Veterinary Integrative Sciences 2024; 22(3): 847 - 856 DOI; 10.12982/VIS.2024.057

Vet Integr Sci

Veterinary Integrative Sciences

Faculty of Veterinary Medicine
Chiang Mai University

ISSN; 2629-9968 (online)

Website; www.vet.cmu.ac.th/cmvj

Research article

Toxoplasma gondii contamination in fresh vegetables destined for human and livestock consumption in Thua Thien Hue, Vietnam

Pham Hoang Son Hung[1], Tawin Inpankaew[2], Le Dinh Duong[3], Tran Nguyen Thao[1], Ho Thi Dung[1], Nguyen Xuan Hoa[1], Bui Thi Hien[1], Nguyen Thi Hoa[1], Tran Thi Na[1], Tran Quang Vui[1], Nguyen Pho Nguyen Nhung[4], Nguyen Thi Giang[4], Nguyen Thi Thuy[1,*]

[1]Faculty of Animal Science and Veterinary Medicine, University of Agriculture and Forestry, Hue University, Hue City 52000, Vietnam.
[2]Department of Parasitology, Faculty of Veterinary Medicine, Kasetsart University, 50 Paholyothin Road, 10900, Bangkok, Thailand .
[3]Faculty of Public Health, Hue University of Medicine and Pharmacy, Hue City 52000, Vietnam.
[4]Student, Faculty of Animal Science and Veterinary Medicine, University of Agriculture and Forestry, Hue University, Hue City 52000, Vietnam

Abstract

Toxoplasmosis caused by *Toxoplasma gondii* is an important zoonosis that shows severe symptoms in immunocompromised patients. High seroprevalences of toxoplasmosis were found in cats, humans, and pigs in traditional farms in Thua Thien Hue province. The main sources of infections remain unknown, thus making toxoplasmosis neglected and uncontrollable in this region. This study aimed to determine *T. gondii* contamination in vegetables used for human and livestock consumption and its spatial distribution in Thua Thien Hue rural areas. A cross-sectional study was conducted to investigate *T. gondii* contamination in vegetables grown in households in three different geographic regions. The pathogen DNA was detected from vegetable samples using a primer pair that is highly specific for the 529-bp repetitive element found in *T. gondii* genome. In this study, 55 out of 221 (24.9%) vegetable samples were positive for *T. gondii* using PCR. Factors, including location, presence of fence in the garden, owning cats, and treatment of feed for cats, were not significantly associated with *T. gondii* contamination in vegetables according to the logistic regression. However, vegetables collected in households that have no fence for vegetable gardens, own cats, and feed cats raw or undercooked foods tend to be more heavily contaminated than others. Furthermore, a wide distribution of infected vegetables was observed in all investigated districts, showing a high infection pressure in these residential areas. This is the first report of vegetables contaminated with *T. gondii* in Vietnam, which reveals an overlooked health risk for humans and animals.

Keywords: Contamination, Infection, Thua Thien Hue, *Toxoplasma Gondii,* Vegetable

***Corresponding author:** Nguyen Thi Thuy, Faculty of Animal Science and Veterinary Medicine, University of Agriculture and Forestry, Hue University, Hue city 52000, Vietnam. Email: ntthuy.huaf@hueuni.edu.vn

Funding; SEAOHUN

Article history; *received manuscript: 15 June 2023*
revised manuscript: 10 Novermber 2023
accepted manuscript: 15 December 2023
published online: 12 January 2024
Academic editor: *Nguyen Trong Ngu*

INTRODUCTION

Toxoplasmosis, caused by the obligate intracellular protozoan *Toxoplasma gondii,* is an important zoonosis that can lead to a severe neurological sequel, ocular toxoplasmosis, and encephalitis in immunocompromised patients (Pleyer et al., 2019). Approximately one-third of the world's population is infected with *T. gondii*, making it one of the most common parasitic organisms (Djurković-Djaković et al., 2019). The infection rate is even higher in rural areas, where water is often contaminated with the pathogen and has not been effectively treated before use (Montoya and Liesenfeld, 2004). Another contributing factor to the prevalence of *T. gondii* is the fact that the cat, a common pet in Vietnam, is the definitive host of this parasite. Feces of infected cats containing oocysts can contaminate water and food. This has been considered one of the main routes of *T. gondii* transmission in humans and animals (Webster, 2010).

