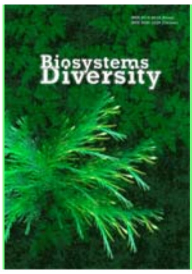

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Changes in the viability of the eggs of *Ascaris suum* under the influence of flavourings and source materials approved for use in and on foods

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One of the most common parasitic diseases of pigs globally is ascariasis. It is caused by the nematode *Ascaris suum* Goeze, 1782 (Nematoda, Ascaridata), which parasitises the small intestine of pigs in its mature form and the respiratory system at the larval stage. This helminthiasis causes immense damage to swine-rearing. Control of the ascariasis pathogen in the host's organism and in the environment is essential for the health of the animals and successful swine-rearing. The results of studying the effect of flavourings and source materials approved for use in and on foods – cinnamaldehyde (0656 Codex Alimentarius), benzoic acid (E_{210} Codex Alimentarius) and methylparaben (E_{218} Codex Alimentarius) on the viability of invasive eggs of *A. suum* are useful for determining the minimum concentration of solution of these substances for use against eggs of *A. suum* (10 g/l). The lowest efficiency against invasive eggs of *A. suum* was obtained for methylparaben (LD_{50} = 3850 ± 2130 mg/l), the highest was obtained with cinnamaldehyde (LD_{50} = 2437 ± 864 mg/l), and benzoic acid (LD_{50} = 1240 ± 680 mg/l).

Keywords: nematodes; antiparasitic activity; flavouring agents; cinnamaldehyde; benzoic acid; methylparaben

Introduction

Swine rearing is one of promising and developed spheres of livestock breeding in the world, and in Ukraine in particular (Feshenko, 2008; Evstafieva, 2010). There are a number of factors which negatively affect its development. First of all, these factors include parasitic diseases, such as ascariasis, trichocephalosis, oesophagostomiasis, strongyloidiasis, echinococcosis, cysticercosis, sarcoptosis, balantidiasis and others. The most widespread disease of pigs globally is ascariasis (De Velásquez et al., 2004; Feshenko, 2008; Evstafieva, 2010; Katakam et al., 2016). It causes great damage to swine-rearing. The prevalence of the disease on pig farms can reach 100%. The disease is especially harmful for young (pigs up to one year old). The intensity of their infestation can reach several hundred eggs in 1 g of feces.

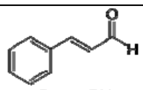
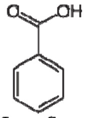
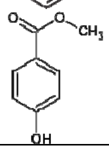
Ascaris suum Goeze, 1782 affects not only the digestive system, but also the respiratory system. Infestation can cause intestinal disorders; high intensity of infestation among piglets causes intestinal blockage, rupture, therefore peritonitis and death. Hepato-pulmonary migrations of *A. suum* lead to liver disorders and bronchopneumonia (Bila, 1999; Evstafieva, 2010). According to Ponomar and Soroka (2008), helminths produce special substances, which prevent the proliferation by T- and B-lymphocytes. Under the influence of helminths' antigens, a great number of T-suppressors appear in the host's organism, which decreases the activity of other subpopulations of T-lymphocytes. This leads to development of immunological tolerance among young pigs. Deficiencies in the immune system are a key factor which determines the onset and duration of the invasive process. Nematodes block the host's defense processes. One of the mechanisms of such immunosuppression is the phenomenon of antigen competition, when T-lymphocytes, which were activated by the helminths' antigens, suppress the ability of B-lymphocytes to produce antibodies to antigens of other infestation agents. This explains the complications during the infection process of *A. suum* infestation and the decrease in the level of postvaccinal immunity against infections, and often causes collapse in the immune system (Ponomar and Soroka, 2008).

Today, scientists all around the world are developing methods of utilization of swine manure, its disinfection, elimination of eggs of *A. suum* in the environment, and treatment of pigs against ascariasis. Recently veterinary specialists have tended to prefer complex injectional antihelmintic preparations, which are efficient both against endo and ectoparasites, and also against different stages of helminths' development. In this respect, preparations of macrocyclic lactone, which are efficient against nematode infestation, appear to be promising. Also there are antihelmintic preparations, which are efficient both at the larval and mature stages of parasites' development. Many Ukrainian and foreign authors (Belœil et al., 2003; Veneziano et al., 2004; Fthenakis et al., 2005; Gudkova et al., 2006; Antipov, 2010; Artemenko, 2011) have reported the high antihelmintic efficiency of preparations of the benzimidazole group (albendazole, phenbendazolum). A number of researchers have conducted experiments on the influence of herbal preparations and flavourings upon the viability of nematode larvae (Chiang et al., 2005; Sato et al., 2006; Somolinos et al., 2008; Si et al., 2009; Belletti et al., 2010).

