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Research Article

Effects of Microwave Cooking on the Infectivity of *Toxocara canis* (Werner, 1782) Larvae in the Liver of Paratenic Host Mice

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Abstract: This study was conducted to determine the effect of microwave cooking on the infective stages of *Toxocara canis* larvae found in the tissues of paratenic hosts. For this purpose, 3 series of experiments were carried out using a household microwave oven (2450 MHz, model: Arçelik MD551) at medium, medium-high and high levels. A total of 54 mice were used in the 3 experiments. *Toxocara canis* eggs were collected from the faeces of naturally infected dogs and incubated at room temperature for 4 weeks for development of infective larvae in eggs. In the first experiment, 6 mice were each infected with 3000 larvated eggs of *T. canis* orally. Infected mice were sacrificed on the second day of infection and each liver was taken out, minced with a scalpel, mixed to provide homogeneity and separated into 3 equal parts after weighing. All mice livers weighed approximately 1 g. One-third of each liver was given to one mouse raw and another third of each liver was digested in a 1% pepsin-HCl solution at 37 °C for 3 days to search for *T. canis* larvae. The remaining thirds of the livers were fed to one mouse after microwave cooking at medium level for 5 min. Since the mice livers were so small they were covered with stretch cooking film and put inside chicken livers (each approximately 52 g, 1.7 cm thick) to obtain a normal sized cooking sample before microwave treatment. Samples were put inside the microwave oven in the centre and cooked separately. Then the livers were taken out of the oven and the temperature inside the liver was measured immediately. The mice fed on raw liver and microwave-treated liver were sacrificed after 2 days. The lungs, livers, carcasses, hearts, spleens and kidneys of each mouse were put into pepsin-HCl digestion solution and examined for *T. canis* larvae under a stereomicroscope. The second and third series of experiments were carried out just like the first experiment except for the cooking levels of the microwave, which were medium-high and high. Various numbers of larvae were found in the organs of mice fed uncooked infected liver, but no larvae were found in the mice fed microwave-processed infected livers.

Key Words: Microwave cooking, *Toxocara canis*, infectivity

Mikrodalga Fırında Pişirme İşleminin Paratenik Konak Olan Farelerin Karaciğerindeki *Toxocara canis* (Werner, 1782) Larvalarına Etkisi

Özet: Bu çalışma paratenik konak dokularında bulunan infektif *Toxocara canis* larvalarına mikrodalga pişirme işleminin etkisini ortaya koymak amacıyla yapıldı. Bu amaç için üç seri deney, medium, medium-high, high fonksiyonları olan ev tipi mikrodalga fırın (2450 MHz, Arçelik MD551) kullanılarak yürütüldü. Üç deneyde toplam 54 adet fare kullanıldı. *Toxocara canis* yumurtaları doğal enfekte köpeğin dışkısından toplandı ve içinde infektif larvaların gelişmesi için oda sıcaklığında 4 hafta süreyle inkübasyona bırakıldı. Birinci deneyde 6 farenin her biri 3000 larvalı *T. canis* yumurtası ile oral olarak enfekte edildi. Enfekte edilen fareler, enfeksiyonun 2. gününde açılarak karaciğerleri alındı, skalpel ile kıyıldı, homojeniteyi sağlamak için karıştırıldı ve tartıldıktan sonra üç eşit parçaya ayrıldı. Her bir farenin karaciğeri yaklaşık 1 gramdı. Her bir karaciğerin 1/3'ü çiğ olarak bir fareye yedirildi ve 1/3'ü *T. canis* larvası aranmak üzere % 1'lik pepsin-HCl solusyonunda 37 °C'de 3 gün sindirime bırakıldı. Karaciğerin geri kalan 1/3'ü mikrodalgada medium seviyesinde 5 dakika pişirildikten sonra bir fareye yedirildi. Fare karaciğerlerinin çok küçük boyutta olmaları nedeniyle normal pişirme boyutunu sağlamak için mikrodalgada pişirmeden önce fare karaciğerleri streç pişirme filmleri ile sarıldı ve tavuk karaciğerinin (her biri yaklaşık 52 g, 1,7 cm kalınlığında) içine kondu. Örnekler tek tek mikrodalga fırının pişirme tablasının ortasına yerleştirilerek 5 dakika süreyle pişirildi. Mikrodalga fırından çıkarılan örneklerin iç sıcaklığı hemen termometre ile ölçüldü. Çiğ ve mikrodalga ile pişirilmiş karaciğerleri yiyen fareler 2 gün sonra sakrifiye edildi. Her farenin akciğer, karaciğer, karkas, kalp, dalak ve böbrekleri pepsin-HCl solusyonunda sindirime bırakıldı ve stereo mikroskopta *T. canis* larvaları arandı. İkinci ve üçüncü deneyler de mikrodalganın medium-high ve high olan pişirme seviyeleri dışında tamamen birinci deneyin dizaynında yürütüldü. Karaciğerlerin içerisindeki sıcaklık mikrodalga uygulamasından sonra üç deneyde de 70 °C'nin üzerindeydi. Çiğ infektif karaciğer ile beslenen farelerin organlarında çeşitli sayılarda larva bulunurken mikrodalgada pişirilmiş infektif karaciğerle beslenen farelerde larvaya rastlanmadı.