In *T. gondii* life cycle, all three stages are infectious: tachyzoites, bradyzoites in tissue cysts, and sporozoites in oocysts (Dubey et al., 1998). When a definitive *T. gondii* host (members of the Felidae family) ingests tissue cysts, the parasite goes through its entire life cycle, and oocysts are shed in the feces of the animal for 3 to 10 days post-infection. A single cat may shed more than 100 million unsporulated oocysts into the environment after ingesting as few as one bradyzoite (Templeton, 2007). After sporulation in the environment (from 1 to 5 days, depending on the temperature), oocysts become infectious. All warm-blooded animals, including felids, are considered intermediate hosts (Miller, 2008). They can be infected by consuming oocysts or tissue cysts. After a primary infection, tachyzoites multiply rapidly in the intermediate host cells. After a few multiplication cycles as tachyzoites (1 to 3 weeks), tachyzoites transform into bradyzoites within intracellular cysts. Thus, tissue cysts contain hundreds of infectious bradyzoites (Sullivan and Jeffers, 2012). *T. gondii* can be transmitted to humans through vertical or horizontal routes. Vertical transmission involves the tachyzoite infection of the fetus from its mother through the placenta, which can cause congenital toxoplasmosis. Horizontal transmission to humans occurs through the ingestion of tissue cysts in infected meat or sporulated oocysts in contaminated water, raw fruit, and vegetables (Shapiro et al., 2019).

Infection with *T. gondii* is more prevalent in the rural areas of Vietnam, where livestock such as cattle, poultry, and pigs are kept in open backyard stalls close to residential areas. In these traditional small-scale farms, cats are often raised to catch rats. These cats are allowed to roam freely around vegetable gardens, grazing pastures, livestock pens, water wells, and kitchens. As a consequence, humans and livestock may easily become infected with *T. gondii* after close contact with an infected cat or ingestion of oocyst-contaminated feed or water source (Marín-García et al., 2022). A study conducted in Thua Thien Hue (Vietnam) demonstrated that 72.1% of cats in small-scale pig farms were seropositive with *T. gondii*. In addition, antibodies against the parasite were also found in 41.3 – 69.1% of pigs raised on these farms and in 30% of people who were taking care of the livestock (Dinh et al., 2009; Hosono et al., 2009).

There are many possible routes for *T. gondii* infection. However, the direct cause leading to a high infection rate in the countryside of Vietnam can be eating raw contaminated fresh vegetables. Vegetables infected with *T. gondii* have been reported previously in other countries. Nevertheless, the presence of *T. gondii* in vegetables has not been investigated before in Vietnam. Therefore, this study aims to achieve two objectives: (i) investigate the spatial distribution of *T. gondii* infection sources from fresh vegetables destined for human and livestock consumption in Thua Thien Hue rural areas, and (ii) suggest appropriate preventive measures to reduce the potential infection for humans and livestock in the areas.

MATERIALS AND METHODS

Sample collection

The fresh vegetables, particularly ones that are often destined for human and livestock consumption as raw vegetables, such as lettuce, coriander, and basil, were collected from households in 3 districts located in 3 different geographical regions (mountain – A Luoi; plain – Phong Dien; and coastal/ Lagoon – Phu Vang) within Thua Thien Hue province, Vietnam. In total, 221 vegetable samples containing vestigial amounts of soil were transported to the laboratory at 4 °C and processed within 24 h after collection. Additionally, the demographic data were collected using a questionnaire during vegetable collection, consisting of location, presence of cats, and fence garden positivity.

