Effects of acetic acid on the viability of *Ascaris lumbricoides* eggs

Is vinegar reliable enough to clean the vegetables?

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ABSTRACT

الأهداف: للتحقيق من تأثير حمض الخليك على بيض Ascaris lumbricoides (A. lumbricoides) لتحديد التركيز الفعال للخل وفترة التنفيذ لجعل استهلاك الخضار النيئة أكثر أماناً.

الطريقة: أجريت هذه الدراسة التجريبية في مايو 2015م في مختبر علم الطفيليات بكلية الطب، جامعة يوزونجويل ، اسطنبول، تركيا. تم تقسيم بيض A. lumbricoides إلى مجموعتين. تم علاج البيض في مجموعة الدراسة بنسبة تركيز 1، 3، 5، 10% من حامض الخليك، وتم علاج البيض في المجموعة المرجعية بيوزين. وتم متابعة قابلية تحمل البيض "في النقاط التالية خلال وقت التجربة: 0، 10، 15، 20، 30، 45، و 60 دقيقة.

النتائج: نسبة حمض الخليك 1% غير كافية لمقاومة بيض الإسكارس. في الدقيقة ال30، أظهر حمض الخليك 3% فعاليته 95%، وعند تركيز 5%، فقدت كل البيض حيويتها. يتطلب علاج حمض الخليك عند نسبة 4.8% في 30 دقيقة، أو نسبة 4.3% في 60 دقيقة لتحقيق النجاح الكامل من العلاج.

الخاتمة: يحتوي بيض الإسكارس على 3 طبقات مقاومة جدا، ويعتقد أن تركيز حمض الخليك، والتي يمكن أن تكون فعالة على البيض قد تكون فعالة أيضاً على مقاومة العديد من العوامل الطفيلية الأخرى. لتحقيق الحماية الضرورية، يفضل غسل الخضروات وتعريضها المباشر للخل محتوياً على حامض الخليك 5% لمدة 30 دقيقة .

Objectives: To investigate the effects of acetic acid on durable *Ascaris lumbricoides (A. lumbricoides)* eggs to determine the effective concentration of vinegar and the implementation period to render the consumption of raw vegetables more reliable.

Methods: This experimental study was performed in May 2015 in the Parasitology Laboratory, Faculty of Medicine, Yuzuncu Yil University, Van, Turkey. The *A. lumbricoides* eggs were divided into 2 groups. Eggs in the study group were treated with 1, 3, 5, and 10% acetic acid concentrations, and eggs in the control group were treated with Eosin. The eggs' viability was observed at the following points in time during the experiment: 0, 10, 15, 20, 30, 45, and 60 minutes.

Results: The 1% acetic acid was determined insufficient on the viability of Ascaris eggs. At the 30th minute, 3% acetic acid demonstrated 95% effectiveness, and at 5% concentration, all eggs lost their viability. Treatment of acetic acid at the ratio of 4.8% in 30 minutes, or a ratio of 4.3% in 60 minutes is required for full success of tretment.

Conclusion: Since *Ascaris* eggs have 3 layers and are very resistant, the acetic acid concentration, which can be effective on these eggs are thought to be effective also on many other parasitic agents. In order to attain an active protection, after washing the vegetables, direct treatment with a vinegar containing 5% acetic acid for 30 minutes is essential.

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Parasitic diseases are witnessed all over the world, it originally emerges in certain areas where people have the habit of defecation in random places; where human stool is used as manure and infrastructure in undeveloped areas. Approximately one billion humans are infected with *Ascaris*, 800 million humans are

