

Ecological and Pathological Study of *T. gondii* Egyptian Rat Isolates Reference to Biological & Genetic Typescripts

El Fadaly H. A¹; Sylvia O. Ahmad²; Barakat A. M. A¹;
Soror A. H³; Mona S. Zaki⁴

¹Zoonotic Diseases Department, National Research Centre, 33 Bohouthst. Dokki, Affiliation I.D. 60014618, Postal Code 12311, Giza, Egypt.

²Department of animal Hygiene and Zoonosis, Faculty of Veterinary Medicine, Assiut University Egypt.

³Animal Reproduction Department, National Research Centre, 33 Bohouthst. Dokki, Affiliation I.D. 60014618, Postal Code 12311, Giza, Egypt.

⁴Hydrobiology Department, National Research Centre, 33 Bohouthst. Dokki, Affiliation I.D. 60014618, Postal Code 12311, Giza, Egypt.

Abstract: *Toxoplasma gondii* is zoonotic opportunistic protozoan of public health impact. It has usual clonal population of three virulent human and animal types: I, II, and III. The commercial rats are frequently harboring the dormant *T. gondii* tissue cysts in their tissues. So, rats consider the major prey for outdoor cats that regularly stimulating it to shed and contaminate the environment with oocysts infective stage which maximizes human and animals' toxoplasmosis. The aim of the present study is to identify the biological and molecular typescripts of naturally infected commercial rats along with serological, mice viability and histo-pathological assays for clarifying to how extent the indirect zoonotic bio-hazards sequence to rats harboring *T. gondii* virulent types. A total number of 278 commercial rats were recognized as *R. norvegicus* (n=74), *Rattus rattus rattus* (n=108) and *Rattus rattus frugivorus* (n=96), were collected from different rural and urban sites from Cairo and Giza governorates. Blood and tissue samples were exposed to serological and mice viability test along with histo-pathological exam. Also, the tissues of inoculated mice were exposed to kitten's viability test for detecting oocyst shedding characters of rat isolates. Parasite load, LD50 and LD100 of the rat isolates were detected for bio-typing. Plus to geno-typing via SAG2 PCR amplification products by electrophoresis analysis using tissue digest from inoculated mice. The results of microscopic and histo-pathological exam of rat tissues and inoculated mice were recorded. The percentages of successes rat isolates through mice inoculation were; (14.9), (3.7), (2.1). while, the overall prevalent of biological and genetic typescripts were; type I (17.6%), type II (52.9%) and type III (29.4%) corresponding to *R. norvegicus*, *Rattus rattus rattus* and *Rattus rattus frugivorus*. We were concluded that the examined commercial rats often were holding *T. gondii* tissue cysts corresponding to the human virulent strains I, II, and III, reflecting their role in toxoplasmosis ecology through indirect human hazard via recurrently exciting oocysts shedder cats.

Keywords: Biological & Genetic typescripts- *T. gondii* -Egyptian Rat Isolates, PCR detection; SAG2 genotype.

Introduction

Toxoplasmosis is ideal zoonosis, *Toxoplasma gondii* is a latent opportunistic zoonotic protozoan; signify one of the most prevalent abortifacient human pathogen, with varies complicated pregnancy¹. *T. gondii* exists in three infective stages; the acute tachyzoite stage responsible for cell invasion and materno-fetal passes. While chronic bradyzoite (tissue cyst) persists viable as tissue dormant stage for the rest of the host survives and can reactivate to acute tachyzoites in immunosuppressed individuals as latent opportunistic character².

Toxoplasma gondii has exceptional cat - rat link: whererats are the most *T.gondii* tissue cysts reservoir and the major prey which regularly stimulating feline's gut for shedding the fecal oocyst stage. Oocysts shed unsporulated and couldn't induce host infection until it environmentally sporulated³.However, theratioof *T. gondii* infection within rat colonies is seriously diverse according to the common available food sources and dissimilarity in omnivorous and carnivorous feeding habit, plus to the difference in rat species, in addition of varied levels of oocysts environmental contamination.

Dusting of sporulated oocysts directly maximizes humans' exposure via polluted food or water, and indirectly through infected meat producing animals which will harbor tissue cysts and infect consumers later on⁴.In addition, dead rodents harbor tissue cysts may be eaten via wastage nourished poultry or ducks. However, their undercooked or unfrozen meat will harbor viable tissue cysts which infect human consumers. Ever since unusual human feeding behavior was adapted in consuming rodents in some poor communities (India, Fleabanes, Congo & Zaire), potentially maximize human exposure to bradyzoite stage, The former study in the Egyptian circumstances: brains from 4 types of commercial rodents caught in the Cairo area of Egypt , the virulent latent tissue cysts was successfully isolated from 8% of *R. norvegicus*, 23% of *A. cahirinus*, 27% of *R. rattus frugivorus* and 35% of *R. rattus alexandrines*⁵. The incidences of congenital toxoplasmosis in Fischer rats infected between the 8th and 12th days of pregnancy with three different strains of *Toxoplasma gondii* (RH, 76K, and Prugnauud) were 58.2, 35.2, and 62.8%, respectively, denote higher diffusion ratio trough rat generation progenyand maximize Toxoplasmosis in rat colonies⁶.

