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EVALUATION IN WHITE MICE OF THE INFECTIVITY OF EGGS OF *LAGOCHILASCARIS MINOR* (NEMATODA: ASCARIDIDAE), INCUBATED BY DECORTICATION WITH SODIUM HYPOCHLORITE (NaOCl). [1]

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SUMMARY

White mice were used to study the infectivity of the eggs of **Lagochilascaris minor** Leiper, 1909 after incubation in liquid media, with or without preservative substances. Potassium bichromate ($K_2Cr_2O_7$) at 1% restrict hatching, while 1% formalin gave a greater larval yield. Incubation of eggs in distilled water, in Roux or Falcon flasks gave a good yield, whether the eggs were obtained from human feces or from experimentally infected cats. Treatment of eggs with Sodium hypochlorite (NaOCl) at 5.25% for 2 min prior to inoculation, produced a notable increment of the larval yield in the infections.

KEY WORDS: Lagochilascaris minor; Infectivity of eggs; Modifying substances; Models for evaluation.

INTRODUCTION

Oviposition without segmentation is characteristic of the Superfamily Ascaridoidea Railliet & Henry, 1915 [11]; within its taxons the periods of embryogenesis are characteristic, but conditioned by the variations in oxygenation, humidity, and temperature in the environment of the eggs [1,7].

In **Lagochilascaris minor** Leiper, 1909, it has been reported that lack of oxygen prolongs maturation of the eggs for months [8] . However, in relation with temperature, eggs from purulent secretions of a patient, maintained in refrigeration develop larvae in 30 days [4], while eggs incubated at room temperature, develop infective capacity after 30 days [2]. At the same time has been registrated that eggs incubated in 1% potassium bichromate for 30 days at 25° C infect the rodent **Dasyprocta leporina** Linnaeus, 1758, but not to **Mus musculus** Linnaeus, 1766, suggesting that preservative substances may act on the structures of the eggs, thus altering their infectivity to the hosts [10].

The present work reports the results of techniques designed to determine the minimum time necessary for development of infectivity, and to evaluate the larval yield from infections of the intermediate hosts.

MATERIALS AND METHODS

Maturation of *Lagochilascaris minor's* eggs.

Eggs were derived from an human case, or from experimentally infected cats [9]. Feces from the human case or the cats were washed with distilled water and filtered various times, and the sediment resuspended in aqueous solutions of potassium bichromate (PB) or formalin (F) at 1%, or distilled water without preservative (DW). The suspensions of eggs were then incubated for 30 days or more at 25° C in Roux or Falcon flasks.

Previous treatment of inocula

For each experiment, the samples of eggs were divided in two aliquots. One was washed by centrifugation and decantation to remove the preservative substances. The other aliquot was also washed in the same manner, then resuspended in an aqueous 5.25% solution of commercial grade sodium hypochlorite (SH) for 1 min, afterward it was centrifuged at 1100 g for 1 min, then resuspended and washed 6 times in distilled water.

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Experimental animals

Groups of 8 adult white mice per cage were used, 126 in total, each group being considered as one experiment. The mice were fed with commercial laboratory animal food and water **ad libitum**. Each animal received a dose of 200 to 600 eggs by stomach tube.

Evaluation of infectivity of eggs

Experiment Nº 1 - For study of the spontaneous eclosion of the eggs, the mice were held fasting for 24 hr previous to infection. Groups of 8 mice each were inoculated with eggs treated with PB, F, or DW without preservative. At the same time, equivalent groups of mice were inoculated with eggs that had been treated additionally with sodium hypochlorite (PBSH, FSH, DWSH). Pairs of mice were sacrificed by cervical dislocation and decapitation for bleeding out, at 15, 30, 60, and 120 min post-inoculation. The stomach and the proximal 20 cm of small intestine were extracted, and washed with 2 ml 0.85% saline. The washings were examined with a Diavert-Leitz microscope, in order to detect eggs larvae.

