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▣ BRIEF COMMUNICATION

Effects of Kimchi Extract and Temperature on Embryostasis of *Ascaris suum* Eggs

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Abstract: To determine the effects of kimchi extracts at different temperatures on larval development, *Ascaris suum* eggs were mixed with soluble part of 7 different brands of commercially available kimchi and preserved at either 5℃ or 25℃ for up to 60 days. *A. suum* eggs incubated at 25℃ showed marked differences in larval development between kimchi extract and control group. While all eggs in the control group completed embryonation by day 21, only 30% of the eggs in the kimchi extract group became embryonated by day 36 and about 25% never became larvated even at day 60. At 5℃, however, none of the eggs showed larval development regardless of the incubation period or type of mixture group. To determine the survival rate of *A. suum* eggs that showed no embryonation after being preserved at 5℃, eggs preserved in kimchi extracts for 14, 28, and 60 at 5℃ were re-incubated at 25℃ for 3 weeks in distilled water. While all eggs in the control group became larvated, eggs in the kimchi extract group showed differences in their embryonation rates by the incubation period; 87.4 % and 41.7% of the eggs became embryonated after being refrigerated for 14 days and 28 days, respectively. When refrigerated for 60 days, however, no eggs mixed in kimchi extract showed larval development. Our results indicate that embryogenesis of *A. suum* eggs in kimchi extract was affected by duration of refrigeration, and that all eggs stopped larval development completely in kimchi kept at 5℃ for up to 60 days.

Key words: *Ascaris suum*, embryonation, kimchi, temperature, embryostasis

The human roundworm, *Ascaris lumbricoides*, is one of the most common parasites, infecting an estimated one-sixth of the global human population [1]. The pig roundworm, *Ascaris suum*, mainly infects pigs but can also infect humans by food items contaminated with swine manure [2]. Sporadic zoonotic infections with *A. suum* have been reported even in developed countries, such as North America [3], Denmark [4], and the United Kingdom [5]. *Ascaris* eggs are resistant to most adverse environmental conditions [6], and pigs and humans become infected by ingestion of fecally excreted eggs through contaminated food, water, or soil [2]. Thus, ascariasis is usually common in rural developing countries with poor sanitation, especially in regions where pig and human feces are used as organic fertilizers. However, infections can occur even in industrialized, urban areas, whenever proper hygiene and sanitation is not improved, such as washing raw vegetables and fruits thoroughly.

In Korea, the prevalence of human ascariasis decreased remarkably due to the national parasite control campaigns that had started from 1969 [7]. By contrast, infection of pigs with *A. suum* continues to be a concern among swine producers. In a recent report from Korea [8], 17.6% of pigs raised on rural farms in a province were infected with *A. suum*. The presence of relatively high percentage of pigs infected with *A. suum*, along with increased demand of organic farming products these days, may increase the risk of human exposure to *A. suum* eggs through vegetables and vegetable products contaminated by using pig feces as a fertilizer. Thus, more attention may need to be paid to the risk of human infection with *A. suum*.

Contamination of kimchi imported from China with parasite eggs hit the Korean society in October 2005, because kimchi is one of the most important side dishes for Koreans served almost every meal. Accordingly, the Korea Food and Drug Administration investigated 502 domestically produced kimchi and cabbages and reported that 3.2% (16 of 502) of kimchi harbored parasite eggs, most of which (81%, 13/16) were as-

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caris eggs originated from animals (http://article.joinsmsn.com/news/article/article.asp?ctg=12&Total_ID=1715222). Since kimchi is made of raw and uncooked vegetables, many studies have been conducted on the possibility of such contamination in kimchi in Korea; survival of *A. lumbricoides* [9-11] and *Giardia lamblia* [12], and the presence of index microorganism [13]. However, no study has been conducted on the survival of *A. suum* eggs in kimchi at different temperatures. The aim of this study was, therefore, to determine the survival rates of *A. suum* eggs after being mixed with soluble extracts of commercially-available kimchi products and preserved at either room or refrigerated conditions for a certain period of time.

Female gravid worms of *A. suum* were collected from the intestines of naturally infected pigs at a slaughterhouse in Gwangju, Korea. Eggs isolated from the uterus of the female worms (Fig. 1A) were incubated in 4% sodium hypochlorite (Clorax, Youhanyanghaeng, Korea) for 3 min to remove the outer acid mucopolysaccharide/protein uterine layer. The uterine layer of egg surface was decorticated to facilitate embryonation and to prevent adherence to each other. The decortication step also allowed for easier viewing of the developmental stage of the larva under the microscope [14]. Then sodium hypochlorite was washed away from the medium by centrifugation in distilled water 3 times and the decorticated eggs were stored at 5℃ in distilled water for further use.