Most *T. gondii* strains are categorize into three types I, II and III refer to their virulence in mice. Wherever, Type-I is acute lethal non-cyst forming strains and led to death of mice less than10 days post inoculation of < 10 tachyzoites; in contrast, types II and III are considerably less virulent cyst-forming strains with neurological symptoms occur a few weeks after inoculation. Variability of human toxoplasmosis was associated with different strains, mainly from type II, but type I was established cause severe human congenital and ocular toxoplasmosis⁷.

Toxoplasmosis isbenign in the humanpopulation except in immunosuppressed patients or during pregnancy, leading to abortion, still birth, fetus hydrocephalus,congenital malformations, mental retardation and loss of vision. High incidence (6.6%) of toxoplasmic abortion was detected in Egyptian women suffering complicated pregnancy⁸. Also, the opportunistic latenthuman toxoplasmosis can be flair up to acute stage sequence to sharp gestation estradiol hormonal shift⁹, or orders to gravidity hyperglycemia and in diabetics as latent opportunistic character¹⁰. Moreover, opportunistic relapse possibly series to anti-inflammatory corticosteroids therapy¹¹.

The objective of the current study is using the biological and molecular typescript of virulent *T.gondii* of naturally infected commercial rats to clarify the possible cyclo-zoonotic bio-hazards as ecological factor maximize human toxoplasmosis through constant stimulating oocyst shedder cats.

Material and Methods

A. Collection sites and identification of *Rattus* spp:

Rats were collected all over the long course of the study (7/2014 to 10/2015) from different rural and urban sites within Cairo, Giza governorates from indoor stores and houses plus to outdoors agriculture fields and gardens situates, by using indoors and outdoors wire traps that were set shortly before sunset and were checked in the morning. The rats caught were typing according to their physical and morphometric features¹². A total of (n=278) rats were recognized as (n=74) brown or Norway rat (*R. norvegicus*), (n=108) black or roof rat (*Rattusrattusrattus*)and (n=96) fruit rat (*Rattus rattusfrugivorus*).

B. Serological assay:

Serum separation:

Rats were euthanatized with chloroform, and ten mL of the available blood samples were collected from each scarified left to clot at room temperature for 30 min, and centrifuged at 1500 rpm for one min. Serum samples were store at -20°C until serologically assayed.

Preparation of RH strain soluble crude antigen:

Purified mice peritoneal exudate of *T. gondii* RH strain tachyzoites was suspension in phosphate buffer saline (PBS) and centrifuged at 2000 g for 15 minutes, retain the pellet and re-suspend it in nine times of its volume distilled water. Then the mixtures was sonicated for 20 seconds at 4°C at amplitude of 20 microns and any cellular debris was removed by centrifugation at 10,000 rpm for 30 minutes at 4°C. The supernatant was stored at [-20°C] until required. Protein estimation was carried out according to¹³.




Latex agglutination test [LAT]:

The latex agglutination test (LAT) was performed according to the manufacturer's instructions (Toxocheck-MT; Eiken Chemical, Tokyo, Japan). It was considered positive when agglutination was observed at dilutions of Anti-*T. gondii* antibody titers ranged from $\geq 1:164$ to 1:2048.

Detection of IgM/IgG ELISA titers:

The original ELISA according to the method described by with the prepared RH strain soluble crude antigen and the collected rat sera was carried out. Antibodies of IgM and IgG were detected using ELISA diagnostic kits (VIRO, Germany), sensitivity and specificity of kits for IgM and IgG were (100 and 98.1%) and (100 and 99.1%), respectively the procedure was done according the methods described by¹⁴. The serum sample not considers positive unless it positive with LAT and confirmed ELISA.

Table (1): The collected numbers and the morphometric differences of the examined *rattus spp.*

<i>Rattus spp.</i>	NO.	morphometric differences					Feeding behavior	Main habitat and collection situate
		Tail	Body	Coat	Ears & Eyes	Muzzle		
 <i>Rattus norvegicus</i>	74	Shorter than head + body	Thick heavy	Mainly brown gray	small	Broad blunt	Omnivores, but tend to be carnivores	Outdoors in lakes, Nile and desert lands and may be house visitor via sewage current
 <i>Rattus rattus</i>	108	Longer than head + body	Slender thin	Mainly black brown	large	Narrow pointed	Omnivores, but tend to be herbivores	Indoors Stores and houses resident
 <i>Rattus frugivorus</i>	96	Longer than head + body	Round heavy	gray	large	Round	Herbivores	Outdoors in agriculture fields and gardens
Total	278							

C. Microscopic exam:

Brainhomogenate and tissue digestion:

