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Short Communication

An Insight into the Behavior, Course and Kinetics of Acute Infection of *Toxoplasma gondii* Human RH Strain in Experimentally Infected Murine Model

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Abstract

Background: *Toxoplasma gondii*, an apicomplexan parasite, is capable of infecting a broad range of intermediate warm-blooded hosts including humans. The parasite seems to be capable of altering the natural behavior of the host to favor its transmission in the environment. The aim of this study was to evaluate the course, alterations in behavior along with normal kinetics of the abnormally induced experimental acute toxoplasmosis in murine models.

Methods: Ten Swiss albino mice were intraperitoneally inoculated with 100 virulent RH strain tachyzoites and finally, the alterations in behavior were described and compared with other known alterations in humans and animals.

Results: The behavior and the other symptoms of the acute toxoplasmosis were recorded. Such mice showed typical symptoms like normal coat, severe ascites with pendulous abdomen and tachypnoea exhibited by resting fore legs either on walls of the cage, or nozzle of water bottle or other resting mice and yielded a creamy colored cloudy natured peritoneal fluid on aspiration.

Conclusions: Finally the alterations in behavior were described and compared with other known alterations in humans and animals. The study has generated some important data related to possible causes of behavioral alterations and generation of suitable strategies for control of these alterations in behavior vis-à-vis better understanding of the effect of acute infection of parasite on normal behavior of infected intermediate host.

Introduction

The unique ability of *Toxoplasma gondii* to multiply in virtually all the nucleated cells of the host body and its subsequent encystment in the various tissues, coupled with its wide host range consisting of all the warm blooded animals including humans, makes the parasite a matter of serious concern for both animal and human health in the modern times. In humans, the parasite is known to cause births with congenital anomalies (1, 2) coupled with ocular involvement (3, 4), acute encephalitis (5) particularly in immunocompromised individuals suffering from AIDS, transplant recipients and those under cancer therapy (6) while in animals particularly small ruminants, the parasite accounts encephalitis (7), abortions (8), neonatal infections (1, 2) coupled with weight loss, hepatomegaly and enlarged kidneys (9).

The human RH strain, originally isolated from a boy with his name initials RH (10), is considered as the referral virulent strain of the parasite. The inability of this strain to form either oocysts in definitive host or bradyzoites in intermediate hosts (11) due to its prolonged passage in the mice (12) further compels the researchers to opt for this as the referral strain for various immunological and molecular biological studies. Rats and mice are routinely used as biological models for isolation, passage and diagnosis of *T. gondii* across the globe (12).

Therefore, an attempt was made to study the behavior, course of toxoplasmosis and its actual kinetics in mice using the standard RH strain.

Materials and Methods

Selection of the parent stock and experimental model

Ten Inbred Swiss albino mice of either sex, 6-12 weeks of age and weighing about 25-30

grams were obtained from the Laboratory of Animal Resource Section, Indian Veterinary Research Institute (IVRI), India. Animals were kept in polypropylene cages and acclimatized for a period of 15 days prior to experimentation under standard temperature, humidity and light cycle conditions. Animals were fed on a balanced diet *ad libitum*, consisting of crushed 62% wheat, 30% maize, 7% wheat bran and 1% common salt. Fresh potable water was made available *ad libitum*.

Preparation of parasitic strain

Tachyzoites of *T. gondii* (RH strain) were obtained from the cryopreserved stock maintained at the divisional laboratory, Indian Veterinary Research Institute. The parasite strain had been maintained in the divisional laboratory in the cryopreserved form for over a decade by continuous serial passage in murine models since time over and over again.

Inoculation of cryopreserved stock strain into mice

The cryopreserved stock of the RH strain was firstly inoculated into the mice through intraperitoneal route. The infected mice were later euthanized by chloroform anaesthesia upon the development of symptoms of toxoplasmosis. After cutting the abdominal skin aseptically, 7-10 ml of Phosphate buffered saline (pH 7.2) was inoculated slowly into the peritoneal cavity and the peritoneal lavage was aspirated without causing any damage to the other organs. The process was repeated till the lavage became clear. Enumeration was carried out as per the standard protocol (13).

Experimental set up of infection in mice

After adjusting the live cell count, ten healthy mice of were injected intraperitoneally with 100 virulent tachyzoites. Behavior of mice along with their feeding pattern and general body condition coupled with symptoms

of acute toxoplasmosis were regularly monitored and recorded. Similarly, ten mice were intraperitoneally injected with sterile PBS and served as uninfected negative control.

The experiments to the laboratory animal were done as per the approval of University Ethical Committee.

Results

The infected mice appeared more or less same as before infection up to 36-48 hours. Thereafter, symptoms like dullness reduced feed and water intake suggestive of anorexia, disinclination to move on their own and moved only when excited through external stimuli and raising of hair coat started to appear by day 3 post infection (PI) (Fig. 1).

They got huddled at the centre of the cage with closed eyes, tucked-up abdomen and arched back. From day 3 PI ascities resulting from pendulous abdomen began to appear and by day 4 PI all the mice developed signs of ascities and were seen having large pendulous abdomen resulting into respiratory distress as suggested by tachypnoea and gasping movement was seen (Fig. 1, 2). Some of the mice with pendulous abdomen were found resting or hanging by keeping one or both the forelegs on the walls of animal cage or on the nozzle of the water bottle or on other lying mouse. By the end of day 4 PI few of the mice begin to die and by day 6 PI all the mice were dead because of acute toxoplasmosis. The peritoneal lavage collected from mice revealed creamy, cloudy, watery liquid which upon Giemsa staining revealed a large number of crescent shaped tachyzoites (Fig. 3). Few of the macrophages were also seen which were filled by tachyzoites from inside, in some of the peritoneal lavages.

