

## The reduction rate of invasive *Ascaris suum* eggs number in slurry and humus layers of selected soil types

H. OLSZEWSKA<sup>1</sup>, K. SKOWRON<sup>2</sup>, K. J. SKOWRON<sup>3</sup>, A. KACZMAREK<sup>2</sup>

<sup>1</sup>Department of Animal Hygiene and Microbiology of the Environment, University of Technology and Life Sciences, Mazowiecka 28, 85-084 Bydgoszcz, Poland, E-mail: [haol@wp.pl](mailto:haol@wp.pl); <sup>2</sup>Department of Microbiology, Faculty of Pharmacy, Nicolaus Copernicus University in Toruń, Collegium Medicum of L. Rydygier in Bydgoszcz, M. Skłodowskiej-Curie 9, 85-094 Bydgoszcz, Poland; <sup>3</sup>Department of Microbiology and Food Technology, University of Technology and Life Sciences, Bernardyńska 6-8, 85-029 Bydgoszcz, Poland

### Summary

The aim of this study was to determine under laboratory conditions the percentage and rate of reduction in invasive *Ascaris suum* eggs, as well as the maximal time of invasiveness retaining by them in swine slurry and 3 soil types. *A. suum* eggs were introduced into perforated perlon bags which then were placed in samples of slurry and in humus layers of the soils: podsolic soil, black earth and browned black earth. The experiment was carried out for 44 weeks at 4 and 20 °C. Finally, at 4 °C a reduction in the number of invasive eggs from 41 % (the humus layer of podsolic soil) to 65 % (the humus layer of black earth) was observed. At 20 °C the fluctuations were smaller and the percentage of elimination ranged from 89 % (slurry) to 96 % (the humus layer of browned black earth).

Keywords: *Ascaris suum*; percentage of invasive eggs; slurry; soil

### Introduction

*Ascaris suum* is one of the most common parasites occurring in pigs. The spread of these nematodes is a serious problem in pigs with access to enclosures (Roepstorff *et al.*, 2001). *A. suum* is relatively best adapted for the modern conditions of pig keeping and as one of few species, it can complete the whole developmental cycle indoors (Rekomendacje Weterynaryjne).

The number of daily produced eggs by a mature female of *A. suum* amounts to 200 000, and in extreme cases can even reach up to 1 600 000 eggs per day (Boch & Supperer, 1983). Eggs of *A. suum* are regarded as the most resistant to unfavourable environmental conditions, where they can survive for months, and even for years (Crompton, 2001).

*A. suum* eggs may occur in all types of environments, but the main source of their transmission is the agricultural usage of swine slurry for fertilization, its transport and spray

irrigation after previous dilution (Crompton, 2001; Venglovsky *et al.*, 2006). Especially high risk is found in the vicinity of large pig farms (Caballero-Hernández, 2004).

The aim of this study was to estimate under laboratory conditions the percentage and rate of reduction of invasive *A. suum* eggs, as well as their maximal time of invasiveness retaining in swine slurry and humus layers of 3 types of soils (podsolic soil, black earth and browned black earth).

### Material and methods

Fragments of about 2 cm in length, counting from the bifurcation, were cut off from uteri prepared out of sexually mature females of *A. suum*. Then eggs were squeezed of them and placed on Petri dishes filled with saline solution. As a result, a suspension with a concentration of *A. suum* eggs equal to about  $4 \times 10^3$  eggs  $\times$  ml<sup>-1</sup> was obtained. Next, a 1 ml of *A. suum* eggs suspension was placed in each perforated perlon bags with a pore diameter of 28  $\mu$ m. Such procedure guaranteed that *A. suum* eggs were subject to all the environmental factors, and at the same time prevented their escaping out of the perlon carrier.

Perlon bags were placed in swine slurry samples and in humus layers of soils: podsolic soil, black earth and browned black earth. Basic parameters characterized the slurry and soils are presented in Table 1 and 2. The samples were stored at 4 °C and 20 °C. The experiment was carried out for a period of 44 weeks, removing bags from each environment after 2, 6, 10, 14, 18, 22, 26, 30, 36, 40 and 44 weeks. The experiment was carried out in 3 replications for each sample. The suspensions of *A. suum* eggs in sterile water, stored respectively at 4 °C and 20 °C, were used as a control.