Anahtar Sözcükler: Mikrodalga, *Toxocara canis*, infektivite

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Introduction

In recent years, the use of microwave ovens for household food cooking processes has increased; therefore, it is of great importance to clarify the effects of microwave cooking on infectious agents in food products.

Studies have been done on the bactericidal effect of microwave ovens on contaminated foods (1,2). A microwave oven has been used for inactivation of 8 viruses suspended in drinking water (3). Another study examined the effect of a microwave oven for the decontamination of diagnostic samples and sterilisation of contaminated materials in the laboratory (4).

Several studies have been carried out to determine the effect of microwave ovens on the inactivation of some parasites found in food (5-7). Lunden and Uggla (6) applied microwave heating to mutton infected with *Toxoplasma gondii* cysts and found that the parasite remained infective, possibly due to uneven heating of the meat. Kotula et al. (5) have reported that cooking pork chops including *Trichinella spiralis* larvae rapidly in a microwave oven to 77 or 82 °C did not completely destroy the infectivity of the meat. Microwave irradiation was reported to be extremely effective in killing or preventing the development of *Eimeria nieschulzi, Strongyloides ratti* and *Taenia taeniaeformis* (4). *Anisakis simplex* larvae in fish fillets were shown to be killed at a minimum of 77 °C (7).

Toxocara canis (Werner, 1782) is a parasite that lives in the intestine of canidae. Larvae of this nematode cause an infection referred to as Visceral Larva Migrans (VLM) in humans and other paratenic hosts (8). Humans become infected not only by the ingestion of infective eggs, but also by consuming the raw or undercooked liver or meat of chickens or cows that contain *T. canis* larvae (9,10). Bouchet (11) demonstrated that a brief exposure to microwave heating interrupted the development of *T. canis* eggs and showed the altered ultrastructure of the eggs after microheating in another study (12). The effects of microwave cooking on the infectivity of *T. canis* larvae in the tissue of paratenic hosts have not been reported.

This study was carried out to show whether microwave cooking had any effect on the infectivity of *T. canis* larvae in the liver of mice, the paratenic host. Mice were used as a model to show the possibility of VLM infection in humans by eating infected organs of paratenic hosts after microwave cooking.

Materials and Methods

Animals: A total of 54 Balb-C mice, 8 weeks old, were used in this study. Mice were kept on a 12-h light/dark photoperiod and fed on a standard mice diet and tap water ad libitum.

Obtaining and preparation of *T. canis* eggs: *T. canis* eggs were obtained from the faeces of naturally infected dogs. Collected eggs were incubated in 1% formalin-saline at room temperature for 4 weeks until larval development occurred (13).

Microwave treatment: A household microwave oven (2450 MHz, model: Arçelik MD551) was used to cook mouse liver containing *T. canis* larvae. The oven had 5 process functions: warm, defrost, medium, medium-high and high. For this study, 3 functions, i.e. medium, medium-high and high, were used for cooking the mouse livers. The livers were placed in the centre of the oven one by one and were exposed to microwave heating for 5 min. to be cooked properly. The temperatures inside the livers were measured immediately with a thermometer after the microwave treatment.

Experimental design: The first experiment was established to determine the effect of microwave heating on the infectivity of *T. canis* larvae in the mouse liver at medium level heating. Six mice were each infected with 3000 larvated eggs of *T. canis* orally. Based on the information that larval trapping and accumulation of the migrating larvae in the liver occurred 42 h after infection (14,15), infected mice were sacrificed on the second day of infection. Each liver was taken out, minced with a scalpel and mixed to provide homogeneity and separated into 3 equal parts after weighing. The mouse livers weighed approximately 1 g.

