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Research Article

Physico-Clinical and Haematological Changes in Olive Baboon (*Papio anubis*) Model of Latent Toxoplasmosis and Toxoplasmic Encephalitis

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ABSTRACT

Toxoplasmosis is a neglected parasitic disease caused by the protozoan *Toxoplasma gondii*, which infects virtually all warm blooded animals. In humans, presentation ranges from an acute asymptomatic self-limiting infection in immunocompetent individuals to a devastating and potentially fatal disease in congenitally infected infants and immunocompromised persons. Although a lot is known about the parasite life cycle, many aspects of pathophysiology of toxoplasmosis remain unclear. The main objective of this study was to evaluate suitability of olive baboon as a nonhuman primate model for toxoplasmosis. The effects of *T. gondii* experimental infection on physical, clinical and hematological presentation, and blood and Cerebrospinal F7id (CSF) parasitaemia in olive baboons were determined. We inoculated, intravenously, four adult baboons with 5 x 10^6 *T. gondii*tachyzoites. On day 46 Post Infection (PI), immunosuppression with tacrolimus, administered at 0.2 mg/kg orally, was commenced. They developed an asymptomatic latent infection characterized by blood and CSF parasitaemia, detectable by mouse bioassay throughout the experiment but by direct cytology only between days 7 and 28 PI. The animals gradually gained body weight, most significantly ($p < 0.05$) on days 35 and 42 PI, followed by a decline to pre-infection values from day 49 PI. One baboon developed bilateral ocular opacity on day 21PI. Following onset of immunosuppression, the baboons developed a neurological syndrome clinically characterized, in two, by hyperactivity, aggression and reappearance of parasitaemia detectable by cytology and mouse bioassay while one baboon became dull, withdrawn and oblivious of the surroundings. Hematology showed significant ($p < .05$) increases in RBC counts and PCV and a concomitant leucopenia with significant ($p < 0.05$) declines in total WBCs. These results demonstrate the olive baboon develops an infection that mimics the human form of toxoplasmosis, providing anon-human primate model for investigating the disease pathogenesis, pathophysiology and evaluation of candidate anti-*T. gondii* vaccines and therapeutics.

Keywords: Toxoplasmosis; Latent toxoplasmosis; Toxoplasmic encephalitis; Animal models; Non-human Primates; Olive baboon

INTRODUCTION

Toxoplasmosis is an anthropozoonosis caused by an obligate intracellular protozoan parasite, *Toxoplasma gondii.* It is one of the most successful parasites in the world as illustrated by its global distribution [1], with one third of human population being infected [2]. Only 10-20% of immunocompetent individuals show mild symptoms of a flu-like illness with lymphadenopathy and low grade fever [3]. Severe disease arises following congenital infection, in patients with immunosuppressive disorders (e.g. HIV/AIDS) and those undergoing immunosuppressive therapy for conditions such as organ transplantation [4].

Congenital transmission usually occurs when mothers who become infected during pregnancy pass the infection to the fetus [5]. Reactivation of an infection acquired before pregnancy can also lead to congenital transmission by immunosuppressed women [6,7] though factors associated with the reactivation are yet to be elucidated. Although congenitally infected infants may be born without any symptoms, a significant proportion later develops chorioretinitis, cardiac anomalies and toxoplasmic encephalitis following reactivation [8]. Toxoplasmic encephalitis, with or without CNS lesions, is the most common manifestation of toxoplasmosis in individuals with AIDS [9,10] and may occur in up to 50% of those with other forms of immunodeficiency. Brain infection results in several specific clinical manifestations involving modifications of host behavior [11-13].

Management of toxoplasmosis however faces numerous challenges ranging from lack of reliable screening tests [14,15] to lack of effective prevention and treatment options [16,17]. Mitigating these challenges calls for development of a high-fidelity and homologous animal model showing the infection pattern and symptoms identical to those of humans. Old world monkeys, being phylogenetically close to humans, are valuable models for studying human diseases. The cynomolgus monkey (*Macaca fascicularis*) has been used to study pathogenesis of ocular toxoplasmosis [18] and the Rhesus monkey (*Macaca mulata*) to study pathogenesis of congenital toxoplasmosis [19].

The baboon has been described as an ideal model for biomedical research [20] due to its physiological, immunological, and biochemical similarities to humans, and the fact that all parameters in human physiology can be measured with the same or equal technical equipment [21]. Besides the advantage of their phylogenetic proximity to humans, their body sizes make them well-suited to serial extraction of sufficient blood and CSF volumes during studies. The main objective of this study was to evaluate suitability of the olive baboon as a non-human primate model for toxoplasmosis. This monkey is widely distributed throughout Africa and has been reported to naturally acquire toxoplasmosis in the wild [22].

MATERIALS AND METHODS

Ethics statement

This study was performed at the Institute of Primate Research (IPR, Nairobi, Kenya). IPR is locally and widely recognized in Africa as a Center of Excellence in preclinical research. Prior to commencement of the study, all protocols and procedures used were reviewed and approved by the Institutional Animal Care and Use committee of the Institute of Primate Research in Kenya (approval number: IRC/21/11).

Experimental animals

Baboons: Four healthy adult olive baboons (*Papio anubis*), two males and two females, sourced from the Institute of Primate Research (IPR, Nairobi, Kenya), were used in this study. The animals had earlier been trapped from the wild and quarantined according to the established protocol at IPR. We subsequently screened them for previous exposure to toxoplasmosis by Nested Polymerase Chain Reaction (nPCR) as described by [22] and they tested negative. Throughout the study period, the animals were maintained in individual cages. They were fed with commercial monkey chow, supplemented with fruits and vegetables. Water was availed *ad libitum*.

Swiss white Mice: Seventy four adult Swiss white mice were obtained from the rodent breeding facility at the Institute of Primate Research, Nairobi, Kenya. They were 6-8 weeks old and weighed 20-30 g. They were housed under standard laboratory conditions, in plastic cages (medium size cages; length 16.9 inches, width 10.5

inches, and height 5 inches) and were provided with wood shaving bedding and nesting material. Feed (Mice Pellets®, Unga Feeds Ltd, Kenya), and drinking water were provided *ad libitum*.

Toxoplasma gondii isolation and expansion

The *T. gondii* isolate used in this study was obtained from the brain of free range chicken from Thika sub-county, Kenya as described by [23]. In summary, the chicken were sacrificed by cervical dislocation and brain tissue collected under sterile conditions. The brain was ground in a pestle and mortar; 1ml of Phosphate Buffered Saline (PBS) added and homogenized using tissue homogenizer [24]. Presence of tissue cysts was confirmed by direct microscopy and aliquots of the brain suspension preserved in liquid nitrogen. At the commencement of this study tissue cysts were recovered from the liquid nitrogen and allowed to thaw on the bench for about 30 minutes and then vortex mixed. Cysts were enumerated by transferring three aliquots of 20 µl of the brain suspensions onto microscopic slides, a coverslip placed over each sample and number of cysts counted in the entire sample at X20 magnifications directly without staining. The brain suspension was serially diluted with PBS (pH 7.2) to adjust to a desired final concentration of 15 tissue cysts/200µl [25]. To obtain tachyzoites for baboon infections, 3 Swiss white mice were intraperitoneally injected each with 15 tissue cysts. The mice were euthanized on the fourth day using CO_2. 5 ml of PBS was injected intraperitoneally into the mice, the bellies gently massaged and the peritoneal fluid aspirated using a gauge 21 needle. Tachyzoites were enumerated using a Neubauer chamber and serially diluted with PBS to a final concentration of 5 x 10^6/ml.