After removing the bag, it was cut up and its content was transferred to a Petri plate filled with water. The plates were incubated for 30 days at 28 °C.

Table 1. Basic characteristic of swine slurry

Parameters	Value
pH	7.03
Dry matter	5.08 %
Organic dry matter	4.01 %
Total suspended solids	811.20 mg/l
Total nitrogen	2621.42 mgN/l
Dissolved solids	6035.09 mg/l
Ammonium nitrogen	1836.21 mgN-NH <sub>4</sub> /l
Nitrite nitrogen	16.49 mgNO <sub>2</sub> /l
Organic nitrogen	6.20 mgN <sub>org</sub> /l
Phosphorus phosphate	97.70 mgPO <sub>4</sub> /l
Total phosphorus	30.64 mgP/l
ChOD <sub>Cr</sub>	17206.20 mgO <sub>2</sub> /l
ChOD <sub>Mn</sub>	9014.03 mgO <sub>2</sub> /l
BOD <sub>5</sub>	7603.10 mgO <sub>2</sub> /l
Potassium (K)	916.36 mgK/l
Sodium (Na)	1078.24 mgNa/l
Lead (Pb)	0.17 mgPb/l
Zinc (Zn)	2.70 mgZn/l
Mercury (Hg)	b.d.t.m.*
Cadmium (Cd)	b.d.t.m.
Copper (Cu)	0.01 mgCu/l
Nickel (Ni)	b.d.t.m.
Chrome (Cr)	b.d.t.m.

\* b.d.t.m. - below the detection threshold of the method

After the incubation, a percentage of invasive eggs in each sample was estimated based on the microscopic observation of 300 eggs. Additionally, the percentage of invasive eggs in the initial suspension before its exposure to environmental effects was determined, in this way obtaining information about the initial concentration of live eggs.

The obtained results were subjected to the statistical analysis using the program SAS 9.1.

## Results

Percentage of invasive *A. suum* eggs in the initial suspension accounted for 93 %. A gradual reduction in the percentage of live *A. suum* eggs at 4 and 20 °C in each of the studied environments and in control sample occurred in the course of the experiment, and its fluctuations were observed during the study (Figs. 1 – 5).

In control samples, the percentage of invasive *A. suum* eggs decreased to 65 % at 4 °C and to 28 % at 20 °C, during 44 weeks of the experiment (Fig. 1). In case of swine slurry the percentage of invasive *A. suum* eggs decreased to a level of 49 % in samples stored at 4 °C, and in those stored at 20 °C to 11 % (Fig. 2). The smallest amount of invasive eggs in liquid excrements at 4 °C was observed in the 40<sup>th</sup> week of the study (35 %), and at 20 °C in the 36<sup>th</sup> week of the experiment (5 %) (Fig. 2). In samples of the humus layer of podsolic soil stored at 4 °C, at the end of research, the percentage of live eggs was 59 %, whereas at 20 °C only 7 % (Fig. 3). In the humus layer of podsolic soil at 4 °C the lowest percentage of invasive eggs of *A. suum* during the experiment, amounting to 36 %, was observed in the 26<sup>th</sup> week (Fig. 3). In samples of the humus layer of black earth the percentage of invasive eggs of *A. suum* in the last week of the study was, respectively, 35 % at 4 °C and 5 % at 20 °C (Fig. 4). In browned black earth (the humus layer) the final percentage of invasive *A. suum* eggs in the 44<sup>th</sup> week of the experiment in samples stored at 4 °C amounted to 43 %, whereas in those stored at 20 °C

Table 2. Basic characteristic of soils

Parameter	Podsolic soil	Black earth	Browned black earth
P [% a.d.m.]	0.04	0.06	0.05
K [% a.d.m.]	0.04	0.11	0.43
Mg [% a.d.m.]	0.04	0.23	0.27
pH	6.7	7.7	7.2
Organic matter [%]	1.7	7.9	3.5
Granulometric fractions [%]:			
Sand 2,0-0,05 mm	92.0	80.1	64.2
Dust:			
thick 0,05-0,02	3.0	7.2	9.8
fine 0,02-0,002	4.0	8.5	14.0
loam <0002	1.0	4.2	12.0
Textural group	light sand	light loamy sand	light loam