T. gondii oocysts recovery

T. gondii oocysts were recovered and concentrated as previously described with some slight modifications (de Souza et al., 2016). Fifty grams of each vegetable sample was transferred into a stomacher bag containing 100ml of 1% Tween 80 for 20 minutes and was manually agitated every 3 mins. The washing liquid was transferred to 15ml tubes and centrifuged at 2500 rpm for 20 minutes. The supernatant was discarded until a volume of 1 ml was reached. The *T. gondii*-like oocysts were detected under a light microscope using ×40 magnification and identified according to the description by Lalonde and Gajadhar (2016). The remaining volume of suspension was kept for DNA extraction.

Toxoplasma gondii DNA extraction

The *T. gondii* oocysts in 200 µl of the suspension were washed three times with 200 ml of PBS 1X (3500 rpm for 5 min at room temperature). The washed pellet suspended in 200 ml of PBS 1X was subjected to a freeze-thaw cycle (−196 °C/4 minutes and 100 °C/4 minutes), then taken out to cool down at room temperature. DNA was extracted by the phenol-chloroform method: The samples were mixed with 400 µl of extraction buffer (10 mM Tris pH 8.0, 10 mM EDTA, 1% sodium dodecyl sulfate [SDS], 100 mM NaCl) and 20 µl of proteinase K. They were then incubated at 65 °C overnight after mixing. After cooling to room temperature, 400 µl of Phenol:Chloroform: Isoamyl alcohol (25:24:1) and 20 µl NaCl 5M were added, and the samples were mixed and centrifuged at 16000 rpm/4 °C/15 minutes. The aqueous phase mixture was

transferred to a new Eppendorf tube. Four hundred µl of Chloroform: Isoamyl alcohol (24:1) was added, mixed, and centrifuged as above. The supernatant was transferred to a new tube, and then 20 µl of 3M sodium acetate and two volumes of ice-cold ethanol 100% were added. The mixture was manually stirred 10 times and incubated at -20 °C / 30 minutes. It was then centrifuged at 16000 rpm/4 °C/30 minutes to pellet precipitated DNA. The supernatant was removed, and 500 µl of 70% ethanol was used to wash the DNA (16000 rpm/4 °C/15 minutes). The supernatant was discarded, and the pellet was air-dried at room temperature for 30 minutes. DNA was preserved by adding 50 µl of TE solution and stored at -20 °C until used (Bourdin et al., 2014; Escotte-Binet et al., 2019).

PCR

Polymerase chain reaction primers TOX4 (CGCTGCAG GGAGGAAGACGAAAGTTG) and TOX5 (CGCTGCAGACACAG TGCATCTGGATT) were selected from the 5' and 3' end of the 529 bp repeated sequence found in T. gondii genome, respectively. The PCR was performed in a reaction mixture encompassing 1 µl of DNA template, 12.5 µl of GoTaq Green Master Mix 2X (Promega, Promega corporation, 2800 Woods Hollow Road Madison, USA), 0.5 µl of each primer (10 µM), and distilled deionized water up to 25 µl. Amplification was performed on an Eppendorf™ Mastercycler™ Nexus Thermal Cycler by 7 min incubation at 94 °C, followed by 35 cycles of 1 minute at 94 °C, 1 minute at 55 °C, 1 minute at 72 °C, and a final 10 minutes of incubation at 72 °C (Homan et al., 2000). Extracted DNA from cultured tachyzoites of T. gondii (RH strain) was used as a positive control and was kindly provided by the Department of Parasitology, Faculty of Veterinary Medicine, Kasetsart University (Thailand).

Statistical analysis

Statistical evaluation of the data was carried out using RStudio version 1.3.1093 on MacOS (Posit, PBC, USA). Univariate logistic regression was employed to analyze the association between the appearance of T. gondii oocysts in vegetable samples and three categorical variables: (i) Location (A Luoi, Phong Dien, Phu Vang), (ii) Owning cat (yes, no) and (iii) Fence garden positivity (yes, no). P values ≤0.05 were considered to be statistically significant.