Study design

Baboon infection, follow up and sampling: Baboons were experimentally infected with 5 x 10^6 tachyzoites through inguinal venipuncture. Every day the animals were monitored for behavioral and clinical presentation. Every week they were anaesthetized with a mixture of ketamine hydrochloride at 10 mg/kg and xylazine hydrochloride at 2 mg/kg body weight. A thorough physical and clinical examination and full blood count using an automated blood cell counter were performed during the whole experimental period. Before inoculation, weekly, for a period of three weeks, 5 ml blood and 2 ml Cerebrospinal Fluid (CSF) samples were collected to provide pre-infection baseline data. Following infection, 10 ml of inguinal blood and 2 ml lumbar CSF were collected on days 0, 7, 14, 21, 28, 35, 42, 49, 56 and 63 for hematology and parasitaemia determination by direct cytology and mouse bioassay. 2 ml of the blood for hematology was collected in heparinized vacutainers while 8 ml was left on the bench overnight and then centrifuged the following morning at 800 g for 10 min and serum separated. The serum was stored at -20°C for biochemical analyses. On day 42 PI, one animal, a female (PAN 4107), was randomly selected and sacrificed and various tissues harvested for histopathology. The remaining three animals (PAN 4080, PAN 4092 and PAN 4104) were, from day 46 PI, immunosuppressed using tacrolimus (PanGraf 1.0 Panacea Biotec Ltd, Malpul, Baddi, Tehsil Nalagah, India) administered orally daily at 0.2 mg/kg body weight, given as two divided doses, in the morning and in the evening. At the end of the experiment (day 63 PI) all animals were euthanized and relevant tissues harvested for histopathology.

Hematologic procedure: An automated coulter counter (Ac. T 5diff CP, Beckman Coulter, USA) was used to determine Parked Cell Volume percent (PCV %), Red Blood Cells (RBC), Mean Cell Volume (MCV), Mean Cell Haemoglobin (MCH), MCH concentration, White Blood Cells (WBC), neutrophils, lymphocytes, eosinophils, basophils and platelets.

CSF collection: The lumbar region of the baboon was cleanly shaven and cleaned with an antiseptic. A gauge 23 needle was used to harvest 2 ml of clean CSF from a lumbar spinal segment into a curvet. The CSF was centrifuged at 1500 rpm for ten minutes and the supernatant decanted and stored at -20°C for biochemical analysis. The cell pellet was reconstituted in 1 ml PBS, vortex mixed and used for preparation of wet smears in a Neubauer chamber for parasite identification and quantification. The rest of the reconstituted CSF pellet solution was used for mouse bioassay via intraperitoneal inoculation.

Mouse bioassay: Seventy four Swiss white mice were used for the determination of parasitaemia in baboon blood and CSF (37 mice each) starting from the day of infection. Eight mice were used every week, two for every baboon. One mouse was inoculated with blood and the other with CSF and monitored for eight weeks after which they were euthanized using CO_2. Brains were harvested and divided symmetrically into two. One half was gently ground using pestle and mortar and a suspension made by adding 1 ml PBS. Examination for tissue cysts was done using a Neubauer chamber and slides of the brain suspension made and stained with H&E. The other half was cryopreserved.

Data management and statistical analysis

Data was recorded in notebooks and keyed into Microsoft Excel which acted as the database. The data was analyzed using the paired Student's *t*-test to compare pre-infection and post-infection means. Microsoft Excel 2013 was used to perform the Student *t*-test. The *t*-test was considered significant when the $p < 0.05$.

RESULTS

The pre-infection data at day 0 comprised of means of four weekly pre-infection samples collected on days, -21, -14, -7 and 0. These data were compared with subsequent weekly post-infection means. Three baboons developed toxoplasmosis characterized by CSF and blood parasitaemia. It was not possible to draw CSF from one baboon at any time due to her extremely narrow lumbar spaces. However, we demonstrated blood parasitaemia throughout the acute phase of the disease at the end of which she was sacrificed.

Parasitaemia in Baboon CSF and blood via mouse bioassay

The establishment of toxoplasmosis was confirmed by parasitaemia, in CSF and blood, determined by cytology and mouse bioassay. Some mice developed an acute disease and died soon after inoculation. The others developed a very rough hair coat and became cachexic but went through the whole experimental period. Brain suspensions from all mice showed heavy presence of tissue cysts when visualized microscopically using a Neubauer chamber (Figure 7). Giemsa stained slides showed tissue cysts containing numerous bradyzoites (Figures 8,9). Some slides showed tissue cysts located within neuronal processes (Figure 10). The tissue cysts within the axons were usually organized in a bead-like arrangement (Figure 11).

Parasitaemia in CSF via direct cytology

Toxoplasma gondii parasites appeared in CSF within seven days of infection. The parasites were not detectable cytologically, using a Neubauer chamber, from day 28 Post Infection (PI). The parasites were

however detectable on 56th day PI, 10 days after immunosuppression with tacrolimus till the end of the experiment (Table 1, Figure 1).

Physico-clinical and hematological data

Cerebrospinal fluid parasitaemia changes: Following inoculation, rapidly swimming tachyzoites were detectable in CSF from day 7 PI, peaked on day 14 PI and waned off on day 28PI. After immunosuppression with tacrolimus on day 46 PI, the tachyzoites reappeared consistently in CSF till the end of experiment (figure 1).

Physico-clinical changes: Only one baboon, (PAN 4104), developed a rough hair coat from day 14 post infection till the end of the experiment. This baboon also developed ocular opacity in both eyes from day 21 PI (Figure 12). PAN 4092 developed moderately enlarged axillary and inguinal lymph nodes from day 14 PI.

Between 5- 10 days after commencement of immunosuppression, PAN 4092 and PAN 4080 became hyperactive, exhibited erect mane hairs, overt aggression and continuous yawning, gnashing/grinding of teeth and tongue rolling. They displayed hyperactivity by continuously moving in their cages. This behavior remained till the end of the experiment. PAN 4107 however exhibited extreme fear. He developed a dull demeanor, always very quiet appearing oblivious of the surroundings and perched at one corner of the cage. When approached, he would make sudden jumps towards the topmost corner of the cage knocking his head hard on the roof.

Mean body weights: Mean body weights increased gradually throughout the experiment. The most statistically significant increases were noted on days 35 ($p = 0.005$) and 42 ($p = 0.049$) post infection as depicted in the Figure 2 below.

Mean body temperature: Mean body temperature values fluctuated evenly throughout the experimental period but without statistical significance ($p > 0.05$) as depicted in Figure 3.

Total RBC count and PCV: There was a gradual statistically significant rise in total RBC counts ($p = 0.040$) and PCV % ($p = 0.010$) throughout the acute phase of the infection. After immunosuppression the values declined to pre-infection values (Figures 4,5).