infected with *Trichuris*, 200 million humans are infected with *Taenia* in the world, and each year, millions of humans lose their lives due to parasitic infections.[1-4] Food borne diseases are receiving increasing attention recently.[5] One of the important ways that humans are infected of these diseases is the consumption of raw, or poorly washed vegetables.[6] Vegetables growing in soil and frequently used in salads, such as lettuce, parsley, radish, cress, garden rocket, green onion, carrot, and cucumber also play an important role in the transmission of parasitic infections, in addition to many bacterial and viral agents. *Giardia, Entamoeba, Cryptosporidium, Hymenolepis, Ascaris, Trichuris, Toxocara, Taenia* and *Fasciola* are the parasites on top of the list, which are transmitted by such vegetables.[3,6-10]*Ascaris lumbricoides* (*A. lumbricoides*) is the largest nematode that lives in the human small intestine. The *A. lumbricoides* eggs are of 2 types: fertilized and unfertilized. Fertilized eggs have an oval shape and a shell of 3 thick layers. The outermost layer is a hackly protein layer, beneath this, is the semipermeable layer, and at the lowermost, there is a non-permeable lipoidal vitellin membrane layer. Fertilized eggs do not have this last layer and have no infection capacity, as well. This layered structure of the egg enables its durability against different chemical and environmental factors. Thus, it could be argued that *A. lumbricoides* eggs are more resilient than many other parasitic eggs. It has been reported that the *A. lumbricoides* eggs could carry their infectious disposition when preserved in a refrigerator for 2 years; and that they keep their viability for up to 40 days at temperatures between 18 and 27°C.[11-14] The objective of the study was to investigate the effects of acetic acid (CH_3CO_2H) on the viability of durable *A. lumbricoides* eggs, to determine the effective concentrations of vinegar, and the time required to treat raw vegetables for safe consumption.

Methods. In this experimental study, several *A. lumbricoides* eggs were counted in the stool sample, which was directed to at the Parasitology Laboratory, Faculty of Medicine, Yuzuncu Yil University, Van, Turkey in 2015 May. After the stool was diluted with 0.9% physiological saline solution (PSS) in order to purify it from particulates, it was strained with a sieve of 150 µm. Later on, the sedimentation technique using PSS was applied to the stool sample until it became clear at room temperature, and the eggs were accumulated at the bottom residue. A 1, 3, 5, and 10% concentrations of pure commercial CH_3CO_2H (Sigma-Aldrich, St. Louis, MO, USA) were prepared. The *A. lumbricoides* eggs in the control group were examined only using 0.1% Eosin, and in the study group, they were treated with 4 differentCH_3CO_2H concentrations (1%, 3%, 5%, and 10%) and Eosin. Selected eggs that are viable and embryonated was the inclusion criteria, and the contrary were the exclusion criteria. Viability survey of the eggs was conducted at the following times: 0, 10, 15, 20, 30, 45, and 60 minutes under x40 magnification and approximately 19.8 (11-25) eggs were counted for each group. The eggs which were colored were identified as dead, and eggs which were not colored, were considered alive (**Figure 1**). This research was carried out solely on fertilized eggs, the unfertilized eggs were ignored.

Statistical assessments were conducted using the Statistical Package for Social Sciences version 15 (SPSS Inc., Chicago, IL, USA) software and Probit Regression Analysis. The level of statistical significant differences was set at $p \leq 0.05$.

Results. It has been observed that after the 45th minute, 10% of the parasitic eggs lost their viability in the control group. The effect of 1% CH_3CO_2H was found to be insufficient, even in the 90th minute, only 35.3% of the eggs (6/17) lost their viability. While 3% CH_3CO_2H showed 95% effectiveness at the 30th minute, this ratio reached 100% at the 60th minute. A 5% concentration started to demonstrate an effect at the moment of application (21/24), and all eggs lost their viability at the 30th minute (**Table 1**). Probit analysis demonstrates that the difference between the results was significant (p=0.001). The "Probit Equality value = A* + 0.78 (B) x concentration" formula has been ascertained, and A* coefficients, which change according to time are presented in **Table 2** (ß=regression coefficient). The CH_3CO_2H concentrations of lethal dose (LD) 10, LD25, LD50, and LD99 that statistically kills the parasitic eggs based on time are presented in **Table 3**. In accordance with the method, it must be treated either with 4.8% CH_3CO_2H for 30 minutes, or with 4.3% CH_3CO_2H for 60 minutes to lose viability of all existing parasitic eggs (**Table 3**).