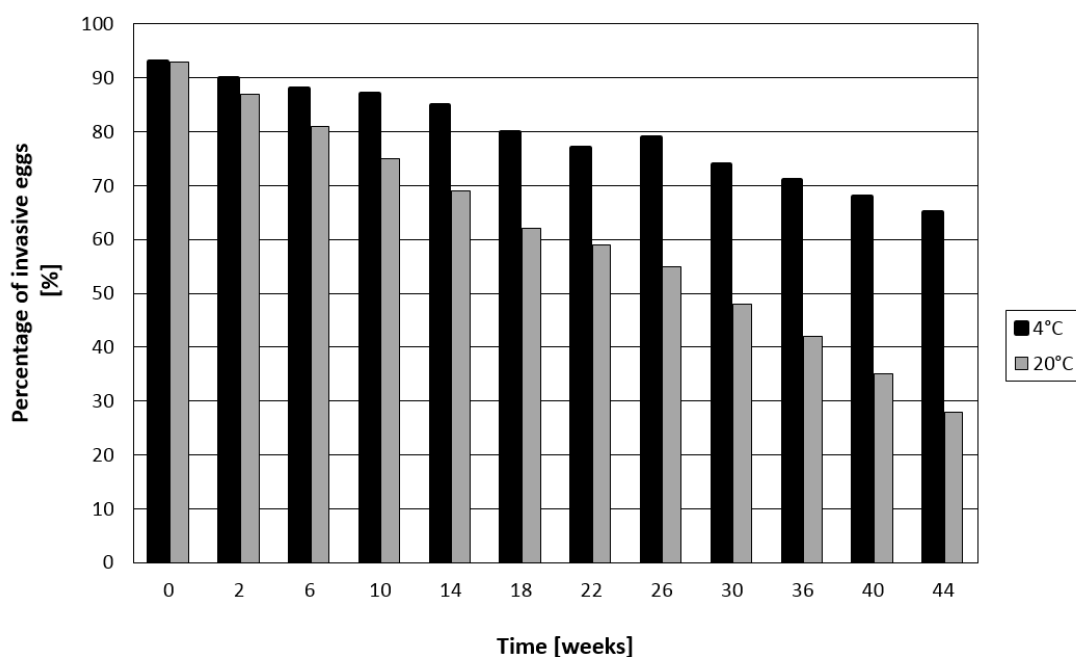


Fig. 1. Percentage of *Ascaris suum* invasive eggs in sterile water storage at 4 and 20°C (control sample)

it was 4 % (Fig. 5). The lowest percentage of live eggs in the samples stored at 4 °C was found in the 40<sup>th</sup> week (29 %), whereas in those stored at 20 °C in the 26<sup>th</sup> week (3 %) (Fig. 5).

Theoretical times of invasiveness retaining by *A. suum* eggs calculated on the basis of obtained results, were highly statistically significantly longer ( $p \leq 0.01$ ) at 4 °C than at 20 °C in each of the studied environments and in control samples. In control samples, the *A. suum* eggs were able to retain invasiveness for highly statistically longer time than in other sample. This time was equal to 143.89 weeks at 4 °C and 64.77 weeks at 20 °C, with elimination rate 1.39 and 0.60 %  $\times$  week<sup>-1</sup>, respectively. In case of

slurry the invasiveness eggs of *A. suum* could be theoretically recovered for 84.80 weeks at 4 °C and for 44.21 weeks at 20 °C, at the elimination rate amounting to 1.14 and 1.67 %  $\times$  week<sup>-1</sup>, respectively. In samples of humus layers of the studied soils stored at 4 °C the theoretical time of invasiveness retaining of *A. suum* eggs stayed at a level from 72.31 weeks in browned black earth to 100.80 weeks in podsolic soil, at the elimination rate amounting to 1.36 do 0.85 %  $\times$  week<sup>-1</sup>. Theoretical time of invasiveness retaining calculated for samples from the humus layer of browned black earth was highly statistically significantly shorter than for the podsolic soil and black earth, and the differences between the podsolic soil and black earth were

