C for 3 and 2 hours, while the larvae were dead in 60°C for one hour (Table 1).

As indicated in Table 2, Ascaris larvae were recovered from liver of mice infected with eggs exposed to 50°C for 20 minutes, 60°C for 10 minutes, and controls (eggs not exposed to the heat).

No larvae were recovered from the liver of mice infected with Ascaris eggs exposed to 60°C for 15 and 20 minutes. In the lungs the larvae has been found in animals who were infected with eggs exposed to 50°C for 20 minutes, and the controls.

1.2. Chemicals.

The effect of various chemicals on the eggs are shown in Tables 3 and 4. As is shown in Table 3 Ascaris larvae were found in the liver of mice exposed for 48 hours to 1/1000 concentration of Levamisole. Larvae were not found in the lungs of mice infected with Ascaris eggs kept for 48 hours in 1/1000 solution of Levamisole. No eggs were found in the liver or lungs of the mouse infected with Ascaris eggs kept for 48 hours in a concentration of 1/1000 of Thiabendazole . However, the number of larvae found in both livers of animals were much less than the number found in the same organs of control mice.

In Table 4, the results of quantitative approach is summarized. Per cent recovery of larvae was much less in mice infected with eggs with fully developed larvae exposed to chemicals than in the controls.

2- Experiments on the effect of various temperatures and chemicals on the development of larvae:

2.1. Temperature:

As shown in Table 5, no larvae have been developed when incubating the eggs in 60°C for 15, 20 and 25 minutes, but 52% of eggs incubated in 60°C for 10 minutes developed larvae. In 65°C for 5 minutes, 60% of eggs have developed larvae, while in 10 minutes no larvae has been developed. In 70°C for 3 and 5 minutes, 47% and 10% of eggs developed larvae respectively, while no development occurred in 70°C for 10 minutes. Eggs kept in Decalite and exposed to 50°C for 15, 20 and 25 minutes and larvae, indicating that this temperature cannot inhibit the development of larvae in the eggs (Table 6).

2.2 Chemicals:

As is shown in Table 7, of various concentrations of Levamisole, none have been effective in inhibition of the development of the larvae in the eggs, while Thiabendazole in concentrations of 1/100,000 or more and Mebendazole with cocentrations of 1/10,000 and 1/100,000 have been effective.

DISCUSSION

Review of the literature during this period has shown that of several different chemicals, drugs, food additives and other substances tried for at least 4 decades for inhibition of the development of larvae, or destruction of larvae inside the eggs, none except a few have been satisfactory.(4)

Works of other authors indicated that of 4 layers of the egg-shell of Ascaris, the inner membrane i.e. vitelline layer

is impermeable to most of the compounds. Therefore, a solution to the problem might be the use of a chemical with the property of increasing the permeability of the inner layer, permitting the direct contact of chemicals with the larvae and the embryo of the parasite inside ova. (5)

The present preliminary study indicates that the eggs with developed larvae are more resistant to the compounds used but a lot more work is needed to prove this hypothesis.

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Table 1: The effect of various temperature on the eggs of Ascaris with fully developed larvae

Temperature (centigrade)	Time (hour)	Percent of eggs with Motile larvae
40	3	42
50	2	44
60	1	0

Table 2: Results of studies on the effect of temperature on the eggs of Ascaris lumbricoides with fully developed larvae

temper. (cent.)	time (minut)	no. mice infect.	intervals between inf. & exam. (days)	lavae found in	
				liver	lungs
60	10	2	5	+	-
60	15	2	5	-	-
60	15	1	11	-	-
60	20	2	4	-	-
50	20	1	5	++	-
50	20	1	11	1 larv.	4 larv.
control	-	1	3	++	-
"	-	1	10	++	+++

Table 3: Results of the study on the effect of various chemicals with different concentrations on the fully developed eggs of Ascaris lumbricoides

CHEMICALS	timex concen	No. mice infec.	No. mice autop.	days between infec. & autop	larvae found in:	
					liver	lungs
Levamisole	48hX 1/1000	2	2	5	++	-
"	"	1	1	11	-	-
Thiabenda zole	48hX 1/1000	1	1	4	-	-
control	-	1	1	3	+++	-
"	-	1	1	10	++	+++

Table 4: Recovery rates of Ascaris larvae when eggs were incubated in various chemicals for 8 days and used for infecting white mice.

Chemicals used	No. of mice	No. of eggs used	days between infect. & autop.	No. of larvae found in:		% larvae recov.
				liver	lungs	
sodium nitrite 1/1000	1	2500	4	45	-	1.8
"	1	2500	12	5	15	0.8
Sodium nitrite 1/1000 + Calcium superphos-phate 8/100	1	2500	4	72	-	2.
"	1	3500	11	-	3	0.1
control	2	2500	5	297	1	11.9
Mebendazole 1/100,000	1	400	5	-	3	0.9
Control	1	400	5	26	4	0.75

Table 5: The effect of various temperatures on the development of the eggs of Ascaris lumbticoides.

Days kept in room temp. perior to exoeriment	temp. (Cent.)	time (minut)	days between collec. of egg & examination	% with larvae	% motile larvae
4	control	-	18	85	74
4	60	10	18	52	30
4	60	15	18	0	0
4	60	20	18	0	0
4	60	25	18	0	0
10	control	-	22	78	65
10	65	5	22	60	43
10	65	10	22	0	0
10	70	3	22	47	34
10	70	5	22	10	5
10	70	10	22	0	0

Table 6: The effect of exposure of eggs of Ascaris to 50°C for various periods on the development of larvae (eggs were kept for 4 days in the room temperature before and 25 days after exposure to the heat, during which they were kept in Decalite^{R})

time (minutes)	% egg with larvae	% with motile larvae
control	82	70
15	72	58
20	50	35
25	42	28

Table 7: The effect of various chemicals on the development of the eggs of Ascaris lumbricoides

Chemicals used	Period of exposure (day)	Concentration	% eggs with larvae	% of eggs with motile larvae
Potassium di-chromate	20	2.5%	75	65
Normal saline	20	-	72	63
Levamisole	20	1/1000	42	36
Levamisole	20	1/10,000	54	32
Levamisole	20	1/100,000	58	30
Levamisole	20	1/1000,000	60	38
Thiabendazole	20	1/1000	0	0
Thiabendazole	20	1/10,000	0	0
Thiabendazole	20	1/100,000	0	0
Thiabendazole	20	1/1000,000	23	12
Mebendazole	30	1/10,000	0	0
Mebendazole	30	1/100,000	0	0
Urea	30	1/10,000	78	72
Urea	30	1/1000	88	61
Iodine	25	7/10,000	40	32
Iodine	25	7/100,000	64	43