Serological and Hormonal Assays of Murine Materno-Fetal *Toxoplasma gondii* Infection with Emphasis on Virulent Strains

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Abstract: The present investigation dealt with studying of *T. gondii* materno-fetal skill within rat progenies that were borne from acute and chronic experimentally infected pregnant dams. A total number of 108 pathogen and toxoplasma free Sprague-Dawley female rats were used. Ninety dams were used for experimental induced acute and chronic trials. Both acute and chronic infected rats were exposed to the three *T. gondii* strains; type I, II and III. ELISA IgM / IgG as well as, progesterone / estrogen assays were done parallel to female rats with their survived litters. The results revealed that type I possessed the higher percentage of fetal degeneration, while cystogenic II and III showed lower values. We can conclude that successful recognition of *T. gondii* materno-fetal transmission resulted in abortion and parasite survival.

Key words: *Toxoplasma gondii* · Rats · Materno-fetal transmission · Sex-steroids hormones

INTRODUCTION

Epidemiological studies clarifying the zoonotic bio-hazard of *Toxoplasma gondii* that may be set as a consequence to congenital flow within rat generations costumes permanent source for tissue cyst supplying and stimulating cats for shedding the environmental sporulated oocysts. *T. gondii* is an obligate intracellular opportunistic coccidian protozoan, exists in three infective stages; the acute tachyzoite stage responsible for the materno-fetal diffusion, while the chronic bradyzoite stage (tissue cyst) persists viable for the rest of the host survive and can reactivate to acute tachyzoites producing latent infection and the third infective stage is the environmental resistant sporulated oocysts [1, 2].

The severity of human congenital toxoplasmosis and toxoplasmosis in immune-compromised patients is of public health impact and the latent infection is potentially serious during pregnancy as it carries the risk of fetal transmission in about 30% of cases [3].

Most *T. gondii* strains are categorized into three types I, II and III referring to their virulence in mice, but they differentiate dissimilar pathogenicity in different hosts [4, 5]. According to such biological and epidemiological diversity, we could expect diverse materno-fetal course corresponding to the different types through rat progenies that must be compared during and prior to pregnancy.

Toxoplasma isolates from human are mainly from type II, but type I and atypical genotypes are found only in severe cases of human toxoplasmosis [6, 7]. The materno-fetal variations between acute and chronic infected groups that exposed to types I, II &III are related to the difference in infiltration and pathogenesis cycle between the administrated bradyzoites and sporozoites.

The sharp exploits of progesterone and estrogen levels during pregnancy could be altered and the dissimilar effects on the immune system may induce resistance or susceptibility to different parasite attacks. The sharp elevated sex steroids could worsen toxoplasmosis; mainly through suppressing host immune-endocrine network (IEN) and progressing parasite replication [8-10].

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Also, higher incidence of Toxoplasma encephalitis was recorded within AIDS-defining females than in males, this support that female hormones possibly predispose latent toxoplasmosis and was confirmed to stimulate higher parasite load in guinea pigs [11]. The actual dynamics stimulating latency are still unknown; however various stimuli were studied including hormonal factor. The higher ability of latency during pregnancy may be related to sharp progesterone and estradiol levels deviation that suspect to stimulate silent bradyzoites to liberate from tissue cysts and motivate the recurrent acute symptomatic stage through tachyzoites re-conversion [11, 12].

The objective of the present study was to evaluate the possibilities of congenital toxoplasmosis within rat progenies that has been born from acute and chronic experimentally infected dams. The results may impersonate possible parallel women materno-fetal skill at similar steroids hormonal path.

MATERIALS AND METHODS

***Toxoplasma gondii* Strains:** Three strains were secured in the Zoonotic Diseases Department, National Research Center, Egypt. Tachyzoites of highly virulent RH strain (type I), mice brain cysts of the mildly virulent ME-49 strain (type II) and Cs Cl purified oocysts of the slightly virulent Prugniaud (PRU) strain (type III).

Experimental Animals (Rats): A total number of 108 pathogen and toxoplasma free Sprague-Dawley female rats was obtained from the Animal House, National Research Center, Egypt and has been used in acute (trial-1) and chronic (trial-2) infections. The experimental dams were housed in individual cages and grouped according to the time, strain and route of inoculation and fed with standard diet and labium.