Seven different brands of commercial kimchi products made with either Chinese cabbage (Jonggajip Pogi [DaeSangFNF Co., Seoul, Korea], Pulmuone Pogi [Pulmuone Co., Eumsung, Korea], Homeplus Pogi [Homeplus Co., Seoul, Korea], Imasi Soonchang [Sunggajung Food, Soonchang, Korea], Hanool GGoma [Hanul, Cheongyang, Korea]) or young radish (Jonggajip Chonggak [DaeSangFNF Co., Seoul, Korea], Homeplus

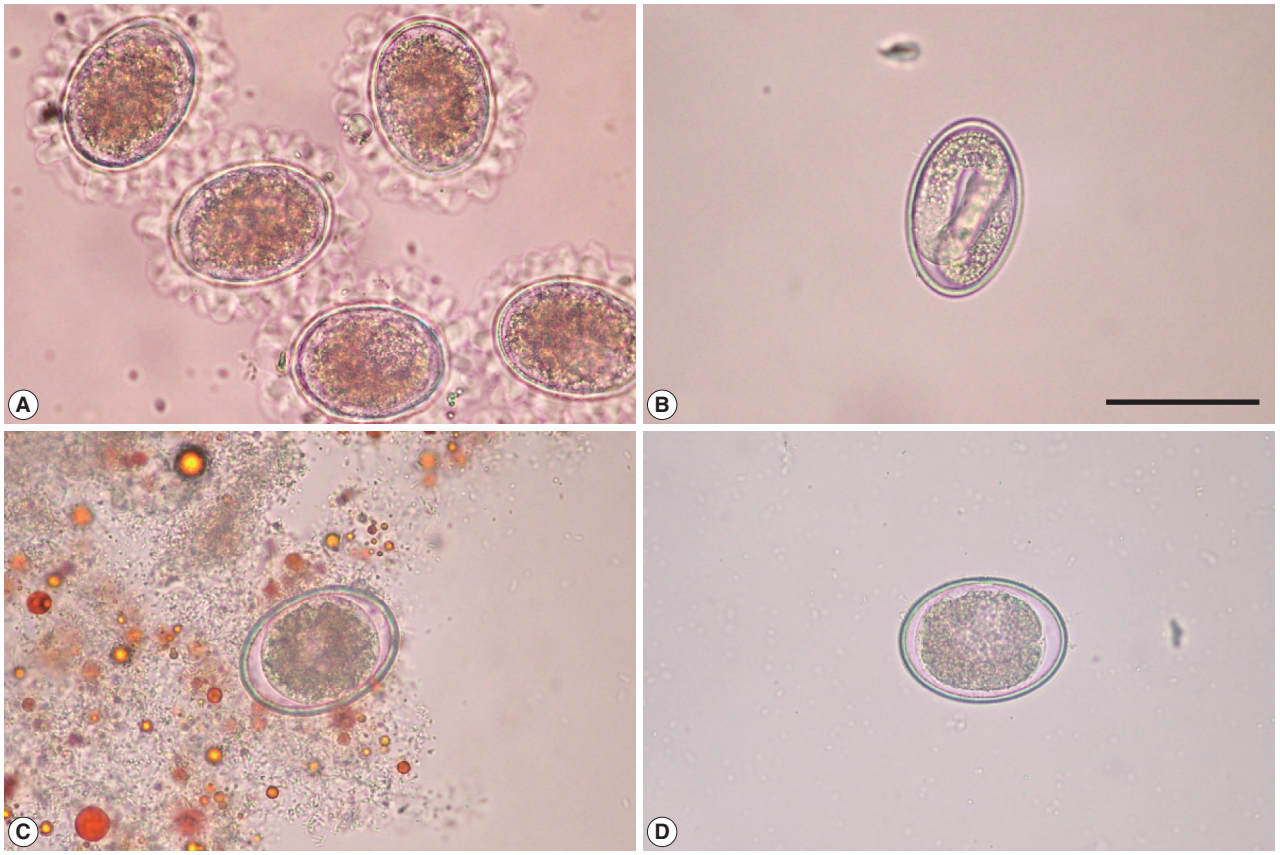

Fig. 1. *Ascaris suum* eggs in various conditions. (A) Fertilized eggs freshly isolated from the uterus of an adult female. They have conspicuously lamillated protein coats on their surface. (B) A fully embryonated egg of *Ascaris suum* which had been incubated in distilled water at 25°C for 3 weeks. The outer acid mucopolysaccharide/protein uterine layer of egg surface was decorticated to facilitate embryonation and to prevent adherence to each other. (C) An unembryonated egg which had been stored with kimchi extract at 5°C for 3 weeks. Colored materials are ingredients of kimchi incubated with eggs. (D) An unembryonated egg which had been stored with kimchi extract at 5°C for 60 days and then incubated in distilled water at 25°C for 3 weeks. Bar=50 μm.