RESULTS

In this study, we collected a total of 221 vegetable samples from households to test for *T. gondii* infection in vegetables. Other information was also collected by interviews during the sampling process to determine the correlation between *T. gondii* infection and factors such as the presence or absence of cats, whether the cat feed is treated or not, and whether the farmer has a fence separating the vegetable area from the surrounding environment or not.

Fifty-five out of 221 vegetable samples (24.9%) showed positivity for *T. gondii* using PCR. The positive samples originated from all three different geographic regions (mountain, plain, and coast) in Thua Thien Hue. No significant risk factor associated with *T. gondii* contamination in vegetables has been found using logistic regression (data not shown).

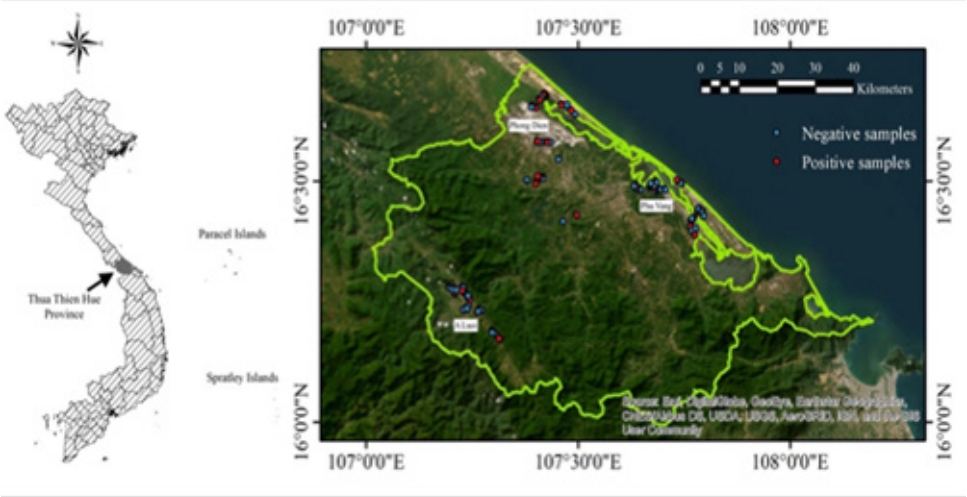

Figure 1 Map of the spatial distribution of *T. gondii*-contaminated vegetables

Vegetables collected in households that have no fence for the farming gardens, own cats, and feed cats with raw or undercooked foods tend to be more heavily contaminated than others. For more detail, 116 out of the total 221 households do not have a fence separating the vegetable-growing area from the surrounding places. Forty-four vegetable samples (27.33%) collected from the 116 households mentioned tested positive for *T. gondii*. This percentage is higher than that in the group of households having fences installed around the gardening places (18.33%) (Table 1).

Table 1 The infection rate of *T. gondii* in vegetables

Categories		Test (Sample)	Positive (Sample)	Negative (Sample)	Percentage (%)	*P* value
Location	A Luoi	86	20	66	23.26	0.42
	Phong Dien	69	21	48	30.43	
	Phu Vang	66	14	52	21.21	
Owning cat	No	86	20	66	23.26	0.77
	Yes	135	35	100	25.93	
Treatment of feed cat	No	68	21	47	30.88	0.51
	Yes	67	14	53	20.90	
Presence of fence in the garden	No	161	44	117	27.33	0.23

A majority of households own at least one cat, accounting for 135 out of 221 households selected for sampling. The prevalence of *T. gondii* infection on vegetables collected from households with cats is 25.93%. Among households that have cats, the numbers of households that feed their cats with treated and untreated food are similar (68 and 67, respectively). However, in the households that treat cat food (cats are fed with hygienic foods), the prevalence of *T. gondii* infection is lower than that of households feeding cats with untreated food, 20.90%, and 30.88%, respectively. Besides, vegetable samples were taken from the group of households without cats (86 samples). We have found that the infection rate was similar to the group of households with cats, which is 23.26%.

