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Isolation of *Toxoplasma gondii* from Horse Meat in Egypt

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Abstract: Portions of heart, liver, skeletal and diaphragmatic muscles obtained from 150 slaughtered horses at Giza-Zoo abattoir were used for bioassays in mice and cats. *T. gondii* tachyzoites were isolated successfully from the peritoneal exudates of the inoculated mice 6-8 days post inoculation with pooled horse tissues. Whereas, *T. gondii* tissue cysts containing bradyzoites were detected in the impression smears of mice brain on the 45th days or more post infection. The oocysts were detected in feces of cats 3-6 days post feeding on horse tissues containing tissue cysts. The oocysts became sporulated within 3-5 days in 2.5% Potassium dichromate. A total of 79 out of 150 horse meat samples were found to be infected with an incidence rate of 52.6 %. This is the first trial for isolation of *T. gondii* infective stages from horses in Egypt. Moreover, this study pointed out to the high infection rate of *T. gondii* in horse meat which may be considered as an important source of infection to wild zoo-animals in Egypt and humans in some countries if consumed raw or insufficiently cooked.

Key words: *Toxoplasma gondii*, isolation, horse meat, Egypt

INTRODUCTION

Toxoplasma gondii is a protozoan parasite of warm-blooded animals including man. It has a worldwide distribution. Cats, including all felines, are the definitive hosts which excrete environmentally-resistant oocysts in their feces. Hosts become infected by ingestion of food or drink contaminated with oocysts or by ingesting undercooked meat from infected animals with *T. gondii*. It causes mental retardation and loss of vision in congenitally-infected children and abortion in pregnant women and livestock (Dubey *et al.*, 2005).

Among the major food animals, viable *T. gondii* has been found in pork, mutton, chicken and horse meat (Dubey *et al.*, 1999). Munday (1970), in Australia, isolated *T. gondii* from the diaphragm of one of four horses. AL-Khalidi *et al.* (1980), in the USA, isolated *T. gondii* from tissues (heart, diaphragm, spinal cord, brain, tongue, skeletal muscles, liver and kidney) of 4 out of 9 ponies experimentally fed infective *T. gondii* oocysts by mouse inoculation and feeding of cats. Dubey (1985), recovered *T. gondii* from tissues of equids (tongue, intestine, muscles, brain and heart) when were bioassayed in mice and cats and proved that *T. gondii* can persist in edible tissues of living equids up to 476 days. Turner and Savva

(1990) reported the presence of *T. gondii* DNA in lesions from placenta of a mare in UK. While evidence that *T. gondii* causes neurologic disease in horses is not definite, there are reports of *T. gondii* infection in the eyes of aborted foals in UK (Turner and Savva, 1991 and 1992) and in CNS of horses in Brazil (Macruz *et al.*, 1975).

Although many studies have been reported in many countries on isolation of *T. gondii* from horse tissues, there is no any previous trial on isolation of *T. gondii* from horses in Egypt. Therefore, the main objectives of this study are isolation of *T. gondii* infective stages from meat and tissues of the slaughtered horses by mouse inoculation and cat feeding and also detection the rate of *T. gondii* infection within horse's tissues in Egypt.

MATERIALS AND METHODS

Isolation of *Toxoplasma gondii* strain from horse meat

Collection and preparation of meat samples: Meat and tissue samples were obtained from 150 slaughtered horses at Giza-Zoo abattoir, prepared from heart, liver, skeletal and diaphragmatic muscles as described by Kotula *et al.* (1983); a pooled sample, about 50 to 100 g, from the tissues and organs of the slaughtered horses, cut into small cubes about 5×5×5 cm and stored at 4°C for few hours until used for bioassays in mice and cats.

Experimental animals

Mice: Swiss albino laboratory breed mice about one month old, their body weights vary from 25 to 35 g, were kept in clean cages and fed on pelleted food and water was offered. These mice were used for bioassay of the suspected infected tissues in order to isolate *T. gondii* tachyzoites from the peritoneal cavity or obtaining the *T. gondii* tissue cysts containing bradyzoites from their brain. Also, these mice were used to maintain the tachyzoites of the isolated *T. gondii* equine strain in the laboratory.

Cats: Three kittens about one month old were isolated from their mothers, kept separately and fed only on boiled or pasteurized milk and bread before using in the experiment. These kittens were used to isolate the *T. gondii* local strain oocysts. Meanwhile their feces were parasitologically examined daily for 7 days to insure that they were free from any of coccidian infection.