While the uninfected mice remained healthy and did not show any of these abnormal signs and remained fully alert and active throughout the experiment. None of the uninfected mice died during the study period.



Fig. 1: Experimentally infected mice showing dullness, closed eyes, timid behavior, raised hair coat and pendulous abdomen (Original)



Fig. 2: Experimentally infected mice clubbed together at the periphery of cage showing altered behavior (Original)

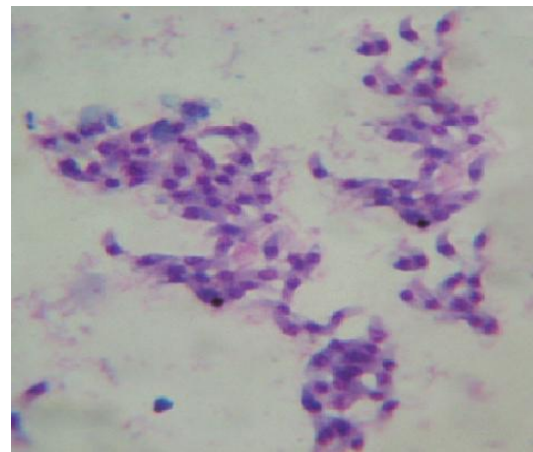


Fig. 3: Giemsa stained peritoneal lavage showing numerous tachyzoites (Original)

Discussion

Toxoplasmosis is one of the most common zoonotic parasitic invasions worldwide, caused by the intracellular protozoan parasite *T. gondii*. The acute invasion of the parasite is a transient stage which is usually followed by chronic invasion when the parasites resides within tissue cysts localized mainly in the central nervous system, muscle, and eye tissues (14). It has proven that *T. gondii* invasion may alter the indefinite host natural defensive behavior in order to increase the risk of rodent predation by cats, the definite parasite hosts (15, 16). This could be attributed to the presence of the parasite in the specified regions of the brain in rodents affects its physiological functions often results in such behavioral changes which are supposed to favor the spread of the parasite to the definite host (17). These behavioral changes are highly specific as parasite invasion blocks rodent aversion toward cat predator odor (18, 19) but do not affect recognition of the non-feline predator scent (20). RH strain of was isolated in USA (21) and has since been passaged continuously in laboratories either in vitro or in vivo throughout the world. Researchers prefer to use it as a standard referral stain mainly due to its inability to form bradyzoite stage (11), lethal effects on mice and good yield of tachyzoites in short time intervals.

In the present study as all the mice experimentally infected with acute toxoplasmosis showed increased body size and pendulous abdomen. This could be explained because of significant increase in brain, spleen and body weight gain during acute phase of toxoplasmosis (22). The increase in brain mass can further be explained on the grounds of induction of inflammatory processes, which is further driven by many pro-inflammatory cytokines like IL-1 β and IL-6 produced in brain during acute infection (23) coupled with accumulation of inflammatory cells into the brain tissue (24). Spleen is involved with the development of specific immunological response against the parasite antigens owing to its role as the pre-

dominant secondary lymphoid organ. This leads to intense accumulation of inflammatory cells, which is further reflected by pronounced increase in spleen weight (22).

Some of the mice, particularly those with huge pendulous abdomen, were found resting or hanging by keeping one or both the fore-legs on the walls of animal cage or on the nozzle of the water bottle or on other lying mouse. Initially all the infected mice showed exploratory behavior but as the symptoms of acute toxoplasmosis developed infected animals exhibited decreased climbing and rearing especially in the central part of the arena and usually remained confined to the periphery of cage in the later stages. It is a matter of common observation that in unfamiliar surroundings mice first explore the peripheral parts and then they move to the central, open part of the arena (25) as this is the most basic means of gathering information about the environment used by nocturnal species with poor vision (26). The change in behavior might be attributed to *T. gondii* infection that inhibited the natural course of exploration (22, 27). This further strengthens the manipulation theory stating that the parasite alters the behavior of the intermediate host to increase a chance of predation by the definitive host. Noteworthy, most of these behavioral changes were mostly pronounced during acute invasion (22, 27). Nevertheless the exact mechanism involved in behavior changes is yet to be fully understand; nevertheless factors like disturbance in the level of neurotransmitters in the brain (28, 29) can prove to be vital plot in this regard. Likewise behavioral changes are also observed in humans like differences personality features between *Toxoplasma* infected (30), associations with schizophrenia (31), Parkinson disease (32), epilepsy (33), or psychosis (34).

Conclusions

The study has generated some important data related to behavioral alterations because of the effect of acute infection of parasite on

normal behavior of infected intermediate host. A lot of research is still warranted to be done regarding behavioral alterations and to investigate through well planned studies the extent and true nature of behavioral alterations in toxoplasmosis and hence fortifying the research to adopt suitable strategies for coping up these alterations in infected individual at the behavioral level for animals in general and humans in particular.

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