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Brief Communication

Ascaris suum: Influence of Egg Density on in Vitro Development from Embryonated Egg to Infective Stage

Large numbers of embryonated eggs can be obtained by dissection of adult female *Ascaris suum (Costello* 1961, *Costello et al.* 1963, *Williams & Soulsby* 1970). Infective larvae develop within eggs when incubated in moisture for 4 to 6 weeks. The author has observed that the rate of development to the infective stage is very slow when egg concentration in a given suspension is high. Furthermore, it has been observed, that some weeks after dilution of such a batch of slowly developing eggs, the rate of development again raised, as evidenced by increased infectivity for mice.

The present experiment was designed to study the development of *A. suum* eggs to the infective stage when incubated at 2 different egg concentrations.

Embryonation of eggs was performed as earlier described (*Eriksen* 1981). Briefly, adult *A. suum* worms were collected from baconers at slaughter. The eggs were obtained by dissection and were decoated by stirring for 5 min at room temperature in 0.5 % sodium hypochlorite. After washing, the eggs were suspended in 0.1 N sulphuric acid in 2 different concentrations and placed in Petri dishes with lid to a liquid depth of 0.5 cm. The egg concentration per microlitre was 25 in egg batch A, and 1250 in egg batch B. Eggs were incubated at room temperature and aerated 2–3 times weekly by stirring.

The eggs were examined once weekly to determine the stage of development. Three hundred eggs were counted to determinate the proportion of eggs with less than 8 cells, of eggs with 8 cells or more and of eggs with viable larvae. After 8 weeks of incubation, the egg hatch test and the mouse (BALB/c mice) inoculation technique were performed as earlier described (*Eriksen* 1981). Infectivity was evaluated by counting the number of larvae recoverable from mouse lungs by a modified Baermann technique on day 8 post oral inoculation with 5000 eggs per mouse.

To determine if substances inhibiting egg development were produced in egg batch B during the 8 weeks of incubation, the egg suspension was centrifuged 10 min at 1500 G and the egg-free supernatant was then used as an incubation medium for a newly collected batch of eggs (batch C) having a concentration of 25 eggs per microlitre. The egg pellet of batch B were, diluted in 0.1 N sulphuric acid to a concentration of 25 eggs per microlitre and reincubated. As a control, another newly collected batch of eggs (batch D) having a concentration of 25 eggs per microlitre was incubated in 0.1 N sulphuric acid. Subsequently the development of batch B, C and D was examined once weekly as described above. After 8 weeks of incubation, the egg hatch test and the mouse inoculation techniques were performed.

Statistical analysis was performed by Student's t-test.

Results are shown in Fig. 1. In batch A (low concentration) eggs with larvae (the infective stage) developed within 4 weeks. In contrast, no larval development was observed in batch B eggs (high concentration). Mice