Furthermore, a wide distribution of positive vegetable samples was observed in all three investigated districts, showing a high infection pressure in these residential areas. The highest positivity was found in Phong Dien, where the plain region with 11.9%; A Luoi and Phu Vang represent 2 other regions, mountain and coast, showed similar prevalence rates (23.26% for A Luoi and 21.21% for Phu Vang) (Table 1).

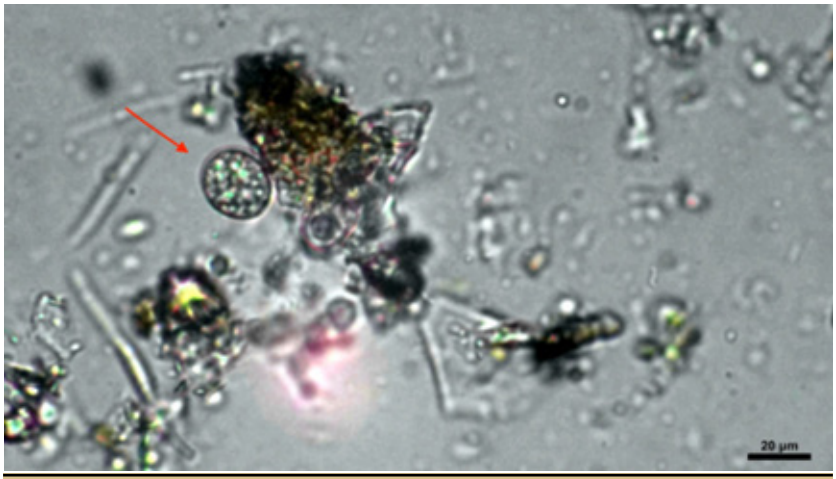

Figure 2 *T. gondi*-like oocyst

Figure 3 PCR amplification of *T. gondii* in agarose gel

DISCUSSION

In this study, *T. gondii* DNA was detected in 55 out of 221 vegetable samples collected from households in three different geographic regions (mountain, plain, and coast) in Thua Thien Hue province of Vietnam. This result indicates that about 25% of fresh vegetables grown in the rural area of Thua Thien Hue province were contaminated with *T. gondii*. To the best of our knowledge, this is the first report of *T. gondii* found in vegetable samples collected in Vietnam. Previously, antibodies against *T. gondii* have been detected in serums of humans, cats, and dogs at Thua Thien Hue (Dinh and Huynh, 2009). In the research related to the seroprevalence of *T. gondii* in cats and pigs from Thua Thien Hue Province (Hosono et al., 2009), the authors indicated that 72.3% of sera samples in cats, 41.3% in live pigs, and 69.1% in slaughtered pigs tested positive for *T. gondii* antibodies. Also in Thua Thien Hue province, the seropositive proportion of antibodies against *T. gondii* in epileptic children was 25.8% among 62 selected pediatric patients with epilepsies (Duc and Anh, 2014). Together with our findings, this implies that *T. gondii* can be transmitted to animals and human beings by consuming contaminated vegetables. This may pose a potential threat to public health safety in the countryside of Vietnam.

Compared with several studies conducted in other parts of the world, our findings reveal that the prevalence of *T. gondii* contamination in vegetables in the rural areas of Vietnam is substantially higher (25%). The percentage of vegetable samples contaminated with *T. gondii* is 5.6% in Pakistan (Adeela et al., 2013), 16.2% in Saudi Arabia (Al-Megrm, 2010), 17.07% in Poland (Lass et al., 2012), 3.8% in Brazil (Marchioro et al., 2016), and around 4.4-8.6% in the Czech Republic (Slany et al., 2019). Many factors possibly contribute to this worrying percentage. First of all, the hot climate and high humidity in Central Vietnam may prolong the viability and facilitate the infectious ability of *T. gondii*. It is known that climate has a significant impact on the circulation of *T. gondii* oocyst in the natural environment (VanWormer et al., 2013). Besides, the cultural habits of the residents may accidentally increase the rate of cross-contamination between animals and the environment. In poorer areas of Vietnam, livestock manure that is not treated properly is frequently used as fertilizer for farming. Also, the habit of eating raw vegetables is very common in Vietnam, which is a real threat to human health. Vegetable by-products are used to feed livestock. These habits together may be the causes of the high prevalence of *T. gondii* found in vegetables, humans, and pigs in Vietnam (Robert-Gangneux and Dardé, 2012).

