**Advanced Studies on Toxoplasma in Buffalo Meat**

**Abstract**

Since buffalo meat, has been demonstrated to be a potential source of human infection, a careful evaluation of the prevalence of Toxoplasma infection in this meat is needed. Tissue cysts of *Toxoplasma gondii* are frequently found in the skeletal muscles of buffaloes. This study evaluated the prevalence of *Toxoplasma gondii* in the meat juice of buffalo meat samples via several diagnostic techniques to protect public health. Pepticdigestion, histopathology, and serology were performed on meat juice from 100 buffalo meat samples from local butchers and retail beef markets. Eighteen samples (18%) were suspected of the presence of bradyzoites after digestion, and were subjected to histopathology which illustrated that only six samples (6%) were suspected to be Toxoplasma tissue cysts. After that periodic acid Schiff (PAS) stain confirmed that only three samples (3%) were Toxoplasma. Finally, enzyme-linked immunosorbent assay (ELISA) asserted that those three samples were *Toxoplasma gondii.* This study provides significant evidence about the risk of human exposure to Toxoplasma through the consumption of raw or undercooked buffalo meat potentially contaminated with infectious tissue cysts.

**Keywords:** Buffalo meat; Toxoplasma gondii; Meat juice; Serology.

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1. **INTRODUCTION**

Toxoplasma is a typical meat borne zoonotic coccidian protozoa, where most human infections occur through the bradyzoite stage that present in the edible meat and exceeding opportunistic human impact sequence to toxoplasmosis (Abd El-Razik *et al.*, 2014). Infection with *Toxoplasma gondii* (*T. gondii*), an obligate intracellular protozoa, may cause abortion in food animals, cerebral and ocular lesions in children with perinatal infection, and fatality in immunocompromised individuals (Taiching Fuh *et al.*, 2013).

One of the main routes for human infection by *T. gondii* is the consumption of raw or undercooked meat of infected intermediate hosts, containing tissue cysts (Belluco *et al.*, 2018; Pinto-Ferreira *et al.*, 2019; Robert-Gangneux and Dardé, 2012). Other risk factors as oocysts dusting and placental diffusion come after meat consumption where amplifying awareness against undercooked meat minimizes human prevalence world-wide (Abd El-Razik *et al.*, 2014). Therefore, the disease is considered to be a public health issue in areas where beef has been destined for broad scale human consumption (Singh *et al.*, 2023).

Overall, the worldwide prevalence of *T. gondii* infection in humid tropical areas is generally higher than in hot and dry areas, and cooler areas (Tenter *et al.*, 1992). It is likely that the hot and humid climate in the south provides better conditions than the colder and drier climate in the northeast to support the circulation of *T. gondii* in the natural environment (Inpankaew *et al.*, 2021).

Cysts have been isolated and detected from 3–22% of fresh and frozen buffalo meat samples obtained from retail stores, markets and abattoirs in many regions (EL‐TRAS *et al.*, 2012) (Gencay *et al.*, 2013) (Bărburaș *et al.*, 2019). A rural settlement location near to the grazing areas had led to the abandonment of domestic cats. These stray animals now feed on birds or small wild mammals, including rodents in that area, and this is likely to increase their chances of contracting toxoplasmosis (Albuquerque *et al.*, 2011) (de F. Santos *et al.*, 2013). Consequently, there is an increased risk that they transfer the parasite to cats and livestock animals (Meerburg and Kijlstra, 2009) (Yan *et al.*, 2016).

One distinct characteristic of *T. gondii* in immune -competent hosts, the tissue cysts were able to persist for several years (life of the host) after infection and the immunity does not eliminate an established infection (Waree, 2010). The formation of tissue cysts under certain circumstances is an important aspect of the pathogenesis of Toxoplasmosis. The cyst wall may considerably reduce the availability of exogenous materials to the bradyzoites. Thus, the switch from tachyzoites to bradyzoites was done (Filisetti and Candolfi, 2004). However, the parasite persists in its bradyzoite form, inside the intracellular cysts. The periodic rupture of these cysts was thought to be the origin of maintained immunity against Toxoplasma (Innes *et al.*, 2009).

Based on various serological tests and convenience samples, the current situation of toxoplasmosis in Egypt is not clear. There is no central laboratory or group of researchers actively investigating toxoplasmosis in humans or animals, and no reports on the national level are available (Abbas *et al.*, 2020). As a result, clinical toxoplasmosis in humans from Egypt needs further investigations using definitive procedures.

Although there are many serological surveys for *T. gondii* in animals, data on infections of buffalo meat are lacking. Hence, we critically focus on the status of Toxoplasma infections in buffalo meat in Egypt, which should be useful to biologist, public health workers, veterinarians and physicians.

1. **MATERIALS AND METHODS**
	1. **Sample collection:**

A total of 100 samples of buffalo meat (from different meat cuts) were purchased from local butchers and retail beef markets. Each sample weighed approximately 250 *g*, and transferred aseptically in ice box to laboratory as soon as possible.