Chonggak [Homeplus Co., Seoul, Korea]) were purchased from grocery stores in Gwangju. They will be indicated as alphabet letters randomly rather than their brand names in the rest parts of this article. All purchased kimchi products were sieved with mesh (1,600 mesh) to remove coarse organic materials that would interfere with the recovery of eggs and facilitated microscopic examination of each egg condition, and were centrifuged for 20 min at 520 *g*. The supernatant of kimchi (hereafter referred to as kimchi extract) was stored at 5℃ for further use.

To test the effects of temperature and kimchi extracts on the larval development of *A. suum* eggs, 3,500 eggs/ml mixed in 6 ml of either kimchi extracts, distilled water, or saline in disposable plastic tubes (Sewonyanghaeng, Seoul, Korea) were incubated at either 5℃ or 25℃ for up to 60 days. A total of 10 eggs per each sample were observed microscopically and the number of embryonated eggs was counted 6 times during the experimental period (days 7, 14, 21, 28, 35, and 60). This counting procedure was repeated 5 times on each sample. The larval development rate was calculated using the following equation:

Embryonation rate (%) = Number of eggs with fully developed larvae/Number of eggs counted × 100.

To determine the survival of *A. suum* eggs after refrigeration in kimchi extracts, eggs preserved for up to 60 days in kimchi extracts at 5℃ were washed 3 times in distilled water and re-incubated at 25℃ for 3 weeks. A total of 10 eggs per each sample were observed microscopically and the number of embryonated eggs was counted 3 times during the experimental period (days 14, 28, and 60). This counting procedure was repeated 5 times on each sample. The larval development rate was calculated as mentioned above. Data analysis was performed with Microsoft Excel 2010 (Microsoft Corp.). Significances were tested using the non-paired Student's t-test to compare between treatment groups. Differences were considered significant if $P < 0.05$.

The effects of temperature and kimchi extracts on the larval development of *A. suum* eggs are presented in Table 1. Eggs incubated at 25℃ showed marked differences in larval development rate between the kimchi extract group and the control group: While some of the eggs in the control group showed larval development as early as day 14 (29.0%), similar degree of embryonation was only observed by day 36 in the kimchi extract group (30.0%, $P = 0.887$). While all eggs completed embryonation by day 21 in the control group (Fig. 1B), about 25% of the kimchi extract group never became larvated even at day 60 ($P = 0.0009$). Meanwhile, none of the eggs incubated at 5℃ showed larval development regardless of incubation period or type of mixture group (Fig. 1C, data not shown).

To determine the survival of *A. suum* eggs that showed no embryonation after being preserved at 5℃, eggs were washed 3 times with distilled water at days 14, 28, and 60, respectively, and re-incubated at 25℃ for 3 weeks before microscopic observation was made on their larval development. As shown in

Table 1. Embryonation rate of *Ascaris suum* eggs in kimchi extracts at 25℃

Group	Kimchi products	Embryonation rates by stored days[a]					
		7	14	21	28	36	60
Kimchi extract	A	0.0	0.0	0.0	0.0	40.0	100.0
	B	0.0	0.0	0.0	0.0	0.0	100.0
	C	0.0	0.0	0.0	0.0	38.0	100.0
	D	0.0	0.0	0.0	0.0	0.0	0.0
	E	0.0	0.0	0.0	0.0	0.0	26.0
	F	0.0	0.0	0.0	0.0	54.0	100.0
	G	0.0	0.0	0.0	22.0	78.0	100.0
	Average	0.0	0.0	0.0	3.1	30.0	75.1
Control	Saline	0.0	30.0	100.0	100.0	100.0	100.0
	Distilled water	0.0	28.0	100.0	100.0	100.0	100.0
	Average	0.0	29.0	100.0	100.0	100.0	100.0

[a]Decorticated eggs mixed in kimchi extracts of 7 commercial products (3,500 eggs/ml) were incubated at 25℃ for up to 60 days. Ten eggs per each sample were observed microscopically for embryonation, and the counting procedure was repeated 5 times on each sample. The larval development rate was calculated using the following equation: Embryonation rate (%) = Mean number of eggs with fully developed larvae/Number of eggs counted × 100.