Discussion. Millions of people have been infected with parasitic agents in the world, and most of them live in underdeveloped, or developing countries. In our country, the parasitosis ratio is approximately 10-85%,

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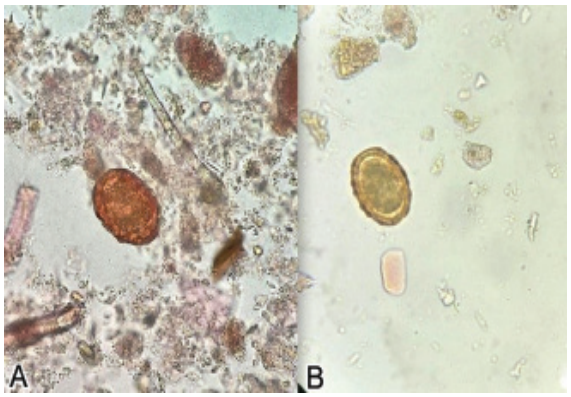

Figure 1 - Photograph showing the **A)** dead eggs (colored eggs) and **B)** non-colored eggs considered as alive.

Table 1 - Colored (dead) and uncolored (alive) eggs count in the control and study groups according to time.

Time, minutes	Control group	Study group Acetic acid concentration			
		1%	3%	5%	10%
0	0/25	1/20	7/23	21/24	23/24
10	0/25	2/20	17/20	20/21	21/21
15	0/20	3/20	20/24	19/20	20/20
20	0/20	4/21	21/25	21/22	18/18
30	0/20	6/24	19/20	19/19	19/19
45	2/20	4/21	17/19	18/18	14/14
60	2/20	7/19	17/17	16/16	13/13
90	3/21	6/17	14/14	15/15	11/11

Table 2 - A* coefficient values depending on time by probit equality analysis in this study.

Time, minutes	A* coefficient
0	-2.930
10	-1.839
15	-1.746
20	-1.674
30	-1.391
45	-1.393
60	-1.036
90	-1.007

Table 3 - The effective concentrations of acetic acid to *Ascaris lumbricoides* eggs obtained by statistical method.

Time, minutes	Effective concentration (g/100 ml)			
	LD10	LD25	LD50	LD99
0	2.113	2.890	3.755	6.736
10	0.715	1.493	2.357	5.338
15	0.596	1.374	2.238	5.219
20	0.503	1.281	2.145	5.126
30	0.140	0.918	1.782	4.763
45	0.143	0.921	1.785	4.766
60	-0.315	0.463	1.488	4.308
90	-0.351	0.427	1.291	4.272

LD - lethal dose

although it varies based on the region and sanitary conditions.[4]

Diseases caused by contaminated food are one of the most common health problems in the world.[3,15] Food borne parasitic diseases are not known much, but they are becoming more and more serious for human health.[5] Vegetables and fruits have much significance in the diet of an individual, since they are sources of various vitamins and minerals. However, they carry the risk of transmission of diseases, since they could get contaminated by many bacterial, viral, and parasitic organisms.

In recent years, the number of reported parasitic infections is increasing.[8,9] Globalization in food supply, the increasing number of international travels, increase in the population susceptible to diseases, changes in dietary habits, and the developments in the diagnostic methods are reported as reasons for the increase in parasitic diseases caused by food originating from all over the world.[5] This situation poses a great risk for humans susceptible to parasitic diseases, who are old, and are immunosuppressive.[16] The agricultural use of water infected with parasitic eggs, unhealthy draining of human stool, and use of animal stool as manure are the main reasons for the contamination of vegetables and fruits.[1-3] It was reported that 19 different food-borne parasitic outbreaks occurred in the United States between the years 1993 and 1997.[17] Furthermore, it was reported that 10% of *Cryptosporidium* and *Giardia* infections, and 50% of *Toxoplasma* infections were originated food ingestion.[18]

