Effects of Some Pesticides on Development of Ascaris suum Eggs

Yong-Man Yu¹, Jin-Won Kim²*, Won-Seok Na³, Young-Nam Youn¹, In-Wook Choi³, Young-Ha Lee³*

¹Department of Applied Biology, College of Agriculture and Life Sciences, Chungnam National University, Daejeon 305-764, Korea; ²Department of Environmental Horticulture, The University of Seoul, Seoul 130-743, Korea; ³Department of Infection Biology, Chungnam National University School of Medicine, Daejeon 301-131, Korea

Abstract: To evaluate the effects of pesticides to parasite eggs, Ascaris suum eggs were incubated with 5 different pesticides (1:1,500-1:2,000 dilutions of 2% emamectin benzoate, 5% spinetoram, 5% indoxacarb, 1% deltamethrin, and 5% flufenoxuron; all v/v) at 20°C for 6 weeks, and microscopically evaluated the egg survival and development on a weekly basis. The survival rate of A. suum eggs incubated in normal saline (control eggs) was 90±3% at 6 weeks. However, the survival rates of eggs treated with pesticides were 75-85% at this time, thus significantly lower than the control value. Larval development in control eggs commenced at 3 weeks, and 73±3% of eggs had internal larvae at 6 weeks. Larvae were evident in pesticide-treated eggs at 3-4 weeks, and the proportions of eggs carrying larvae at 6 weeks (36±3%-54±3%) were significantly lower than that of the control group. Thus, pesticides tested at levels similar to those used in agricultural practices exhibited low-level ovicidal activity and delayed embryogenesis of A. suum eggs, although some differences were evident among the tested pesticides.

Key words: Ascaris suum, egg, pesticide, larval development, embryogenesis

Ascaris suum is an intestinal roundworm of pigs, but can also infect humans [1]. A. suum eggs are resistant to most adverse environmental conditions. Pigs and humans are infected by ingestion of fecally excreted eggs on contaminated vegetables, fruits, food, soil, or in water [1-3]. Zoonotic infections of A. suum have been reported in many countries, including Japan and UK [1,4,5]. Pig manure, which is principally feces, is a valuable and widely used organic fertilizer. The longevity of infective A. suum eggs in pig feces is an important public health issue [2,3]. In Korea, the rate of A. suum infection of pigs by fecal examination was about 17.6% in rural areas [6], thus posing a risk of environmental contamination. Food safety is a major concern of agricultural producers to minimize the contamination of food by microorganisms including parasites.

The eggs of Ascaris species remain infective for a long period of time under laboratory and environmental conditions [7] and are extremely resistant to the actions of common disinfectants [8,9]. For example, a quaternary ammonium salt (alkyl-dimethyl-benzyl ammonium chloride) 2.5% benzenthionium chloride, and a povidone-iodine solution failed to inactivate A. suum eggs [8], which alone survived for up to 48 hr when submerged in absolute ethanol, acetone, xylol, mercuric chloride, or pure Lysol [9]. A pesticide is any substance (or mixture of substances) formulated with the aim of preventing, destroying, or controlling any pest, to be harmful to plants or animals; or insects or arachnids. Although there have some drawbacks using the pesticides during cultivation of agricultural products, such materials have various agricultural benefits and their application is widespread. Some reports indicated that disinfectants exerted ovicidal effects on the eggs of various nematodes [8,9]; however, the effects of pesticides on A. suum eggs have received little attention. Thus, we isolated A. suum eggs from female worms and incubated them with 5 different kinds of pesticides (2% emamectin benzoate, 5% spinetoram, 5% indoxacarb, 1% deltamethrin, and 5% flufenoxuron), diluted to 1:1,500-1:2,000 times (all v/v) used in agricultural applications, at 20°C for 6 weeks. We then microscopically evaluated egg survival and development on a weekly basis.