Experiment Nº 2 - For a preliminary study of the larvae in transit into the lungs, groups of 4 mice each, which had been inoculated with eggs as described above, were sacrificed 72 hr post-inoculation. The animals were sacrificed as above and the cardio-pulmonar block extracted. Fifteen ml of a cold citrated salina solution was injected[12] into the right ventricle to flush out the blood, then the organs were sectioned into small portions. These were left 8 hr in saline solution at room temperature in vessels with transparent bottoms in order to be examined with an inverted microscope, larvae which got out from tissue spontaneously and compared with the number of eggs inoculated (larval yield).

Experiment Nº 3 - For a longer-term study, groups of 4 mice each were inoculated in the same way, and maintained for 30 days, after which they were sacrificed, skinned and examined with the naked eye to localize and extract nodules with larvae of the parasite, comparing the yield as above.

Determination of the minimum time necessary for the eggs to become infective

Fresh cat feces containing only eggs of **L. minor**, were washed as above, resuspended and incubated in 1% potassium bichromate. Samples of the eggs were taken every 3 days to determine their development. On the 12th day of incubation, the samples taken were treated sodium hypochlorite as described above, and inoculated by stomach tube in groups of 2 mice, which were sacrificed 30 days post-inoculation for searching of nodules containing larvae into the skeletal muscles and subcutaneous tissues. The procedure was repeated in further pairs of mice until the eggs had been incubated for 30 days.

RESULTS

The use of containers with ample free liquid surfaces for gaseous exchange permitted maturation of a higher percentage of the eggs within 30 days. For eggs incubated without preservative, 70% of the eggs matured within this period, being required three or four changes of the medium. For eggs that were incubated with PB or F, no changes of the media were needed, but maturation was above 90%.

Experiment Nº 1 - For all the inocula that had been treated with sodium hypochlorite, the number of larvae found in the washings from stomach and small intestine were equal or greater than the number of intact eggs observed. One stomach sample showed 19 and the ones small intestine 45 larvae. The average for all the mice, in the 3 groups, was 10 larvae per mouse. On the contrary, the inocula which had not been treated with sodium hypochlorite infected only one mouse, where 2 larvae were obtained from the small intestine of this animal.

Experiment Nº 2 - All mice inoculated with eggs treated with NaOCl became infected, with variability on numbers of larvae obtained from each sample, inclusive between each of them.

The highest larval yield was of one mouse inoculated with PBSH which had 270 larvae, 40% of the eggs inoculated. The maximum yield from DWSH inoculations was 26.3%, and from FSH, 25.4%. The inocula not treated with NaOCl produced far fewer larvae in comparison to the eggs administered: DW, 19%; F. 12%; and PB only 0.62%. The yields were also variable.

Experiment Nº 3 - Modules containing a single larva were visible to naked-eye 30 days post-in-

oculation, with parasites measuring 3 mm in lenght approximately. Among mice inoculated with PB incubated eggs, one developed 2 nodules in the proximal part of a hind paw; while the one with F incubated eggs had an average yield about 4.6%, and those inoculated with DW incubated eggs increased their yield to 10.6%. Treatment of the eggs with NaOCl increased the yield markedly: 30% for PBSH, and 20% for FSH and DWSH. The results of Experiments 1, 2 and 3 are presented in Table I.

Results of the experiment on the minimum time necessary for eggs to become infective - Eggs sampled during the first week showed the progressive celular division and on seventh day they showed an immobile spindle-shaped form. At 9 days, a larva was seen in rapid movement within the egg; on being pressed out of the egg, it disintegrated, with refringent globules of various sizes emerging from its body. At 12 days, there were eggs with mobile larvae which survived being expelled from the egg, maintaining their form and movement. Their posterior extremity showed a short filiform process with a terminal thickening. At 14 days, the larvae appeared less fragile, and the posterior process was longer, with the terminal thickening far from the body, this form is observated successive days. Only mice inoculated with eggs incubated for 15 days revealed nodules in the skeletal muscle and adipose tissue. The yield of nodules per egg inoculated increased from 1% to 12% after 30 days of incubation. The rise in the parasitosis with eggs' incubation time is presented in Figure 1.