One-third of each liver was given to one mouse raw, and another third after exposure to microwave heating for 5 min at medium level. Since the livers of the mice were so small, they were covered with stretch cooking film and put inside commercially available chicken livers measuring approximately 52 g and 1.7 cm thick, to exemplify the normal size of a meal before heating. The mouse livers within the livers of the chickens were put in a glass dish separately, covered with stretch film again

and were placed in the microwave oven in a marked central position one by one and were treated at medium level for 5 min. Then the samples were taken out of the microwave oven, and the temperature inside was measured with a thermometer. Then the livers of the mice were taken out of the chicken livers, left to cool for 5 min and each sample fed to one mouse.

The remaining third of each liver was incubated at 37 °C for digestion in pepsin-HCl solution (13) and *T. canis* larvae were examined under a stereomicroscope after 3 days. The presence and the number of larvae in this part of the liver was important to prove that the mice liver was harbouring the larvae and to estimate the number of larvae that the mice had ingested by eating the raw and cooked liver.

Mice fed raw liver and microwave-treated liver were sacrificed after 2 days. Their lungs, livers, carcasses, hearts, spleens and kidneys were taken out and put into pepsin-HCl digestion solution. The tissues were incubated at 37 °C for digestion and examined for the presence of *T. canis* larvae under the stereomicroscope.

The second and the third experiments were performed to determine the microwave effect on the infectivity of *T. canis* larvae in mouse liver at medium-high and high levels. The same design and techniques were used as given in experiment one, only the levels of the microwave were medium-high and high. In all 3 experiments the temperatures in the cores of the livers were above 70 °C. The organs were examined for the presence of *T. canis* larvae after the 3 experiments had been completed.

Results

The larvae found in the thirds of the liver of mice inoculated with *T. canis* eggs previously were counted and the average numbers of the larvae ingested by experimental mice were calculated (in the second columns of Tables 1-3). All mice that have been fed on raw infected liver were found to have been invaded by *T. canis* larvae. The numbers and distribution of the larvae in the organs of the mice are shown in Tables 1-3. No larvae were seen in the mice fed on infected livers that were cooked by microwave at medium, medium-high and high levels (the last columns of Tables 1-3).

Discussion

Bactericidal effects of microwave heating on some micro-organisms in broth and in food have been studied and this type of cooking was found to be ineffective on spore forming bacteria (1). Fresh, skinless chicken meat inoculated with *Escherichia coli* K12 and *Campylobacter jejuni* was exposed to microwaves, and consequently microwave treatment showed only a minimal effect on bacterial numbers, and bacteria counts were reported to be higher in some samples after treatment as well (2). Mahnel et al. (3) reported that 8 certain viruses, except bovine parvovirus, were inactivated by microwave

Table 1. Medium-level experiment: the numbers of larvae ingested by the mice, distribution and the numbers of larvae in the organs.

Mouse no.	Number of ingested larvae	Larva distribution in the organs of the mice fed raw liver					LRR[2] %	Number of larvae in the mice fed microwave-treated liver (Whole body)
		Liver	Lungs	Carcass	Other[1]	Total		
1	163	13	6	0	0	19	11.65	0
2	76	9	3	0	0	12	15.78	0
3	85	7	6	0	0	13	15.29	0
4	164	7	11	0	6	24	14.63	0
5	135	5	4	0	3	12	8.88	0
6	135	6	3	0	0	9	6.66	0
Total	758	47	33	0	9	89	11.77	0

[1] Heart, spleen and kidney
[2] Larva recovery rate: Ratio of recovered larvae to ingested larvae

Table 2. Medium-high level experiment: the numbers of larvae ingested by the mice, distribution and the number of larvae in the organs.

Mouse no.	Number of ingested larvae	Larva distribution in the organs of the mice fed raw liver					LRR[2] %	Number of larvae in the mice fed microwave-treated liver (Whole body)
		Liver	Lungs	Carcass	Other[1]	Total		
1	49	15	7	0	0	22	44.89	0
2	87	17	5	0	0	22	25.28	0
3	54	15	3	0	0	18	33.33	0
4	41	12	7	0	1	19	46.34	0
5	20	7	3	0	0	10	50	0
6	31	8	2	0	0	10	32.25	0
Total	282	74	27	0	1	102	36.17	0

[1] Heart, spleen and kidney
[2] Larva recovery rate: Ratio of recovered larvae to ingested larvae

Table 3. High-level experiment: the numbers of larvae ingested by the mice, distribution and the number of larvae in the organs.

