Journal of Animal and Veterinary Advances 5(6) : 456-458, 2006

Detection of *Toxoplasma gondii* Tachyzoites in the Milk of Experimentally Infected Lactating She-Camels

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Abstract: The excretion of *Toxoplasma gondii* tachyzoites in milk of she- camels was studied by IP inoculation of nine albino mice with milk of three lactating she-camels experimentally infected with *Toxoplasma* sporocysts. *Toxoplasma* tachyzoites and cysts were detected in the brains of all inoculated mice and suckling calf- camels. *Toxoplasma* antibodies were detected in the sera of mice and calves with in a titres that ranged between 1/16 to 1/32. Our communication may indicate that the number of *Toxoplasma* tachyzoites excreted in the milk of she-camels was low.

Key words: *T. gondii*, infected lasting, she- camels

INTRODUCTION

Milk was implicated as a source of infection of *Toxoplasma gondii* in several reports. Jackson and Hutchison[1] found *T. gondii* tachyzoites in the milk of ewes and cows. Sacks *et al.*[2] detected tachyzoites of *T. gondii* in milk of goats, while Bonametti *et al.*[3] described toxoplasmosis in breast fed child whose mother had recently acquired toxoplasmosis. infection by ingestion of raw goat milk has been documented in humans[4]. Experimentally there are reports of chronically infected lactating mice excreting *T.* tachyzoites in theire milk[5]. One of the most recent studies showed that tachyzoites of *Toxoplasma* may occasionally survive for two hours in acid Pepsin solutions[6]. This may be of public health significance for nomads who consume raw milk. The purpose of this study is to determine the presence of *Toxoplasma gondii* tachyzoites in the milk of camels experimentally infected with *Toxoplasma gondii.*

MATERIALS AND METHODS

Toxoplasma gondii oocysts were isolated from naïve kittens that had been fed raw cameline meat as described by Manal[7].

Four lactating she-camels with their calves were obtained from a camel's market. All camels were specific pathogen free as determined by latex agglutination test. The camels were kept in animal pens at the CVR Laboratories, they were fed alfa hay and concentrates during the experimental period (nine weeks).

Three lactating she-camels (1, 2 and 3) were inoculated orally with $5X10^5$ sporulated *Toxoplasma gondii* oocysts. One she-camel was left as a non infected contact control. Serum samples were collected from each camel at weekly intervals throughout the experimental period for detection of *Toxoplasma* antibodies by latex agglutination test using Toxo-latex kit (Spinreact Comp., Spain). Each serum sample was screened at dilutions ranging from 1/8 to 1/2048. Two weeks after infection, one ml of milk obtained from each she-camels and inoculated IP into each of three albino mice as described by Dubey[8]. Latex agglutination test was used to detect *Toxoplasma* antibodies in mice. Inoculated mice were killed 30 DAI and their brains were fixed in formalin 10% for histopathological examination. Suckling calf-camels were killed 50 days after infection of their mothers,tissue samples (lung, heart, diaphragm, tongue, oesophagus, kidney, liver, small and large intestine, mesentric lymph nodes, brain, eyes and skeletal muscles) were fixed in formalin 10%. Paraffin embedded tissues were cut at 5 μm, stained with haematoxylin and eosin (H&E) and examined by light microscope for detection of histopathological changes[9].

RESULTS

Toxoplasma tachyzoites and cysts were found in six mice that were inoculated with milk obtained from two

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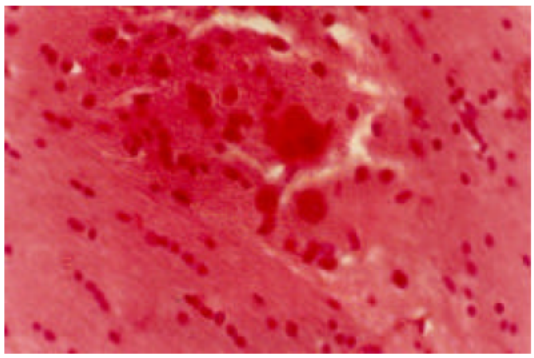

Fig. 1: A photomicrograph of *Toxoplasma* cysts in a brain of suckling calf-camel x 400 (H and E). Notice the lymphoid cells infiltration

infected she-camels (1 and 3), *Toxoplasma* antibodies were detected in the mice sera. On the other hand, three mice that were inoculated with milk obtained from she-camel (2) and the contact control mice did not show *Toxoplasma* infection; no tachyzoites were detected microscopically nor antibodies were detected serologically.

Suckling calf-camels remained clinically normal during the experimental period although latex agglutination tests revealed the presence of *Toxoplasma gondii* antibodies in their sera 7-9 days after infection of the mothers. The antibody titres were low (1/8-1/16). Postmortem examination for all suckling calf-camels showed enlargement of mesentric, inguinal and subscapular lymph nodes. Peticheal hemorrhages were found in brain and lungs. Microscopic examination revealed the presence of *Toxoplasma gondii* cysts in brains (Fig. 1) and tachyzoites in lungs, livers, kidneys and hearts. No gross nor histopathological changes were detected in tissues of the contact contol.

DISCUSSION

This study confirmed the excretion of *Toxoplasma gondii* tachyzoites in the milk of she-camels. Suckling calf-camels acquired toxoplasmosis from milk of their experimentally infected mothers. Histopathological examinations revealed the presence of *Toxoplasma* tachyzoites and cysts in the brains of suckling calf-camels and the mice that were inoculated with milk of infected she-camels.

The number of *Toxoplasma gondii* tachyzoites excreted in milk of infected she-camels was low, although the camels received a high dose of infective *Toxoplasma gondii* oocysts ($5X10^5$). This was shown by the low titres of *Toxoplasma* antibodies detected in the suckling calf-camels sera and by the degree of mice infection ; mice inoculated with milk from she-camel (1) and (3) became infected while those inoculated with milk from she-camel (2) remained non infected.These results are in agreement with those of Dubey[8] who found that the chance of *Toxoplasma gondii* being in the milk of naturally infected goats is very small *Toxoplasma gondii* tachyzoites were detected in the heart, lungs, liver and kidneys of infected calf-camels, but no clinical signs were observed in those animals.

Although tachyzoites are sensitive to proteolytic enzymes and are destroyed by gastric digestion, a recent study showed that tachyzoites survived for up to two hours in acid pepsin solutions; and that oral application of tachyzoites might have resulted in an infection[6,4,2] suggested that tachyzoites may enter the host by penetration of mucosal tissue and thereby gain access to the host's circulation or lymphatic system before reaching the stomach.

There are many factors that have an impact on the epidemiology of *Toxoplasma gondii* infection of camels, such as the type of camel management, the density of cats or wild felines in the enviroment, the environmental conditions that have an infllluence on the sporulation of oocysts, the hygienic standards of abattoirs as well as the different habits of human consumers.

The high seroprevalence of *Toxoplasma gondii* reported in pastoral camels in the Sudan (61.7%); 68% in El Gedarif and 60% in North Kordofan[10] and the high sero-reactivity of *Toxoplasma* in camels' herders, (100%) in Butana area and (30%) in North Kordofan state[11], may warants a closer look into its economic impact as well as its public health significance especially among nomads who consume raw cameline milk.

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