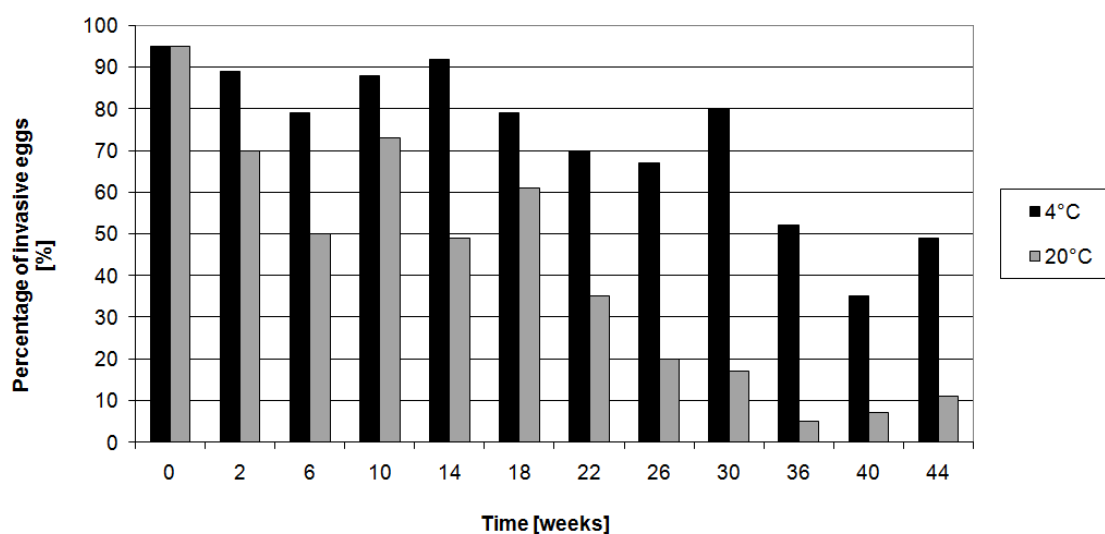


Fig. 2. Percentage of *Ascaris suum* invasive eggs in slurry storage at 4 and 20°C

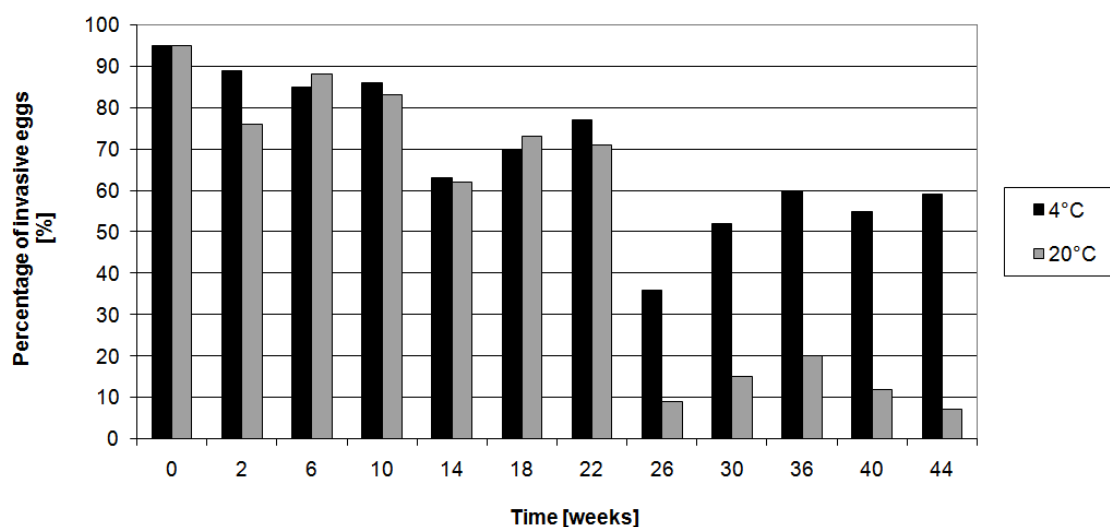


Fig. 3. Percentage of *Ascaris suum* invasive eggs in humus layer of podsol soil storage at 4 and 20°C