Apart from human activities, the household cat is also a key transmission factor of *T. gondii*. It has been reported that *Toxoplasma* seroprevalence in humans is highly associated with living in close proximity to *Toxoplasma* seropositive cats (Bawm et al., 2020). Cat owners can reduce the risk of *T. gondii* infection by not allowing their cats to roam in the gardening areas (Udosom et al., 2021). However, in this study, the infection rates of *T. gondii* in households with and without raising cats are not significantly different (P=0.77). On the other hand, many stray cats in the investigated areas were seen roaming freely around the neighborhood, according to interviewed families. This could be the reason why we did not observe a statistical difference in this case. The presence of infected stray cats can be an important yet-neglected piece contributing to the prevalence of *T. gondii* in these local areas of central Vietnam.

Treatment of foods for cats has also been considered in the present study. Although there is no statistical difference between the variants in this case (P=0.51), vegetables collected in households having cats fed with untreated foods (undercooked or raw foods) seem to have a higher percentage of contamination with *T. gondii* (30.88%), compared to vegetables in households that prepared cooked foods for cats (20.90%). There could be a slight correlation between the high rate of Toxoplasmosis occurrence in cats and the poor treatment of cat foods in rural areas of Vietnam. No literature has mentioned this correlation before.

According to Hosono et al. (2009), cats can be the keys to horizontal transmission to pigs raising in small-scale farms where biosecurity management is poorly practiced. Cats carrying oocytes of *T. gondii* can also enter vegetable gardens, which increases the chance of *T. gondii* presence in vegetable products that are later used for humans and livestock. According to the result of the interview in our study, 72% (161/221) of vegetable gardens have no fence. We observe a higher percentage of *T. gondii* contamination in vegetables collected in these gardens (27%) compared to samples collected from gardens with fences (18%). This difference is not statistically significant (P=0.23). Perhaps this could be due to the fact that the security level of fences has not been considered in our study. Many types of fences are very insecure, like those made from bamboo trees. These fences partially prevented, but did not completely block, cats from entering the gardening places, hence biasing the result.

CONCLUSIONS

In conclusion, it is hard to conclude which route is the main transmission route to humans. Cats that can roam freely and easily enter vegetable gardens, livestock pens, and human-living houses may introduce oocytes of *T. gondii* to these places. Cats, humans, and livestock can also be infected with *T. gondii* by eating raw and infected vegetables and other foods. This cycle means *T. gondii* reservoir could be exchanged and preserved via contaminated vegetables, infected cats, pigs, and human beings. To prevent toxoplasmosis, we think that measures should be implemented to prevent *T. gondii* presence in the food chains. One of the feasible measures could be raising awareness of parasitic diseases among populations, which can subsequently lead to behavior changes for better disease control.

ACKNOWLEDGEMENTS

We would like to thank SEAOHUN 2022 Small Grants financially supported by the United States Agency for International Development (USAID).

AUTHOR CONTRIBUTIONS

Pham Hoang Son Hung; Investigation, methodology, formal analysis, manuscript preparation, editing, and finalization
Tawin Inpankaew; contributed to the design of the research and field diagnosis of the disease

Le Dinh Duong; contributed to the design of the research and field diagnosis of the disease
Tran Nguyen Thao; Manuscript preparation, editing, and finalization
Ho Thi Dung; Manuscript preparation, editing, and finalization
Nguyen Xuan Hoa; Conceptualization and design of the experiment, research, and field diagnosis of the disease
Bui Thi Hien; Conceptualization and design of the experiment, research, and field diagnosis of the disease
Nguyen Thi Hoa; Sampling and analyzed
Tran Thi Na; Sampling and analyzed
Tran Quang Vui; Conceptualization and design of the experiment
Nguyen Pho Nguyen Nhung; Sampling and analyzed
Nguyen Thi Giang; Sampling and analyzed
Nguyen Thi Thuy; Investigation, methodology, formal analysis, manuscript preparation, editing, and finalization

CONFLICT OF INTEREST

We have no conflict of interest.