DISCUSSION

Classically, toxoplasmosis in immunocompetent individuals begins with an acute phase associated with rapid tachyzoite proliferation followed by a chronic stage, characterized by the presence of latent cysts within the central nervous system and skeletal muscles [26] with subsequent immunosuppression resulting in reactivation and development of toxoplasmic encephalitis [9]. The present study managed to successfully establish the disease in olive baboons, as evidenced by parasitaemia and physico-clinical changes; the acute stage from infection to appearance of parasites in CSF (7-21 DPI), latent stage from 28 DPI to day 56 PI and a toxoplasmic encephalitis stage characterized by reappearance of CSF parasites and associated neurological signs after commencing immunosuppression.

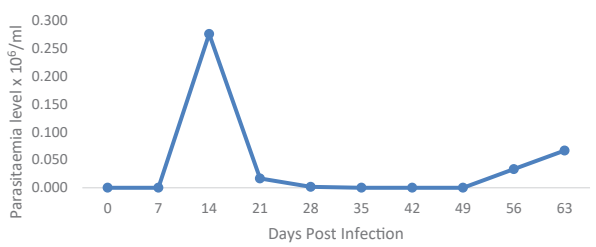

Figure 1: This chart shows the changes in CSF parasitaemia in baboons following infection and after immunosuppression with tacrolimus. There was a sharp rise in parasitaemia which peaked on day 14 PI, then fell to zero. Parasites were detectable, from day 56 PI, after immunosuppression.

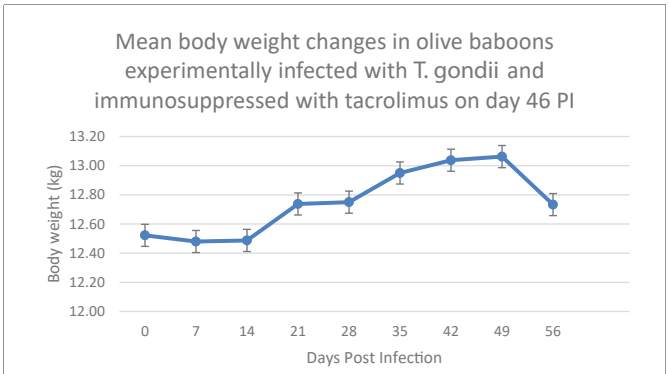

Figure 2: Day 0 data is the pre-infection value. The figure depicts gradual increase in mean body weights throughout the experiment. PI, Post Infection.

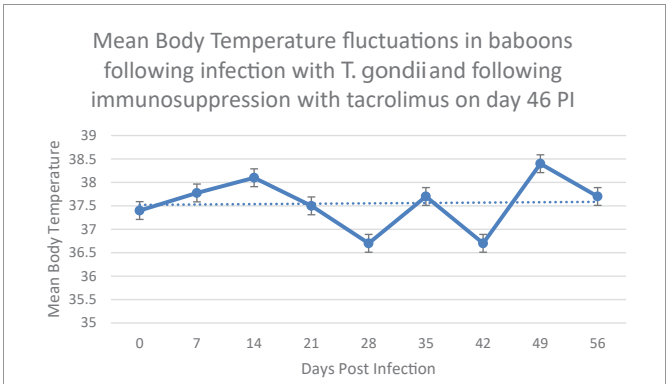

Figure 3: Shows fluctuation of body temperature throughout the experimental period.

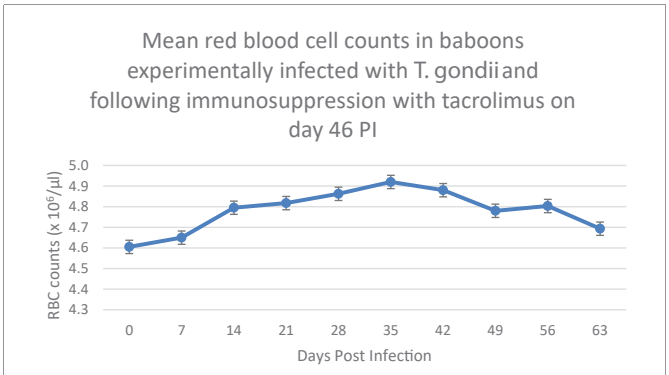

Figure 4: RBC counts in olive baboons. They were infected on day 0. Standard error bars highlight significance of the differences between pre-infection and post-infection values.

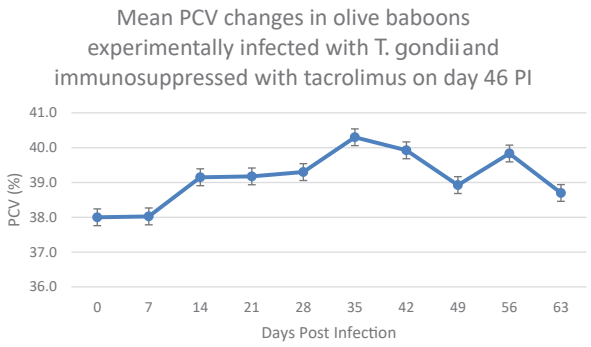

Figure 5: Mean PCV changes in baboons before (Day 0) and after experimental infection with *T. gondii* tachyzoites. Standard error bars highlight significance of the differences between pre-infection and post-infection values.

We recorded significant body weight gains throughout the latent stage of infection. According to our knowledge, this could be the first experiment to demonstrate a causal relationship between toxoplasmosis and obesity. This phenomenon, referred to as "infectobesity", suggests the potential role of environmental organisms in the pathogenesis of obesity [27,28]. Latent toxoplasmosis has been associated with weight gain/obesity in rats [29] and humans [30]. The peripheral and/or central mechanisms responsible have not been clearly elucidated but parasite induced biochemical [31] and behavioral alterations may play significant roles. Clinically one animal showed moderately swollen inguinal and axillary lymph nodes. There were no behavioral changes reported during the latent phase of the infection.

This study also reports development of bilateral ocular opacity in one baboon suggesting the establishment of ocular toxoplasmosis following experimental postnatal infection. Ocular lesions occur in up to 25% of individuals infected with *T. gondii* though in some countries up to 50% of all cases of posterior uveitis in certain populations are attributable to toxoplasmosis [32,33]. As a rule of thumb, ocular toxoplasmosis has commonly been attributed to congenitally acquired infection [34,35] or reactivation [36,37] except where clear evidence exists to show that infection has been acquired postnatally. Our study reinforces available evidence which confirms that a significant proportion (at least two thirds) of ocular toxoplasmosis is caused by primary acute postnatal infections [38-40]. Indeed ocular lesions have been reported to appear in patients as early as 2 months after the onset of infection [41]. The role of different *T. gondii* strains in the causation of either acquired or congenital infection is still not clear. Murine model virulence studies [42] have described three clonal lineages of *T. gondii*, namely types I, II and III. While traditionally the type II clonal lineage has been incriminated in the majority of postnatal ocular lesions, and type I in congenital toxoplasmosis [42], more recent studies have shown that type I and some atypical strains may also play a significant role in postnatal ocular infection [42,43]. The parasite strain used in this study needs to be ascertained.