Experimental Infection:
Trial-1 (Acute Infection): Forty five confirmed pregnant dams were classified into three groups; 15 each, experimentally inoculated with *T. gondii* in mid gestation period (10-12 days of pregnancy) as follows; The first one was inoculated intra peritoneally (IP) with 10^3 RH tachyzoites *T. gondii* strain, the second one was orally inoculated with 10^2 mice brain tissue cysts of ME-49 strain and the last one was orally inoculated with 10^2 purified Cs C_1 oocysts of Prugniaud strain, respectively corresponded to the three virulent types I, II and III.

Trial-2 (Chronic Infection): Forty five non pregnant dams were classified into three groups; 15 each, inoculated by the same strains and routes as trial-1; at 45 days before their mating.

Control Animals: Eighteen rats; nine each, non inoculated non pregnant and pregnant served as control groups for trial 1 and 2, respectively.

Blood Samples: Sera were collected from rats at 7DPI (days post infection) and from scarified progenies at 15 DPD (days post its delivery) in trial-1. While in trial-2, the blood samples were taken from dams at 45 DPI and from scarified progenies at 15 DPD.

Serological Assay: All dams and their litters were serologically assayed by ELISA to detect IgG & IgM titer according to procedures described by Lind *et al.* [13].

Hormonal Assay: Estrogen and progesterone hormonal assays in trial-1&2 were done in the central lab, of Egyptian National Research Center by radioimmunoassay according to Cabrera-Muñoz *et al.* [8]. Highly specific anti progesterone & anti estrogen were used, the minimum sensitivity of the assay was 0.1pg/ml for estrogen and 0.2ng/ml for progesterone.

Detection of Viable Parasite: Along the course of experiment; ninety acute and chronic infected dams and their newborns corresponding to the three virulent types were sacrificed for tissue cysts detection in their organs by pepsin digestion [14] followed by microscopic examination and inoculation for viability bio-assay in susceptible mice[15].

Statistical Analysis: Data were statistically analyzed using the MSTAT and STATISTICA (6.0) computer programs. The average and standard deviation among the different parameters were determined as well according to Freed *et al.* [16]. Only differences with a probability of less than 0.05 were considered significant.

RESULTS

Trial-1 (Acute Infection): The experimental rats during the acute course of toxoplasmosis showed no deaths in the three pregnant rat groups and completed the trial. The obtained data were represented in Table 1 and Fig 1, 2, 3, 4 & 5.

Table 1: Serological, tissue cyst and reproductive assays of acute *T. gondii* infected pregnant murine dams and their off springs

Trial-1		Acute infected pregnant dams/ inoculated during mid gestation phase			Off springs from acute inoculated dams			
Strain type & Route	NO	IgM/ IgG 7DPI	failed to labor	Succeeded to labor NO(%)	Total NO & Average litters/dam	Succeeded to grow (%)	Scarified NO at 15DPD(IgM/ IgG)	NO of +ve T. cyst(%)
type I RH tachzoites (IP)	15	(0.360±0.22 /0.290±0.20)	11(73.3%)	4(26.7 %)	13(3.3)	7(54%)	7(0.404±0.23 /0.341±0.22)	2(28.6%)
		*14.7 ±0.29	*23.2 ±0.33	*11.3 ±0.26				
		**45.2 ±0.41	**21.7 ±0.31	**58.5 ±0.43				
type II ME-49 (Oral mice brain cyst)	15	(0.280± /0.132±)	5(33.3%)	10(66.7%)	42(4.2)	29(69%)	13(0.298±0.20/0 .243±0.19)	6(46.2%)
		*10.6 ±0.23	*18.2 ±0.22	*8.9 ±0.21				
		**39.8 ±0.34	**17.4 ±0.22	**60.1 ±0.45				
Type III Prugniaud (Oral purified oocysts)	15	(0.210±0.21/0 .108±020)	7(46.7%)	8(53.3%)	29(3.7)	18(62%)	7(0.307±0.23 /0.219±0.20)	4(57.1%)
		*13.2 ±0.22	*16.4 ±0.23	*10.5 ±0.22				
		**36.3 ±0.33	**23.6 ±0.31	**48.8 ±0.40				

