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THE EFFECT OF LOW TEMPERATURES ON THE DEVELOPMENT OF EGGS OF *ASCARIS SUUM* GOEZE, 1782

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ABSTRACT. Eggs of *A. suum* were kept at -10 and -20°C for 12 weeks and subsequently incubated in a thermostat at 28°C. During the incubation, their development was checked every 5 days. It was found that storage at low temperatures slows down their development and reduces the number of eggs successfully completing their embryogenesis.

Key words: *Ascaris suum*, cleavage, eggs, gastrulation, larval stage.

INTRODUCTION

It is estimated that the number of people around the world infected with the giant intestinal roundworm, *Ascaris lumbricoides* Linné, 1758, is about 1.5 billion (Crompton 1999). In the countries where meadows and pastures are fertilized with swine manure, cattle, sheep, chicken, and humans become increasingly infected with *A. suum* (cf. McCraw and Lautenslager 1971, McLennan et al. 1974, Brown et al. 1984, Murrell et al. 1997, Permin et al. 2000, Arimura et al. 2001, Olsen et al. 2001).

The aim of the study was to determine how the storage at low temperatures effects embryonic development in eggs of *A. suum*.

MATERIAL AND METHODS

The material used for this experiment were eggs of *A. suum* obtained from the terminal sections of the uterus of sexually mature females of this nematode. After being isolated and cleaned from uterine debris, the eggs were suspended in a small amount of tap water. All eggs were divided into 3 samples. Sample No. 1 – the control – was placed in an incubator at 28°C. Sample No. 2 was placed in a freezer at –10°C, and sample No. 3 was placed in a freezer at –20°C. After 12 weeks, all samples were taken out of the freezer and after bringing it up to the room temperature, 5 droplets of the suspension were taken and the development of eggs was evaluat-

ed under a microscope. Subsequently, the eggs were placed in an incubator at 28°C. The solutions were replaced and the development of eggs was observed every 5 days. To this end, 5 droplets of egg suspension were taken from a sample and under a Biolar microscope, at 20 x 12.5 magnification, the percentage of eggs in consecutive developmental stages was determined. Non-fertilised eggs were not taken into account in the calculations. Three following stages were distinguished as development: cleavage, gastrulation, and the larval stage. The culture continued for 30 days.

RESULTS

The results are presented in Table 1. After the period of keeping eggs in sub-zero temperatures, no development of eggs was observed. After 5 days of incubation in an incubator, 55% of control sample eggs had reached the stage of cleavage. In the samples kept at -10°C and -20°C, 24 and 23% of eggs, respectively, reached the stage of cleavage after 5 days of incubation. After 10-day incubation, 47% of eggs in the control sample had reached the larval stage, while in the experimental samples, the percentage of eggs in this stage was much lower and did not exceed 23-27%. In subsequent days, the percentage of eggs, which reached the stage of larva, increased and reached 72% on day 30 for control, whereas for the experimental samples it ranged from 26 and 30%, respectively. The percentage of eggs with arrested development was about 20% in control sample, while those values in experimental samples were 56 and 71%, respectively.

DISCUSSION

The results obtained in this experiment under laboratory conditions are consistent with observations by numerous authors conducting environmental research (Piątkowska 1966, Stevenson 1979, Mizgajska 1993, Wagner and Polley 1999), who found that in winter, when the temperature drops below 0°C, the development of eggs is inhibited. Wagner and Polley (1999) observed the inhibition of development of *A. suum* eggs in a pigsty under the climatic conditions of Canada. Similar results were obtained by Stevenson (1979) in samples placed in pigsties in Britain. Piątkowska (1966) found that after a year-long keeping of *A. suum* and *Toxocara canis* eggs under natural conditions near Gdańsk, about 60% of *A. suum* eggs and 80% of *T. canis* eggs retained their potential for development. A significant decrease of the number of *A. suum* eggs, which were able to develop, was also observed by Mizgajska (1993) after they were kept for 17 months in soil near city of Poznań. According to these authors, the decrease in the number of eggs able to develop resulted from a low temperature during the winter period.

Table 1. The development of *Ascaris suum* eggs after storage at low temperature

Day of incubation	The stage of development*	Mean % and standard deviation Storage at temperature Control	-10°C	-20°C
5	Z	45± 4.6	76 ± 6.3	77 ± 9.7
	C	55 ± 5.5	24 ± 1.5	23 ± 2.7
10	Z	30 ± 2.5	68 ± 8,5	73 ± 9.8
	C	10 ± 3.5	2 ± 3.6	3 ± 2.4
	G	13 ± 1.8	3 ± 0.2	1 ± 0.1
	L	47 ± 6.2	27 ± 2.3	23 ± 1.5
15	Z	25 ± 2.1	59 ± 8.6	73 ± 8.1
	C	8 ± 2.6	13 ± 0.3	2 ± 2.6
	G	18 ± 4.5	1 ± 0.5	1 ± 1.7
	L	49 ± 3.5	27 ± 0.9	24 ± 4.1
20	Z	26 ± 2.8	57 ± 5.7	72 ± 9.5
	C	9 ± 0.6	8 ± 0.7	1 ± 0.4
	G	9 ± 1.8	7 ± 1.5	2 ± 0.2
	L	56 ± 5.6	28 ± 4.8	25 ± 3.8
25	Z	23 ± 1.8	57 ± 9.2	71 ± 9.9
	C	6 ± 0.3	3 ± 0.5	3 ± 0.5
	G	8 ± 0.3	12 ± 1.7	1 ± 0.5
	L	63 ± 4.7	28 ± 4.6	25 ± 3.6
30	Z	20 ± 2.3	56 ± 3.6	71 ± 5.6
	C	5 ± 1.2	7 ± 1.7	2 ± 0.7
	G	3 ± 1.4	7 ± 1.2	1 ± 0.5
	L	72 ± 5.6	30 ± 2.6	26 ± 2.7

*Z – zygote, C – cleavage, G – gastrula, L – larval stage

It was found in this experiment that prolonged exposure of the eggs to low temperatures affects their viability rate. This may have been caused by an energy deficiency resulting from a prolonged cold storage. The development of *A. suum* embryo is made possible by substrates and their precursors accumulated in yolk. One of the stock carbohydrates is trehalose. Apart from its energy-supplying function, trehalose protects nematodes from a temperature stress (van Leeuwen 1995) and from being frozen (Wharton 1995), making possible for many parasites of cold climatic conditions to complete their life cycle (Wharton 1995). The largest concentration of trehalose in the body of *A. suum* was found by Fairbairn and Passey (1957) in the muscles, reproductive system, and in the pseudocoelomic fluid. Prolonged keeping of *A. suum* eggs under extreme conditions may have influenced the decrease of the level of this carbohydrate, thus reducing their development potential.

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