Toxoplasma gondii tachyzoites were detected using direct CSF microscopic examination from day 7 PI and persisted up to day 28PI. They were subsequently undetectable microscopically throughout the rest of the acute phase. The disappearance of the parasites from blood and CSF suggests conversion of tachyzoites to bradyzoites and subsequent formation of tissue cysts [44]. However mice inoculated with blood and CSF collected from the baboons at all sampling points throughout the acute phase and beyond developed toxoplasmosis, indicating existence of a source of a sustained "low dose" parasitaemia. This confirms that while tissue cysts are long-lived, they episodically rupture at a low but continuous frequency without reactivation since the bradyzoites released are readily killed by the host immune factors, especially cell-mediated immunity [45]. The episodically released parasites were however detected by mouse bioassay confirming it as the "gold standard" for detection of infective stages of *T. gondii* [46,47]. Appearance of tachyzoites in CSF just 7 days PI indicates this rapid migration into the brain as a means of escaping the hostile host immune response within circulation into the less immunologically robust brain where they encyst and predominantly reside in neurons [48]. The tropism of *T. gondii* parasites for neurons is attributed to the fact that neurons are not capable of efficiently clearing the parasites [49] because they are somewhat immunologically naive and lack full immune-response capabilities [50].

Within 10 days of commencement of immunosuppression, tachyzoites were detectable by both direct microscopy and mouse bioassay once again, indicating massive release of parasites from latent tissue cysts, and reactivation of the disease. Reappearance of CSF parasitaemia coincided with onset of the neurological syndrome characterized by aggression, continuous pathological yawning, and tongue rolling/chewing, gnashing of teeth, extreme fear, dull demeanor, withdrawal and being oblivious of the surroundings. This syndrome marked the onset of Toxoplasmic Encephalitis (TE), a potentially fatal condition that most frequently presents in immunocompromised individuals. TE is one of the most common Central Nervous System (CNS) disorders seen in AIDS patients [51,52]. *T.gondii* may play a role in the etiopathogenesis of psychiatric disorders by affecting neurotransmitters, especially dopamine, that are implicated in the emergence of psychosis and behavioral abnormalities like schizophrenia [53], bipolar disorder [54], self-directed violence and suicidal tendencies [55,56] and by inducing brain inflammation through direct stimulation of inflammatory cytokines in the central nervous system [57-59].

This study has demonstrated hematological changes generally characterized by upregulation of erythropoiesis and a concurrent

Table 1: CSF parasitaemia in baboons experimentally infected with *Toxoplasma gondii* and then immunosuppressed on day 46PI using tacrolimus administered orally. PAN, *Papio anubis*; DPI, Day Post Infection; NS, No sample; Tac, Tacrolimus immunosuppression; Parasitaemia in x 10^6.

Animal Number/DPI	0	7	14	21	28	35	42	46	49	56	63
PAN 4080	0	5.125	4.375	0.035	0.005	0	0	Tac	0	0.030	0.010
PAN 4092	0	3.575	2.390	0.010	0	0	0	Tac	0	0.010	0.150
PAN 4107	0	1.355	0.150	0.005	0	0	0	Tac	0	0.060	0.040
PAN 4104	0	NC	NS	NS	NS	NS	NS	EUTH			

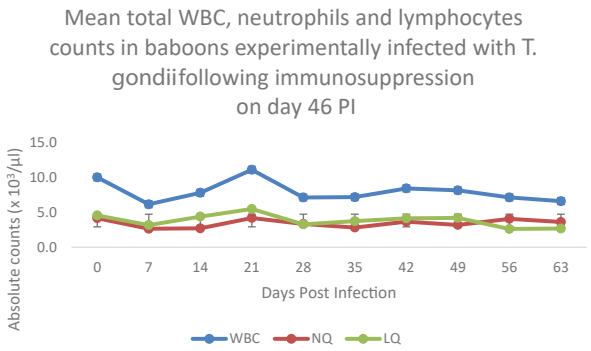

Figure 6: Mean WBC, neutrophils and lymphocytes counts. WBC = White Blood Cells, NQ = Neutrophils, LQ = Lymphocytes.

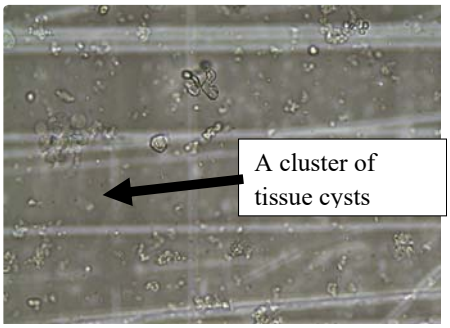

Figure 7: (X 40 mag): Plain/unstained Neubauer chamber slide of brain suspension of mouse infected with CSF of PAN 4107 harvested on day 14 post Infection. The slide shows numerous tissue cysts with some clustered together.

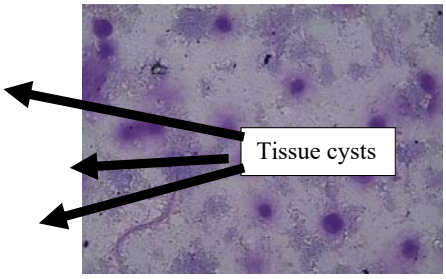

Figure 8: (X 40): Giemsa-stained slide of brain suspension of mouse infected with CSF of PAN 4104 harvested on day 7 Post Infection. The slide shows numerous tissue cysts dispersed in brain suspension.

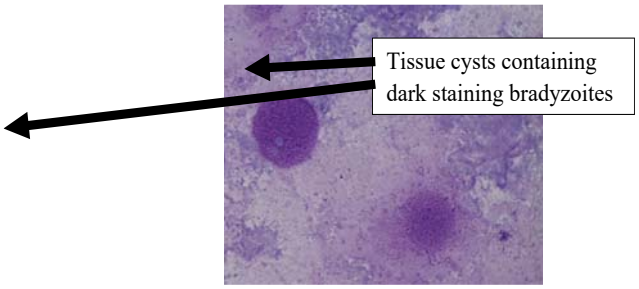

Figure 9: (X 40): Giemsa-stained slide of brain suspension of mouse infected with blood of PAN 4107 harvested on day 7 post Infection. The slide shows a tissue cyst containing numerous dark staining bradyzoites.

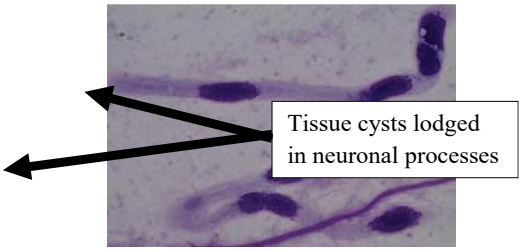

Figure 10: (Oil Immersion): Giemsa-stained slide of brain suspension of mouse infected with CSF of PAN 4092 harvested on day 14 post Infection. The slide shows tissue cysts lodged within neuronal processes.

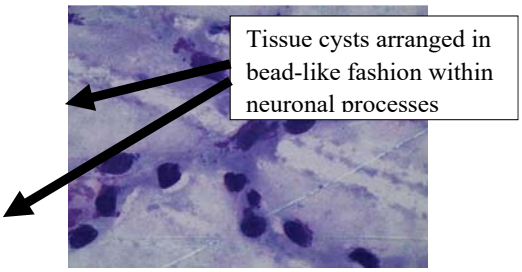

Figure 11: (Oil Immersion): Giemsa-stained slide of brain suspension of mouse infected with CSF of PAN 4092 harvested on day 14 post Infection. The slide shows tissue cysts arranged in a bead-like fashion within neuronal processes.

