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Full Article

Evaluation of Humoral Immune Response of Cats to the Experimental Infection with the different Clonal Types of *Toxoplasma gondii* by Measurement of IgG Antibodies

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ABSTRACT

Toxoplasma gondii is one of the most prevalent parasitic infections in world. Rh, NED and Me49 are of the most prevalent clonal types of the parasite isolated till now. Differences in pathogenicity and virulence of different types have been investigated in different studies. No controlled study was performed to compare the ability of different types to initiate humoral immune response. We investigated IgG antibody responses of kittens infected with each of these three clonal types. For this, experimental infection was performed using ME49 clonal type of *T. gondii* and humoral immune response (by measurement of IgG) was detected and compared with the other two clonal types of the parasite. No antibodies were detectable at least until 7 days post infection for types Rh and NED while this period of no response was 19 days for ME49. Serum ELISA indices were significantly higher in kittens infected with Rh and NED tpes in comparison with ME49. The results of this study showed that humoral immune response of cats to ME49 starts with delay and are weaker than two other clonal types.

Keywords: Cats, Toxoplasma gondii, NED, ME49, Rh

INTRODUCTION

Infection with intracellular apicomplexan parasite; *Toxoplasma gondii* is one of the most common parasitic infections in human and all warm blooded animals including birds, livestock and marine mammals. Worldwide distribution of the parasite and wide range of hosts necessitate adaptation capacity of the parasite to various ecological systems and having a high genetic diversity (Sibley *et al* 2009).

Types I, II and III are the most isolated clonal types of *T. gondii* isolated from North America and Europe. Differences in the virulence of these clonal types have been established in mice. While clonal Type I always caused a lethal infection in outbred mice, clonal Types II and III were shown to be significantly less virulent. Atypical genotypes of *T. gondii* were found in association with generalalised severe diseases in some patients (Carme *et al* 2009). *Toxoplasma gondii* infection is very common in cats throughout the world. Most cats are subclinically infected and potentially fatal clinical disease occurs in some of them. Cats are both

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the intermediate and definitive hosts for *T. gondii*; they can get the clinical disease due to multiplication of tachyzoites in their different cell types or they can shed oocysts that may be infectious for the other intermediate hosts (Frenkel *et al* 1970, Mosallanejad *et al* 2007). Encountering to the different clonal types of *T. gondii* may surge a different serological response in infected cats. This study was aimed to investigate how do cats responses to the experimental infection with *T. gondii*, Rh, NED or ME49 strains when they infect to them.

MATERIALS AND METHODS

Parasites and Animals. ME49, reference strain of *T. gondii* was isolated from Vero cells monolayers using DMEM with 2% FCS and 1% antibiotic solution (10,000 IU Penicillin and 10,000 µg Streptomycin/ml solution) at 37 °C and 5% CO2.Tachyzoites were isolated and purified as described previously (Hosseininejad *et al* 2009). Six kittens of about two months old and their relevant queens were examined to be seronegative for *T. gondii* antibodies by indirect fluorescent antibody test (IFAT) as described previously (Hosseininejad 2013b). Clinical examinations were also performed and survey radiographs were taken from thoracic cavity of cats to ensure their clinical health. Kittens were de-wormed using an advised dose of Mebendazole for five days. Kittens received a diet of cooked food while caged in separate cages.

Experimantal Infection. 10^4 harvested tachyzoites were suspended in sterile normal saline and inoculated intra-peritoneally to four kittens. Two other kittens received an equal volume of sterile Vero cell lysates suspended in normal saline.

Sampling and Investigations. Serum samples were collected every other day after inoculation and frozen until used. Serum samples were also collected previously from sero-negative kittens infected in the same way by Rh and NED reference strains of *T. gondii* isolates derived from bioassays and maintained in Vero cell monolayers (Hosseininejad 2013a, b).

Serological investigation of collected serum samples was performed using both IFAT and an indirect ELISA test that was developed previously based on an affinity purified surface antigen; SAG1 (Hosseininejad 2013b). In both of the test IgG was measured. Sample index values were calculated by the formula SIn= (Sn-N)/(P-N) where SIn is the individual ELISA index value, Sn is the OD value obtained for a single sample, N is the OD value obtained for the negative serum, and P represents the OD values obtained for the positive serum. Data expressing IFAT titers and serum ELISA indices (SIn) were collected in different days post infection. Humoral Immune responses were compared between kittens received each of three T. gondii clonal types. Mean serum ELISA indices were compared between samples with positive IFAT titers between three clonal types infected kittens. Kruskal-Wallis and Bonferroni tests were used to assess the differences between ELISA SIn of these groups (SPSS16 software, SPSS Inc. Headquarters USA). The experiment was performed based on the ethic codes prepared by the Iranian animal welfare committee for studies on laboratory animals (Aledavoud *et al* 2006).

