

Histological Detection of *Toxoplasma gondii* in *Gallus gallus domesticus*

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ABSTRACT

*Toxoplasmosis is one of the most common parasitic infections that affect both human and warm-blooded animals. The infection is caused by *Toxoplasma gondii*, an obligate protozoan that is found to be prevalent worldwide. Human contraction occurs through ingestion of the infective stage of *T. gondii* which came from food and drinks contaminated with oocysts acquired from exposure to felines, which act as a definitive host. Infection in *gallus domesticus* is an important indicator of soil contamination and aids in the identification of the epidemiology of the parasite. This study sought to determine the presence of *Toxoplasma gondii* in different parts of chickens which are intended for human consumption. Histopathological technique was utilized to detect the presence of the morphological form of the parasite in four female chickens raised for four months in free-range and caged conditions. Based on the findings, there was no presence of *Toxoplasma gondii* in 39 histopathologic slides obtained from organs of the chickens intended for human consumption. The study indicated that regardless of raising confinement of the chickens there was no *Toxoplasma gondii* seen and reported.*

Key words: *Gallus gallus domesticus*, Histopathological technique, Soil contamination, *Toxoplasma gondii*, Toxoplasmosis

INTRODUCTION

Toxoplasmosis is an infection caused by *Toxoplasma gondii*, an obligate apicomplexan protozoan which may be found worldwide, since a large variety of animals may harbor this parasite. Humans may contract this parasite through ingestion of oocysts from contaminated food and drinks and through exposure to felines, which act as a definitive host while bird species may serve as an intermediate host. Bird species are considered the most important host of *Toxoplasma gondii* (Saadatnia & Golkar, 2013).

Domestic cats and other members of the family Felidae are the only known definitive host for *Toxoplasma gondii*. These ingest rodents or birds which can serve as an intermediate host that may possess infective oocysts from ingesting of soil, water or plant material. The oocysts transform into cyst in the brain or muscle tissue. The definitive host can acquire the infective stage from consuming an intermediate host and the cycle will reoccur continuously. Development of tissue cysts can also be present in humans and food animals after ingestion of sporulated oocysts in the environment (World Health Organization, 2015). Infection caused by *Toxoplasma gondii* cannot be passed from direct contact of human, but in instances of congenital (mother-to-child) transmission and in cases of blood transfusion or organ transplantation. In 2014, Centers for Disease Control and Prevention (CDC) stated that typically, people may contract this by eating meat contaminated by the tissue form of parasite and animal-to-human (zoonotic) transmission.

The primary diagnostic method in the determination of toxoplasmosis in humans is typically made by serologic testing, wherein this technique quantifies immunoglobulin G (IgG) titles to identify if infection is present. Generally, in order to detect the time of infection, detection of immunoglobulin M (IgM) is particular especially in pregnant patients. Furthermore, diagnostic process also includes direct observation of the morphology of parasite in stained tissue samples or histological examination. In terms of detecting congenital transmission, molecular techniques are used to isolate the DNA of the parasite in the amniotic.

In the prevention of the infection from *Toxoplasma gondii*, proper hand washing technique must always be observed and always avoid consuming undercooked meat (WHO, 2015).

Chicken (*Gallus gallus domesticus*) is also known as domestic fowl. This plays as a significant model animal in biomedical research (Miao, Peng, Wu, Ouyang, Yang, Yu, & Palanichamy, 2013). They have a broad function, such as providing raw materials for industrial merchandise, agricultural necessity and especially for food product.

The Food and Agriculture Organization (FAO) stated that the Philippines is the fastest growing meat consuming nation and annually, an average Filipino consumes about 11.6 kg of chicken. Furthermore, 2013 Food Consumption Survey by the Food and Nutrition Research Institute of the Department of Science and Technology showed that chicken and egg ranked nine in the top 20 commonly consumed food products among Filipino households. There are various sources of feather, organic fertilizer, egg and meat; however, chicken is the most common and available domestic animal serving as a primary source (Dar & Tanveer, 2013). In lieu of this, isolation of possible pathogens from the *Gallus gallus domesticus* is essential in determining the epidemiology of the infection, specifically, toxoplasmosis caused by *Toxoplasma gondii*.

The absence of study on *Toxoplasma gondii* infection in chickens in the Philippines compelled the researchers to embark on this study. The possible identification of *T. gondii* in several parts of the chicken such as heart, liver, intestine, skin, breast, gizzard, brain, kidneys, and spleen that undergo histological examination will provide a good indication of possible consumption of contaminated food unknown to the public. The researchers also aim to gain additional information about the epidemiology of *Toxoplasma gondii* in *Gallus gallus domesticus* as possible host of infection.

