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▣ BRIEF COMMUNICATION

Effects of Some Pesticides on Development of *Ascaris suum* Eggs

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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 20ºC 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% benzethonium 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℃ 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

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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℃ 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 granulated 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℃ 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℃ for 6 weeks

Types of pesticides	Dilution factor	Week after incubation at 20℃						
		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.

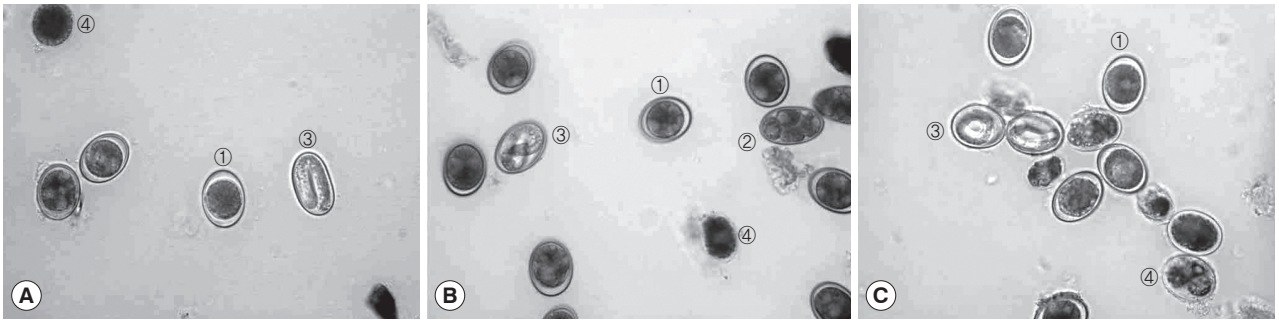

Fig. 1. Microscopic findings of *Ascaris suum* eggs incubated with various kinds of pesticides at 20℃ for 6 weeks. *A. suum* eggs were incubated with (A) normal saline for 4 weeks, (B) 2% emamectin benzoate for 5 weeks, and (C) 5% spinetoram for 6 weeks. The number means one cell stage (①), 2-8 cell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

dilution of 5% indoxacarb (85 ± 3% survival).

We also assessed the morphological characteristics of *A. suum* eggs, and the extent of embryo development, over 6 weeks at 20℃ (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

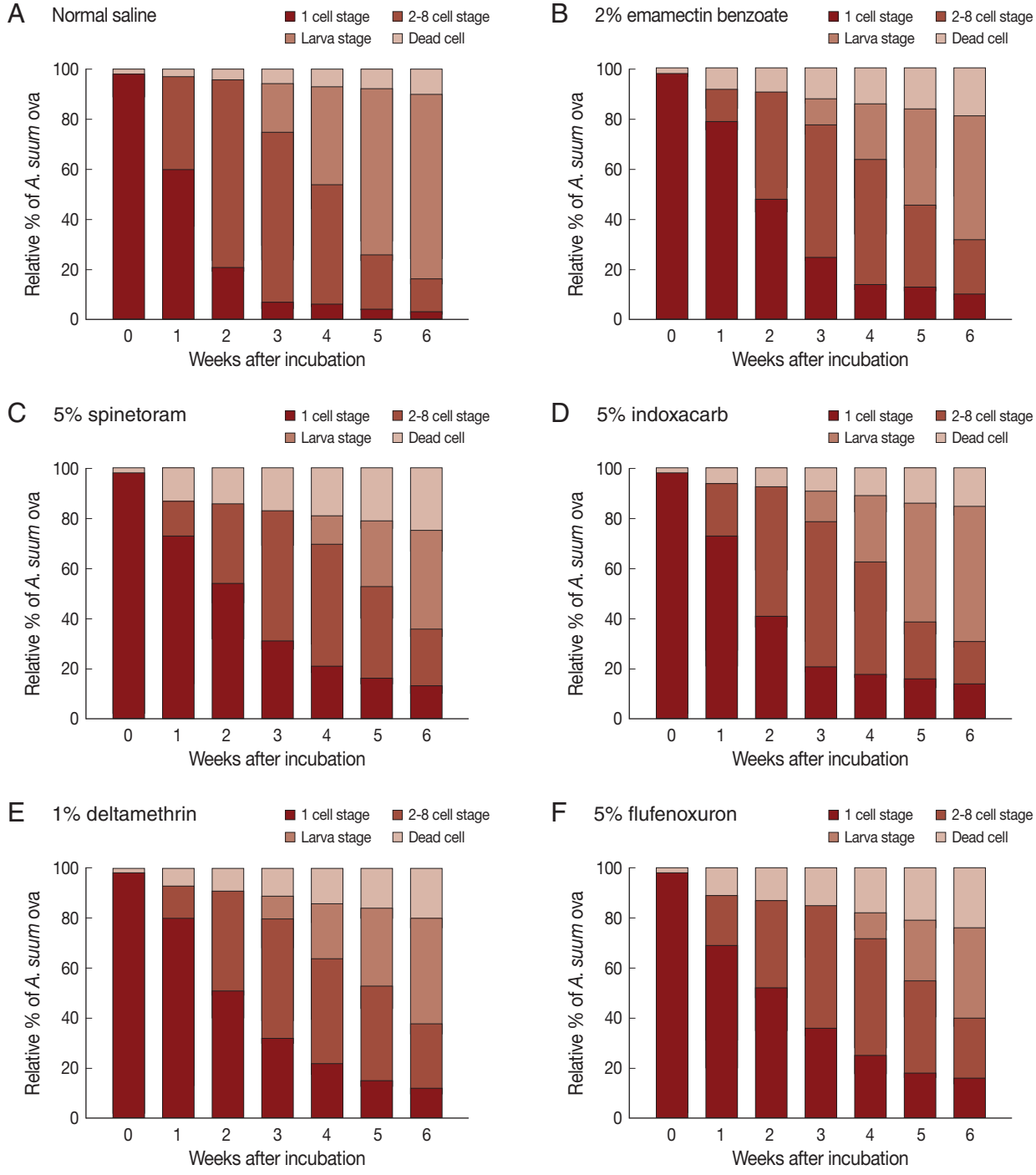

Fig. 2. The effect of exposure to pesticides at 20℃ for 6 weeks on development and embryogenesis of *A. suum* eggs. Two hundred eggs were randomly sampled on a weekly basis and microscopically observed. *A. suum* eggs were incubated with (A) 0.9% NaCl (control group), (B) 2% emamectin benzoate, (C) 5% 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 *Ascaris* 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 *Ascaris* 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℃ in this study, which was similar to the 87.5% observed after 21 days of incubation at 28℃ [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 ovicidal 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 H_2SO_4 at 28℃ [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℃ [10]. However, our developmental timeline was somewhat delayed because we treated eggs at 20℃, and higher temperature (not exceeding 35℃) is known to accelerate the development of *Ascaris* eggs [16]. Pesticide treatment delayed egg development and embryogenesis. When dilutions of 5% spinetoram and 5% flufenoxuron were used, larvae were noted 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 ovicidal 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 *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 *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 *A. suum* eggs exposed to pesticides can be infected into animal hosts or not.

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CONFLICT OF INTEREST

We have no conflict of interest related with this work.

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