Three portions from each sample were taken; the first part was exposed to peptic digestion. The second part was kept in a 10% formalin solution for the histopathological examination, and the third was preserved in ethyl alcohol 70% for the molecular analysis. All samples’ remnants were frozen at - 20°C until the end of the experiment.

* 1. **Peptic digestion:**

Digestion was carried out to all of the 100 meat samples according to the method described by *Hussein et al.*(Hussein *et al.*, 2017),and *Dubey*(Dubey, 1998). The appearance of clear bradyzoites under the microscope, as a characteristic indication of coccidian parasites, was considered a positive result.

* 1. **Histopathological examination:**

Only the tissue samples positive for bradyzoites were fixed in 10% neutral-buffered formalin for 24 hours then dehydrated in ascending grades of ethanol and inserted in paraffin blocks. 4–5 µm thickness serial sections were cut and mounted(Bancroft and Gamble, 2008). Some slides were stained with hematoxylin and eosin (H & E) and others were stained with periodic acid Schiff (PAS) stain to distinguish Toxoplasma from Sarcocystis (Hill and Dubey, 2018). Two independent observers performed the histopathological evaluation blindly.

* 1. **Serology:**

Antigen-antibody (Ag-Ab) rapid test was performed to confirm the positive meat samples infected with toxoplasma resulted from the previous two methods. This test was done using Atlas Toxo Latex Kit®, Germany; a rapid latex agglutination test for qualitative detection of Toxoplasma gondii antibodies according to method described by *Wallander et al.*(Wallander *et al.*, 2015),as shown in Figure (1). This method illustrated that serum drained from meat samples (at 4 °C for 24 h) called ‘‘meat juice’’ could be used in serological assays when diluted to about tenth of the serum dilution.

1. **RESULTS**

The digestion of all of the 100 meat samples revealed the presence of bradyzoites in 18 of them indicating the existence of coccidian protozoa. Those 18 samples were subjected to histopathology which illustrated that 12 samples were infested with Sarcocystis only, 4 samples had a mixed Sarcocystis and Toxoplasma infection, and only two samples were infested with toxoplasma only as observed in Figure (2).

PAS stain was used to differentiate between Toxoplasma cysts and Sarcocystis in the 4 mixed samples, as only one of them was PAS positive having pink bradyzoites (+ve Toxoplasma), while the other 3 samples contained Sarcocystis with violet bradyzoites when stained with PAS.

From the previous results, three samples out of a total of 100 buffalo meat samples were suspected of Toxoplasma infection (3%) and subjected to serology where they were confirmed to be infected with *Toxoplasma gondii* as demonstrated in Figures (4, 5).

1. **DISCUSSION**

Generally, the existence of toxoplasmosis in buffaloes and cattle is subclinical and this complicates disease identification when it is based only on clinical presentation. Hence, the only option to estimate the risk of transmission to humans from raw and undercooked meat is to employ laboratory-based approaches that offer data on the prevalence of *T. gondii* infection in these animals (Dubey, 2021). Several serological methods have been utilized globally to detect the seroprevalence of *T. gondii* infection (De Barros *et al.*, 2020).

Recently, *Shaapan et al.*(Shaapan *et al.*, 2021)concluded that the development of an effective Enzyme-linked immunosorbent assay (ELISA) test for the detection of *T. gondii* in meat juice could be considered as a promising tool for monitoring Toxoplasmosis in meat and meat products of cattle in large-scale. Also, the meat juices of small ruminants were analyzed by *Gazzonis et al.*(Gazzonis *et al.*, 2020)with commercial [ELISA](https://www.sciencedirect.com/topics/immunology-and-microbiology/elisa) and *T. gondii* antibodies were detected in 28.6% sheep and 27.5% goats. In addition, *Felin et al.*(Felin *et al.*, 2017)confirmed that ELISA methods helped in detecting *Toxoplasma* antibodies in the meat juice, and also for slaughterhouse-based serological monitoring of toxoplasmosis in pigs to identify positive farms.

To diagnose the existence of *Toxoplasma* tissue cysts in buffalo meat in the present study, we used peptic digestion, followed by histological inspection and serology. In the current investigation, we discovered that only 3% of buffalo meat samples tested were positive for *T. gondii* tissue cysts. These findings disagreed with *Almashhadany*(Almashhadany, 2020), whoobserved a higher prevalence of anti-*T. gondii* antibodies among meat juice of red beef meat in Iraqi markets, which was 15.2% (19 positives out of a total of 125 samples) according to the Latex agglutination test (LAT), and 13.6% (16 positives out of a total of 125 samples) by ELISA.

However, the infection of water buffaloes with *T. gondii* has been investigated worldwide in many continents, with prevalence ranging from 0 to 88% reported using different serological techniques (De Barros *et al.*, 2020). Moreover,*Gencay et al.,*(Gencay *et al.*, 2013) investigated frozen buffalo meat in Turkey and found tissue cysts of *T. gondii* in 15% of the samples that analyzed by light microscopy of percoll dilutions.