MATERIALS AND METHODS

The researchers raised 10 native female chickens for up to four months in summer season. Five of which had been kept under free range condition wherein they had free access to water and can feed on ground aside from the food given by the researchers while the rest had been put into a cage wherein, they were given the same food every morning and afternoon. The area where the chickens were kept is covered with canopy and the soil has a pH of 5.9 along with different animals such as dogs, ducks, turkeys, birds, and cats where husbandry is also present.

Routine necropsy

Routine necropsy was observed in order to recognize and record if abnormalities were present in the chicken. First, gross examination was done to evaluate the general body condition of the samples. The American Veterinary Medical Association has approved a method of using carbon dioxide (CO₂) to euthanize animals. This can be used as an analgesic at 7.5% and can be used as an anesthetic which causes rapid loss of consciousness with no struggle or distress at 30%-40%.

The whole chicken has been immersed into a bucket of hot water for the easy removal of feathers. This decreased the chance of skin to be aerosolized which helped keep the scalpel free of small feathers for the dissection. In dissecting the body, the wings were reflected back and then a cut has been made through the skin between the legs and breast. The skin was removed from the ventral surface of the chicken by cutting across the tail and then pulling the skin cranially and caudally to expose the muscular body wall.

The abdominal viscera was exposed using a scalpel blade where an incision was made behind the breast bone and the abdominal muscle was pulled caudally. The breast muscle was removed by incising the pectoral muscles on each side of the keel and cutting through the ribs. Heavy poultry shears have been used to cut through the ribs in order to remove the keel and breast muscles entirely. This allowed the full view of the internal organs from the oral cavity to the rectum. It is easier to begin removing the abdominal viscera first then going back to the thoracic organs. Since the liver occupied a larger space in the abdomen, it was removed first. Spleen has been isolated next since it is difficult to locate when all other organs were already removed. It resembles a spherical shape and is in the junction of the right side proventriculus and

ventriculus. By pulling the proventriculus aside, the spleen should show up, then removed and set in a clean dry spot. Locate the junction site of the esophagus and stomach, cut throughout it and pull the digestive tract out together with the cloaca. The heart and kidneys were carefully removed. For the collection of the brain, smaller scissors were used to chip away the skull from the foramen magnum (Bruce-Gregorios, 2012).

Fixation

The heart, liver, intestine, skin, breast meat, gizzard, brain, kidneys, spleen which were freshly obtained from the chickens were immediately immersed in a 10% neutral buffered formalin for approximately 24 hours under 30°C to prevent decomposition and to preserve the morphological, chemical, and cellular composition of organs (Wang, 2015). Fixation alters the tissue by stabilizing proteins that allows the tissue to be resistant for further changes. Specimens harvested have been cut for approximately three millimeters (mm) thick by 15 mm wide for optimal fixation to ensure that the formalin can penetrate well into the tissue, cross-link proteins, and for autolysis to stop quickly as possible (El-Razik et al., 2014).

Dehydration

After appropriate fixation and before staining, the cassettes were subjected to dehydration wherein water was entirely removed from tissues. This allowed tissues to be enveloped in a paraffin wax which is not miscible in water using an increasing concentration of alcohol starting from 70%, 95%, and 100% alcohol for 1 hour, respectively (El-Razik et al., 2014).

Clearing

After dehydration, the cassettes were put on a clearing agent using Xylene for one hour and another solution of it for another hour in order to remove the excess alcohol from the tissue making it optically clear (El-Razik et al., 2014).

Infiltration

After clearing the cassettes, the tissues were impregnated with paraffin wax at 56°C in an oven for 15 minutes then repeated for 4 times in a different paraffin wax (El-Razik et al., 2014).

Embedding and sectioning

The specimens were embedded in a mold. In this process, tissues were arranged in a precise position in a mold, then cooled in preparation for blocking and trimming. Excess amount of wax was removed; thus, truncated pyramid was obtained. This allowed easy sectioning of the specimen. Embedded tissues were trimmed and cut into uniformly thin slices using rotary microtome that can cut the tissue in 10-12 micrometers thin (Wang, 2015).