Table 2. Embryonation rate of *Ascaris suum* eggs re-incubated in distilled water at 25℃ for 3 weeks after preserved at 5℃ for up to 60 days

Group	Kimchi products	Embryonation rates by stored days[a]		
		14	28	60
Kimchi extract	A	100.0	76.0	0.0
	B	100.0	0.0	0.0
	C	100.0	0.0	0.0
	D	92.0	96.0	0.0
	E	98.0	20.0	0.0
	F	36.0	0.0	0.0
	G	86.0	100.0	0.0
	Average	87.4	41.7	0.0
Control	Saline	100.0	100.0	100.0
	Distilled water	100.0	100.0	98.0
	Average	100.0	100.0	99.0

[a]Decorticated eggs incubated in kimchi extracts of 7 different commercial products (3,500 eggs/ml) at 5℃ were washed 3 times with distilled water at days 14, 28, and 60, respectively, and were re-incubated at 25℃ for 3 weeks before microscopic observation was made.

Table 2, almost all eggs in the control group became larvated after 14, 28, or even 60 days of incubation at 5℃. In contrast, eggs in the kimchi extract group showed significant differences in their embryonation rates by the incubation period; only 87.4%, 41.7%, and 0% of eggs refrigerated for 14, 28, and 60 days became embryonated, respectively ($P < 0.004$).

Although *A. suum* infection in humans has not been very common, the risk of infection continues to rise as demand for organic products continues to increase these days. Humans are usually infected when they eat fresh vegetables grown in soil fertilized with porcine excrement contaminated with *A. suum* eggs. Results of this study showed that both kimchi extract and low temperature have effects on larval development period and survival rates of *A. suum* eggs. In particular, low temperature (5℃) seemed unfavorable for larval development and survival. None of the eggs preserved at 5℃ showed larval development regardless of the presence of kimchi extract, although most of them resumed embryonation when they were re-incubated at 25℃ for 3 weeks after 14 days of incubation at 5℃. Even after 60 days of incubation at 5℃, most eggs in the control group became embryonated, while none of the eggs in kimchi extract group did. This result indicates the adverse effect of kimchi extract on the survival of *A. suum* eggs especially in low ambient temperature.

Meanwhile, the unfavorable effect of kimchi extract on the larval development rate varied markedly among the kimchi extracts originated from different commercial products: when preserved at 25℃ for 60 days, the larval development rate ranged from 0 to 100%. The gap among the kimchi extracts might be due to the kinds and amounts of ingredients contained in each kimchi product such as garlic, onion, and ginger, which are all known to have anticancer or antimcirobial effect [15-17]. The effect of kimchi ingredients on parasitic eggs have been previously investigated in Korea: Soh [9] reported that garlic and mustard were the most effective ingredients in retarding the larval development of ascarid eggs. Kim et al. [12] also reported that *Giardia lamblia* cysts were killed in salt, garlic, red pepper and ginger. In addition, there have been many reports of anticancer, antifungal, or antimcirobial effects of kimchi [18,19].

Although *A. suum* eggs mostly retain the outer acid mucopolysaccharide/protein uterine layer in nature, we used decorticated eggs in this study to facilitate embryonation and to prevent adherence to each other as well as for easier viewing of the developmental stage of the larva under the microscope. The decortication of outer layer does not affect the permeability of the egg shell because the inner lipoprotein layer which consists of a unique mixture of 25% protein and 75% lipid-containing ascarosides is responsible for the impermeability of the shell [20]. Since it is likely that *A. suum* eggs in pig manure were already embryonated in nature, it would have been much better if we evaluated the effect of kimchi extract and temperature on the infectivity of embryonated eggs to experimental animals such as rats and mice, instead of their *in vitro* effects on embryogenesis. In spite of this limitation, our study still provides valuable information regarding the effect of kimchi extract at different temperatures on embryogenesis of *A. suum* eggs.

Since kimchi is made of a variety of raw vegetables, it can be easily contaminated with different kinds of microorganisms in the process of cultivation and handling of vegetables and ingredients. Thus, many studies on parasitic and bacterial contamination in kimchi has been conducted in Korea [9-13], although no study has been reported on the synergistic larvicidal effect of temperature and kimchi extract on *A. suum* eggs. The results obtained in this study are not in agreement with those of previous studies, in which larval development and survival of *A. lumbricoides* eggs were not affected by the ingredient of kimchi [9], radish water kimchi extract [10], or young radish kimchi extract [11]. The disparity between those studies and ours could be due to many factors, including different type of kimchi, the duration of experimental period, and the different condition of incubation, especially in low temperature.

In conclusion, our results indicated that embryogenesis of *A. suum* eggs in kimchi extract was affected by duration of refrigeration, and that all eggs stopped laval development completely in kimchi kept at 5℃ for up to 60 days.

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