Female gravid adult worms were collected from the intestines of naturally infected pigs processed in a slaughterhouse.
in Daejeon, Korea. Eggs were collected from the uteri of adult female worms and shaken for 3 min in 4% sodium hypochlorite (Sigma, St. Louis, Missouri, USA) to remove the outer proteinaceous coating. After washing in normal saline (0.9% NaCl), eggs were transferred to 50-ml tubes and incubated with working concentrations of pesticides (1:1,500-1:2,000 dilutions in normal saline) at 20˚C for 6 weeks. The pesticides studied were 2% emamectin benzoate (Syngenta Korea, Seoul, Korea), 5% spinetoram (Dongbang Agro, Seoul, Korea), 5% indoxacarb (Dongbu Farm Hannong, Seoul, Korea), 1% deltamethrin (Sungbo Chemical Co., Goyang, Korea), and 5% flufenoxuron (Sungbo Chemical Co.).

About 200 eggs of each group were examined weekly, and survival and development of eggs were microscopically evaluated by analysis of morphological changes in germinal cells and the presence of viable larvae in the eggs. Each survival rate was calculated as follows: survival rate (%) = number of viable eggs/number of viable and nonviable eggs × 100. Each test was performed in triplicate. The viability criteria used were those of Cruz et al. [10]. Nonviable eggs were microscopically identified as follows: 1) if the egg structure was poorly defined; 2) if contraction, rupture, and loss of membrane continuity was evident; 3) when no larval movement was observed, even upon light stimulation; and, 4) if vacuolization of the cytoplasm was evident, as was unicellular cellular condensation accompanied not only by cytoplasmic vacuolization, but also by development of a granular appearance [10]. All data were presented as means ± SDs. The significance of observed among-group differences was evaluated using analysis of variance (StatView; Abacus Concepts Inc., Berkeley, California, USA). A P-value of <0.05 was considered to reflect significance.

We evaluated the survival rate and morphological alterations of unembryonated *A. suum* eggs incubated in normal saline or pesticide solutions at 20˚C for 6 weeks (Table 1). Most (98 ± 2%) eggs were viable at the commencement of the experiment. Control eggs exhibited 90 ± 3% viability after 6 weeks of incubation. However, the survival rates of pesticide-treated eggs at 1 week (87-93%) were 4-11% lower than the baseline (P < 0.05), and differed somewhat according to the pesticide type. The survival rates declined slowly thereafter, but 75-81% of eggs survived 6 weeks of incubation with dilutions of 2% emamectin benzoate, 5% spinetoram, 1% deltamethrin, and 5% flufenoxuron. Survival rates were thus significantly lower than that of control eggs (P < 0.05), except those eggs treated with a

Table 1. Survival rates of *Ascaris suum* eggs incubated with various types of pesticides at 20˚C for 6 weeks

| Types of pesticides | Dilution factor | Week after incubation at 20˚C |
|---------------------|----------------|-----------------------------|
|                     |                | 0  | 1  | 2  | 3  | 4  | 5  | 6  |
| Control (0.9% NaCl) | 1:1            | 98 ± 2 | 97 ± 2 | 96 ± 2 | 94 ± 2 | 93 ± 3 | 92 ± 3 | 90 ± 3 |
| 2% emamectin benzoate | 1:2,000 | 98 ± 2 | 92 ± 3 | 91 ± 4 | 88 ± 3 | 86 ± 3 | 84 ± 3 | 81 ± 4 |
| 5% spinetoram       | 1:2,000       | 98 ± 2 | 87 ± 3 | 86 ± 3 | 83 ± 4 | 82 ± 4 | 79 ± 4 | 75 ± 4 |
| 5% indoxacarb       | 1:2,000       | 98 ± 2 | 94 ± 4 | 93 ± 3 | 91 ± 3 | 89 ± 3 | 86 ± 3 | 85 ± 4 |
| 1% deltamethrin     | 1:1,500       | 98 ± 2 | 93 ± 3 | 91 ± 3 | 89 ± 3 | 86 ± 3 | 84 ± 3 | 80 ± 4 |
| 5% flufenoxuron     | 1:1,500       | 98 ± 2 | 89 ± 4 | 87 ± 3 | 85 ± 4 | 82 ± 4 | 79 ± 4 | 76 ± 4 |

Pesticides were diluted to 1:1,500-1:2,000 with normal saline. At each time, about 200 eggs were evaluated under a microscope on the basis of morphological changes in triplicate. Data are presented as mean ± SD of 200 eggs.