REFERENCES

Adeela, A., Azhar, M., Muhammad, F.Q., Kamran, A., Aftab, A.A., 2013. Detection of Toxoplasma gondii in environmental matrices (water, soil, fruits, and vegetables). Afr. J. Microbiol. Res. 7(16), 1505–1511.

Al-Megrm, W.A.I., 2010. Prevalence intestinal parasites in leafy vegetables in Riyadh, Saudi Arabia. Int. J. Trop. Med. 5(2), 20–23.

Bawm, S., Phyu, A.Z., Chel, H.M., Htun, L.L., Nakao, R., Katakura, K., 2020. Seroprevalence of Toxoplasma gondii in household cats in Myanmar and molecular identification of parasites using feline faecal oocysts. Food. Waterborne. Parasitol. 20, e00094.

Bourdin, C., Busse, A., Kouamou, E., Touafek, F., Bodaghi, B., Le Hoang, P., Mazier, D., Paris, L., Fekkar, A., 2014. PCR-based detection of Toxoplasma gondii DNA in blood and ocular samples for diagnosis of ocular toxoplasmosis. J. Clin. Microbiol. 52(11), 3987–3991.

de Souza, C.Z., R afael, K., Sanders, A.P., Tiyo, B.T., Marchioro, A.A., Colli, C.M., Gomes, M.L., Falavigna-Guilherme, A.L., 2016. An alternative method to recover Toxoplasma gondii from greenery and fruits. Int. J. Environ. Health Res. 26(5–6), 600–605.

Dinh, T.B.L., Huynh, V.C., 2009. Detection of antibodies to Toxoplasma gondii in humans and livestock in Thua Thien Hue province. Hue Univ. J. Sci. 55, 149155.

Djurković-Djaković, O., Dupouy-Camet, J., Van der Giessen, J., Dubey, J.P., 2019. Toxoplasmosis: overview from a One Health perspective. Food. Waterborne. Parasitol, 15, e00054.

Dubey, J.P., Lindsay, D.S., Speer, C.A., 1998. Structures of Toxoplasma gondii Tachyzoites, Bradyzoites, and sporozoites and biology and development of tissue cysts. Clin. Microbiol. Rev. 11(2), 267.

Escotte-Binet, S., Da Silva, A.M., Cancès, B., Aubert, D., Dubey, J., La Carbona, S., Villena, I., Poulle, M.L., 2019. A rapid and sensitive method to detect Toxoplasma gondii oocysts in soil samples. Vet. Parasitol. 274, 108904.

Homan, W.L., Vercammen, M., De Braekeleer, J., Verschueren, H., 2000. Identification of a 200- to 300-fold repetitive 529 bp DNA fragment in Toxoplasma gondii, and its use for diagnostic and quantitative PCR. Int. J. Parasitol. 30(1), 69–75.

Hosono, H., Ito, S., Kono, H., Xuan, X., 2009. Seroprevalence of toxoplasma gondii in cats and pigs from Thua Thien Hue Province in Vietnam. J. Vet. Epidemiol. 13(2), 100–106.

Lalonde, L.F., Gajadhar, A.A., 2016. Detection of Cyclospora cayetanensis, Cryptosporidium spp., and Toxoplasma gondii on imported leafy green vegetables in Canadian survey. Food. Waterborne. Parasitol. 2, 8–14.

Lass, A., Pietkiewicz, H., Szostakowska, B., Myjak, P., 2012. The first detection of Toxoplasma gondii DNA in environmental fruits and vegetables samples. Eur. J. Clin. Microbiol. Infect. Dis. 31(6), 1101–1108.