DISCUSSION

The object to research the experimental susceptibility of white mice into parasitic disease is owing to get an easy process for reproduction, manipulation and low price maintenance. On the other way, preservative substances used as formalin, potassium bichromate is well known in lab in order to conservate Helminthes' eggs (by their antibacterial action).

It has been reported diverses results of the susceptibility of white mice, inoculated with **L. minor's** eggs incubated in 1% formalin; so, **CAMPOS ET AL.**[3], doesn't get sucess using this substance. However, **CAMPOS & FREIRE** communicated excellent results subsequently[2]. Meanwhile we fall to infect white mice with **L. minor's** eggs incubated in potassium bichromate[11].

This work confirm the formalin advantages and potassium bichromate restricted action on matured eggs eclosion. In effect, while Experiment Nº 1 did not show differences about of the perma-

Table 1

Evaluation in the white mice of the infectivity of eggs of **Lagochilascaris minor** incubated with ou without preservative (Potassium bichromate or formalin), and its comparison with infectivity of eggs treated afterward with sodium hypochlorite, determining the ratio of larvae obtained to the eggs in the inoculum.

Experiment	Nº 1*		Nº 2** yield (%)		Nº 3*** yield (%)	
Medium	Without NaOCl	with NaOCl	without NaOCl	with NaOCl	without NaOCl	with NaOCl
Potassium Bichromate (1%)	-	+	0,62	40,00	E	30,00
Formalin (1%)	-	+	12,00	25,40	4,60	20,00
Distilled Water	E	+	19,00	26,30	10,60	20,00

* = Presence (+) or absence (-) of larvas in stomach or small intestine in the first 2 hr post-inoculation

** = Ratio of the number of larvas in the lungs 72 hr post-inoculation to the number of eggs in the inoculum

*** = Ratio of the number of parasitic nodules in the tissues 30 days post-inoculation to the number of eggs in the inoculum

E = Occasional appearance

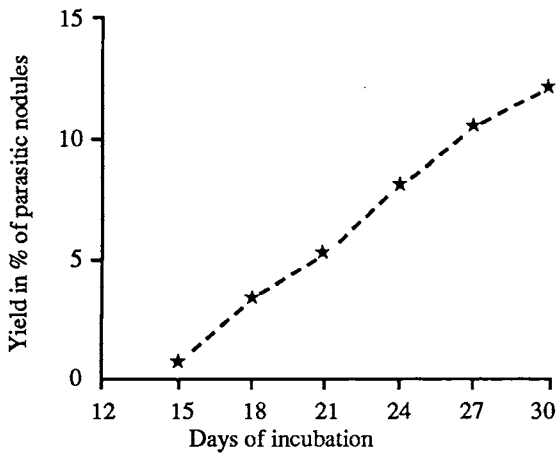

Figure 1: Progressive increase in the yield (%) of parasitic nodules in the tissues of mice inoculated with eggs of **Lagochilascaris minor** incubated in 1% potassium bichromate and treated with sodium hypochlorite, from day 12 to day 30.

nence of the incubated eggs with both preservative substances, together with mice's digestive enzymes for a short time; the Experiment Nº 2 & Nº 3 inoculated animals with potassium bichromate incubated eggs, reported sucess occasionally in spite of its permanence reached maximal physiological time inside mice's bowels while the one inoculated formalin reached 12% and 4.6% of yield respectively (Table 1).

Meanwhile, confronting the final results between the one obtained in similar experiments where **L. minor**'s eggs were incubated into distilled water, we noted the increment reached by Experiment Nº 2 & Nº 3 was almost twice: 19% and 10.6% respectively (Table 1) suggesting that formalin, in less grade than potassium bichromate, restrict eclosion of eggs if we think over that distilled water incubation without preservative substance may be considered as the normal pattern in this class of experiments.