Brain tissue was processed for chronic bradyzoites stage, as was macerated and homogenized in 0.95% sterile phosphate buffer saline (PBS) solution, at a ratio of 1 gm brain tissue/ 2.5 mL PBS. Also, for not avoiding the presence of acute stages; a total 15gm of liver, kidney and heart were automatically homogenized in 2.5% acid-pepsin PBS solution, and incubated at 37°C for 60 min with continuous shaking¹⁵. Following

incubation the large particles were removed by filtering with fine mesh gauze, for avoiding bacterial contamination, per 20 mL of brain or tissue homogenate, 10 units of G-penicillin and 100 mg of streptomycin were added¹⁶. Each brain homogenate or tissue digest was microscopically examined at low and high powers, and the suspected positive microscopic simple was subcutaneously injected into 3 SPF mice of about 1ml/mouse for viability bio-assay.

D. Viability bio-assay:

Viability bio-assay in mice:

A total number of fifty five pathogen free Swiss Webster albino mice weighing 30 gm were procured from Laboratory Animals House, National Research Center, Egypt were used along the course of experiment, fifty one were intra-peritoneal inoculated for each sample of microscopically confirmed tissue cysts into three mice plus to two mice are control positive (RH injected) and two mice are uninfected control negative¹⁷. The animals were inspected daily for signs of a febrile response that may indicate acute toxoplasmosis. Mice which showed signs of illness (tottering gait, hunched appearance alongside evidence of early emaciation and dehydration) were culled immediately and a sample of peritoneal exudates removed and inspected for tachyzoites by microscopic examination, followed by serological confirmation. Mice were considered *T. gondii* negative when antibodies were not found at dilutions of 1:64 and greater of their sera and notachyzoites or tissue cysts were isolated⁹.

Reference parasite stain:

The highly virulent tachyzoites RH strain were maintained in Zoonotic Diseases Department, National Research Center, Egypt, through successive intra-peritoneal tachyzoites - tachyzoites passages in Swiss mice, the obtained tachyzoites from mice ascetic fluid was intra-peritoneal infected in positive control mice consequence with the inoculated rat isolates after counting and dilution as necessary (10^3 factor dilution), the harvest store at 4c containing penicillin (100 U/ml) and streptomycin (50 mg/L).

Parasite load (BL/100mg) and lethal doses (LD50&LD100) estimation:

One drop of the brain homogenate or tissue digest was spread on a slide and the parasite was microscopically counted in average of every 100 mL using haemo-cytometer. The average parasite load (PL/100mL) was estimated by calculating the average number of tissue cysts multiplying in the average number of bradyzoites per cyst. Thus, the practical evaluation of parasite load was done by counting the total number of cyst-free bradyzoites/100mL brain homogenate or tissues digest⁴. Also, lethal doses (LD50&LD100) that kill the half or total number of inoculated mice respectively were identified.

Viability bio-assay in kittens:

14 sero-negative kittens were used; twelve kittens two each were inoculated with the corresponding rat isolates, while the two remaining kittens still un-inoculated control ones. Laboratory kittens were fed mice tissues and/or orally inoculated with the peritoneal washes of mice confirmed to harboring tachyzoites or bradyzoites. According to¹⁸ Kittens were housing in separate cages, feed on toxoplasma free supplement and their fecal matter were microscopically examined daily up to 30 (DPI) by salt or sugar flotation technique for oocysts determination. Positive samples containing *T. gondii* oocysts were purified from fecal debris by centrifugation for 5 min at 1800 r.p.m and then purified samples were subjected to cesium chloride (CsCl) solution, this technique gives a > 96% recovery of pure un-sporulated oocysts¹⁹. The purified samples were stored at -20°C. Oocyst characters concerning their numbers (NO/100 mg), prepatent periods (PP/DPI) and shedding periods (SP /D) were monitored for each isolates.

Biological typing:

The virulent isolates will represent by significant pathogenicity in experimental injected sero-negative mice, where parasite load (BL/100mg) is the number of bradyzoites per 100mg of tissue, and (LD50) the lethal dose that kill half number of inoculated mice, plus to (LD100) the lethal dose that kill the total number of inoculated mice will be determined. While oocysts shedding properties were determine by orally inoculated sero-negative kittens. Biological typing of isolates will done according to²⁰, who classified the vast majority of

isolates studied until now belong to only 3 clonally lineages designate types I, II and III based on the proprieties of data monitoring for each isolates that referred to the used control isolates (Table.4), represented by LD, ability of tissue cyst formation and oocysts characters. Biological typing of virulent isolates was determined by mice clinical signs together with cat shedding skills and reference to the three types I, II, III.