Materials and methods

In the experiment, we used fresh eggs of *A. suum* nematodes from swine feces, taken from farms in Dnipropetrovsk region. Samples of pigs' feces were delivered in plastic containers at the temperature of +17...+19 °C to the laboratory of the Parasitology and Veterinary-Sanitary Examination Department at the Dnipro State Agrarian-Economic University. At the beginning of experiment, *A. suum* eggs (Fig. 1) were detected by the McMaster method (Pereckiene et al., 2007; Zajac et al., 2011). A thick brown tuberous shell is typical for these eggs. Females of this nematode species lay grey eggs in the small intestine. During their way through the intestines, they obtain a brown colour due to the feces. The eggs detected were not mature: inside they had bleached blastomeres in the process of fragmentation, which is typical for eggs of this species of nematode. Average intensity of *A. suum* infestation in the selected material was 475 eggs/g of feces. Later in the study, swine feces were weighed by portions of 10 g. The samples were put in glasses, by addition of water, the eggs of *A. suum* were "washed" from the feces. The "washed" eggs were then put in a Petri dish and left for 21 days in a thermostat at the temperature of +28 °C for cultivation of larvae inside the eggs before the infestation stage (Fig. 2). Such eggs contained sedentary larvae, twisted in form larvae. At the same time, for preventing the breeding of bacteria and fungi in the sample, 2% solution of formalin was added. Then the solution with eggs was uniformly stirred and poured in 0.1 ml portions into plastic test-tubes of 1.5 ml capacity. Then 1 ml solution of flavourings was added: cinnamaldehyde (0656 Codex Alimentarius), benzoic acid (E_{210} Codex Alimentarius) and methylparaben (E_{218} Codex Alimentarius). The experiment used three concentrations of the substances (10, 1 and 0.1 g/l), and also the control (distilled water); each experiment was conducted with eight replications (Fig. 3). The duration of the experiment was 24 hours at the temperature of +22...+24 °C. The viability of the larvae in the eggs was determined by their mobility when the temperature of the fluid containing the eggs was raised to +37 °C in the solutions of the studied substances.

Table 1
Properties and usage of flavouring agents* which were used for establishing the viability level of *A. suum* larvae

Name of the substance	Chemical formula	Structural formula	Properties	Content	Usage	
					in food industry	in medicine
Cinnamaldehyde	C_9H_8O		Colourless or pale-yellow liquid with strong smell of cinnamon	Is present in brown and cassia essential oil, and also is present in small amounts in oil of patchouly, hyacinths and others	As a component of food essences	As a fungicide, insecticide
Benzoic acid	$C_7H_6O_2$		White crystals, poorly soluble in water	Is present in the content of many plants (about 0.05% in berries) and animals (in the urine of mammals, especially herbivores)	When canning food products	For treating skin diseases, as an external antiseptic and fungicidal agenda
Methylparaben	$C_8H_8O_3$		White crystalline substance with a typical smell	Is found in the roots of *Oxalis tuberosa*	As a preservative	As an antiseptic

Note: * – Official Journal of the European Union, L 354/46 (2008), Sun (2007), Smirnov (2008).