Staining

Ribbon sectioned tissues were put in a tissue floating bath at a temperature of 45-50°C temperature or 10°C below the melting point of the paraffin wax to preserve the integrity of the specimen. Using clear glass slides, the tissues were fished out and were incubated at paraffin oven with 2-5°C temperature. The samples were stained using Hematoxylin and Eosin for better visualization of the morphology of parasite (Wang, 2015).

Routine microscopy

The specimens were examined using a light microscope at 400x magnification aiding in the reporting of the possible morphological findings in the experiment. Digital photographs were taken using a Canon EOS Rebel T2i camera.

RESULTS AND DISCUSSION

As demonstrated, there was no presence of any morphological forms of *T. gondii* in parts of free-range and caged chickens destined for human consumption (Table 1). Detecting *Toxoplasma gondii* oocysts in the environment is difficult because cats that excrete these bury their feces in warm and moist soil; however, chickens are considered to be the best indicators for contamination of the parasite's infective stage because they naturally feed on the ground wherein soil might be contaminated with oocysts. This may lead to infection and then possible transmission to cats which is an important host in the epidemiology of Toxoplasmosis (Ayinmode & Dubey, 2013). The prevalence of toxoplasmosis varies according to geographical factors, transmission route and social and cultural habits (Aboelhadid et al., 2013).

Table 1. Microscopic Analysis of *Toxoplasma gondii* in tissue biopsy of *Gallus gallus domesticus*

PARTS	Tissue Biopsy of Chicken			
	FREE RANGE CHICKENS		CAGED CHICKENS	
	A	B	C	D
Brain	Negative	Negative	Negative	Negative
Breast	Negative	Negative	Negative	Negative
Gizzard	Negative	Negative	Negative	Negative
Heart	Negative	Negative	Negative	Negative
Intestine	Negative	Negative	Negative	Negative
Kidney	Negative	Negative	Negative	Negative
Liver	Negative	Negative	Negative	Negative
Skin	Negative	Negative	Negative	Negative
Spleen	Negative	Negative	Negative	Negative

According to Wang (2015), there was no presence of tissue cysts in histopathological sections of brain, heart, liver and other organs of chickens even if there are antibodies and DNA of *T. gondii* detected. Possible reason is that cysts have been formed but the numbers are rare and could not be observed in tissue sections. The specimens used by the researchers were female chickens, suggesting that gender was not a risk factor for toxoplasmosis but *T. gondii* can change the steroid hormone concentration. This enhance the susceptibility of males or females to infection but there is a greater probability of exposure to oocysts in male roosters because of its active characteristic (Feng, Wang, Liu, Zhang, & Yang, 2016).

The variation in prevalence may be due to female animals, compared to male, are more susceptible to protozoan parasites. However, some reports show that there is a higher prevalence in male chickens compared to females. This is due to hormonal differences in males and females which is important in determining their susceptibility to parasitic infections. Estrogen enhances the production of antibody, however, the immunity of chickens may be broken down by certain factors such as age, nutrition, and reproductive and environmental factors (Mose, Kagira, Karanja, Ngotho, Kamau & Njuguna., 2016).

On the other hand, there is a significant relationship of prevalence of *T. gondii* between age of chickens and revealed higher prevalence in chickens older than 2 years old than in younger chickens less than 1 year old. This is because as they get older, they became more susceptible to the infection than the younger ones (Severance, Xiao, Jones-Brando, Sabuncian, Pletnikov & Yolken, 2016). In the recent study, immune host response might also be considered, since chicken macrophages are crucially involved with high expression of pro-inflammatory Th1 cytokines which is typically related to macrophage function in *T.*

gondii infection. Generally, cytokines such as IFN- γ and tumor necrosis factor α (TNF- α) play a vital role in immunity of chicken against toxoplasmosis (Zhang, Thatbet, Hiob, Zheng, Daugschies & Bangoura, 2018).

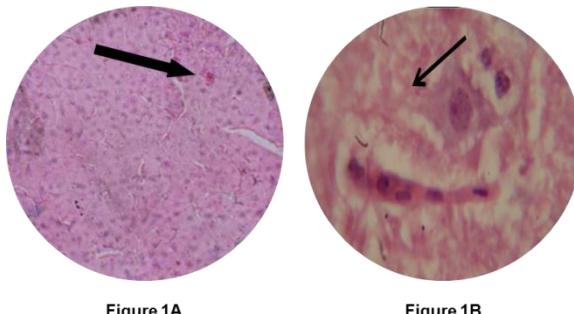


Figure 1A

Figure 1B

Figure 1: Specimen taken from Hematoxylin and eosin (H&E) stained brain tissues of *Gallus gallus domesticus*.