E. Genotyping:

T. gondii DNA was extracted from infected mouse tissues as described by¹⁸. The PCR-RFLP genotypes of the SAG2 locus were used to determine the genetic type. According to²¹ samples were analyzed at the SAG2 locus by using a nested PCR approach that separately amplified the 5' and 3' ends of the locus. The 5' end of the locus was amplified by standard PCR for 40 cycles with the primers SAG2.F4 (5' GCTACCTCGAACAGGAACAC 3') and SAG2.R4 (5' GCATCAACAGTCTTCGTTGC 3') at an annealing temperature of 65°C. The resulting amplification products were diluted 1/10 in water, and a second amplification of 40 cycles was performed with the internal primers SAG2.F (5' GAAATGTTT CAGGTTGCTGC 3') and SAG2.R2 (5' GCAAGAGCGAAC TTGAACAC 3') by using 1 ml of the diluted product as the template. The amplified fragments were purified with Quia Clean (Qiagen Inc.) and digested with *Sau3AI*, and the restriction fragments were analyzed by agarose gel electrophoresis. The 3' end of the locus was similarly analyzed with the primers SAG2.F3 (5' TCTGTTCTCCGAAGTGACTCC 3') and SAG2.R3 (5' TCAAAGCGTGCA TTATCGC 3') for the initial amplifications and the internal primers SAG2.F2 (5' ATTCTCATGCCTCCGCTTC 3') and SAG2.R (5' AACGTTTCACGAAGG CACAC 3') for the second round of amplification at an annealing temperature of 63°C. The resulting amplification products were purified with quiaClean, digested with *HhaI*, and analyzed by agarose gel electrophoresis. The amplified products were digested with *Sau3AI* (5'-end products) and with *HhaI* (3' -end products). The PCR products and the restriction fragments were analyzed by 2% agarose gel electrophoresis. Restriction digestion of 5'-end-amplified products with *Sau3AI* distinguished the type III strain from types I and II strains and *HhaI* digestion of the 3'-end-amplified fragments differentiated types I and III strains from type II strains.

F. Histo-pathological methods:

Tissue samples were taken from brain, liver, kidney and uterus of the three groups of rats pp. Samples were fixed in 10% neutral buffered formalin. Washed, dehydrated and embedded in paraffin wax. The tissues were sectioned at 4-5 thickness and stained with haematoxylin and eosin (H and E) according to²². Periodic acid-Schiff reaction "PAS" and Toluidine blue stains were used were for detection of *T. gondii* within the tissue.

G. Ethical Approval:

The work was approved ethically by the Medical Research Ethics Committee-National Research Centre, Al Tahrir St. Dokki, Giza,Egypt under registration number 1-2 /0-2-1.2014.

Results

All results were full in table (2, 3&4).Corresponding and respectively to a total of 278 collated rats as;*R. norvegicus* (n=74), *R. rattusrattus* (n=108) and *R. Rattusfrugivorus*(n=96); where, the sero-positive rats were 45.9% (34/74) and 19.4% (21/108).Plus to 13.5% (13/96), and atotal of 24.5% (68/278). The Average IgM / IgG were (0.523± /0.321±),(0.336± /0.287±) and(0.314± /0.245±).while the varied percentages of positive Microscopic exam were; (24), (6.5), (4.2) and the total value was (10.4). Also, the percentages of successes mice isolates were; (14.9), (3.7), (2.1) and the total percent were (6.1) respectively in (table.2).Table (3) insulated the results of mice and kittens oocysts characterizes, plus to the bio-types and geno-Typescript. While table (4) was collected the overall prevalent of strain isolates of *Rattus spp.* were; type I; 3/17 (17.6%) only detected in *R. norvegicus*, type II; 9/17(52.9%) and type III; 5/17 (29.4%).Microscopic and histo-pathological exam of *T.gondii* in rat tissues and inoculated mice were detected. Also, lethal doses that kill half number of inoculated mice (LD50/ 10⁽³⁾) and lethal doses that kill all number of inoculated mice (LD100/10⁽³⁾), plus to parasite load (BL/100mg) were identified. The obtained virulent isolates have marked biological differences concerning their virulence to mice: Type I strain led to death of mice less than 10 days after inoculation of < 10 tachyzoites; in contrast, mice survived to infection with a type II strain (50 percent lethal dose (LD50) > 10). Type III is also considered low virulent in mice, particularly with neurological symptoms, can occur a few

weeks or months after inoculation. Diffuse leukocytes infiltration of mononuclear inflammatory cells mainly lymphocytes and macrophages were detected in brain, liver, kidney and lung of inoculated mice. Uterus showed focal desquamation of surface epithelium (Fig.5) associated with diffuse edema and reduction in the uterine glands. Some of the uterine glands were atrophied and less branched with narrow lumen. Diffuse leukocytes infiltration of mononuclear inflammatory cells mainly lymphocytes and macrophages were seen in the endometrial stroma. Endometrium of female mice infected by *T.gondii* Type I strain tachyzoites showing atrophy of uterine glands with periglandular mononuclear inflammatory cells infiltration associated with diffuse edema (Fig-5). Agarose gel electrophoresis analysis of SAG2 PCR amplification products (Fig-6) and restriction digests from *Toxoplasma gondii*-infected clinical samples. PCR detect the target regions for amplification was shortened to encompass only the relevant polymorphisms. Primers were selected to separately amplify the 5' and 3' ends of the *T. gondii* SAG2 locus as 94bp products. Digestion of the 5' amplification products with Sau3AI distinguished allele 3 (type III strains) from alleles 1 and 2 (type I and II strains), and digestion of the 3' amplification products with HhaI distinguished allele 2 (type II strains) from alleles 1 and 3 (type I and III strains).