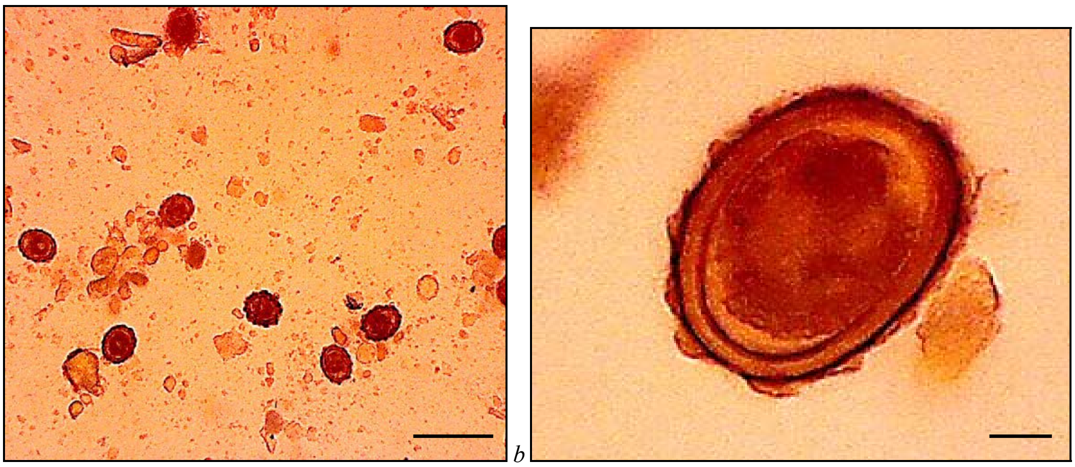


Fig. 1. Eggs of *A. suum* on the first day of the experiment: bar = 100 µm *(a)* or 10 µm (*b*)

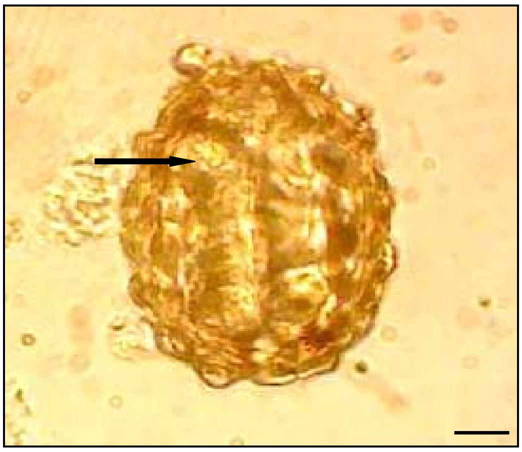
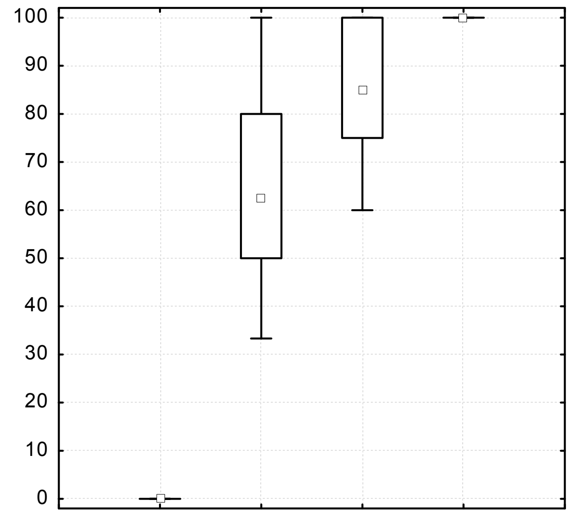


Fig. 2. Mature egg of *A. suum* on 22nd day of the experiment: pointer indicates the formed invasive larvae, bar = 10 µm

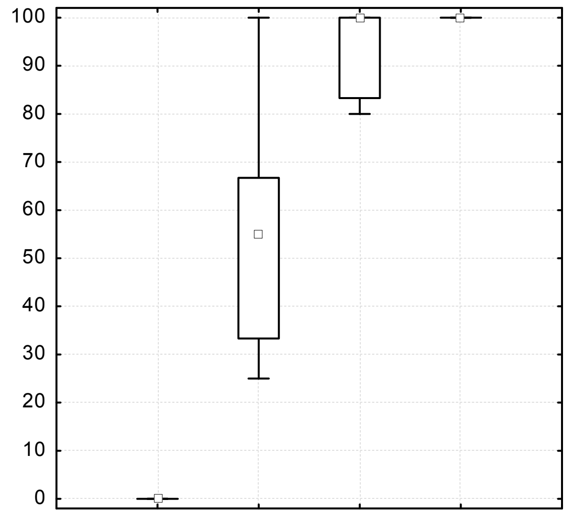
Results

Laboratory experiments on the influence of flavourings and source materials approved for use in and on foods (cinnamaldehyde, benzoic acid, methylparaben) upon the viability of larvae and eggs of nematodes indicate that the concentration of these substances significantly contributed to the mortality of parasites. After raising the temperature of fluid containing the eggs to +37 °C in the solution of 10 g/l cinnamaldehyde concentration, no mobility of *A. suum* larvae in eggs was registered. Only 39% and 16% of *A. suum* larvae died under the concentrations of 1 and 0.1 g/l respectively. LD_{50} for invasive eggs was 2 437 ± 864 mg/l (Fig. 3*a*).