RESULTS

Clinical examination and survey radiographs revealed no clinical signs in examined cats. IFAT examination of sera showed no detectable anti-*T. gondii* antibodies in kittens and relevant queens at the first sampling time in titer of at least 1:16. In kittens that Rh strain of *T. gondii* was inoculated, antibodies were detectable from day 7 afterwards. Serum dilutions, in which IFAT titers were positive, were started from 1:32 to more than 1:512 during sampling days (table 1). As the final IFAT titer was 512 all of four kittens in this group experienced the final titer; expressing a relatively high antibody surge. Humoral immune responses were also evaluated in kittens received NED strain; antibodies were detectable from 7 days PI. Humoral immune responses were started from titer of 1:32 and reached to more than 1:512 during the

Table 1. Evaluation of IFAT titers and Serum ELISA Indices in Kittens Infected with Rh Clonal Type of *T. gondii.*

Days Post Infection	Cat 1		Cat 2		Cat 3		Cat 4	
	IFAT	SIn	IFAT	SIn	IFAT	SIn	IFAT	SIn
1	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0
7	0	0.1	0	0	32	0.11	0	0
9	32	0.2	0	0	32	0.11	0	0
11	64	0.28	32	0.3	32	0.11	32	0.15
13	256	0.39	64	0.35	32	0.18	128	0.3
15	≥512	0.43	256	0.4	128	0.32	256	0.33
17	≥512	0.50	≥512	0.45	256	0.4	≥512	0.4
19	≥512	0.55	≥512	0.55	256	0.4	≥512	0.4
21	≥512	0.64	≥512	0.6	256	0.4	≥512	0.4
23	≥512	0.75	≥512	0.71	256	0.45	≥512	0.44
25	≥512	0.78	≥512	0.81	≥512	0.54	≥512	0.44

Table 2. Evaluation of IFAT titers and Serum ELISA Indices in Kittens Infected with NED Clonal Type of *T. gondii.*

Days Post Infection	Cat 1		Cat 2		Cat 3		Cat 4	
	IFAT	ELISA	IFAT	ELISA	IFAT	ELISA	IFAT	ELISA
1	0	0.05	0	0.06	0	0.06	0	0.06
3	0	0.07	0	0.06	0	0.06	0	0.07
5	0	0.07	0	0.07	0	0.07	0	0.07
7	32	0.12	0	0.09	0	0.09	0	0.08
9	128	0.28	0	0.09	0	0.09	32	0.11
11	128	0.57	128	0.23	32	0.12	32	0.12
13	128	0.63	128	0.31	64	0.24	64	0.24
15	128	0.51	128	0.51	128	0.51	64	0.30
17	≥512	0.91	≥512	0.98	256	0.60	128	0.40
19	≥512	0.72	≥512	0.77	≥512	0.79	256	0.60
21	≥512	0.63	≥512	0.91	≥512	0.85	≥512	0.85
23	≥512	0.97	≥512	1.02	≥512	0.90	≥512	0.90
25	≥512	0.85	≥512	1.05	≥512	0.90	≥512	0.90

Table 3. Evaluation of IFAT titers and Serum ELISA Indices in Kittens Infected with ME49 Clonal Type of *T. gondii.*

Days Post Infection	Cat 1		Cat 2		Cat 3		Cat 4	
	IFAT	ELISA	IFAT	ELISA	IFAT	ELISA	IFAT	ELISA
1	0	<0.1	0	<0.1	0	<0.1	0	<0.1
3	0	<0.1	0	<0.1	0	<0.1	0	<0.1
5	0	<0.1	0	<0.1	0	<0.1	0	<0.1
7	0	<0.1	0	<0.1	0	<0.1	0	<0.1
9	0	<0.1	0	<0.1	0	<0.1	0	<0.1
11	0	<0.1	0	<0.1	0	<0.1	0	<0.1
13	0	<0.1	0	<0.1	0	<0.1	0	<0.1
15	0	<0.1	0	<0.1	0	<0.1	0	<0.1
17	0	<0.1	0	<0.1	0	<0.1	0	<0.1
19	128	0.13	128	0.14	128	0.13	128	0.13
21	128	0.17	128	0.18	128	0.17	128	0.13
23	128	0.19	128	0.26	128	0.26	128	0.17
25	128	0.21	128	0.26	256	0.27	128	0.26