Mouse no.	Number of ingested larvae	Larva distribution in the organs of the mice fed raw liver					LRR[2] %	Number of larvae in the mice fed microwave-treated liver (Whole body)
		Liver	Lungs	Carcass	Other[1]	Total		
1	40	6	3	0	0	9	22.5	0
2	57	10	4	0	1	15	26.31	0
3	65	11	6	0	0	17	26.15	0
4	51	7	2	0	0	9	17.64	0
5	27	5	1	0	0	6	22.22	0
6	53	16	9	0	0	25	47.16	0
Total	293	55	25	0	1	81	27.64	0

[1] Heart, spleen and kidney
[2] Larva recovery rate: Ratio of recovered larvae to ingested larvae

treatment at 50-65 ℃ and bovine parvovirus was inactivated as well when the temperature was elevated to 90 ℃.

Conder and Williams (4) showed that microwave radiation affected the developmental and infective stages of *E. nieschulzi, S. ratti* and *T. taeniaeformis*. Bouchet (11) reported that a brief exposure to microwave heating interrupted the development of *T. canis* eggs. Bouchet et al. also (12) reported that exposure of *T. canis* eggs to microwave heating caused changes in the structure, shell and cytoplasmic organelles of the eggs. The effects of microwaving on fish fillets (each 126-467 g, 0.5 to 1.75 cm thick) containing larvae of *A. simplex* at different temperatures were studied by Adams et al. (7) and 77 ℃ was proved to be necessary to inactivate the larvae.

Lunden and Uggla (6) reported that *T. gondii* tissue cysts in mutton were not inactivated after microwave heating at 65 ℃ and the relevant finding was considered to be associated with uneven heating and undercooking of the mutton. Kotula et al. (5) found that pork chops infected with the larvae of *T. spiralis* and cooked rapidly in a microwave oven to 77 or 82 ℃ still had infective larvae inside.

In the present study, mouse livers containing *T. canis* larvae were exposed to microwaves at medium, medium-high and high levels in a commercial, domestic-type microwave oven for 5 min and all applications were found to effectively inactivate the larvae. The inner temperature of the livers was above 70 ºC at all cooking levels. The success of microwave cooking in our study is thought to be related to the small size of the cooking sample and the matrix of the cooking sample, the liver, which has high water content and homogeneous composition. In addition, cooking time was sufficiently prolonged to cook the liver entirely and covering the liver samples with stretch film before microwave heating spread the heat uniformly. The heat resistance of helminths has been reported as unimpressive and temperatures as low as 56-60 ºC for several minutes have been suggested to eliminate the infectivity in many instances (16). Orlandi et al. (17) have claimed that the heat must uniformly penetrate the entire food matrix because some parasites may be encysted deep inside the tissues. It was also reported that microwave cooking was not 100% effective in killing *T. spiralis* larvae in large pieces of meat because of the unavoidable "cold spots" in the patterns of microwave beams (18).

Many parasites have infective stages in the organs of intermediate or paratenic hosts in their life cycle and consumption of these organs raw or undercooked causes important parasitic diseases in humans, such as anisakid infections, taeniosis, toxoplasmosis, trichinosis and visceral larva migrans (19). *Toxocara canis* is an important parasite that causes VLM in humans, who get the infection by accidental consummation of the eggs or raw and undercooked infected organs of paratenic host (8-10). Min (20) reported that the larvae of *T. canis* obtained from the paratenic host were more pathogenic than the larvae from eggs. Therefore, the cooking style and conditions of foods, especially for meat dishes, are important for human health to prevent the transmission of infectious diseases. In recent years, the microwave oven has become widespread as a household cooking utensil, and in this study the efficacy of microwave cooking in the destruction of *T. canis* larvae in the liver of a paratenic host was studied at different cooking procedures supplying 70 ºC cooking heat for 5 min.

In conclusion, further studies should be carried out to test this cooking method at different temperatures and cooking periods, as well. As reported in the literature (4-7), the effects can vary according to the species of the parasite and infected tissue, and the effect of this cooking method should be evaluated separately on different species of parasite found in food.

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