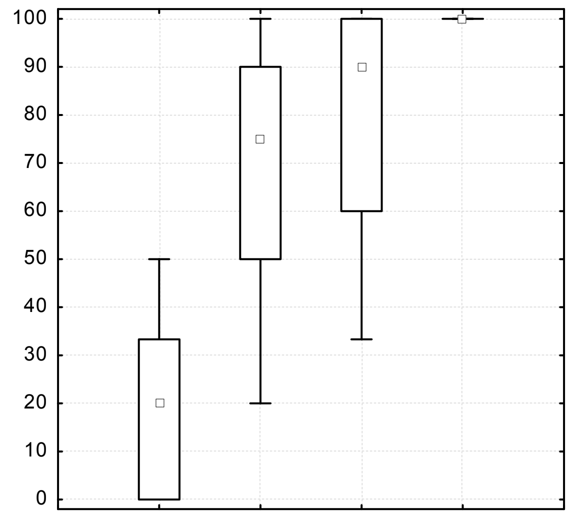
Similar results were registered for pathogens of ascariasis after using benzoic acid. About 55% of larvae in eggs survived at 1 g/l concentration of this substance. With smaller concentrations of benzoic acid, we observed a 100% survival rate of larvae in the eggs of *A. suum*. Only at 10 g/l concentrations of benzoic acid did 100% of eggs die. For *A. suum* at the infestation stage LD_{50} =1 240 ± 680 mg/l (Fig. 3*b*).



a



b



c

Fig. 3. The effect of flavourings and source materials approved for use in and on foods on the vitality of larvae in eggs of *A. suum*: *a* – cinnamaldehyde (LD_{50} = 2 437 ± 864 mg/l), *b* – benzoic acid (LD_{50} = 1 240 ± 680 mg/l), *c* – methylparaben (LD_{50} = 3 850 ± 2 130 mg/l); along the abscissa axis – concentration of active substance (mg/l), along the ordinate – percentage of larvae which survived in the experiments during 24 hours; n = 8

Methylparaben was the least effective. Under 10 g/l concentration of methylparaben 20% of larvae in eggs of *A. suum* survived. At smaller concentration (1 g/l) the substance killed 25% of larvae in the eggs of nematodes. Methylparaben solution at 0.1 g/l concentration killed about 10% of larvae in eggs of *A. suum*. LD_{50} for methylparaben at the infestation stage of *A. suum* eggs was 3 850 ± 2 130 mg/l (Fig. 3*c*).

Discussion

The search for the most effective methods of controlling ascariasis of swine continues to this day. For deactivation of the eggs of these nematodes, ammonia is used (Nordin et al., 2009; Katakam et al., 2014; Fidjeland et al., 2015). The temperature of NH_3 is an essential factor: increase in temperature speeds up the deactivation of *A. suum* eggs. Therefore Fidjeland et al. (2015) recommend adding urea and other alkaline material when processing feces of pigs with ascariasis, and also advise raising the temperature. Similar experiments were also conducted by Katakam et al. (2014). According to the results of their research, following an increase in temperature, the time needed to deactivate *A. suum* eggs decreased. Nordin et al. (2009) proved the lethal action of urea and temperature +34 °C on eggs of *A. suum*. Analysis of their publications proves that the deactivation of nematode eggs in such conditions takes less than 10 days. For deactivating the eggs of *A. suum* in the environment, Nelson et al. (2001) recommend using a mixture of reagents: the observed deactivation was attributed primarily to the 35% ethanol content of the acid-alcohol solution. De Velásquez et al. (2004) have reported the lethal action of ozone on the eggs of *A. suum*. Under the influence of ozone, 90% of eggs became nonviable already after the first 60 minutes. Another 10% of the number of surviving eggs died after the second hour of the experiment. The concentration of dissolved ozone in the liquid phase (in the solution of feces) was from 3.5–4.7 mg/l.