Fig. 1. Microscopic findings of *Ascaris suum* eggs incubated with various kinds of pesticides at 20˚C for 6 weeks. *A. suum* eggs were incubated with (A) normal saline for 4 weeks, (B) 2% emamectin benzoate for 5 weeks, and (C) 5% spinetoram for 6 weeks. The number means one cell stage (1), 2-8 cell stages (2), larval stages (3) of *A. suum* eggs, and dead eggs (4).
Yu et al.: Effects of pesticides on development of A. suum eggs

We also assessed the morphological characteristics of A. suum eggs, and the extent of embryo development, over 6 weeks at 20°C (Figs. 1 and 2). Initially, all A. suum eggs were non-embryonated and at the 1-cell stage. Control eggs developed from the 1-cell stage to the 2-, 4-, and 8-cell stages from week 1. On dilution of 5% indoxacarb (85 ± 3% survival).

Fig. 2. The effect of exposure to pesticides at 20°C for 6 weeks on development and embryogenesis of A. suum eggs. Two hundred eggs were randomly sampled on a weekly basis and microscopically observed. A. suum eggs were incubated with (A) 0.9% NaCl (control group), (B) 2% emamectin benzoate, (C) 5% spinetoram, (D) 5% indoxacarb, (E) 1% deltamethrin, and (F) 5% flufenoxuron. The relative proportions of eggs at each developmental stage at each time point are shown.
week 2, morulae and gastrulae were evident. Larvae appeared from 3 weeks and 73 ± 2% of eggs contained larvae at 6 weeks (Fig. 2A). The effects of pesticides on the development of A. suum eggs are shown in Fig. 2B-F. Various pesticides exerted different effects on the egg development. Although development of pesticide-treated eggs was evident from week 1, the proportions of developing eggs (87-93%) were significantly lower than that of the control group (97%, P < 0.05). Larvae appeared inside the eggs after 3 weeks of treatment with dilutions of 2% emamectin benzoate, 5% indoxacarb, and 1% deltamethrin, whereas eggs treated with dilutions of 5% spinetoram and 5% flufenoxuron (both v/v) became embryonated from 4 weeks. At 6 weeks, the proportions of pesticide-treated eggs exhibiting larvae (36-54%) were significantly lower than that of the control group (73 ± 2%, P < 0.01). The greatest extent of larval development was evident in the 5%-treated indoxacarb group (54 ± 3%), and the lowest in the 5%-treated flufenoxuron group (36 ± 3%). At 6 weeks, the proportions of eggs treated with dilutions of 2% emamectin benzoate, 5% spinetoram, and 1% deltamethrin that developed larvae were 49 ± 3%, 39 ± 2%, and 42 ± 2%, respectively.

A. suum is an intestinal roundworm of pigs, and the life cycle is identical to that of Ascaris lumbricoides [1]. When humans or other mammals ingest A. suum egg-contaminated vegetables, fruits, food, water or soil, the larvae reach the liver and lung alveoli via the bloodstream, causing eosinophilic pneumonia, liver lesions, myelitis, and visceral larva migrans [1,11]. Thus, inactivation of A. suum eggs is a important aim of sewage treatment [7,12]. Pecson et al. [7] reported that the presence of ammonia is important in A. suum egg inactivation although temperature, pH, and ammonia all contributed to egg inactivation. Also, we can find reports about the effects of temperature, Kimchi extract, UV radiation, disinfectants in the development and embryogenesis of A. suum eggs [8,9,13-16]. Phenol (5%) and cresol (3%) completely inactivated Ascaris eggs, but a quaternary ammonium salt did not [8], and the extent of embryogenesis in A. suum eggs exposed to Kimchi extract was affected by the duration of refrigeration [13]. Temperature is a critical regulator of development and inactivation of A. suum eggs [15,16]. However, no previous report has studied inactivation of A. suum eggs in the agricultural context.

