

DETECTION OF TOXOPLASMOSIS IN ENVIRONMENTAL SAMPLES AT A WET MARKET OF A CAPITAL CITY CENTRE

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Summary: The local Chow Kit market is the largest wet market in the city of Kuala Lumpur. It is very close to the biggest government hospital in the city centre. However, the level of cleanliness in this area is always questionable and a matter of concern. The aim of this study was to identify the prevalence of *T. gondii* oocyst in water samples used by hawkers in that market and tissue cysts in rats' brains captured from the same area.

Water samples were taken to the parasitology laboratory at the National University of Malaysia and a sugar flotation concentration method was used. Supernatant microscopical examination was then performed. A total of 752 slides were screened for the presence of *T. gondii* oocyst. A hundred rats wandering in the same area were also captured by the hawkers using mousetraps. After each animal was sacrificed, and an electric microtome was used to cut out serial sections 5 μ thick from the rat brains. The de-waxed tissue sections were stained by the progressive Haematoxylin and Eosin (H&E) stain for microscopical examination.

A total of 1000 slides were screened under a light microscope to detect the presence of *T. gondii* brain cysts. All the water samples were found to be negative for *T. gondii* oocyst. Out of the 100 rats captured, three rats were found to possess *T. gondii* cysts in their brains.

Water samples reflect minimal or no solid food contamination, while the 3% of positive brain cysts influence the researchers to broaden their investigations for future projects.

Key words: *T. gondii*; Transmission; Environmental samples; Intermediate host; Water contamination

Introduction

Toxoplasma gondii infections are widely prevalent in human beings and animals worldwide. Its definitive hosts are cats and other Felidae. People typically become infected by three principal routes of transmission: food borne, animal-to-human (zoonotic) and mother-to-child (congenital). Presently, there are no tests to identify the source of infection in an individual, and evidence is based exclusively on epidemiological surveys.

Toxoplasmosis is common and has a high prevalence rate among the Malaysian population. The majority of infections are mild, with most people being asymptomatic (chronic/latent infection). From 1985 to present, the trend of toxoplasmosis prevalence has shown a similar figure of 20–30% (8, 9, 16).

Toxoplasmosis, until recently, was not considered a water-borne zoonosis. Some results show an association with *T. gondii* in municipal waters and marine mammals, suggesting that the contamination of seawater with *T. gondii* may be more common than actually identified (3). In certain areas of Brazil, approximately 60% of 6–8-year-old children have antibodies to *T. gondii* linked to the ingestion of oocysts in a heavily contaminated environment with

T. gondii oocysts (1). It is believed that the domestic cat is essential for the maintenance of the transmission cycle, and added to this is the unhealthy habits of meat consumption (6, 10, 12, 15). Interestingly, the Atlanta outbreak was linked to nasopharyngeal ingestion of oocysts aerosolized in the riding stable where *T. gondii* infected cats were present (11).

T. gondii oocysts are extremely resistant to environmental influences and can sporulate and survive in soil or water at 4 °C for months. However, at any given time only 1% of cats shed oocysts (5). Therefore, for epidemiological surveys, detection of *T. gondii* oocysts in cat feces is impractical and less informative. To the best of our knowledge, there are no commercial reagents available to detect *T. gondii* oocysts in the environment.

The aim of this study was to identify the prevalence of *T. gondii* oocyst in water samples that are widely used by hawkers in a local market in Kuala Lumpur and in tissue cysts in rats' brains captured from the same area.

Materials and methods

The study was performed between August 2008 and March 2009. The study design is a prospective laboratory

based study. Prior ethical approval from the National University of Malaysia University was given for the study.

There are 177 workers and 153 small shops in the Chow Kit market. Only 133 shops were included in the study (owners of the remaining shops refused to be enrolled in this study). The selection criteria planned to be according to hygienic state, but we found that all shops possess a poor hygienic state. Therefore, no specific selection criteria were established, and all shops selling vegetables and fruits were enrolled after getting the owners' consent.