In "organic" swine rearing, the usage of chemical substances against the pathogens of infections and infestations is strictly limited. Therefore pigs in such farms are more heavily affected by *A. suum* infestation. "Organic" farms decrease the level of environmental contamination with *A. suum* eggs by regular mechanical cleaning of the pigs' living area from feces with further compulsory composting (Katakam et al., 2016). Katakam et al. (2014) in their experiments studied the relationship between viability of *A. suum* eggs in deep litter and the litter's humidity and temperature. At a temperature of +43.6 °C and relative humidity of 43% the viability of *A. suum* eggs was no higher than 5%, and only 0.004% of eggs developed to the larval stage. The influence of temperature on the development of *A. suum* eggs was described by Kim et al. (2012). *A. suum* eggs were unable to develop for a month at a temperature of 5 °C. The optimum temperature for the development of eggs of this nematode species is +35 °C: the larvae of *A. suum* develop in eggs already on the 17th day of incubation.

Decreasing the number of *A. suum* eggs in the environment is possible not only by processing the pigs' feces. This can also be achieved by increasing the resistance of the pigs' organism by using a balanced diet dominated by protein. Lack of iron and also the decrease of protein content in the diet leads to malnutrition, loss of weight, decreased resistance in the pigs' organism to *A. suum* (Pedersen et al., 2001, 2002).

Williams et al. (2015) recommend using purified extract of *Cichorium intybus* Linnaeus, 1758 for phytotherapy of *A. suum* larvae. It can affect the viability of larvae of these nematodes *in vitro*. The extract inhibits glutathione-S-transferase activity of *A. suum*.

As an alternative to using synthetic antihelmintic preparations for decreasing the number of eggs which enter the environment through pigs' feces, and also for decreasing the level of pigs' infestation with *A. suum*, Kaplan et al. (2014) recommend using a mixture of essential oils: TTN1013 (a-pinene, linalyl acetate, p-cymene, and thymol octanoate) and TTN1014 (a-pinene, linalyl acetate, p-cymene and thymol acetate).

De Araújo et al. (2008) recommend using fungi-helmintophages *Duddingtonia flagrans* (Dudd.) R. C. Cooke, 1969, *Monacrosporium sinense* Xing Z. Liu & K.Q. Zhang, 1994, *Pochonia chlamydosporia* (Goddard, 1913) Zare et W. Gams, 2001 with domination of the latter species for biological control of the number of *A. suum*. Similar studies have been conducted by Ferreira et al. (2011): they also showed high ovicidal activity of *P. chlamydosporia* fungi against eggs of *A. suum*.

The work of Rojas-Oropeza et al. (2016) covers the study of the influence of volatile fatty acids (VFA) of four different compounds on the viability of eggs of *A. suum*. The compounds included acetic, propionic, butyric, valeric, and isovaleric acids. The studied concentrations of these acids are often found in acidogenic anaerobic digesters. According to the results of the experiment, the viability of *A. suum* eggs decreased after using these acids.

The literature includes data on using flavourings not only in the food industry. These compounds are tested for bactericidal and fungicidal properties (Chiang et al., 2005; Sato et al., 2006; Somolinos et al., 2008; Si et al., 2009; Belletti et al., 2010). The antihelmintic properties of such substances have been studied earlier against *Strongyloides ransomi* (Schwartz and Alicata, 1930) larvae (Nematoda, Rhabditida). LD_{50} ($x \pm SD$) for *S. ransomi* larvae in laboratory experiment: benzaldehyde – 142 ± 64, citral – 97 ± 36 mg/l (Boyko et al., 2017). Thus, chemical compounds used as flavourings in the human diet have a certain antihelmintic potential, which needs to be studied further.

Conclusions

One of the most common parasitic diseases of pigs globally is ascariasis. Control of the ascariasis pathogen in the host's organism and in the environment is essential for the health of the animals and successful swine-rearing. According to the results of study on the effect of cinnamaldehyde, benzoic acid and methylparaben upon the viability of eggs of *A. suum*, we recommend using minimum concentration of solutions of these substances against the eggs of *A. suum*, at 10 g/l.

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