examination period. The maximum IFAT titers were also experienced by this clonal type infection (Table 2). Serological evaluation of sera collected from kittens infected with ME49 clonal type, were different: no antibodies were detected by using ELISA and IFAT during the first 19 days post-infection. Humoral immune responses were started in these cats from day 19 PI. IFAT results showed positive results in titer of 1:128 from day 19 and maximum titer of 1:256 at the end of the experiment (Table 3). Data expressing serum ELISA indices in which IFAT titers were also positive, compared in cats received each of three clonal types. Sin values (mean±SE) were 0.43±0.03, 0.60±0.05 and 0.19±0.01 for serum samples of cats infected with clonal types Rh, NED and ME49 respectively. Statistical analysis showed significant differences between SIn values of three groups ($P<0.05$).

DISCUSSION

Ante-mortem diagnosis of *T. gondii* infection can clarify a part of host-parasite relationship and can show the way in which the animals response to the exposure to the organism. Parasite isolation, fecal examination for oocysts and serologic tests are the ways to diagnose the infection in cats. Isolation of the parasite in living animals is difficult, costly and time consuming and so it is limited to the researches and is not a reasonable routine diagnostic test. Oocyst shedding period is very short (less than 3 weeks, mostly for 1 week) and after this period, most of the cats remain infected without excreting the oocysts (Dubey 1995). Therefore, the most suitable and practical way of detecting the *T. gondii* infection is serology. Knowing the pattern in which cats may response humorally to the infection with different clonal types of *T. gondii* is important especially when typical infections occur. T. gondii infection causes often a humoral immune response with detectable antibody titres, independent of the clinical manifestations (Dubey 2008, Parmley *et al* 1994). There are few studies regarding serological follow after experimental T. gondii infection. In one study, experimental infection in cats has previously been performed using inoculation of ME49 tissue cyst, isolated from an infected dolphin (Dubey *et al* 2007). In one study, serotyping of seropositive field sera was performed in Germany to investigate whether which of the clonal types of *T. gondii* are more prevalent in Germany. The results of this study revealed patterns resembling those observed after a clonal type II *T. gondii* infection (Maksimov *et al* 2013). Serological responses of cats after oral infection with tissue cysts were studied with MAT, IHA, DT, and ELISA. The cats were bled sequentially starting 7 days p.i. and up to 6 yr p.i. Cats seroconverted 10 days p.i. and high titers persisted even after 6 years (Dubey 1995).In one study, feline model of toxoplasmosis was performed and produced antibodies were detected in aques humor of the cats. In this study, total immunoglobulins were calculated to verify intraocular antibody production. Toxoplasma gondii-specific IgM was not detected in the aqueous humor of any cat. Data indicate that cats have transient local production of T gondii-specific IgG in the aqueous humor after primary and secondary oral inoculations with T gondii tissue cysts (Chavkin *et al* 1994). In another study, Serum samples were derived from 7 cats 3 to 950 days after cats had been fed tissue cysts of Toxoplasma gondii. In this study *T gondii* antibody titers remained high (greater than 1:1,000) for up to 29 months after infection.(Dubey & Thulliez 1989). Experimental infection was performed by oral inoculation of *T. Gondii* tissue cysts to sixteen pregnant queens and fetal membranes and offspring were examined for *T. gondii* infection. Trans-placental transmission of Immunoglobulines were investigated in the kittens (Dubey *et al* 1995). Clonal types used in this study were the most isolated ones in Europe and North America and other regions of the world (Herrmann *et al* 2009, Sibley *et al* 2009). The results of this experiments show that infection with *T. gondii* (irrespective of types) is not detectable until at least 7 days PI in experimental infection. This period is longer (19 days PI) in cats infected with type ME49. Initiating an immune response to a specific type of parasite depends on the ability of parasite to immune mediators

such as interleukins (Saeij *et al* 2005). In this study we focused mainly on differences between different types to initiate the immune response and the exact molecular process remains to be assessed. Weaker IFAT titers were also seen in sera infected to ME49 clonal type in compare to other two types. So that antibodies could not be detected in dilutions of more than 1:256 in cats infected with this type. The results of this study shows that different clonal types of *T. gondii* initiate different patterns of humoral responses in experimentally infected cats. Infection with ME49 could surge a more delayed and weaker IgG antibody surge in compare to other two types.

Ethics

I hereby declare all ethical standards have been respected in preparation of the submitted article.

Conflict of Interest

Hereby, I declare "no conflict of interest exists" regarding submitted article.

Acknowledgment

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