Table (2): Serological and microscopic examination along with mice inoculation of digested tissue samples from Rattus spp.

Rat types	NO.	Sero-positive (%)	Average IgM/ IgG	Microscopic exam/ (%)	Mice isolates/ (%)
<i>R. norvegicus</i>	74	34(45.9)	(0.523± /0.321±)	18(24)	11(14.9)
<i>R. rattusrattus</i>	108	21(19.4)	(0.336± /0.287±)	7(6.5)	4(3.7)
<i>R. rattusfrugivorus</i>	96	13(13.5)	(0.314± /0.245±)	4(4.2)	2(2.1)
Total	278	68(24.5)		29(10.4)	17(6.1)

Table (3): Bio and geno- typing through mice and cats passage along with DNA eguance and corresponding to Virulent Types I, II and III.

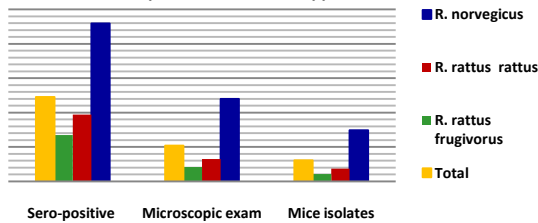
Rat types	Biological Typescript via Mice and kittens passage									Geno-Typescript	
	NO. of Mice isolates (inoculated mice)	Mice characterizes				kittens Oocysts			Bio-types		
		LD50/10(3)	LD100/10(3)	BL/100mg	Virulence	PP/DPI	SP/D	NO/100mg			
<i>R. norvegicus</i>	11	3(9)	3/9 (33.3)	6/9 (66.7)	10	HV	3	5	Free sporozites	I	I
		6(18)	11/18 (61.1)	7/18 (38.9)	50	MV	7	15	30	II or III	II
		2(6)	6/6(100)	0	30	LV	10	21	15	III	II
<i>R. rattusrattus</i>	4	1(3)	1/3(33.3)	2/3 (66.7)	50	MV	7	15	30	II or III	II
		3(9)	3/9 (33.3)	0	30	LV	10	21	15	III	III
<i>R. rattusfrugivorus</i>	2	2(6)	6/6 (100)	0	30	LV	10	21	15	III	III
Total	17(51)										

DPI (Days Post infection), oocyst numbers (NO/100 mg), prepatent periods (PP/DPI) and shedding periods/Day (SP /D), lethal doses that kill half number of inoculated mice(LD50/ 10⁽³⁾), , lethal doses that kill all number of inoculated mice (LD100/10⁽³⁾), parasite load(BL/100mg).

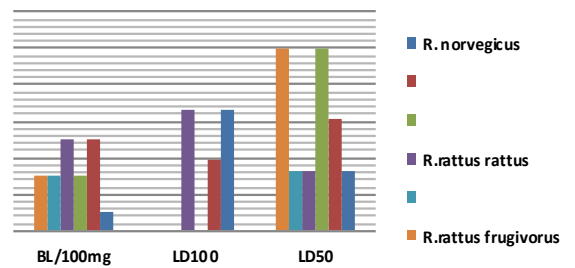
Table (4): Biological Characters of rat isolates reference to virulent types.

Strains	Biological Characters
Type I	Less <i>Rattus</i> spp prevalent type 3/17 (17.6%), only detected in <i>R. norvegicus</i> , highly virulent strain, with an LD ₁ _100 (the dose at which 100% of animals die) of just one parasite. Reduced tachyzoites–bradyzoites interconversion and don't produce typical oocysts in cats. RH strain represent the typical reference type I.
Type II	The more <i>Rattus</i> spp prevalent type 9/17(52.9%), LD ₁₀₀ Mildly virulent, with an LD ₁₀₀ of several thousand parasites. Highly tachyzoites–bradyzoites interconversion and Highly produce typical oocysts in cats.
Type III	Middle <i>Rattus</i> spp prevalent type 5/17 (29.4%), LD ₅₀ Low virulent, with an LD ₅₀ of several thousand parasites. Highly tachyzoites–bradyzoites interconversion and Highly produce typical oocysts in cats.