Spreading of cow manure on agricultural land may be economically efficient. As exposure of agricultural products to animal manure (organic fertilizer) increases, the likelihood that such products are contaminated with various microorganisms, including A. suum, increases. Also, many consumers currently prefer raw or lightly cooked vegetables, which may increase the infective potential from vegetables and fruits [2,3,5]. Pesticides are widely applied during cultivation of agricultural products. In the present study, we tested 5 synthetic pesticides used to kill insects and mites that feed on plants in terms of their activities on eggs of A. suum. In the control group, 90 ± 3% of eggs were viable 6 weeks after incubation at 20°C in this study, which was similar to the 87.5% observed after 21 days of incubation at 28°C [10]. However, eggs treated with pesticides for 6 weeks exhibited reduced viability. When treated with a dilution of 5% indoxacarb, 85 ± 4% of eggs survived, similar to the level seen in the control group. However, treatment with other tested pesticides significantly lowered the survival rates, commencing after 1 week of treatment. Ultimately, 19-25% of eggs were dead after 6 weeks of treatment. Thus, most pesticides tested at levels similar to those used in agricultural practice exhibited a low-level of ovidical activity.

We also evaluated the development and embryogenesis of A. suum eggs after treatment with pesticides. A recent report described morphological changes in A. suum eggs incubated in vitro with 0.1 N H2SO4, at 28°C [10]. A total of 12 developmental stages were described, commencing at the 1-cell stage and concluding with the second-stage larva (L2). By day 14 of incubation, 90% of eggs had developed into the first-stage larvae (L1), and, by day 18, all embryos were at the L2 larval stage [10]. The egg developmental pattern of the control group observed in the present study was similar to that noted by Cruz et al. [10], who incubated non-embryonated A. suum eggs at 28°C [10]. However, our developmental timeline was somewhat delayed because we treated eggs at 20°C, and higher temperature (not exceeding 35°C) is known to accelerate the development of A. suum eggs [16]. Pesticide treatment delayed egg development and embryogenesis. When dilutions of 5% spinetoram and 5% flufenoxuron were used, larvae were not observed within 4 weeks. The proportions of pesticide-treated eggs bearing larvae were 36-53% at 6 weeks, which were significantly lower than that of control eggs. Thus, pesticides affected the development of A. suum eggs, as did lead and zinc ions, in a concentration-dependent manner. The latter ions reduced the development of invasive larval stages by 37-66% [17].

One of the limitations of the current study is that A. suum eggs were incubated with solution containing pesticides at structured experimental conditions in the laboratory, so the ovidical activity and embryogenesis patterns of A. suum eggs
may be different from the eggs in the soil. Another limitation of the current work is that embryonation of \textit{A. suum} eggs were checked only by microscopy, so there was no information about the infectivity of the embryonated eggs to an animal model. In the present study, we evaluated the effects of environmentally applied pesticides on the development of \textit{A. suum} eggs. The tested pesticides exhibited minimal ovicidal activities and delayed egg development somewhat. Further research is needed to find out the mechanisms of ovicidal effects by pesticides. Also, it will be interesting to check whether the embryonated \textit{A. suum} eggs exposed to pesticides can be infected into animal hosts or not.

**ACKNOWLEDGMENTS**

The present research has been performed according to a planning project of Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry, and Fisheries (ARPC, project no. 608010-5) and the support of Rural Development Administration, Republic of Korea (project no. PJ9071142012).

**CONFLICT OF INTEREST**

We have no conflict of interest related with this work.