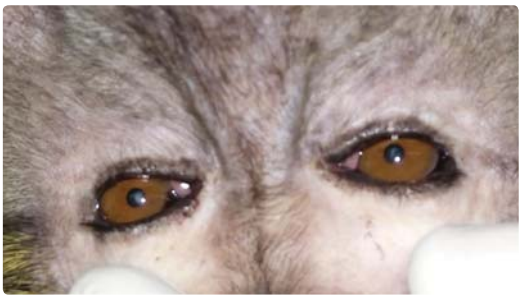

Figure 12: Photograph of PAN 4104 showing bilateral ocular opacity noticed on day 21 post infection.

leukopenia. According to our knowledge, this could be the first report of *T. gondii*-induced upregulation of erythropoiesis, in olive baboons, and is quite confounding. Similar perplexing increases in PCV, RBC and haemoglobin values have been reported in naturally *T. gondii*-infected cats [60,61] and experimentally infected gerbils [62]. Most studies generally report development of anemia characterized by low PCV %, Hgb concentration, and RBC counts [63,64]. Experimental murine studies have demonstrated anaemia as a key erythroid sequel of a variety of protozoal diseases including malaria [65,66] trypanosomiasis [67] and toxoplasmosis [68]. *T.gondii*-induced anemia has been attributed to depressive effects of pro-inflammatory cytokines especially IL-6, IFNγ and TNF-α on erythroid precursor cells [69], and hemorrhage [68]. This study also reports a generalized leukopenia, neutropenia, lymphocytopenia and eosinopenia. The intravenous route of infection is akin to drastic bombardment of immune cells in the first line of defense, which are themselves susceptible to parasite invasion and destruction. The ambush attack could have occasioned serious leukocyte casualties hence the sharp leukopenia on day 7 PI which was soon followed by a

Table 2: The pre-infection and post infection means and respective standard deviations (M, SD). Day 0 data is the pre-infection mean calculated from four weekly pre-infection values. DPI = Days Post Infection, *n* = number of baboons; ND, no data available.

Variable/DPI	0 *n* = 4	7 *n* = 4	14 *n* = 4	21 *n* = 4	28 *n* = 4	35 *n* = 4	42 *n* = 4	49 *n* = 4	56 *n* =3	63 *n* = 3
Body weight(kg)	12.52, 2.668	12.4,2.632	12.49, 2.429	12.73, 2.450	12.75, 2.512	12.95, 2.645	13.04, 2.375	13.06, 2.460	12.73, 3.252	ND
Body temp. (˚C)	37.43, 0.567	37.8,0.340	38.13, 0.618	37.5, 0.638	36.65, 0.532	37.7, 0.391	36.73, 0.618	38.38, 0.075	37.67, 0.208	ND
RBC count (x 10^6/µl)	4.61, 0.179	4.65, 0.176	4.80, 0.066	4.82, 0.135	4.86, 0.102	4.92, 0,217	4.88, 0.180	4.78, 0.155	4.89, 0.077	4.87, 0.180
PCV Relative %	38.0, 1.643	38.0, 1.977	39.2, 0.818	39.2, 1.037	39.3, 1.023	40.3, 1.402	39.9, 0.813	38.9, 1.228	38.9, 0.832	38.7, 0.519
WBC count (x 10^3/µl)	10.01, 1.081	6.15, 0.640	7.8, 1.984	11.1, 4.859	7.13, 1.244	7.18, 1.876	8.43, 1.944	8.15, 2.020	7.13, 1.514	6.6, 1.708
Neutrophils (x 10^3/µl)	4.01, 0.297	2.64, 0.221	2.70, 0.970	4.19, 1.208	3.34, 0.978	2.82, 0.842	3.67, 0.889	3.18, 0.859	4.07, 1.296	3.61, 0.859
Neutrophils; Relative %	48.6, 16.8	43.2,	34.6	40.0	46.1	39.3	43.2	41.5	56.2	54.9
Lymphocytes (x 10^3/µl)	5.34, 1.770	3.18, 0.683	4.38, 1.216	5.48, 2.783	3.28, 0.307	3.73, 1.233	4.14, 1.152	4.21, 1.224	2.62, 0.423	2.67, 0.902
Lymphocytes Relative %	45.2	51.3	56.3	48.2	46.5	51.2	48.7	51.3	37.6	40.4

Table 3: Paired t-test results comparing pre-treatment values for different physical, clinical and haematological variables during different Days Post Infection (DPI). (t (df) = *t* value, *p* - value). For statistical significance, $p < 0.05$. ND, no data.

Variable/DPI	7	14	21	28	35	42	49	56	63
Body weight(kg)	t(3) = 0.80, *p* = 0.480	t(3) = 0.27, *p* = 0.810	t(3) =-1.63, *p* = 0.20	t(3) = -2.48, *p* = 0.080	t(3) = -7.25, *p* = 0.005	t(3) = -3.2, *p* = 0.050	t(3) = -2.75, *p* = 0.070	t(2) = 3.84, *p* = 0.060	ND
Body temp (°C)	t(3) =0.36, *p* = 0.24	t(3) = 0.19, *p* = 0.24	t(3) =0.64, *p* = 0.77	t(3) = 0.53, *p* = 0.12	t(3) = 0.34, *p* = 0.47	t(3) = 0.52, *p* = 0.21	t(3) =0.07, *p* = 0.13	t(2) = 0.21, *p* = 0.54	ND
RBC count (x 10^6/µl)	t(3) = -1.1, *p* = 0.35	t(3) = -2.09, *p* = 0.13	t(3) = -2.1, *p* = 0.13	t(3) = -3.32, *p* = 0.04	t(3) = -3.41, *p* = 0.04	t(3) = -2.5, *p* = 0.09	t(3) = -1.54, *p* = 0.22	t(2) = -2.51, *p* = 0.13	t(2) = -0.14, *p* = 0.9
PCV Relative %	t(3) = -0.07, *p* = 0.95	t(3) = -2.22, *p* = 0.11	t(3) = -3.53, *p* = 0.04	t(3) = -3.32, *p* = 0.05	t(3) = -5.12, *p* = 0.01	t(3) = -3.4, *p* = 0.04	t(3) = -1.24, *p* = 0.30	t(2) = -2.9, *p* = 0.10	t(2) = -0.7, *p* = 0.56
WBC count(x 10^3/µl)	t(3) = 7.03, p=0.006	t(3) = 4.68, *p* = 0.02	t(3) = -0.42, *p* = 0.71	t(3) = 5.46, *p* = 0.01	t(3) = 5.54, *p* = 0.01	t(3) = 1.79, *p* = 0.17	t(3) = 3.65, *p* = 0.04	t(2) = 9.99, *p* = 0.01	t(2) = 4.70, *p* = 0.04
Neutrophils (x 10^3/ µl)	t(3) = 2.96, *p* = 0.090	t(3) = 2.06, *p* = 0.130	t(3) = -0.04, *p* = 0.970	t(3) = 0.93, *p* = 0.420	t(3) = 1.79, *p* = 0.170	t(3) = 0.68, *p* = 0.540	t(3) = -0.04, *p* = 0.970	t(2) = 0.37, *p* = 0.74	t(2) = 1.31, *p* = 0.28
Neutrophils Relative%	t(3) = 0.86, p=0.45	t(3) = 1.58, *p* = 0.21	t(3) = 0.60, *p* = 0.59	t(3) = 0.22, *p* = 0.84	t(3) = 0.92, *p* = 0.42	t(3) = 0.58, *p* = 0.600	t(3) = 0.65, *p* = 0.56	t(2) = -1.26, *p* = 0.34	t(2) = -1.45, *p* = 0.28
Lymphocytes (x 10^3/µl)	t(3) = 2.62, *p* = 0.12	t(3) = 0.34, *p* = 0.760	t(3) = -0.42, *p* = 0.70	t(3) = 1.36, *p* = 0.27	t(3) = 1.34, *p* = 0.27	t(3) = 0.56, *p* = 0.620	t(3) = 0.41, *p* = 0.71	t(2) = 2.89, *p* = 0.10	t(2) = 3.04, *p* = 0.09
Lymphocytes Relative %	t(3) = -0.97, *p* = 0.41	t(3) = -1.40, *p* = 0.26	t(3) = -0.24, *p* = 0.82	t(3) = -0.16, *p* = 0.920	t(3) = -0.71, *p* = 0.53	t(3) = -0.39, *p* = 0.720	t(3) = -0.65, *p* = 0.56	t(2) = 1.20, *p* = 0.35	t(2) = 1.29, *p* = 0.33