Every evening, the researchers collected water that had been used for the whole day by the hawkers. The water samples collected were used by the vendors to wash vegetables, fruits, tableware and hands. Therefore, these samples of water served as a good indication for the presence of *T. gondii* oocyst in solid food in the local Chow Kit market. The used water was kept in buckets, and the researchers collected it daily. The total amount of water collected during the time of study was 77 liters. A total number of 135 drinking water samples were taken. Collected water samples were taken to the parasitology laboratory at the National University of Malaysia and a sugar flotation concentration method was used. This technique separates the oocyst from other debris. The principle of this technique is that the specific gravity (SG: 1.200) used should float the cysts without changing their specific gravity, which might cause swelling or shrinking. The supernatant layer was removed from the tube with a pipette and placed on two clean glass slides for microscopical examination. A permanent Trichrome stain technique was used to help identify the parasites. A total of 752 slides were screened for the presence of *T. gondii* oocyst. The slide examination was done by a technologist who has 30 year experience in identifying protozoa.

One hundred rats wandering in the same area (Chow Kit market) were also captured by the hawkers using mouse-traps. Each morning, the researcher collected the rats and took them back to the Anatomy Department at the National University of Malaysia. Open ether anesthesia was used for 90 seconds on average. After each animal was sacrificed, the whole brain of each rat was removed carefully. The left and right brain hemispheres were divided carefully. For each hemisphere, fixation and paraffin-wax embedding was followed by electric microtome sectioning which cut serial sections 5 μ in thickness. The de-waxed tissue sections were stained by the progressive Haematoxylin and Eosin (H&E) stain for microscopical examination. There were a total of 1000 slides which were screened under light microscope to detect the presence of *T. gondii* tissue cyst.

Results

All the water samples were screened and found to be negative for *T. gondii* oocyst.

Out of the 100 rats captured, three rats were found to possess *T. gondii* tissue cysts in their brain. One of them had

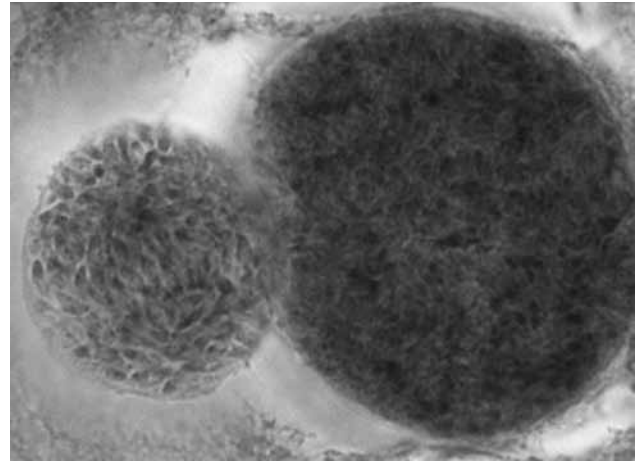


Fig. 1: Photograph showing two tissue cysts in the left hemisphere of rat number 1 ($\times 1000$)

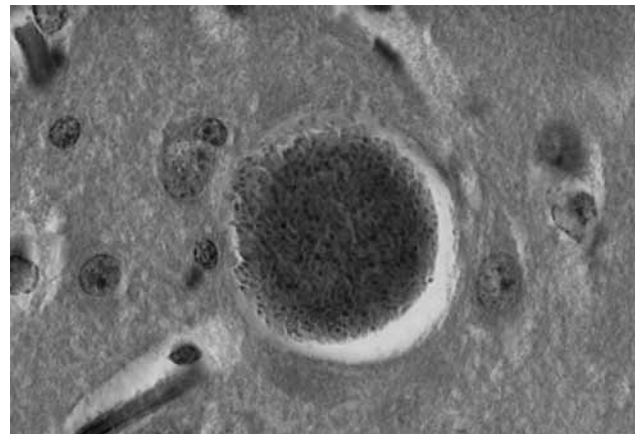


Fig. 2: Photograph showing tissue cyst, neurocytes and red blood cells in rat number 2 ($\times 400$)