DPI (Days Post infection); DPD (Days Post Delivery); (E/Pg) Estrogen-progesterone; *Average [E](pg/ml); **Average [Pg](ng/ml)
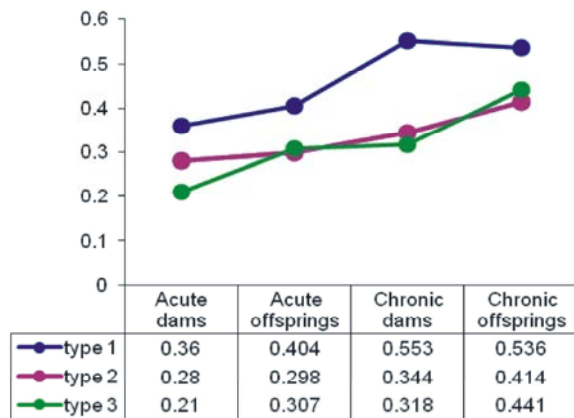

Fig. 1: IgM variation in acute and chronic inoculated murine dams compared with the three *T. gondii* virulent types
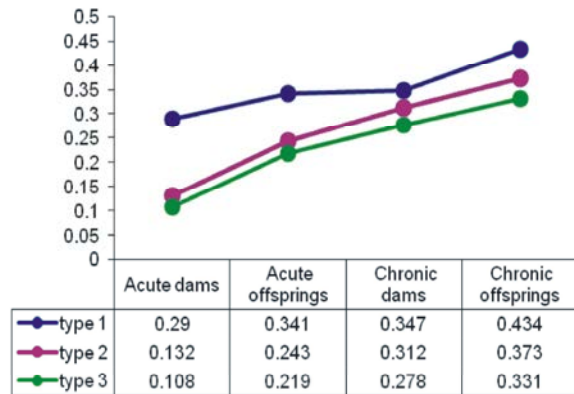

Fig. 2: IgG variation in acute and chronic inoculated murine dams compared with the three *T. gondii* virulent types
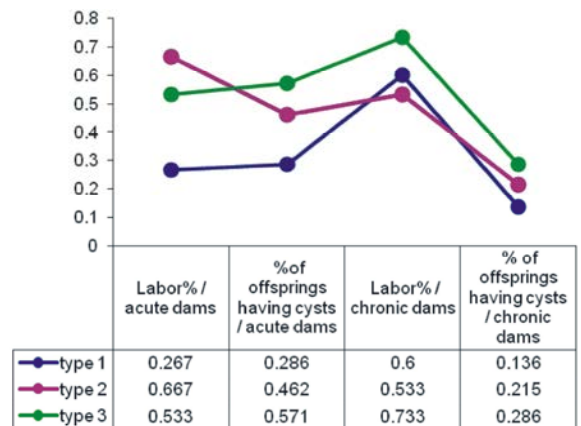

Fig. 3: Labor rate of acute and chronic *T. gondii* inoculated murine dams and the % of their offspring's harboring tissue cysts