homeostatic recovery. Similar leukocyte changes have been reported in *T. gondii* infected cats, dogs and humans [70,71]. The validity of our haematological findings could however be reinforced by using a larger number of baboons in a similar experiment.

CONCLUSION

This study used a *T. gondii* isolate from free range chicken in Kenya. The results have demonstrated that the olive baboon develops an infection that mimics the human form of toxoplasmosis, during both latent toxoplasmosis and toxoplasmic encephalitis, providing an excellent animal model suitable for investigating the disease pathogenesis, pathophysiology and evaluation of candidate anti-*T. gondii* vaccines and therapeutics.

REFERENCES

1. Dubey JP. Toxoplasmosis of animals and humans, 2nd ed. Boca Raton: CRC Press. 2010. https://tinyurl.com/y9clplzm
2. Tenter AM, Heckeroth AR, Weiss LM. Toxoplasma gondii: From animals to humans. Int J Parasitol. 2000; 30: 1217-1258. **PubMed:** https://www.ncbi.nlm.nih.gov/pubmed/11113252
3. HoYen DO, Joss AWL. Clinical features. In: Human Toxoplasmosis, Oxford University Press. 1992. https://tinyurl.com/y7qy42f9
4. Girdwood RW. 'Protozoan' infections in the immunocompromised patient - the parasites and their diagnosis. J Med Microbiol. 1989; 30: 3-16. **PubMed:** https://www.ncbi.nlm.nih.gov/pubmed/2674446
5. Remington JS, McLeod R, Thulliez P, Desmonts G. Toxoplasmosis. In: Infectious diseases of the fetus and newborn infant. Elsevier Saunders, Philadelphia. 2006; 947-1091.
6. Mitchell CD, Erlich SS, Mastrucci MT, Hutto SC, Parks WP, Scott GB. Congenital toxoplasmosis occurring in infants perinatally infected with human immunodeficiency virus 1. Pediatr Infect Dis J. 1990; 9, 512-518. **PubMed:** ncbi.nlm.nih.gov/pubmed/2371084
7. Minkoff H, Remington JS, Holman S, Ramirez R, Goodwin S, Landesman S. Vertical transmission of Toxoplasma by human immunodeficiency virus-infected women. Am J Obstet Gynecol. 1997; 176: 555-559. **PubMed:** https://www.ncbi.nlm.nih.gov/pubmed/9077606
8. Caroline Paquet, RM, Trois Rivieres QC, Mark H. Yudin, Toronto ON.

Toxoplasmosis in pregnancy: Prevention, screening, and treatment. J Obstet Gynaecol Can. 2013; 35: 78-79. https://tinyurl.com/yadr9fca

9. Luft BJ, Conley F, Remington JS, Laverdiere M, Wagner KF, Levine JF, et al. Outbreak of central-nervous-system toxoplasmosis in Western Europe and North America. Lancet. 1983; 1: 781-784. **PubMed:** https://www.ncbi.nlm.nih.gov/pubmed/6132129

10. Israelski DM, Chmiel JS, Poggensee L, Phair JP, Remington JS. Prevalence of Toxoplasma infection in a cohort of homosexual men at risk of AIDS and toxoplasmic encephalitis. J Acquir Immune Defic Syndr. 1988; 6: 414-418. **PubMed:** https://www.ncbi.nlm.nih.gov/pubmed/8455146

11. House PK, Vyas A, Sapolsky R. Predator cat odors activate sexual arousal pathways in brains of Toxoplasma gondii infected rats. PLoS One. 2011; 6: e23277. **PubMed:** https://www.ncbi.nlm.nih.gov/pubmed/21858053

12. Berenreiterova M, Flegr J, Kubena AA, Nemec P. The Distribution of Toxoplasma gondii cysts in the brain of a mouse with Latent Toxoplasmosis: Implications for the behavioral manipulation hypothesis. PLoS One. 20116; 6: e28925. **PubMed:** https://www.ncbi.nlm.nih.gov/pubmed/22194951

13. Dass SA, Vasudevan A, Dutta D, Soh LJ, Sapolsky RM, Vyas A. Protozoan parasite Toxoplasma gondii manipulates mate choice in rats by enhancing attractiveness of males. PLoS One. 2011; 6: e27229. **PubMed:** https://www.ncbi.nlm.nih.gov/pubmed/22073295

14. Stray Pedersen B. Toxoplasmosis in pregnancy. Baillieres Clin Obstet Gynaecol. 1993; 7: 107-137. **PubMed:** https://www.ncbi.nlm.nih.gov/pubmed/8513640

15. Liesenfeld O, Press C, Montoya JG, Gill R, Isaac Renton JL, Hedman K, et al. False-positive results in immunoglobulin M (IgM) toxoplasma antibody tests and importance of confirmatory testing: the Platelia Toxo IgM test. J Clin Microbiol. 1997; 35: 174-178. **PubMed:** https://www.ncbi.nlm.nih.gov/pubmed/8968902

16. Chene G, Thiebaut R. Options for clinical trials of pre and post-natal treatments for congenital toxoplasmosis. Mem Inst Oswaldo Cruz. 2009; 104: 299-304. **PubMed:** https://www.ncbi.nlm.nih.gov/pubmed/19430657

17. Wallon M, Liou C, Garner P, Peyron F. Congenital toxoplasmosis: systematic review of evidence of efficacy of treatment in pregnancy. BMJ. 1999; 318: 1511-1514. **PubMed:** https://www.ncbi.nlm.nih.gov/pubmed/10356003

18. Holland GN. Ocular toxoplasmosis: new directions for clinical investigation. Ocul Immunol Inflamm. 2000; 8: 1-7. **PubMed:** https://www.ncbi.nlm.nih.gov/pubmed/10896456