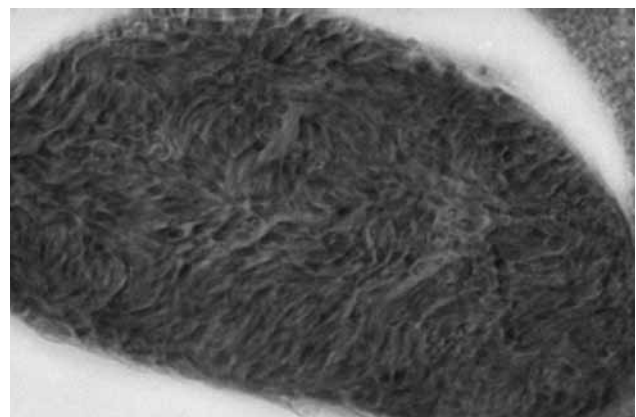


Fig. 3: Photograph showing individual bradyzoites inside tissue cyst in rat number 3 ($\times 1000$)

multiple cysts in the same hemisphere, while the second one had a bilateral infection and the third rat had one single cyst. *T. gondii* cyst was diagnosed according to the morphological appearance. Further molecular study should be performed to confirm the diagnosis.

Discussion

The local Chow Kit market is the largest wet market in the city of Kuala Lumpur. It is very close to the biggest government hospital in the city centre, Hospital Kuala Lumpur (HKL). This market offers good prices and a wide range of options for visitors. However, the level of cleanliness in this area is always questionable and a matter of concern.

Kuala Lumpur is a rapidly developing city. Modernization and industrialization of this capital city influences the health status, especially with the presence of many legal and illegal foreign workers. In addition, those who cannot cope with the challenges of living in urban areas are plunged into social ills. This description gives an overview of the appropriate circumstances around the Chow Kit market, where prostitution and drug dealers are often reported. According to a study performed in 2003 at HKL, the incidence of toxoplasmic encephalitis in AIDS patients and commercial sex workers was 11% and 46.6%, respectively (7). Thus, *T. gondii* prevalence around Chow Kit market reflected the rate of infection of *T. gondii* among the local residents.

All water samples in this study were negative for *T. gondii* oocyst. There might be slight water contamination that went undetected because water concentration and microscopic examination was the only method used for detection. Detection of *T. gondii* oocysts in water is more difficult than that of other coccidian oocysts and there are no standardized methods to perform it. Attempts to recover *T. gondii* oocysts from water samples in the British Columbia outbreak were unsuccessful (4).

It is known that a polymerase chain reaction (PCR) is one of the most sensitive methods to detect *T. gondii* oocyst in environmental samples and, at present, a bioassay is one definitive way to detect viable oocysts. In fact, both methods are not recommended in our country due to high expenditure and lack of experience. However, in order to determine the presence of *T. gondii* oocyst, we used a sucrose flotation method which was known to be quite sensitive for environmental samples (13).

Rats usually get infected from contaminated soil or by consuming meat containing the infective *T. gondii* cyst. Chicken is a strong possible source of infection in this market. *T. gondii* is proved to be lodged in the heart and brain of hens (2). Hawkers usually slaughtered hens, removed their internal organs, and kept them in open containers which were easily reached by scavenging rats.

We speculate that infection may have changed the habits or behaviors of rats in terms of being less afraid of cats. This

may be due to damage of the warning mechanism in the rats through a decreased function of anxiety (14). The amount of stray cats may be one of the reasons for getting a relatively lower number of infected rats in the Chow Kit market. In comparison with the method used in this study, whereby stained histological slides were examined, microscopical examination of squeeze slides from native or homogenized brain tissue is very effective because a bigger volume of brain tissue can be inspected on one slide. The researchers have the intention to use this method in future studies to get more accurate results.

In 2008, the Kuala Lumpur City Hall launched a campaign to encourage hawkers to catch rats on their premises. Free mousetraps have been provided to hawkers, who are paid one Malaysian Ringgit (0.35 USD) for each rat tail. It is hoped that this plan will help to control and prevent the transmission of this parasitic infection. The research group has the intention to do further studies on the isolation of *T. gondii* tissue cysts from hens, chickens, ducks and repeat the testing of rat and other rodent organs in the market.

The authors declare that they have no competing interest. All authors read and approved the final manuscript. The research was sponsored by the Faculty of Allied Science, Universiti Kebangsaan Malaysia (National University of Malaysia).

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