Bioassays of meat and tissues of slaughtered horses

Bioassay in mice: Prepared pooled horse meat samples were digested by pepsin as the procedures described by Sharma and Dubey (1981). The suspended sediment of each digested sample was inoculated separately into two groups of mice (each of 2 mice), one group inoculated intra-peritoneally and the other subcutaneously (1 mL/mouse). After one week from inoculation, the first group was scarified and the peritoneal exudates were examined microscopically to identify the tachyzoites. While after 4-6 weeks, impression smears from brains of the second group were prepared and examined to detect *T. gondii* tissue cysts containing bradyzoites.

Bioassay in cats: Each of the three cats was fed on about 250 g of infected pooled horse meat samples, which previously showed *T. gondii* bradyzoites by pepsin-digestion, in order to isolate *T. gondii* oocysts as the procedures described by Davis and Dubey (1995). Fecal samples from each cat were collected daily from 3-14 days post experimental feeding and examined parasitologically by concentration floatation technique. The recovered oocysts were mixed with 2.5% Potassium dichromate solution, placed in wide Petri dish at room temperature for 5-7 days to allow the sporulation and then stored at 4°C.

Maintenance of the isolated *T. gondii* strain: The virulent locally isolated strain of *T. gondii* obtained from horse meat was maintained in Department of Zoonosis, National Research Center by serial passages in mice. The peritoneal fluid containing about 2×10^6 tachyzoites was

inoculated into 3 to 5 mice (0.2 mL each) and further inoculation was done every 2-4 days according to the method of Johnson *et al.* (1979).

RESULTS

***T. gondii* infection rate in horse meat samples:** A total of 79 out of 150 (52.6%) of horse meat samples were found to be infected with *T. gondii* and viable infective stages were detected from mice inoculated intra-peritoneally with the pepsin-digested meat samples.

Isolation of *T. gondii* infective stages from horse tissues

***T. gondii* tachyzoites:** Viable infective tachyzoites were recovered from the peritoneal exudates of mice inoculated 6-8 days earlier with infected digested horse meat samples. The tachyzoites were found inside leucocytes (lymphocytes and macrophages) or free in the peritoneal exudates after rupture of leucocytes. The isolated tachyzoites were often crescent in shape or banana shape, pointed at one end and rounded or blunt at the other one, their sizes ranged from 2-4 μ in width \times 5-8 μ in length (mean 3 \times 7 μ) (Fig. 1A and B).