Figure 1A shows that brain tissue from Specimen 1 is suspected with *T. gondii* cyst due to uncanny resemblance to the morphology of the parasite with circular appearance and stains well with Hematoxylin and eosin and Oil Immersion Objective (OIO) used to verify that it is not an existence of the said parasite. This morphology is usually spherical and may resemble degenerating host cells (Dubey, 2013).

Figure 1B shows the tissue cyst morphology of the parasite that was isolated in an infected chicken from a research entitled Diagnostic Study of Toxoplasmosis in Domestic Chickens in Sulamani Province. In the study conducted, three out of 9 specimens that undergone histological detection revealed a positive result (Mohammed & Abdullah, 2013).

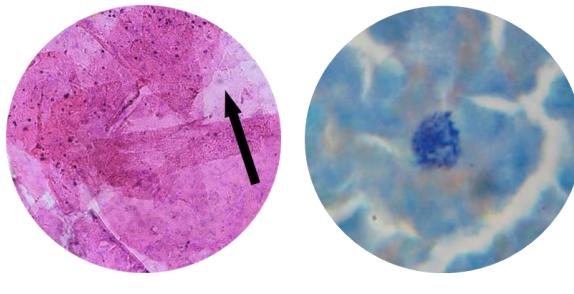


Figure 2A

Figure 2B

Figure 2. Sample from brain tissue stained with Hematoxylin and eosin (H&E)

Figure 2A presents structure suspected to be a cyst of *T. gondii* from Specimen 2 which has remarkable similarities to this infective stage of the parasite which has a circular appearance and the nucleus of cell seems to resemble this stage of *T. gondii*. Oil Immersion Objective (OIO) confirmed that it is not the said morphologic stage of the parasite. Tissue cyst usually localize in neural and muscular tissues wherein they persist longer in there because in these organs, immune responses are less effective (Dubey, 2013). Figure 2B indicates an infected brain tissue with cyst morphology of *T. gondii* that was isolated in an infected chicken from the same study that undergone histopathologic technique in examining 9 specimens that yielded three positive results (Mohammed & Abdullah, 2013)

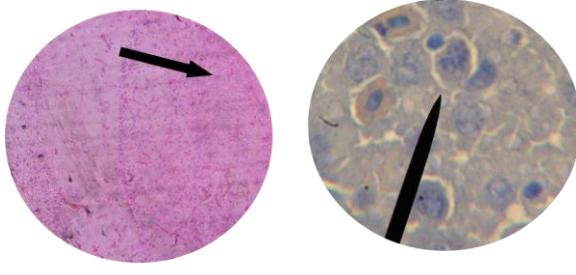


Figure 3A

Figure 3B

Figure 3. Sample from brain tissue stained with Hematoxylin and eosin (H&E)

Figure 3A reveals cyst-like structure in the brain tissue of Specimen 4 since the nucleus of cell exhibits characteristics of *T. gondii* tissue cyst; however, OIO confirmed that this is only a nuclear material of the parasite. The suspicion arised since *T. gondii* is considered to be as neutropic parasite which attacks the brain then forming cysts in the course of infection (Estate, Stipursky, Gomes, Mergener, Teixeira, Allodi & Adesse, 2018). Whereas, Figure 3 (b) shows a brain tissue contaminated with *T. gondii* cyst that was isolated in an infected chicken from the same study that undergone examination by histopathology wherein nine specimens that yielded three positive results (Mohammed & Abdullah, 2013)

CONCLUSION AND RECOMMENDATION

The completion of the study indicated that there was no presence of any morphological forms of *Toxoplasma gondii* in different parts of *gallus domesticus* intended for human consumption that has been examined through histological technique. Results hereby proved that *Toxoplasma gondii* was absent in both free-ranged and caged chickens with same age, gender and feeding pattern and which had been raised in the same locality considering the context of the study.

The researchers believed that this study is informative and beneficial in sanitary food handling of every Filipino household and further research regarding this topic is needed. For the future researchers, they may involve bigger samples to have wider distribution of presence of the parasite. They may also culture chickens in longer period of time to lengthen their exposure to the environment that may have a greater chance of being infected. Future researchers may also perform screening test to detect initial stage of infection by several serological examination like Enzyme-linked immunosorbent assay (ELISA), Modified agglutination test (MAT) and Sabin-Feldman dye test.

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