19. Schoondermark van de Ven EM, Melchers WJ, Galama JM, Meuwissen JH, Eskes TK. Prenatal diagnosis and treatment of congenital Toxoplasma gondii infections: an experimental study in rhesus monkeys. Eur J Obstet Gynecol Reprod Biol. 1997; 74: 183-188. **PubMed:** https://www.ncbi.nlm.nih.gov/pubmed/9306115

20. Laura A Cox, Anthony G Comuzzie, Lorena M Havill, Genesio M Karere, Kimberly D Spradling, Michael C Mahaney, et al. Baboons as a model to study genetics and epigenetics of human disease. ILAR Journal. 2013; 106-121. https://tinyurl.com/yargxvcw

21. Dormehl IC, Hugo N, Beverley G. The baboon: an ideal model in biomedical research. Anesth Pain Control Dent. 1992; 1: 109-115. **PubMed:** https://www.ncbi.nlm.nih.gov/pubmed/1392685

22. Kamau DM, Kagira J, Maina N, Mutura S, Mokua J, Karanja S. Detection of natural toxoplasma gondii infection in olive baboons (papio anubis) in Kenya using nested PCR. The 11th JKUAT Scientific, Technological and Industrialization Conference and Exhibitions Conference Proceedings. 2016. https://tinyurl.com/y8gfp7px

23. John Mokua Mose, John Maina Kagira , Simon Muturi Karanja, Maina Ngotho, David Muchina Kamau, Adele Nyambura Njuguna, et al. Detection of natural toxoplasma gondii infection in chicken in Thika region of Kenya using nested polymerase chain reaction. BioMed Research International. 2016. https://tinyurl.com/y7xu5q9j

24. Beermann F, Orlow SJ, Lamoreux ML. The Tyr (albino) locus of the laboratory mouse. Mamm Genome. 2004; 15: 749-758. **PubMed:** https://www.ncbi.nlm.nih.gov/pubmed/15520878

25. Subauste C. Animal models for Toxoplasma gondii infection. Current Protocols in Immunology. 2012; Chapter 19: Unit 19.3.1-23. https://europepmc.org/article/med/22314833

26. Dubey JP. Advances in the life cycle of Toxoplasma gondii. Int J Parasitol. 1998; 28: 1019-1024. **PubMed:** https://www.ncbi.nlm.nih.gov/pubmed/9724872

27. Vasilakopoulou A, le Roux CW. Could a virus contribute to weight gain? Int J Obes (Lond). 2007; 31: 1350-1356. **PubMed:** https://www.ncbi.nlm.nih.gov/pubmed/17420782

28. Dhurandhar NV. Infectobesity: obesity of infectious origin. J Nutr. 2001; 131: 2794S-2797S. **PubMed:** https://www.ncbi.nlm.nih.gov/pubmed/11584109

29. Hermes-Uliana C, Pereira-Severi LS, Luerdes RB, Franco CL, da Silva AV, Araújo EJ, et al. Chronic infection with Toxoplasma gondii causes myenteric neuroplasticity of the jejunum in rats. Auton Neurosci. 2011; 160: 3-8. **PubMed:** https://www.ncbi.nlm.nih.gov/pubmed/20932812

30. Reeves GM, Mazaheri S, Snitker S, Langenberg P, Giegling I, Hartmann AM, et al. A positive association between T. gondii seropositivity and obesity. Front. Public Health. 2013; 1: 73. **PubMed:** https://www.ncbi.nlm.nih.gov/pubmed/24400300

31. Milovanovic I, Vujanic M, Klun I, Bobic B, NikolicA, IvovicV, et al. Toxoplasma gondii infection induces lipid metabolism alterations in the murine host. Mem Inst Oswaldo Cruz. 2009; 104: 175-178. **PubMed:** https://www.ncbi.nlm.nih.gov/pubmed/19430640

32. Soheilian M, Heidari K, Yazdani S, Shahsavari M, Ahmadieh H, Dehghan M. Patterns of uveitis in a tertiary eye care center in Iran. Ocul Immunol Inflamm. 2004; 12: 297-310. **PubMed:** https://www.ncbi.nlm.nih.gov/pubmed/15621869

33. Vallochi AL, Muccioli C, Martins MC, Silveira C, Belfort R Jr, Rizzo LV. The genotype of Toxoplasma gondii strains causing ocular toxoplasmosis in humans in Brazil. Am J Ophthalmol. 2005; 139: 350-351. **PubMed:** https://www.ncbi.nlm.nih.gov/pubmed/15734002

34. E.S. Perkins. Ocular toxoplasmosis. Br J Ophthalmol. 1973; 57: 1-17. https://tinyurl.com/y94tpcd4

35. Holland GN, Engstrom RE Jr, Glasgow BJ, Berger BB, Daniels SA, Sidikaro Y, et al. Ocular toxoplasmosis in patients with the acquired immunodeficiency syndrome. Am J Ophthalmol. 1988; 106: 653-667. **PubMed:** https://www.ncbi.nlm.nih.gov/pubmed/3195645

36. Nussenblatt RB, Belfort R Jr. Ocular toxoplasmosis. An old disease revisited. JAMA. 1994; 271: 304-307. **PubMed:** https://www.ncbi.nlm.nih.gov/pubmed/8295291

37. Montoya JG, Remington JS. Toxoplasmic chorioretinitis in the setting of acute acquired toxoplasmosis. Clin Infect Dis. 1996; 23: 277-282. **PubMed:** https://www.ncbi.nlm.nih.gov/pubmed/8842263

38. Paul M. Immunoglobulin g avidity in diagnosis of toxoplasmic lymphadenopathy and ocular toxoplasmosis. Clin Diagn Lab Immunol. 1999; 6: 514-518. **PubMed:** https://www.ncbi.nlm.nih.gov/pubmed/10391853/

39. Ruth E Gilbert, Miles R Stanford. Is ocular toxoplasmosis caused by prenatal or postnatal infection? Br J Ophthalmol. 2000; 84: 224-226. https://tinyurl.com/ybymgzry

40. Holland GN. Ocular toxoplasmosis: new directions for clinical investigation. Ocul Immunol Inflamm. 2000; 8: 1-7. **PubMed:** https://www.ncbi.nlm.nih.gov/pubmed/10896456

41. Couvreur J, Thulliez P. Acquired toxoplasmosis of ocular or neurologic site: 49 cases. Presse Med. 1996; 25: 438-442. **PubMed:** https://www.ncbi.nlm.nih.gov/pubmed/8685192

42. Howe DK, Sibley DL. Toxoplasma gondii comprises three clonal lineages: correlation of parasite genotype with human disease. J Infect Dis. 1995; 172: 1561-1566. **PubMed:** https://www.ncbi.nlm.nih.gov/pubmed/7594717

43. Belfort Neto R, Nussenblatt V, Rizzo L, Muccioli C, Silveira C, Nussenblatt R, et al. High prevalence of unusual genotypes of Toxoplasma gondii infection in pork meat samples from Erechim, Southern Brazil. An Acad Bras Cienc. 2007; 79: 111-114. **PubMed:** https://www.ncbi.nlm.nih.gov/pubmed/17401480

44. Carsten G K Luder, Taibur Rahman. Impact of the host on Toxoplasma stage differentiation. Microb Cell. 2017; 4: 203-211. **PubMed:** https://www.ncbi.nlm.nih.gov/pubmed/28706936/