The 1st rats group showed (0.360/0.290) IgM/ IgG ELISA titer and 26.7% of the pregnant dams succeeded to deliver with a total progenies of 13 off springs and 7/13(54%) of litters succeeded to grow and completed their life. At 15 DPD the off springs sera recorded 7/7(100%) sero-positive and (0.404/0.341,) IgM/ IgG ELISA titer. Bioassay using pepsin digestion followed by microscopic examination revealed no visible cysts (0%), but viability bio-assay confirmation in mice recorded 2/7(28.6%) off springs were harboring bradyzoites. Average estrogen-progesterone levels showed dissimilar percentages (14.7 pg/ml -45.2 ng/ml) at 7 DPI, (23.2 pg/ml -21.7ng/ml) for pregnant dams failed to labor and (11.3 pg/ml -58.5ng/ml) for pregnant dams that succeeded to deliver respectively.
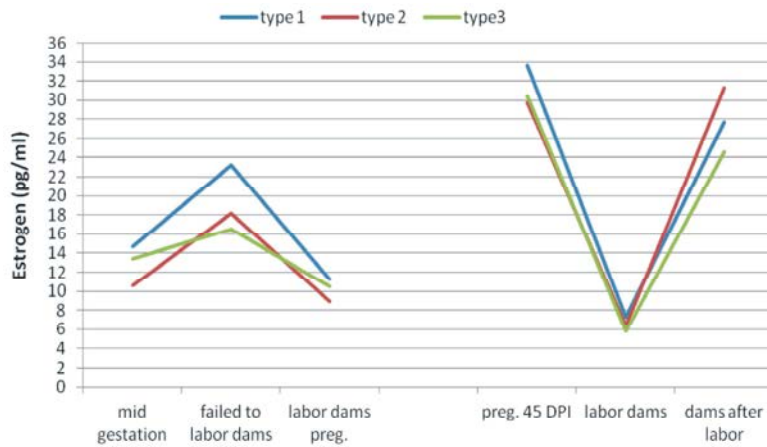
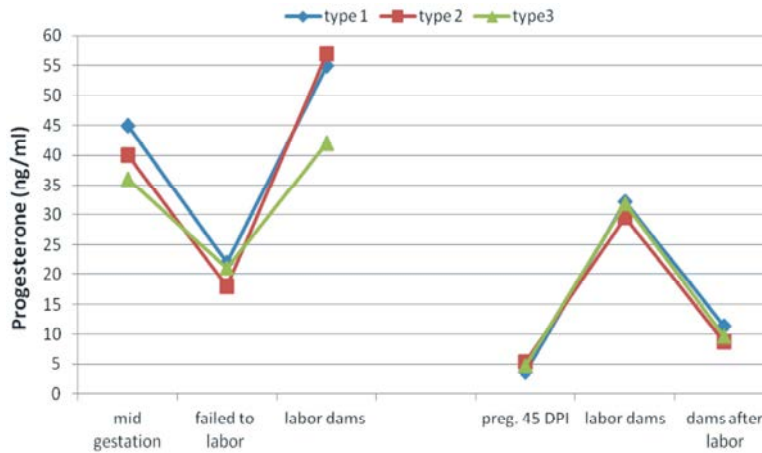Fig. 4: Estrogen (pg/ml) variation in acute (left) and chronic (right) *T. gondii* inoculated murine dams

Fig. 5: Progesterone (ng/ml) variation in acute (left) and chronic (right) *T. gondii* inoculated murine dams

The 2nd rats group showed (0.280/0.132,) IgM/ IgG ELISA titer at 7 DPI and 5 (33.3%) of the pregnant dams failed to labor. While, the remaining 10 (66.7%) were succeeded to deliver with a total progenies of 42 offspring's. Only 29/42 (69%) of litters were succeeded to grow and complete their life. At 7 DPD, the off springs sera recorded 13/29(44.8%) sero-positive and (0.298/0.243) IgM/ IgG ELISA titer. Bio-assay in mice showed 6/13(46.2%) off springs harboring bradyzoites. Average estrogen -progesterone were (10.6 pg/ml - 39.8 ng/ml) at 7 DPI, (18.2 pg/ml -17.4ng/ml) for pregnant dams failed to labor and (8.9 pg/ml -60.1ng/ml) for pregnant dams that succeeded to deliver respectively.

The 3rd rats group gave (0.210/0.108) IgM/ IgG ELISA titer at 7 DPI. 7(46.7%) pregnant dams failed to labor while, the remaining 8(53.3%) pregnant dams succeeded to deliver with a total progeny of 29 offspring. Only 18/29(62%) of litters succeeded to grow and completed

their life. During 15 DPD the off springs sera recorded 7/18(38.8%) sero-positive with average (0.307/0.219,) IgM/ IgG ELISA titer. Pepsin digestion followed by microscopic examination showed 3/7(42.9%) of dams were harboring visible cysts and confirmed by viability bio-assay confirmation in mice which recovered 4/7(57.1%) off springs harboring bradyzoites. Average percentages of Estrogen -Progesterone levels were (13.2 pg/ml - 36.3 ng/ml) at 7DPI, (16.4 pg/ml - 23.6ng/ml) for pregnant dams failed to labor and (10.5 pg/ml - 48.8ng/ml) for pregnant dams that succeeded to deliver respectively.

Trial-2 (Chronic Infection): The results of the rat groups concerned with chronic course of toxoplasmosis in trial -2, were shown in Table 2 and Fig 1,2,3,4&5. The three groups, showed no deaths and completed the experimental trial.

