Factors Affecting Toxoplasma gondii Cyst Survival: A Review for Prevention Strategies

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Introduction

Toxoplasma gondii is an obligate intracellular parasite and a zoonosis. It is an opportunistic parasite in both humans and animals, classified under the phylum Apicomplexa due to its complex secretory organelles at the apical end. These organelles facilitate its penetration into host cells. Human infection typically occurs through the consumption of food contaminated with oocysts or undercooked meat or organs containing cysts. Unlike many other diseases, toxoplasmosis often presents no clinical symptoms in both definitive and intermediate hosts.[1]

Within the intermediate host, Toxoplasma gondii exists in two stages: tachyzoites, which cause acute infection, and bradyzoites, which reside within tissue cysts and can persist for a lifetime. Tachyzoites infect all nucleated cells and multiply by endodyogeny, rapidly spreading throughout the body via the bloodstream or lymphatic system. After approximately two weeks, tachyzoites decrease in number, and bradyzoites develop within host tissues. Bradyzoites can evade the immune system and treatment due to their location within cysts. However, they can revert to tachyzoites if the immune system is compromised, such as in immunosuppressed individuals.[2]

Research conducted by Hartono revealed that approximately 40% of 50 pork samples contained Toxoplasma cysts.[3] These cysts were commonly found in the muscles and internal organs of these animals. Additionally, cysts are prevalent in pets like cats and birds. Cysts are one of the infectious forms of *T. gondii* and play a significant role in the transmission of toxoplasmosis. The high prevalence of cysts in meat, a common food source, contributes to the ease of human infection. To prevent the transmission of this disease through cysts, extensive research has been conducted on methods to inhibit cyst-mediated transmission of toxoplasmosis.

Cysts serve as a defense mechanism for *T. gondii*, enabling it to survive in the environment outside the host. These cysts can infect intermediate hosts, including humans. While this survival ability benefits the parasite's transmission, research has identified various factors that influence the viability of *T. gondii* cysts, such as pH, NaCl concentration, refrigeration and heating temperatures, hydrostatic pressure, and other substances. These findings offer potential avenues for preventing infection by *T. gondii* cysts.

Methods

The literature review for this article was conducted by systematically searching Scopus, Google Scholar, and PubMed using keywords such as "Toxoplasma gondii viability," "cyst survival factors," and "toxoplasmosis prevention." The selected studies investigated the impact of environmental factors and specific treatments on the viability of T. gondii cysts. Studies were categorized based on the type of factor explored, including physical conditions (pH, temperature), chemical agents, and processing technologies like High Pressure Processing (HPP).

Results and Discussion

Viability refers to the proportion of cells that remain alive in a sample.[4] Several factors influence the viability of *Toxoplasma gondii*, including:

1. Heating

   The parasite is rapidly inactivated at temperatures exceeding 66 °C (151 °F) and is killed by boiling. Oocysts in water can be eliminated by boiling or filtration using a 1 μm filter. While the parasite can withstand heating at 50 °C for 30 minutes, tissue cysts are destroyed at 55 °C for 30 minutes.[5] Microwave heating has been shown to be less effective in *inactivating T. gondii* cysts due to uneven heat distribution, which may result in the survival of some cysts. Wrapping meat in aluminum foil before microwaving is recommended to ensure even heat distribution. Smoking, a common method for preserving meat, is ineffective in eliminating *T. gondii* cysts due to uneven heat exposure.

2. pH

   Previous research has shown that infectious cysts are more commonly found in muscles undergoing short-term fermentation compared to those undergoing long-term fermentation. This indicates that changes in pH during fermentation play a role in cyst inactivation. To verify this hypothesis, tests were conducted on cysts present in muscle tissue. The research findings are as follows:

   Table 1. pH factors influencing the inactivation of *Toxoplasma* cysts.[6]

	Tissue sampled:					
	Muscle					
Day of sampling	Micro	PCR	Micro	PCR	Micro	PCR
	pH 5		pH 6		pH 7	
10	+	+	ND	ND	ND	ND
13	+	+	ND	ND	ND	ND
15	+	+	ND	ND	ND	ND
18	+	+	ND	ND	ND	ND
20	−	−	+	+	+	+
22	+	+	+	+	+	+
24	−	+	+	+	+	+
26	+	+	−	−	−	−
28	−	−	−	−	−	−
30	ND	ND	ND	ND	−	−

   In this experiment, various pH gradients ranging from 5 to 7 were applied to cysts found in muscle tissue. The study was conducted over 30 days, with daily observations to monitor the progression. The results revealed the following:

   a. On day 26, the cysts in muscle tissue were inactive at pH 6 and 7, whereas cysts at pH 5 remained active.
   b. By day 28, all cysts across the three tested pH levels (5, 6, and 7) were inactive.

   These findings indicate that *Toxoplasma* cysts can be inactivated within a pH range of 5 to 7 after 28 days of storage. Changes in pH disrupt the cell's ability to synthesize and carry out other metabolic processes. This inability leads to cyst inactivation. Consequently, pH modification could serve as a potential method for preventing the spread of infectious *Toxoplasma* cysts.[7]

3. Freezing

   Freezing at a temperature of −12°C (10°F) for 2–3 days effectively destroys a significant percentage of *Toxoplasma* cysts. The decrease in temperature during microorganism growth disrupts cellular metabolic processes, leading to imbalance and cessation of metabolic activity. Furthermore, the drop in temperature alters the lipid composition of the cell

membrane, affecting its functionality. These changes compromise the integrity of the membrane, causing it to rupture and ultimately leading to cell death. In addition to lipid composition changes, the freezing process also affects gene expression patterns, altering protein synthesis and further inhibiting cellular function.[7]

4. NaCl

   Seasoning meat with salt, sucrose, or other solutions can inactivate tissue cysts, although survival rates many variation.[8,9] Studies have shown that pork products treated with NaCl or lactate solutions reduce microbial contamination, enhance flavor and texture, and extend shelf life. The effectiveness of lactate and NaCl solutions lies in their ability to inhibit spoilage organisms and pathogenic microbes.[10]

   To confirm these findings, an experiment was conducted using mouse muscle tissues.