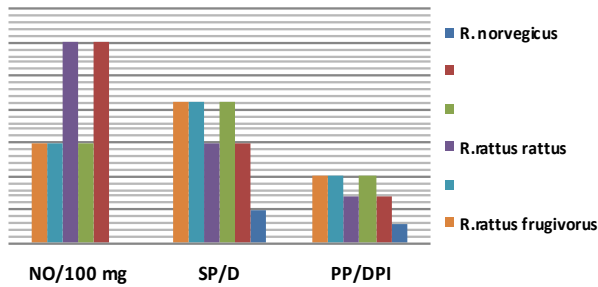
(Fig-1): Results of sero-positive ,microscopic and mice pass isolates of rats spp.



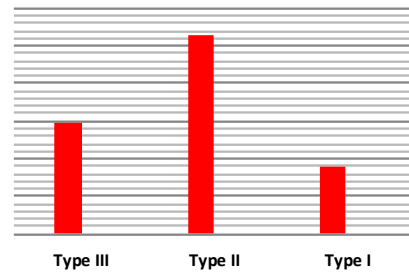
(Fig-2): Parasite load elevation, LD100 and LD50 results of rat spp.



(Fig-3): Differences of oocysts shedding characters



(Fig-4): The varied overall incidence of rat isolates



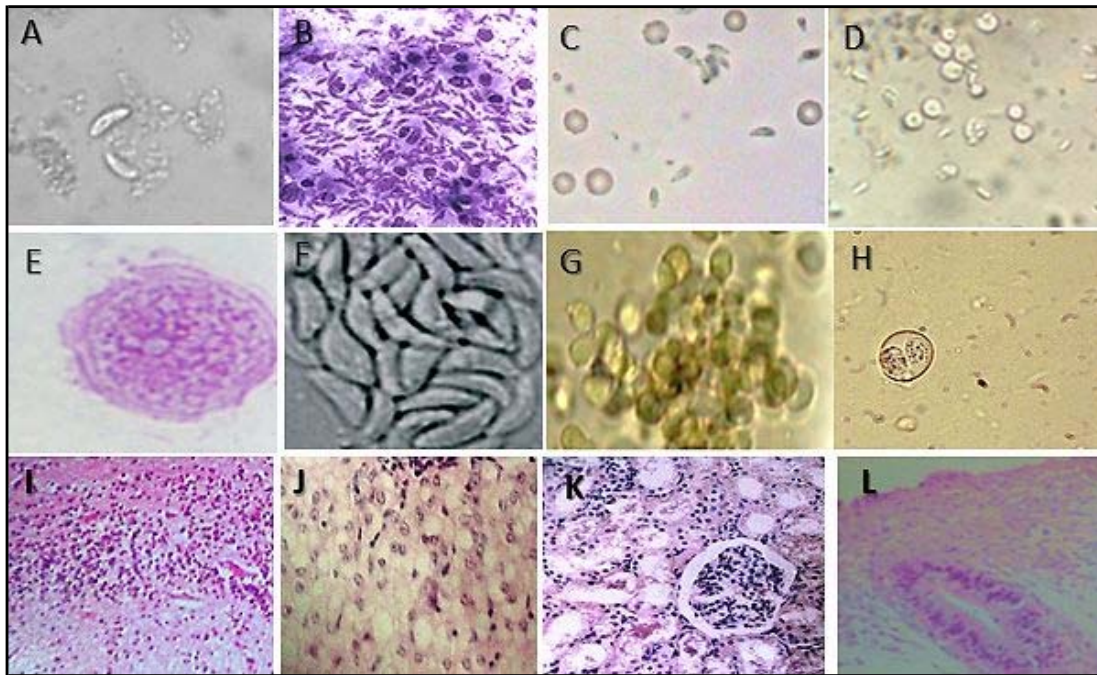


Fig-5: Free bradyzoites in brain homogenate from naturally infected rat/ X400(A). Massive number of RH strain tachyzoites (Control) with leukocytes from mice peritoneal exudate 72 HPI/ X100(B). Little & moderate number of tachyzoites from rat isolates type II or III in mice peritoneal exudates/ X100(C&D) respectively. Ideal brain prints of cystogenic rat types from inoculated mice at 45 DPI/ X100(E). Bradyzoites in brain cyst from inoculated mice at 45 DPI/X1500(F). Cesium chloride (CsCl) purified un-sporulated *T.gondii* oocysts from massive shed inoculated kitten feed mice tissue containing cystogenic rat isolates / X200 (G). Typical Sporulated oocyst in Cesium chloride (11 x 13 µm) in diameter and each contains four sporozoites in each of two sporocysts/ X200(H). Brain of inoculated mice with cystogenic rat isolates, showing gliosis and congestion of blood vessels ;H&E stain/ X100 (I). Liver of inoculated mice with cystogenic rat isolates, showing vacuolar degeneration of hepatic cells associated with mononuclear inflammatory cells infiltration of portal area ;H&E stain/ X100 (J). Kidney of inoculated mice with cystogenic rat isolates, showing vacuolar degeneration and necrobiotic changes of lining epithelium of renal tubules; H&E stain/ X100 (K). Uterus showed focal desquamation of surface epithelium associated with diffuse edema and atrophied with less branched reduction in the uterine glands ;H&E stain/ X100 (L).