The action of sodium hypochlorite upon the eggs ascarids that parasitic man has been employed for various ends. So **GRUBB & OLIVER-GONZÁLEZ**[5] reported ovicidal action on **Ascaris lumbricoides** and **A. suum** at a concentration of 0.0015% in 2 hr, and pHs of 6.5 and 6.7, respectively. **KENNEDY & QURESHI**[6] used a 25% concentration to decorticize eggs of **A. suum** to obtain living larvae for use as antigens. The present study employed a concentration of 5.25% for 2 min, and its action upon the egg of **L. minor** was limited to the outer shell: On the one way, inhibiting the preservative substances effects, and the other way, weakening the shell, and allowing larvae to break it and hatch earlier. In effect, it should be noted that, in Experiment Nº 1, the inocula not treated with NaOCl, whether incubated with preservatives (PB, F) or not (DW), hatching of eggs may be eventual in DW one, during the first 2 hr, while the treated inocula showed numerous hatchings within the first few minutes post-inoculation. Thus, pretreatment with NaOCl allowed the larvae to come into contact with the host mucosa earlier, increasing the chance of larval to get into the blood circulation because of larger area of exposition, in contrast to the untreated inocula, which was affected only by the digestive juices. This caused a higher rate of infection as is supported by the results of Experiment Nº 2, where a recounting of larvae which migrated across the mice's lungs with 72 hours post-inoculation. The larval migration is a continuos process that usually begins to 12 hr post-inoculation and ends after 5 days when is finished their establishment into skeletal muscles and adipose tissue.

Independently of the expected destruction of larvae by host response, the final count of larvae established in nodules confirms the action of NaOCl, and it also shows the inhibition of hatching by potassium bichromate, whose action is limited to the outer shell of the egg.

Sodium hypochlorite at the concentration and time that we have employed, causes a partial digestion of the outer shell (Fig. 2), without affecting the interior. Thus, eggs incubated in potassium bichromate, when treated with NaOCl after 12 days of incubation and then inoculated into mice, would allow larvae of earlier maturation (15 days) to infect the host. With longer periods of incubation, the percentage of infection continually rises (Figure 1), indicating that the eggs liberated daily in the feces of the final host form a heterogeneous population.

In conclusion, despite the wide variations of the infections in mice inoculated at the same time with the same doses of eggs, the application of sodium hypochlorite as described here, leads to a higher yield of infection in white mice, facilitating the study of the parasite, which is the agent of an important disease in the Neotropical Region.

VOLCAN,G.S.; MEDRANO,C.E. & QUIÑONES,D. - Evaluation in white mice of the infectivity of eggs of **Lagochilascaris minor** (Nematoda: Ascarididae), incubated in liquid media with and without preservative substances. Increment by decortication with sodium hypochlorite (NaOCl). **Rev. Inst. Med. trop. S. Paulo, 35** (2): 193-198, 1993.

RESUMEN

Evaluación en ratones Albinos de la Infectividad de huevos de Lagochilascaris minor (Nematoma: Ascarididae), incubados con substancias preservadoras de uso frecuente. Incremento por el descortezamiento con Hipoclorito de Sodio

Fue realizado un estudio para determinar las variaciones en la infectividad de huevos de **Lagochilascaris minor** Leiper, 1909, cuando son incubados en medios líquidos con o sin substancias preservadoras, usándose como modelo experimental al ratón blanco.