**REFERENCES**

1. Dold C, Holland CV. \textit{Ascaris} and ascariasis. Microbes Infect 2011; 13: 632-637.
2. Kłapeć T, Borecka A. Contamination of vegetables, fruits and soil with geohelminth eggs on organic farms in Poland. Ann Agric Environ Med 2012; 19: 421-425.
3. Erdogrula O, Şener H. The contamination of various fruit and vegetable with \textit{Enterobius vermicularis}, \textit{Ascaris} eggs, \textit{Entamoeba histolyca} cysts and \textit{Gardia} cysts. Food Control 2005; 16: 559-562.
4. Arizono N, Yoshimura Y, Tohzaka N, Yamada M, Tegoshi T, Onishi K, Uchikawa R. \textit{Ascaris} in Japan: is pig-derived \textit{Ascaris} infecting humans? Jpn J Infect Dis 2010; 63: 447-448.
5. Bendall RP, Barlow M, Betson M, Stothard JR, Neisum P. Zoonotic ascariasis, United kingdom. Emerg Infect Dis 2011; 17: 1964-1966.
6. Ismail HA, Ieon HK, Yu YM, Do C, Lee YH. Intestinal parasite infections in pigs and beef cattle in rural areas of Chungcheong-nam-do, Korea. Korean J Parasitol 2010; 48: 347-349.
7. Pecson BM, Barrios JA, Jiménez BE, Nelson KL. The effects of temperature, pH, and ammonia concentration on the inactivation of \textit{Ascaris} eggs in sewage sludge. Water Res 2007; 41: 2893-902.
8. Labare MP, Soochoo H, Kim D, Tsio Ky, Liotta JL, Bowman DD. Ineffectiveness of a quaternary ammonium salt and povidone-iodine for the inactivation of \textit{Ascaris suum} eggs. Am J Infect Control 2013; 41: 360-361.
9. Lehnhart JP. New \textit{ascaris} laboratory ovicides. J Parasitol 1972; 58: 364.
10. Cruz LM, Allanson M, Kwa B, Azizan A, Izurieta R. Morphological changes of \textit{Ascaris} spp. eggs during their development outside the host. J Parasitol 2012; 98: 63-68.
11. Sakakibara A, Baba K, Niwa S, Yagi T, Wakayama H, Yoshida K, Kobayashi T, Yokoi T, Hara K, Itoh M, Kimura E. Visceral larva migrans due to \textit{Ascaris suum} which presented with eosinophilic pneumonia and multiple intra-hepatic lesions with severe eosinophil infiltration–outbreak in a Japanese area other than Kyushu. Intern Med 2002; 41: 574-579.
12. Szabová E, Juris P, Papajiová I. Sanitation composting process in different seasons. \textit{Ascaris suum} as model. Waste Manag 2010; 30: 426-432.
13. Kim JS, Oh DS, Ahn KS, Shin SS. Of kimchi extract and temperature on embryostasis of \textit{Ascaris suum} eggs. Korean J Parasitol 2012; 50: 83-87.
14. Brownell SA, Nelson KL. Inactivation of single-celled \textit{Ascaris suum} eggs by low-pressure UV radiation. Appl Environ Microbiol 2006; 72: 2178-2184.
15. Yu YM, Cho YH, Youn YN, Quan JH, Choi IW, Lee YH. Quantitative evaluation of viability- and apoptosis-related genes in \textit{Ascaris suum} eggs under different culture-temperature conditions. Korean J Parasitol 2012; 50: 243-247.
16. Kim MK, Pyo KH, Hwang YS, Park KH, Hwang IG, Chai JY, Shin EH. Effect of temperature on embryonation of \textit{Ascaris suum} eggs in an environmental chamber. Korean J Parasitol 2012; 50: 239-242.
17. Ziółowska K, Bialowas K, Lopieńska E. Influence of zinc and lead ions on the development of eggs of \textit{Ascaris suum} (Nematoda). Wiad Parazytol 2000; 46:501-506.