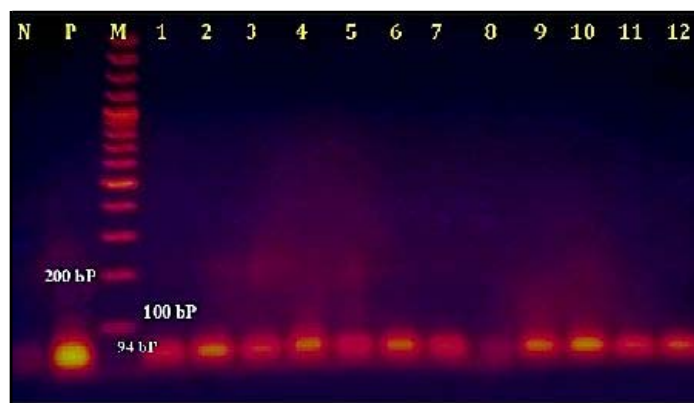


Fig-6: An agarose gel electrophoresis showing PCR amplification product of *T. gondii* using B1 gene. Lane N: Negative control, Lane P: Positive control, Lane M: 100 bp Marker, Lane 1--12: PCR *Toxoplasma* positive from inoculated mice product (94bp).

Discussion

The current study approves *T. gondii* Egyptian isolates corresponding to the three virulent human types within all examined rat types. The percentages of isolates via mice passage (viability test) are lowering than that of both microscopic and sero-positive results (Table.2&Fig.1). However, the higher values of microscopic examination may be related to the possible discordancy among the taken tissue samples and the rat cysts distribution or due to the possible detection of tissue cyst forming protozoa other than *T. gondii*⁴. While, the non-synchronized higher serological values ensure that not all sero-positive rats were harboring bradyzoites. This could be linked to the transferred maternal antibodies or possible cross-reactivity against other parasites as *Trichinella spiralis*²³. Accordingly indicate failure of sero-diagnostic tools to judge any of sero-positive host as a *T. gondii* hazard source.

The mouse inoculation test was confirmed as the most reliable method for isolation of *T.gondii* from brain tissues of rats²⁴, as it was found to be a predilection site for the dormant viable bradyzoites^{25,26}. In the present study the ratio of *T.gondii* infection in Egyptian commercial rats was approved with that reported by²⁷. Also, higher infection prevalence were recounted in latent rats by²⁸ in Costa Rica and by²⁴ in the U. S. A., and by²⁹ in the China and by³⁰ and³¹ in the U. K. Also,³² was identifying *T.gondii* infection form rats that were caught from dissimilar sites at Dasmariñas, Cavite. Infection accounted for 60.0% in *R. norvegicus* and 50.0% in *R. rattusmindanensis*, with anti-*T. gondii* antibodies (Abs) titer ranging from 1:64 to 1:2048. Chronic infection ($\geq 1:256$ anti- *T. gondii* Abs) was detected in 53 (61.0%) rats.

Generally; the superior incidence of *T.gondii* carrier rats is due to: rats are one of the supreme *T.gondii* susceptible species. Naturally catch *T.gondii* through any of the three infective stages; either from meat tissue cysts containing bradyzoites or oocysts contaminated foods or water. Plus to tachyzoitesmaterno-fetal flow that was established to be the predominant transmission mode within rat populations.⁹ was successful recognition *T.gondii* congenital flow within rat progenies. Also, rats are persisting live with toxoplasmosis, where weaned rats are *T.gondii* resistant and do not submit to acute infection even with high dosages of virulent strains that killing mice³³. So, *Rattus spp.* consider the furthestmost *T.gondii* carrier and asymptomatic reservoir. During 1994⁽³¹⁾ had confirmed that *T. gondii* can persist and diffuse within rat colonies even without the sympatric presence of cats' oocyst, and proposed congenital flow as the major transmission route, concluding that rats represent significant and persistent *T. gondii* wildlife intermediate host. Where, healthy animals are adapted to eat the dead ones. So, carnivorous behavior among individuals' rat is potentially secure the dormant tissue cysts within colonies via meat bradyzoites - bradyzoites pass.