Conversely, our results were in disagreement with *Tienthai & Sajjarengpong*(Tienthai and Sajjarengpong, 2013), who affirmed that neither *T. gondii* cysts nor antigens were detected in the imported beef samples by ELISA, although 15% of samples were positive for *T. gondii* using PCR. Also, our study contradicted with *El-Tras et al.*(EL‐TRAS *et al.*, 2012), who didn’t find any *T. gondii* tissue cysts in the imported frozen buffalo meat (0%) compared to 15.4% in the fresh buffalo meat via bioassay of meat samples in cats.

In the current investigation, 18 samples were positive for bradyzoites after digestion, before confirming the suspected Toxoplasma via histopathology. These findings were in accordance with *Abdel-Rahman*(Abdel-Rahman, 2017), who confirmed that the digestion of meat samples in trypsin or pepsin is used to concentrate *T. gondii* in meat. This could be explained by the fact that Bradyzoites of *T. gondii* are more resistant to digestive enzymes, (pepsin and trypsin) than tachyzoites. Therefore, ingestion of viable tissue cysts by a non-immune host will usually result in an infection with *T. gondii* (Dubey *et al.*, 1998).

Instead of digestion, *Gencay et al.,*(Gencay *et al.*, 2013) employed another assay todetect the presence of *T. gondii* tissue cysts using a high speed tissue homogenizer, centrifugation, and percoll dilutions.

Concerning the histological examination, our study revealed that 6 out of the 18 samples were suspected for *Toxoplasma* tissue cysts. These results were endorsed by *Hussein et al.,*(Hussein *et al.*, 2017), who concluded that the histopathology was a successful method for the detection of coccidian tissue cysts in buffalo beef. On contrary, our findings were repudiated by*Tenter et al.*(Tenter *et al.*, 1992), who believed that Toxoplasma was more frequently detected in the brain, and heart than in muscle samples especially in sheep. Furthermore, *Al-Khafagi & Zainab*,(Al-Khafagi and Zainab, 2016)noted that histopathological test showed multiple variable sizes of Toxoplasma cysts embedded and scatter between cardiac muscle.

The reason for the prevalence of *T. gondii* infections in humans and animals from Egypt is the fact that the living circumstances in Egypt favor the transmission of *T. gondii*, as up to 95% of domestic cats, the key host of *T. gondii*, are infected with *T. gondii*; and they are abundant in rural and suburban areas, spreading *T. gondii oocysts* (Abbas *et al.*, 2020).

Regarding blood serum samples from living buffaloes, the same seroprevalence percentage of our study (3%) was reported in buffaloes by *Huong**et al.*(Huong *et al.*, 1998)in Vietnam and *Sharma et al.*(Sharma *et al.*, 2008) in India. While slightly higher percentage of positive samples for *T. gondii* (6.8%) was declared by *Inpankaew et al.*(Inpankaew *et al.*, 2021)in Thailand suggesting that humans and animals living on those farms may be exposed to infection.

In Menoufia province, *Ibrahim et al.*(Ibrahim *et al.*, 2021)examined the seropositivity ofToxoplasma in water buffaloes by estimating the anti- *T. gondii* antibodies (IgM and IgG) via ELISA assays, and observed that the overall seroprevalence was 9.02% for IgM and 8.2% for IgG. Additionally, *Bărburaș et al.*(Bărburaș *et al.*, 2019) documented that the overall seroprevalence of *T. gondii* IgGs in water buffaloes from Romania was 6.6% using a commercial ELISA test.

Besides, the seroprevalence in the present study was lower than those of some investigations carried out in buffaloes from other authors, such as 14% by *Hamidinejat et al.*(Hamidinejat *et al.*, 2010)in Iran, 14% by *Zahra et al.,*(Zahra *et al.*, 2014)in Pakistan, and 18% by *Luo et al.* (Luo *et al.*, 2017)inChina. In addition, *Santos et al.*(de F. Santos *et al.*, 2013) stated that 27.2% of serum samples from Brazilian water buffaloes were positive for *T. gondii* antibodies by indirect fluorescent antibody test (IFAT). Moreover, a study performed in Northern Brazil recorded a much higher prevalence of 41.6% for *T. gondii*-seropositive buffalo (Silva *et al.*, 2013).

On the other hand, much lower anti-*T. gondii* antibodies (1.3%) were reported by*Dubey et al.* (Dubey *et al.*, 1998) in sera in water buffalo from Cairo Province in Egypt. The cause for such a disparity might be attributed to the rarity of cat numbers in the research region.

1. **Conclusion**

Our present study showed that buffalo meat may harbor viable *T. gondii* and serve as a potential infection source. To our knowledge, this is the first study that reported the presence of *T. gondii* tissue cysts in the buffalo meat in Egypt using serological assays on the meat juice. Besides, histopathology and peptic digestion were helpful in the diagnosis of Toxoplasma cysts in the meat samples.

While the world is facing ascending volumes of trade in buffalo meat, the presence of *T. gondii* tissue cysts in buffalo meat needs further investigations, in addition to the diagnosis of these cysts in other meat products or mixtures derived from buffalo meat to maintain buffalo meat safe for human consumption.

**Consent for publication**

Not applicable.

**Availability of data and materials**

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Competing interests**

The authors declare that they have no competing interests.

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