El agregado de Bicromato de Potasio

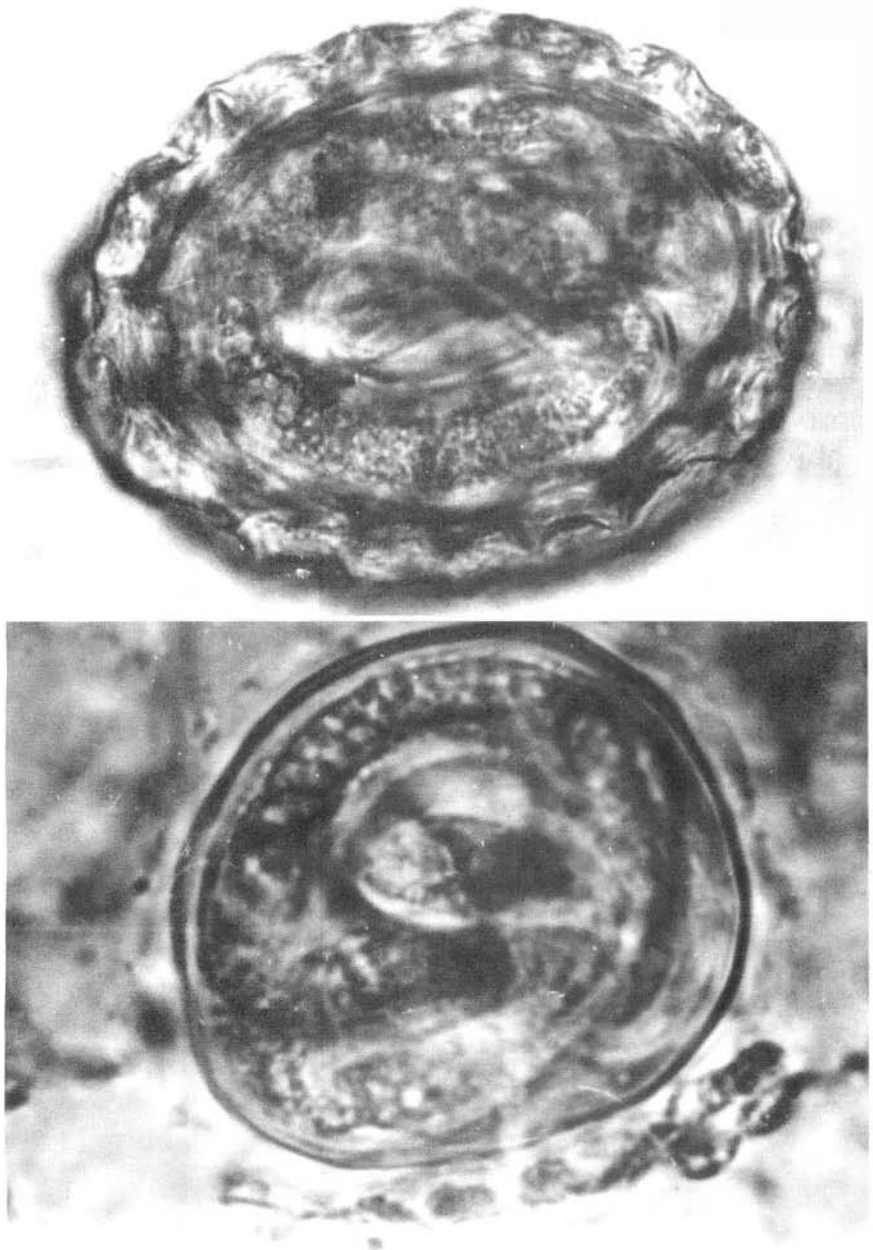

Figure 2: Microphotograph of eggs of **Lagochilascaris minor** incubated 30 days in potassium bichromate at 1% . a) egg untreated prior to inoculation; b) egg treated with 5.25% NaOCl prior to inoculation; observe digestion of outer shell. 1200 X.

($K_2Cr_2O_7$) al 1% en el medio de incubación, restringe la eclosión de los huevos del parásito, mientras que el uso de Formalina al 1% produce un mayor rendimiento. La incubación de huevo del parásito en agua destilada usando recipientes tipo Roux o modelo Falcón, se mostró con un buen rendimiento, aún cuando estas experiencias se realizaron con heces humanas, o de gatos experimentalmente infectados. El uso de Hipoclorito de Sodio (NaOCl) al 5,25% por 2 minutos, antes de la inoculación en los ratones albinos, produjo un notable incremento del rendimiento en las infecciones.

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