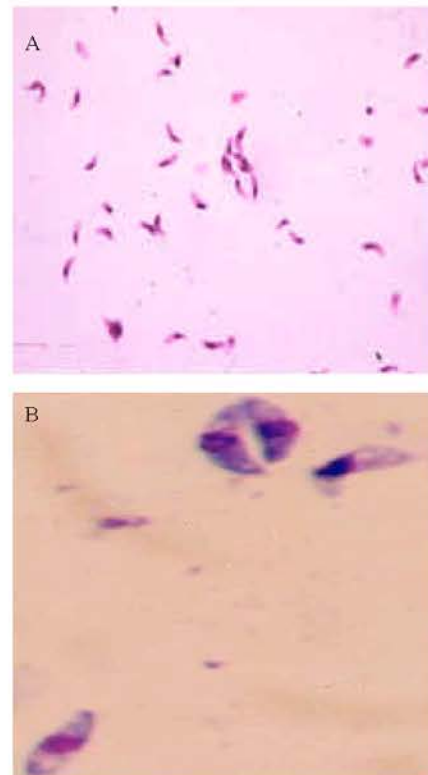


Fig.1: Giemsa stained *T. gondii* tachyzoites; A: \times 400, B: \times 1000

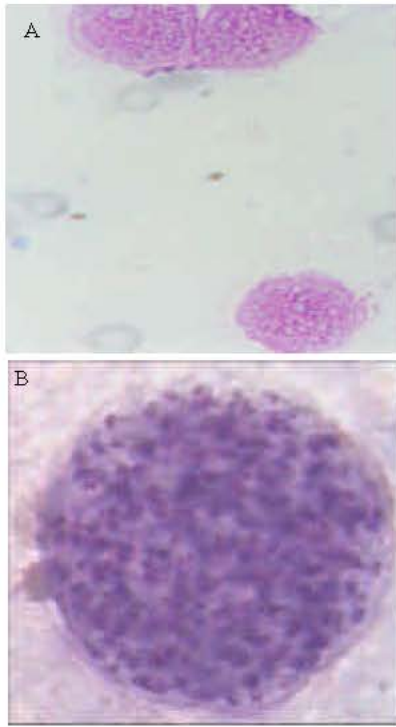


Fig. 2: Giemsa stained *T. gondii* tissue cysts; A:×400, B:×1000

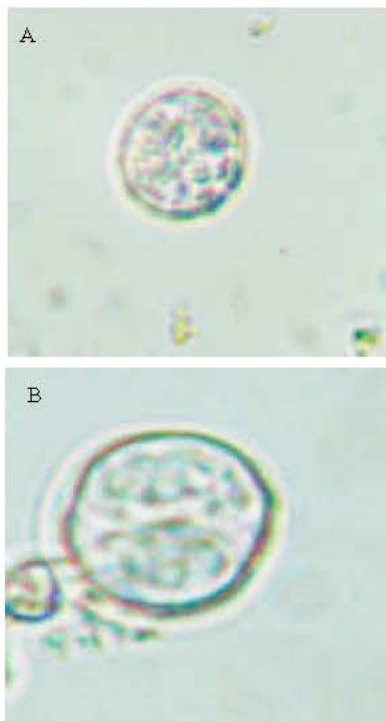


Fig. 3: *T. gondii* oocysts A: Unsporulated, B: Sporulated (×400)

***T. gondii* tissue cysts with bradyzoites:** Tissue cysts of *T. gondii* were noticed in the impression smears of mice brain or prints on the 45th day or more post infection. They were usually sub-spherical to spherical in shape and their cyst walls were thin, elastic and well-defined enclosing crescent shaped bradyzoites. The young tissue cysts may be small (5-10 μ in diameter) contain only 4 to 8 bradyzoites, but the mature older tissue cysts were 50 to 200 μ in diameter and contain several hundreds of bradyzoites (Fig. 2A and B).

***T. gondii* oocysts:** *T. gondii* oocysts were isolated from feces of the three cats 3-6 days post infection with horse tissues containing *T. gondii* tissue cysts (pre-patent period), these oocysts were still released in cat feces for a period of 10-12 days from the beginning of the release (patent period). The fresh recovered unsporulated oocysts were identified by their spherical shapes, their sizes which ranged from 9-11 μ ×11-13 μ (mean 10×12 μ) and the embryonic cells which were in close contact to the cyst wall (Fig. 3A). The sporulated oocysts were spherical to ellipsoid in shape and their sizes were ranged from 10-13 μ ×12-15 μ (mean 11.5×13.5 μ). Each oocyst contains two sporocysts and each contains four sporozoites and sporocystic residual bodies (Fig. 3B). The sporulation of fresh oocysts were observed after 3-5 days under suitable conditions of temperature and oxygenation by using 2.5% Potassium dichromate at room temperature.

DISCUSSION

T. gondii tachyzoites were isolated successfully from the peritoneal exudates in the inoculated mice 6-8 days post inoculation (DPI) with the digested horse tissue samples. Whereas, *T. gondii* tissue cysts containing the bradyzoites were detected in mice brain prints after 45 DPI. Such results are in agreement with that of Dubey and Frenkel (1973), who found *T. gondii* tachyzoites in the impression smears obtained from intestinal lymph nodes on the 6th day post inoculation. However, Dubey and Frenkel (1976) recognized *T. gondii* tissue cysts in brain prints 3-4 weeks post infection, earlier than the findings obtained during this study. Also, Dubey (1997) detected *T. gondii* tachyzoites and cysts in the mesenteric lymph nodes, lung and brain after 3, 7 days and 2 months post infection, respectively. The discrepancy between the results of the present study and those recorded by previous authors might be ascribed to the difference in the strain of the parasite, route of inoculation, age of inoculated mice and the development of their immune system.

T. gondii oocysts were detected in feces of cats 3-6 days post feeding on horse tissues containing *Toxoplasma* tissue cysts and these oocysts were still released in cat feces for a period of 10-12 days. The isolated oocysts were in accordance with that reported by Dubey and Beattie (1988) and Dubey (2001), who declared that cats are very sensitive indicator of *T. gondii* infection because cats fed even a few *T. gondii* bradyzoites can shed a million of oocysts. In the present study, *T. gondii* oocysts ranged between 10-12 μ in case of unsporulated oocysts but the sporulated forms were 11.5-13.5 μ . These were comparatively similar to those measurements of oocysts obtained by Dubey (1998a), who reported that *T. gondii* oocysts ranged from 9 \times 11 μ for unsporulated oocysts and 12 \times 13 μ for sporulated ones. The oocysts became sporulated within 3-5 days in 2.5% Potassium dichromate solution. In this regard Zaman (1970) and Dubey (1998b and 2002) proved that oocysts sporulation is temperature dependent. Moreover, Dubey (1998a) added that good ventilated media as 2.5% Potassium dichromate or 2% H₂SO₄ with slight shaking had remarkable effect on the sporulation period of *T. gondii* oocysts.

In conclusion, this study is the first trial for isolation of *T. gondii* infective stages from horses in Egypt and detection of the incidence rate of infection among horse meat. It is important, therefore, to evaluate the clinical significance of equine toxoplasmosis and to consider the public health aspects, especially if horse meat infected with toxoplasmosis is to be offered for animal or human consumption in some countries.

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