Marchioro, A.A., Tiyo, B.T., Colli, C.M., De Souza, C.Z., Garcia, J.L., Gomes, M.L., Falavigna-Guilherme, A.L., 2016. First detection of toxoplasma gondii dna in the fresh leafs of vegetables in South America. Vector. Borne. Zoonotic. Dis. 16(9), 624–626.

Marín-García, P.J., Planas, N., Llobat, L., 2022. Toxoplasma gondii in foods: prevalence, control, and safety. Foods. 11(16), 2542.

Miller, M.A., 2008. Tissue cyst-forming coccidia of marine mammals, In: Fowler, M.E., Miller, R.E. (Eds.) Zoo and wild animal medicine, (6th edition). W.B. Saunders, Saint Louis, pp. 319-340.

Montoya, J.G., Liesenfeld, O., 2004. Toxoplasmosis. Lancet. 363(9425), 1965–1976.

Nguyen T.H.D., Ton N.V.A., 2014. Study of Toxoplasma gondii infection in epileptic children. J. Med. Pharm. Hue. Univ. Med. Pharm. 4(3), 63.

Pleyer, U., Groß, U., Schlüter, D., Wilking, H., Seeber, F., 2019. Toxoplasmosis in Germany: epidemiology, diagnosis, risk factors, and treatment. Dtsch. Arztebl. Int. 116(25), 435.

Robert-Gangneux, F., Dardé, M.L., 2012. Epidemiology of and diagnostic strategies for toxoplasmosis. Clin. Microbiol. Rev. 25(2), 264.

Shapiro, K., Bahia-Oliveira, L., Dixon, B., Dumètre, A., de Wit, L.A., VanWormer, E., Villena, I., 2019. Environmental transmission of Toxoplasma gondii: Oocysts in water, soil, and food. Food. Waterborne. Parasitol. 15, e00049.

Slany, M., Dziedzinska, R., Babak, V., Kralik, P., Moravkova, M., Slana, I., 2019. Toxoplasma gondii in vegetables from fields and farm storage facilities in the Czech Republic. FEMS Microbiol. Lett. 366(14).

Sullivan, W.J., Jeffers, V., 2012. Mechanisms of Toxoplasma gondii persistence and latency. FEMS Microbiol. Rev. 36(3), 717–733.

Templeton, T.J., 2007. The secretory protein repertoire and expanded gene families of toxoplasma gondii and other apicomplexa, In: Weiss, L.M., Kim, K. (Eds.), Toxoplasma gondii: the model apicomplexan. perspectives and methods. Academic Press, London, pp. 445-463.

Udonsom, R., Buddhirongawatr, R., Nishikawa, Y.M., Fereig, R., Jirapattharasate, C., 2021. Toxoplasma gondii prevalence and risk factors in owned domestic cats from Nakhon Pathom Province, Thailand. Vet. Integr. Sci. 19(3), 557–566.

VanWormer, E., Fritz, H., Shapiro, K., Mazet, J.A.K., Conrad, P.A., 2013. Molecules to modeling: Toxoplasma gondii oocysts at the human–animal–environment interface. Comp. Immunol. Microbiol. Infect. Dis. 36(3), 217.

Webster, J.P., 2010. Toxoplasmosis of animals and humans. In: Dubey, J.P. (Ed.), Parasites & vector. 3, 112.

How to cite this article;

Pham Hoang Son Hung, Tawin Inpankaew, Le Dinh Duong, Tran Nguyen Thao, Ho Thi Dung, Nguyen Xuan Hoa, Bui Thi Hien, Nguyen Thi Hoa, Tran Thi Na, Tran Quang Vui, Nguyen Pho Nguyen Nhung, Nguyen Thi Giang, Nguyen Thi Thuy. *Toxoplasma gondii* contamination in fresh vegetables destined for human and livestock consumption in Thua Thien Hue, Vietnam. Veterinary Integrative Sciences. 2024; 22(3): 847 - 856