Table 2: Serological, tissue cyst and reproductive assays of chronic *T. gondii* infected pregnant murine dams and their off springs

Trial-2	Chronic infected pregnant dams/ inoculated 45days before pregnancy				Off springs from chronic inoculated dams			
Strain types & Routs	NO	IgM/ IgG 45DPI	Labor No /%	+ve Tissue cyst 15D after Labor	Total NO & Average litters/dam	Success of growth /%	Scarified No at 15DPD/ IgM/ IgG	NO of +ve T. cyst/%
type I RH IP peritoneal tachyzoites	15	(0.553±0.36 / 0.347±0.28)	9(60%)	7(46.7 %)	35(3.9)	22(63%)	22(0.536±0.33 /0.434±0.31)	3(13.6%)
Average E(pg/ml)		33.6 ±0.41	7.3 ±0.22	27.7 ±0.33				
P4(ng/ml)		3.6 ±0.25	32.2 ±0.38	11.3 ±0.27				
type II ME-49 oral Mice brain cyst	15	(0.344±0.26 / 0. 312±0.24)	7(46.7%)	5(71.4%)	31(4.42)	20(64.5%)	14(0.414±0.33 /0.373±0.27)	3(21.5%)
Average E(pg/ml)		29.7 ±0.36	6.4 ±0.24	31.3 ±0.36				
P4ng/ml)		5.4 ±0.24	29.6 ±0.34	8.8 ±0.33				
Type III Prugniaud oral purified Oocysts	15	(0.318±0.21 / 0.278±0.20)	11(73.3%)	10(83.4%)	47(4.7)	32(68%)	21(0.441±0.32 /0.331±0.29)	6(28.6%)
Average E(pg/ml)		30.4 ±0.42	5.8 ±0.51	24.6 ±0.40				
Pg(ng/ml)		4.8 ±0.13	31.8 ±0.41	9.6 ±0.34				

DPI (Days Post infection); DPD (Days Post Delivery); (E)= Estrogen- (/P4)=Proogesterone.

The 1st Rats Group: The collected sera recorded 100% sero-positive and (0.553/0.347, mention cut off value??) IgM/ IgG ELISA titer after 45 days post infection (DPI). 6/15 (40%) of females which were exposed to males failed to concept, while the remaining 9(60%) were pregnant and succeeded to deliver after average 21 days with a total progenies of 35 off springs. Only 22/35(63%) of litters succeeded to grow and completed their life. At 15 DPD, the off springs sera recorded 22/22 (100%) sero-positive with (0.536/0.434,) IgM/ IgG ELISA titer. Pepsin digestion followed by microscopic examination showed no visible cysts and was confirmed by viability bio-assay in mice that revealed 3/22 (13.6) of progenies were harboring bradyzoites. Average percentages of Estrogen - Progesterone levels were (33.6 pg/ml - 3.6 ng/ml) at 45 DPI, (7.3 pg/ml - 32.2ng/ml) for pregnant dams that succeeded to labor and (27.7 pg/ml - 11.3 ng/ml) for positive tissue cyst dams 15 days after labor respectively.

The 2nd rats group recorded 100% sero-positive and (0.344/0.312, mention cut off value??) IgM/ IgG ELISA titer after 45 DPI and all served females recorded 8/15 (53.3%) failure to concept. While, the remaining 7(46.7%) females were pregnant and succeeded to deliver after average 21 days with a total progeny of 31 off springs. Only 20

(64.5%) of 31 litters were succeeded to grow and complete their life. During 15 DPD the off springs sera showed 14/20 (70 %) sero-positive and (0.414/0.373, mention cut off value) IgM/ IgG ELISA titer. Pepsin digestion followed by microscopic examination revealed that 3/14(21.5%) of dams showed visible cysts and confirmed by viability bio-assay in mice that recorded the same percent. Average percentages of Estrogen -Progesterone levels were (29.7 pg/ml - 5.4 ng/ml) at 30 day post infection (6.4 pg/ml - 29.6ng/ml) for pregnant dams that succeeded to labor and (31.3 pg/ml - 8.8 ng/ml) for positive tissue cyst dams 15 DPD after labor respectively.