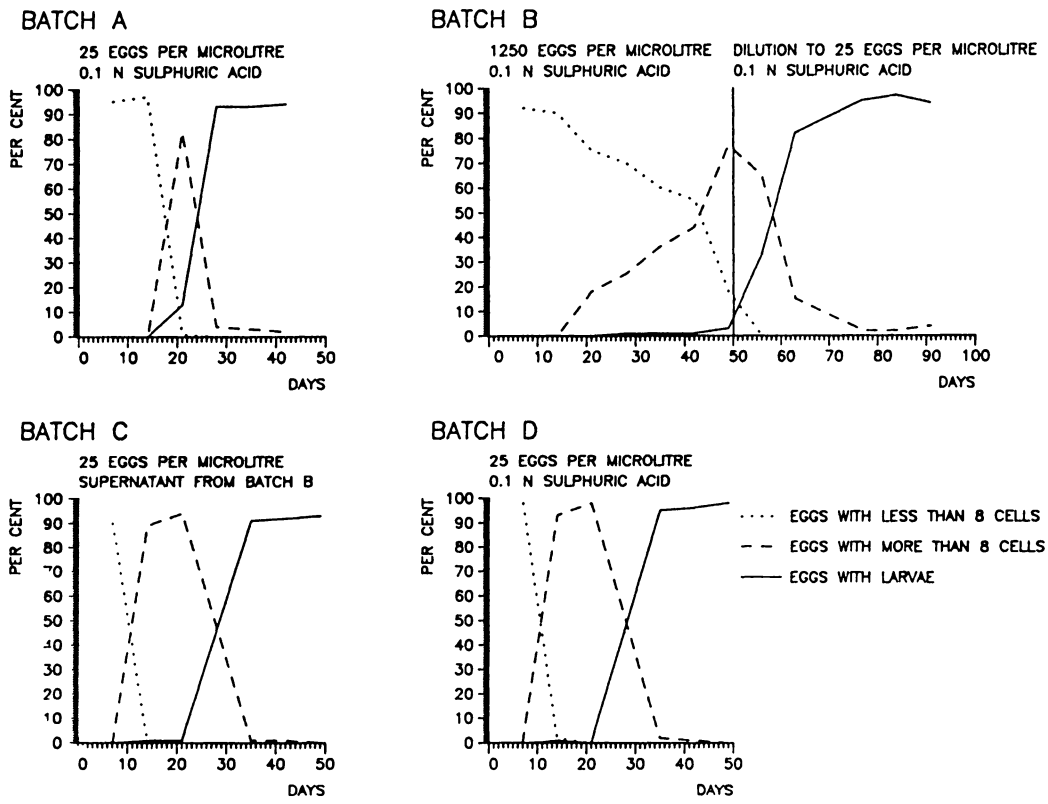

Figure 1. Development of *A. suum* eggs to infective stage during incubation at different egg concentration.

inoculated with 5000 eggs each from batch A yielded a lung larval recovery of 152 ± 65 (average of ten mice ± standard deviation) and all eggs hatched in vitro. In batch B no larval development in the eggs was observed at 7 weeks of incubation and none of the eggs hatched in vitro or were capable of infecting mice. (Ten mice infected each with 5000 eggs).

The supernatant from batch B did not inhibit development of a new batch C of eggs as development in batch C and D (control) was parallel, with regard to larval development, in vitro egg hatch test and infectivity (Table 1).

When the eggs of batch B were diluted at 7 weeks, larval development occurred rapidly within the eggs and 3 weeks later, most of the eggs contained larvae (Fig. 1).

The present experiment demonstrated that embryonation of *A. suum* eggs was temporarily inhibited when the density of the eggs was high during culture. After dilution of the concentrated egg suspension, larvae developed rapidly within the eggs.

The reason why eggs in high concentration have none or retarded development is not clear. *Cleeland* (1963) suggested that a decline in oxygen tension could be responsible, but other mechanisms may also be involved. The culture fluid supernatant from the high egg concentration (batch B) did not seem to

Table 1. In vitro egg hatch test and lung larval recovery (mean ± standard deviation) from mice inoculated orally with 5000 *A. suum* eggs with larvae.

	Batch C supernatant of batch 2 25 eggs/µl	Batch D 0.1 N sulphuric acid 25 eggs/µl
Hatch test, per cent week 7	86	96
Lung larval counts*) week 7	7 ± 5	13 ± 5
Lung larval counts*) week 9	114 ±56	165 ± 81

*) non significant difference between batch C and D (Student's t-test).

contain substances that inhibited development of eggs since comparable development was demonstrated in culture fluid supernatant and 0.1 N sulphuric acid (batch C and D).

It is concluded that suspension of *A. suum* eggs intended for inoculation experiments, should be adjusted to a fixed low concentration of eggs during incubation to ensure optimal development of eggs and a stable inoculum for experimental purposes.

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References

Cleeland R: The effects of different atmospheres and various supplements on the in vitro survival and growth of *Ascaris suum* larvae. J. Parasitol. 1963, *49*, 64–68.

Costello LC: Research Note: A simplified method of isolating Ascaris eggs. J. Parasitol. 1961, *47*, 24.

Costello LC, Oya H, Smith W: The comparative biochemistry of developing Ascaris eggs. Archs Biochem. Biophys. 1963, *103*, 345–351.

Eriksen L: Host parasite relations in *Ascaris suum* infection in pigs and mice. Thesis, Carl Frederik Mortensen, Copenhagen 1981, p. 193.

Williams JF & Soulsby EJL: Antigenic analysis development stages of *Ascaris suum*. I. Comparison of eggs, larvae and adults. Exp. Parasitol. 1970, *27*, 150–162.

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