statistically significant. In samples of soils stored at 20 °C, the eggs of *A. suum* were theoretically able to retain invasiveness for the period from 40.13 weeks in browned black earth (the humus layer) to 54.07 weeks in black earth (the humus layer) at the elimination rate ranging from 2.36 to 1.56 % × week<sup>-1</sup>, respectively. Differences in the theoretical time of invasiveness retaining of *A. suum* eggs observed between the humus layer of browned black earth and the analogical layer of black earth were highly statistically significant, whereas those observed between humus layers of the podsol soil and black earth were statistically significant (Table 3).

## Discussion

In the conducted experiment an effect of both temperature and the kind of environment on the reduction rate of the number of invasive *A. suum* eggs was proved. Analogical regularity was observed in the study by Venglovsky *et al.* (2006), who also claim that apart from the temperature, the factors which considerably affect the elimination rate of invasive eggs from the given environment are: the type of slurry or soil, relative humidity and UV radiation. Reports of those authors (Venglovsky *et al.*, 2006), similarly to the results of the present study, indicated that parasite eggs

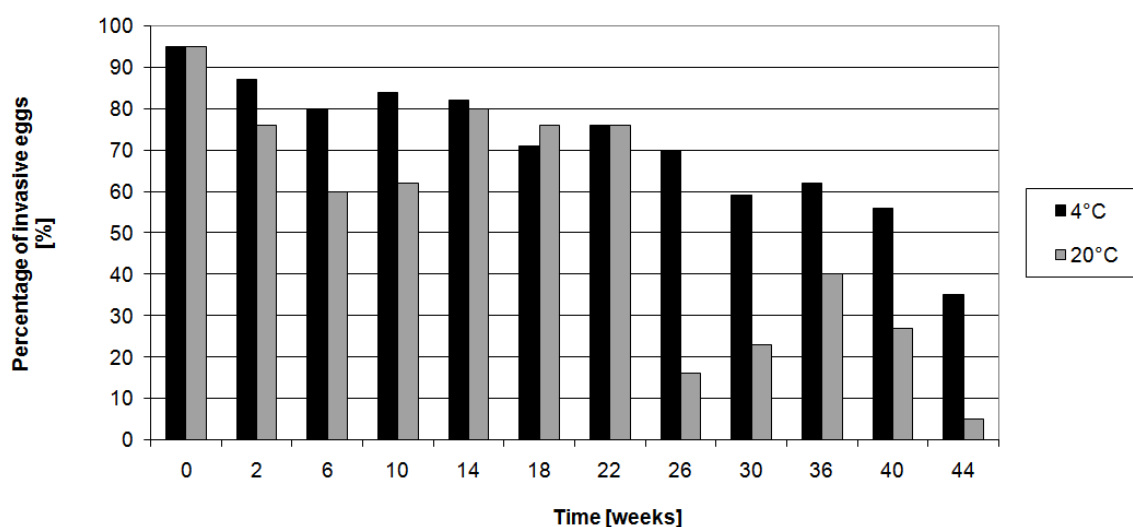


Fig. 4. Percentage of *Ascaris suum* invasive eggs in humus layer of black earth storage at 4 and 20°C