In the present study *R. Norvegicus* found to be the furthestmost type harboring *T. gondii* isolates than the other two rats examined strains (Table.2&Fig.1). However, the main significant factor is the potent and favorable carnivorous feeding habit, potentially exposed it to meat tissue cysts. In contrast, *R.rattusrattus* is omnivorous type tend to be herbivorous, while *R.rattus frugivorus* is absolute herbivorous type, they mainly catch *T. gondii* via oocysts contaminated food or water. Moreover, *R. norvegicus* is the only specie establishes to harboring *T. gondii* (type-I) isolate. The unanswered question is how rats could catch type I and harboring bradyzoites corresponding to this type that was reduced its ability for either oocyst or tissue cysts formation? The explanation answer may concern the possible of high mutation capacity of cystogenic types II or III to type I within rats, especially during materno-fetal pass through their progeny for maximizing *T. gondii* diffusion in these species colonies⁽⁹⁾. Also, higher parasite load (BL) and lethal doses (LD50&LD100) were identified of most rat virulent strains (Table.3 & Fig.2). Symbolize higher *T. gondii* rat susceptibility with suspicion of higher diffusion in thief progenies. This suspicion was confirmed through histo-pathological lesion of uterus of mice which injected with control RH strain or (type-I) from *R. norvegicus* that showed focal desquamation of surface epithelium associated with diffuse edema with atrophied and less branched with narrow lumen in the uterine glands (Fig.5). However, successful recognize of cerebral and uterine toxoplasmosis were recorded with the three *T. gondii* virulent types, and realize the possible match of pathological alters with humans at similar circumstance³⁴. This concept was established through bio-typing and molecular identification of *T.gondii* infection that was mainly (type- II) form aborted Egyptian women, also not all sero-positive women were harboring compatible DNA placental cysts³⁵.

The high overall incidence of infected rats in the current study, consider a bio-markers evaluate the zoonotic hazards of rat colonies which still serve as permanent tissue cysts supply for stimulating oocyst shedder cats. However, during 2008 higher percent (50.8%) of naturally infected Egyptian kittens was detected,

and the study certify that sero-negative kittens were better oocyst shedders (11.34%) than sero-positive ones (3.09%)³⁶. Cats possibly shed and re-shed millions of oocysts to pollute the environment series to the frequently feeding on rat tissues holding *T.gondii* virulent strains, reflect on higher incidence of human toxoplasmosis, directly via consumption on oocysts contaminated food or water, and indirectly sequence to forming tissue cysts in meat producing animals and birds³⁷. The vast majority of virulent Egyptian rat isolates were type II strains (Fig.4), corresponding to the most isolates originating from Egyptian personals, kittens, sheep and birds³⁸. Also, higher incidence of the viable *T. gondii* tissue cysts was recognized in ready to Eat Egyptian meat meals³⁹.

The results in the present study (Fig.3&4) validate varied overall Incidence of rat types and guarantee the possibility of naturally infected rats through cystogenic types II or III which are more adaptive to form oocysts or meat tissue cysts than type I⁴⁰. However, those types share higher in meat-borne toxoplasmosis. Moreover, cystogenic isolates create oocysts in shedder cats, and clear why those types were more prevalent in herbivorous rat. Also ensure the difficulty of herbivorous rats to catch type-I through oocysts. So, the natural primary infection through type-I need further ecological studies and maybe concern its mutation aptitude through cystogenic types II or III. However, former studies weresettle public health hazards of cystogenic type-II strains have moderate virulent in mice, can be very pathogenic in human fetus through stimulate latent congenital toxoplasmosis^{41,42}. It was detected as the most prevalent strain in Egyptian mutton⁴, and in Egyptian free-range chickens⁴³.

Toxoplasma gondii protozoan can manipulates the behavior of its intermediate rat host in order to increase its chance of being predated by cats (feline definitive host), thereby ensuring the completion of its life cycle. *T. gondii* manipulation appears to alter the rat's perception of cat predation risk, in some cases turning their innate aversion into an imprudent attraction. The selectivity of such behavioral changes suggests that this ubiquitous parasite subtly alters the brain of its intermediate host to enhance predation rate, followed by congenital flow through their generation⁴⁴.

Mixed infection of *T. gondii* may be involved more than one type in the same rat sample. Practically the summations of those types were biologically behaved like the more virulent one when bio-assayed together. The higher virulent types hidden the biological properties of less virulent ones, the hidden types still secrete until bio-assayed alone. Support our inspection that; it has been reported that multi-genotype infections are common in animals and humans, although, no cases of mixed human infection have ever been identified by mouse inoculation or in vitro culture⁴⁵, but experimental infection by Type II and III strains could produce different viable progeny in cats⁴⁶. Also, only one infection by two different Type II isolates was reported⁽⁴⁷⁾. Therefore, we draw attention here to the possibility that the parasite isolation process could be biologically passed in a single strain even if several strains were found in the inoculums. Serological data are collaborated by earlier reports of *T. gondii* infection ranging between 23.3 to 41.7% in *R. norvegicus*^{48, 49}, and 11.6-13.0% in *R. rattus*^{50,51}. Live-trapped rodents from 54 localities were tested for toxoplasmosis,(4.1%) of *R. Norvegicus* were seropositive⁵².

Conclusion:

Mice viability bioassay with DNA approves along with microscopic exam were identified *T. gondii* tissue cysts within Egyptian rat colonies corresponding to the three virulent types; indirectly exploit human exposure via frequently exciting cats to shed the environmental resistance oocysts infective stage.

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