Studies conducted across the world demonstrated that lettuce, parsley, cabbage, cress, mint, carrot, garden rocket, cucumber, pepper, radish, green onion, red beet, tomato, and similar raw vegetables are contaminated with parasitic cysts, oocysts, and eggs. In these plants, mostly *Toxocara, Taenia, Ascaris, Enterobius, Trichuris*, hooked worm (*Ancylostoma duodenale, Necator Americanus*) the eggs of *Hymenolepis nana*, cysts of *Giardia, Entamoeba, Balantidium,* and oocysts of *Cryptosporidium* were found.[3,6-10,19,20] Studies conducted in different countries showed that the rate of contamination by parasitic agents were higher: 31.2% (156) of 500 samples in Pakistan,[6] 66.3% in Morocco,[21] 34.7% in Poland,[9] 25% of the vegetables bought from bazaars, and 29% of those picked from the gardens in Iran,[8] and 23.8% bought from bazaars and 36.9% picked from the gardens in Saudi Arabia.[10] In Norway,[16] 2% of vegetables were found positive with *Giardia*. Similar studies were also conducted in Turkey, and 3-14% of the vegetables were found to be contaminated.[7,19,20,22] It is of vital importance that infected vegetables should be freed

from parasitic agents. Properly washing raw vegetables and fruits may remove the parasitic cysts, oocysts and eggs, however, vegetables with leaves are usually difficult to clean.[2] Thus, further precautions must be taken in addition to washing the vegetables. It is more important to implement this in sensitive environments, such as hospitals, schools, restaurants, and hotels where vegetables are consumed in large amounts.

It was noted according to the standards that the total acid content of vinegar (free CH_3CO_2H in water) must be no less than 4 g/100 mL. In a study conducted in Turkey[23] on 12 grape vinegar brands, the acetic acid content was identified to be between 3.96 and 5.36 g/100mL. It was found that in 65 different wine and apple vinegar samples, total acidity varied between 5.4 and 6.6 g/100mL in Italy.[24] Ravdin[25] suggested immersing vegetables into CH_3CO_2H or vinegar for 10-15 minutes for eradication of *Entamoeba histolytica* cysts. It was observed that this period was shorter than our findings. As mentioned above, this could be due to differences between the CH_3CO_2H content of foreign vinegar brands and vinegar brands in Turkey. Since the *A. lumbricoides* eggs have 3 layers and are durable, it is conceived that an CH_3CO_2H concentration, which might be effective on these eggs, could also be effective on other parasitic agents. It was ascertained that after raw vegetables are washed, vinegar with a 5% CH_3CO_2H content must be applied for at least for 30 minutes to ascertain an effective protection, and in cases where the acid content is lower, immersing time must be prolonged (**Tables 1 & 3**).

This study was limited to investigate the CH_3CO_2Hs viability also on other parasitic agents, but it is an advantage that the *A. lumbricoides* eggs are one of the most durable parasitic eggs. It is a common knowledge that vegetables must be immersed into a water-vinegar mixture for health reasons. Thus, a little amount of vinegar is mixed with large amounts of water to clean the vegetation, where the CH_3CO_2H gets diluted, and its concentrations are decreased by 10s/100s of times. Contrary to popular belief, vinegar must not be mixed with water; rather it must be treated directly on the vegetation after they are prewashed.

In conclusion, the public must be informed regarding the parasitic infections that might be contaminated from vegetables and fruits. It must never be forgotten to treat raw vegetables used in salads with vinegar after washing; this would be an effective protection against parasitic infections. As a result of this study, the effectiveness of acetic acid on the viability of Ascaris eggs was demonstrated. These findings are meaningful for further studies that plan to use different medications for decontamination vegetables from parasitic agents.

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