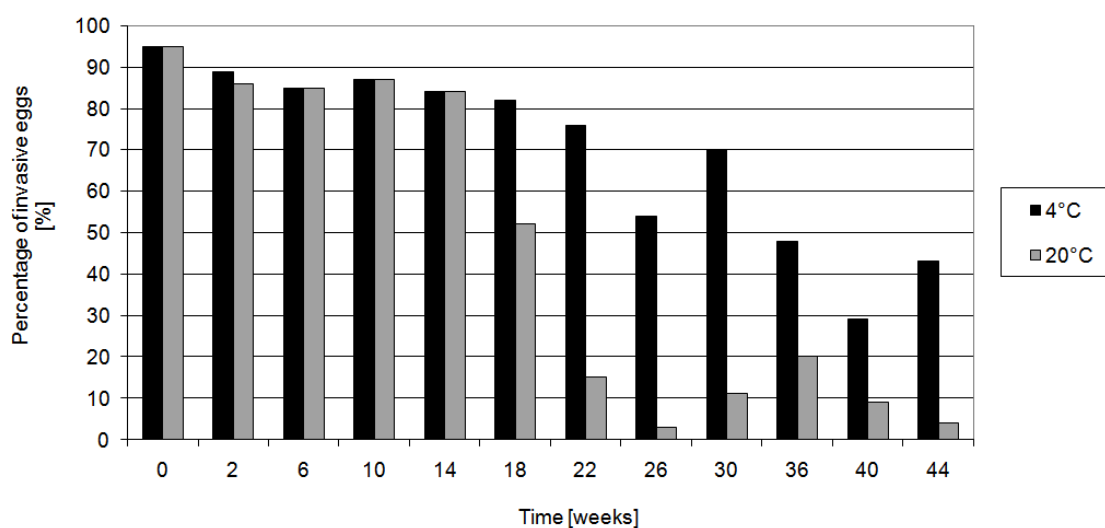


Fig. 5. Percentage of *Ascaris suum* invasive eggs in humus layer of browned black earth storage at 4 and 20°C

subjected to the environmental factors lose their invasiveness, but this process lasts long enough for them to be able to induce infections in people and animals.

Slurry is the most important medium contributing to transmission of *A. suum* eggs from animals having ascariasis to the environment. Results of the present experiment showed that after 44 weeks of storing swine slurry samples at 20 °C there was a reduction in the percentage of live

eggs by 89 %, and at 4 °C by 51 %. Calculated time of invasiveness retaining at 20 °C and 4 °C was equal to 309.47 and 593.6 days, respectively. Quite different results were obtained in the study by Bürger (1984), according to whom the total elimination of invasive eggs of *A. suum* from slurry stored in lagoons occurred after 28 – 75 days, depending on temperature. Similarly, Engik (1980) showed that eggs of *A. suum* are able to survive in swine and cattle

Table 3. The parameters characterizing the rate of elimination of invasive eggs from tested environments at 4 and 20°C

Environment	Temp.	Regression line equation	Theoretical invasiveness time [weeks]	Elimination rate [% × week <sup>-1</sup> ]	R <sup>2</sup> coefficient	Correlation coefficient
Water (control sample)	20°C	$y = -1.3868x + 89.827$	<b>64.77</b> <sup>A,G,a</sup>	1.39	0.99	-0.99
	4°C	$y = -0.6041x + 92.235$	<b>143.89</b> <sup>B,b</sup>	0.60	0.98	-0.99
Slurry	20°C	$y = -1.6703x + 73.841$	<b>44.21</b> <sup>C,F,c</sup>	1.67	0.83	-0.91
	4°C	$y = -1.1391x + 96.591$	<b>84.80</b> <sup>D,d</sup>	1.14	0.74	-0.86
Podsollic soil	20°C	$y = -2.1357x + 95.059$	<b>44.51</b> <sup>C,F,c</sup>	2.14	0.79	-0.89
	4°C	$y = -0.8503x + 85.717$	<b>100.80</b> <sup>E,e</sup>	0.85	0.52	-0.72
Black earth	20°C	$y = -1.5602x + 84.357$	<b>54.07</b> <sup>A,C,f</sup>	1.56	0.78	-0.79
	4°C	$y = -1.0127x + 92.105$	<b>90.95</b> <sup>D,E,g</sup>	1.01	0.82	-0.92
Browned black earth	20°C	$y = -2.3577x + 94.609$	<b>40.13</b> <sup>F,c</sup>	2.36	0.78	-0.89
	4°C	$y = -1.3645x + 98.673$	<b>72.31</b> <sup>G,h</sup>	1.36	0.82	-0.92