Table 2. The Effect of NaCl on the Inactivation of *Toxoplasma* Cysts.[6]

	Tissue sampled:					
	Muscle					
Day of sampling	Micro	PCR	Micro	PCR	Micro	PCR
	2.0% NaCl		2.5% NaCl		3.0% NaCl	
1	–	–	–	–	–	–
2	–	+	–	–	–	–
3	+	+	–	–	–	–
4	+	+	–	–	–	–
5	+	+	–	–	–	–
6	–	–	–	–	ND	ND
7	–	–	ND	ND	ND	ND
8	+	+	ND	ND	ND	ND

In the analyzed samples, varying concentrations of NaCl (2-3%) were used, and cyst inactivation was detected through microscopy and PCR. The results were as follows:

a. On the first day, all *Toxoplasma* samples tested negative; however, with 2% NaCl, positive results were observed between days 2 and 5. This might be due to some cysts remaining viable and not yet fully inactivated on the first day.
b. By the sixth day, all samples tested negative, confirming that salt effectively inactivates *Toxoplasma* cysts.

The inactivation of cysts was attributed to dehydration caused by the presence of NaCl. Dehydration disrupts the cell's ability to perform metabolism, ultimately rendering the cysts inactive.[11]

Tabel 3. The Influence of NaCl on the Inactivation of *Toxoplasma* Cysts[12]

Days stored	% NaCl			
	0.85	2.0	3.3	6.0
7	2†	2	2	0
14	2	2	2	1
21	2	2	2	0
28	2	2	0	0
35	2	2	0	0
42	2	2	0	0
49	2	2	0	0
56	2	0	0	0

From the findings of this research, it is evident that NaCl concentration significantly influences the viability of protist cells. Similar studies have been conducted, incorporating pH adjustments, and the results indicated that higher pH levels accelerate *Toxoplasma* inactivation. Based on these findings, the application of NaCl can be considered an effective method to curb the spread of this protozoan. A combined approach involving NaCl addition and temperature modification in storage conditions has been shown to effectively inactivate *Toxoplasma* cysts. The outcomes of temperature adjustments and NaCl application are as follows:

Tabel 4. Factors Influencing the Inactivation of *Toxoplasma gondii* Cysts [12]

	Temperature and NaCl concentration (%)									
Days	10 C				15 C			20 C		
stored	0.85	2.0	3.3	6.0	0.85	2.2	3.3	0.85	2.2	3.3
3	6/6†	6/6	6/6	0/4	2/2	2/2	2/2	2/2	2/2	2/2
7	4/4	4/4	4/4	0/4	2/2	2/2	2/2	2/2	2/2	0/2
14	6/6	6/6	4/6	0/4	4/4	4/4	2/4	3/4	0/4	0/4
21	4/4	4/4	2/4	ND	4/4	0/4	0/4	0/2	0/2	0/2
28	2/2	2/2	0/2	ND	0/2	0/2	0/2	0/2	0/2	0/2
35	2/2	0/2	0/2	ND	0/2	0/2	0/2	0/2	0/2	0/2

The results of this study showed an increase in the inactivation of Toxoplasma cysts. It was observed that the higher the NaCl concentration and temperature, the more cysts were inactivated. The fastest inactivation of cysts was achieved by storage at 10°C with 6.0% NaCl concentration or on the seventh day at 20°C with 3.3% NaCl concentration.

5. *High Pressure Processing* (HPP)

High Pressure Processing (HPP) is a food processing technology that applies high pressure to solid or liquid foods to enhance food safety by targeting harmful microorganisms. The process begins with packaging the food, which is then placed in a pressure chamber equipped with a seal and filled with water connected to a hydrostatic pump. The pressure is transmitted to the packaged food through the water for a specific period. The effects of HPP on microorganisms include the elimination of certain organisms and the inactivation of specific enzymes without altering the taste or nutritional content of the food.

This technique was tested on Toxoplasma gondii cysts to observe its effectiveness in cyst inactivation. The results demonstrated that:

Table 5. Factors of High Pressure Processing (HPP) Influencing the Inactivation of *Toxoplasma gondii* Cysts [13]

Treatment (MPa)	Exposure time (sec)*	No. of mice inoculated/ No. of mice positive
400	30	4/0
400	60	4/0
400	90	4/0
300	30	4/0
300	60	4/0
300	90	4/0
200	30	4/4
200	60	4/4
200	90	4/4
100	30	4/4
100	60	4/4
100	90	4/4
0	NA	4/4
0†	NA	4/0

* NA = not applicable.
† Negative control sample did not contain tissue cysts.

At a pressure of 300 MPa, a significant effect was observed, with the number of mice testing positive for *Toxoplasma* cysts in their muscles dropping to zero. This indicates the impact of pressure application on the inactivation of *Toxoplasma* cysts. The mechanism of High-Pressure Processing (HPP) involves protein denaturation, which is crucial for the survival of microbial cells, thereby leading to the inactivation of these microorganisms.[14]

Conclusion

Toxoplasma gondii cysts have a high level of environmental adaptability, but their viability can be influenced by pH, temperature, salinity, and treatment technologies such as HPP. A thorough understanding of these factors provides a scientific basis for developing strategies to prevent toxoplasmosis, particularly through safe food processing and preventing the spread of Toxoplasma. Further research is needed to evaluate the efficiency of treatment combinations in inactivating cysts under various conditions.

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