The 3rd rats group showed 100% sero-positive and (0.318/0.278,) IgM/ IgG ELISA titer. After 45 DPI all served females recorded 4/15(26.7%) failed to concept while, the remaining 11/15(73.3%) females were pregnant and succeeded to deliver after average 21 days with a total progenies of 47 offspring. Only 32/47(68%) of litters were succeeded to grow and complete their life. During 15 DPD the off springs sera recorded 21/32(65.7%) sero-positive and (0.441/0.331, mention cut off value??) IgM/ IgG ELISA titer. Pepsin digestion followed by microscopic examination showed 6/21(28.6%) dams with visible cysts and was confirmed by viability bio-assay in mice that

recovered the same percent. Average percentages of Estrogen -Progesterone levels were (30.4 pg/ml - 4.8 ng/ml) at 45 DPI, (5.8 pg/ml - 31.8ng/ml) for pregnant dams that succeeded to labor and (24.6 pg/ml - 9.6 ng/ml) for positive tissue cyst dams 15 days after labor respectively.

DISCUSSION

In the present study, no deaths occurred up to 7 DPI in acute inoculated mature pregnant dams with elevated IgM / IgG ELISA values (0.360 & 0.290). However, highly disseminated RH tachyzoites were identified in acute pregnant dams that failed to labor (73.3%) which might explain degeneration and re-sorption of early formed feti. In contrast, 60% of chronic dams inoculated with RH strain 45 days prior to pregnancy were succeeded to concept and labor. This might explain the parasitemia phase of RH strain that has no long persistence with fast clearance time. Although tissue cysts were demonstrated by 46.7% in chronic dams at > 45 DPI or after labor the tachyzoites were vertically transmitted to offspring's and recorded only 13.6% compared with 28.6% in acute groups. The offspring maternal-fetal diffusion is logic during acute infection, but our surprise is due to the demonstration of tissue cysts parallel to 15 DPD in offspring that have been born in dams infected with RH strain 45 days prior to their pregnancy (chronic trial). The result clarified the power of type I to transmit formed tissue cyst followed by the higher ability of latency via bradyzoites-tachyzoites re-conversion later on [5] and chronic toxoplasmosis predispose to host latency and provokes strain dependent congenital toxoplasmosis [17].

The obtained results may explain why toxoplasmosis was more prevalent in pregnant woman which can be through a fact that sharp elevated progesterone and estradiol levels in pregnant females improve susceptibility to *T. gondii*, mainly through suppressing host immune-endocrine network (IEN) and progressing parasite re-conversion and latency [10, 18]. Also, the higher incidence of Toxoplasma encephalitis was recorded within AIDS-defining females than in males and this support that female hormones possibly predispose to latent toxoplasmosis [19].

The present investigation revealed the higher possibility of abortion during early pregnancy although progesterone and estrogen levels are low and the chance of transmission to the fetus is related to strain virulence. In contrast, during the late stages of pregnancy with high levels of progesterone and estrogen and the diminished NK cell, macrophage and $CD8^+$ may facilitate parasite

survival and increase the higher possibility of latency with subsequent congenital diffusion [20-22].

The study described the disseminated RH tachyzoites were of more significance in acute pregnant dams than that of chronic ones, modifying the ability of their progenies to survive from 54to 63% respectively. These results ensured that strain type is validated as the major latent stimulus during pregnancy. Although, strain types I, II and III were succeeded in placental flow by dissimilar velocity through offspring's at 15 DPD. But the significant higher titer of IgM & IgG in acute and chronic offspring's corresponding to their mothers may reflect the mothers' immunoglobulin stimulation due to parasite immune reaction in further generations [5, 7].

After labor, RH tachyzoites were expressed to have the higher draw back on delivered litters than that of the type II & III during acute infection, represented in the percent of the success growth of litters [9, 18]. Our results agree with the higher persistence in tissue cysts in rat progeny [19] and also revealed that congenital toxoplasmosis occurred in rats irrespective to the stage or the route of inoculated dams [20].

From the obtained results we can concluded that, the materno-fetal variations in acute and chronic infected groups produced by cystogenic types, I & II might be due to the differences in infiltration and pathogenicity of *T. gondii* strains. Congenital and chronic toxoplasmosis depends mainly on stage of pregnancy besides, estrogen /progesterone deviation in pregnant rat reflects on the possible occurrence in women that may be matched with parallel hormonal alter. Rat litters were verified to harboring tissue cysts corresponding to the three virulent inoculated types I, II and III; reflecting the zoonotic impact of controlling rat colonies in toxoplasmosis ecology through serving significant minimizing animals and human exposure.

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