A,B,C, ... - high statistically significant differences ( $p \leq 0.01$ )

a,b,c, ... - statistically significant differences ( $p \leq 0.05$ )

slurry at 10 – 17 °C for more than 3 months, whereas at 18 °C they can survive more than 37 days (Engik *et al.*, 1965) that is considerably shorter than in the present study. Present results are similar to those obtained by Bürger (1984), concerning liquid manure and swine manure, where presence of invasive eggs was observed for 365 and 800 days respectively. Gaasenbeek & Borgsteede (1998) indicated that the percentage of live eggs of *A. suum* in swine slurry after respectively 4, 8 and 16 weeks of storage was 80 %, 40 % and 0 %. For comparison, in the present experiment this percentage after 6, 10 and 18 weeks was equal to 50 %, 73 % and 61 % at 20 °C and 79 %, 88 % and 79 % at 4 °C, respectively. Kachnič *et al.* (2014) observed the increase of devitalized eggs percentage from 15.55 to 26.97 % at 4 °C, from 10.13 to 37.65 % at 20 °C and from 14.90 to 99.65 % at 42 °C during 115 days of slurry storage. Johnson *et al.* (1998) observed that both temperature and the part of lagoon have the effect on the level of invasive eggs elimination. According to the results of their study in the period between 5 and 25 week of the experiment the percentage of *A. suum* live eggs in the middle part of the lagoon decreased from 77 % to 11 %, and in the outflow from 68 % to 48 % (Johnson *et al.*, 1998).

Usage of improperly prepared slurry for agricultural purposes creates a possibility of introducing *A. suum* eggs to the soil environment as well as to ground and surface waters and on field crops. Literature data show that *A. suum* eggs survive in soil for all the growing period, and sometimes they are still present there in the third growing period after their introduction (Gaasenbeek & Borgsteede, 1998). It is essential that eggs of *A. suum* survive better in surface soil layers without the vegetation cover, and that the percentage of invasive eggs decreases faster at the soil surface than deep inside the soil profile (Gaasenbeek & Borgsteede, 1998). The percentage of invasive eggs in soil is also affected by the temperature and amount of UV beams getting to it. Larsen and Roepstorff (1999) and Kraglund (1999) indicated that the eggs undergo a faster elimination in summer than in winter, whereas their development is possible only in the summer period. In the present study, the eggs of *A. suum* during 44 weeks at 20 °C underwent elimination which accounted for 93 % – 96 % of initial concentration, depending on the soil type, whereas at 4 °C the reduction ranged between 41 % – 65 %. According to Kato *et al.* (2004) 95.6 % elimination of eggs from soil occurred already after 60 days, and after 90 days it increased only slightly to 96 %. Based on the present experiment, it was determined that the maximal time of invasiveness retaining, depending on the soil type, ranged from 281 to 379 days at 20 °C and from 506 to 706 days at 4 °C. Strauch (1991), in turn, reported that eggs of *A. suum* do not survive in soil longer than 78 – 107 days, depending on the conditions. The results of research quoted by the American Environmental Protection Agency (U.S. EPA, 1992) prove that eggs of *A. suum* are isolated from soil on average for the period of 2 years, and for 7 years maximum, while on plants grown in the infected soil they can be present for a period from 1 to 5 months.

The assessment of survivability of *A. suum* eggs in slurry and in soil is of the utmost importance because of a possibility of pollution water and plants by way of those environments, which may lead to spreading ascariasis both among animals (mainly pigs), considerably reducing production economics, and among people.

## Conclusions

1. Number of invasive eggs of *A. suum* underwent a gradual reduction in all the studied environments and at each tested temperature, whereas at 20 °C the theoretical maximal time of retaining invasiveness by eggs of *A. suum* was about two times shorter than at 4 °C.
2. Effect of the thermal factor on elimination of live eggs was decidedly higher than the effect of the kind of environment in which they were placed.
3. Highest reduction rate of the percentage of invasive eggs was observed in the humus layer of browned black earth at 20 °C, and the lowest in an analogical sample of podsollic soil at 4 °C.
4. In the experiment the differences in the time of invasiveness retaining by *A. suum* eggs between slurry and soil were similar to those found between different soil types.

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