45. Dubey JP, Ferreira LR, Alsaad M, Verma SK, Alves DA, Holland GN, et al.

Experimental toxoplasmosis in rats induced orally with eleven strains of toxoplasma gondii of seven genotypes: tissue tropism, tissue cyst size, neural lesions, tissue cyst rupture without reactivation, and ocular lesions. PLoS One. 2016; 11: e0156255. **PubMed:** https://www.ncbi.nlm.nih.gov/pubmed/27228262

46. Liu Q, Wang ZD, Huang SY, Zhu XQ. Diagnosis of toxoplasmosis and typing of *Toxoplasma gondii*. Parasit Vectors. 2015; 8: 292. **PubMed:** https://www.ncbi.nlm.nih.gov/pubmed/26017718

47. Burrells A, Taroda A, Opsteegh M, Schares G, Benavides J, Dam Deisz C, et al. Detection and dissemination of Toxoplasma gondii in experimentally infected calves, a single test does not tell the whole story. Parasit Vectors. 2018; 11: 45. **PubMed:** https://www.ncbi.nlm.nih.gov/pubmed/29347971/

48. DJP Ferguson, WM Hutchison. An ultrastructural study of the early development and tissue cyst formation of Toxoplasma gondii in the brains of mice. Parasitology Research. 1987; 73: 483-491. https://tinyurl.com/y8k6uec6

49. Schluter D, Deckert M, Hof H, Frei K. Toxoplasma gondii infection of neurons induces neuronal cytokine and chemokine production, but gamma interferon- and tumor necrosis factor-stimulated neurons fail to inhibit the invasion and growth of *T. gondii*. Infect Immun. 2001; 69: 7889-7893. **PubMed:** https://www.ncbi.nlm.nih.gov/pubmed/11705972

50. Rall GF, Mucke L, Oldstone MB. Consequences of cytotoxic T lymphocyte interaction with major histocompatibility complex class I-expressing neurons in vivo. J Exp Med. 1995; 182: 1201-1212. **PubMed:** https://www.ncbi.nlm.nih.gov/pubmed/7595191

51. Abgrall S, Rabaud C, Costagliola D, Clinical epidemiology group of the French hospital database on HIV. Incidence and risk factors for toxoplasmic encephalitis in human immunodeficiency virus-infected patients before and during the highly active antiretroviral therapy era. Clin. Infect. Dis. 2001; 33: 1747-1755. **PubMed:** https://www.ncbi.nlm.nih.gov/pubmed/11595976

52. Madalyn M McFarland, Maggie L Bartlett, Paul H Davis. Toxoplasmic encephalitis. Encephalitis. 2016. https://tinyurl.com/ya36wlwo

53. Bhadra R, Cobb DA, Weiss LM, Khan IA. Psychiatric disorders in Toxoplasma seropositive patients-the CD8 connection. Schizophr Bull. 2013; 39: 485-489. **PubMed:** https://www.ncbi.nlm.nih.gov/pubmed/23427221

54. Del Grande C, Galli L, Schiavi E, Dell'Osso L, Bruschi F. Is Toxoplasma gondii a trigger of bipolar disorder? Pathogens. 2017; 6: pii: E3. **PubMed:** https://www.ncbi.nlm.nih.gov/pubmed/28075410

55. Arling TA, Yolken RH, Lapidus M, Langenberg P, Dickerson FB, Zimmerman SA, et al. Toxoplasma gondii antibody titers and history of suicide attempts in patients with recurrent mood disorders. J Nerv Ment Dis. 2009; 197: 905-908. **PubMed:** https://www.ncbi.nlm.nih.gov/pubmed/20010026

56. Okusaga O, Duncan E, Langenberg P, Brundin L, Fuchs D, Groer MW, et al. Combined Toxoplasma gondii seropositivity and high blood kynurenine-Linked with nonfatal suicidal self-directed violence in patients with schizophrenia. J Psychiatr Res. 2016; 72: 74-81. **PubMed:** https://www.ncbi.nlm.nih.gov/pubmed/26594873

57. Yolken RH, Dickerson FB, Fuller Torrey E. Toxoplasma and schizophrenia. Parasite Immunol. 2009; 31: 706-715. **PubMed:** https://www.ncbi.nlm.nih.gov/pubmed/19825110

58. Torrey EF, Bartko JJ, Yolken RH. Toxoplasma gondii and other risk factors for schizophrenia: An update. Schizophr. Bull. 2012; 38: 642-647. **PubMed:** https://www.ncbi.nlm.nih.gov/pubmed/22446566

59. Sutterland AL, Fond G, Kuin A, Koeter MW, Lutter R, van Gool T, et al. Beyond the association. Toxoplasma gondii in schizophrenia, bipolar disorder, and addiction: systematic review and meta analysis. Acta Psychiatr Scand. 2015; 132: 161-179. **PubMed:** https://www.ncbi.nlm.nih.gov/pubmed/25877655

60. Cristina Cabanacan Salibay, Janina Karla dela Cruz Advincula, Samira Yaser Perez Iewida. Serologic detection of Toxoplasma gondii infection in stray and household cats and its hematologic evaluation. Scientia Medica. 2010; 20: 76-82. https://tinyurl.com/yamg763w

61. S. Javadi, S. Asri Rezaei, H. Tajik M. Hadian, F. Shokouhi. Haematological changes of cats with Toxoplasma gondii-specific antibodies. Comparative Clinical Pathology. 2010; 19: 307-310. https://tinyurl.com/y7by7tqf

62. Nurgul Atmaca, Miyase Cinar, Bayram Guner, Ruhi Kabakci, Gazyagci A, Hasan Tarık Atmaca, et al. Evaluation of oxidative stress, hematological and biochemical parameters during Toxoplasma gondii infection in gerbils. Ankara Univ Vet Fak Derg. 2015; 62: 165-170. https://tinyurl.com/y73nvux2

63. Lappin MR. Feline toxoplasmosis: interpretation of diagnostic results. Semin Vet Med Surg (Small Anim). 1996; 11:154-160. **PubMed:** https://www.ncbi.nlm.nih.gov/pubmed/8942211

64. E. A. Okewole, M.O. Akpan. Clinical feline toxoplasmosis: parasitological, haematological and serological findings in retro-viral infected and uninfected cats. VETERINARSKI ARHIV. 2002; 72: 67-79. https://tinyurl.com/y9etqqqu

65. Miller KL, Silverman PH, Kullgren B, Mahlmann LJ. Tumor necrosis factor alpha and the anemia associated with murine malaria. Infect Immun. 1989; 57: 1542-1546. **PubMed:** https://www.ncbi.nlm.nih.gov/pubmed/2707858

66. Artavanis-Tsakonas K, Tongren JE, Riley EM. The war between the malaria parasite and the immune system: Immunity, immunoregulation and immunopathology. Clin Exp Immunol. 2003; 133: 145-152. **PubMed:** https://www.ncbi.nlm.nih.gov/pubmed/12869017

67. Malvezi AD, Cecchini R, de Souza F, Tadokoro CE, Rizzo LV, Pinge Filho P. Involvement of nitric oxide (NO) and TNF-alpha in the oxidative stress associated with anemia in experimental Trypanosoma cruzi infection. FEMS Immunol Med Microbiol 2004; 41: 69-77. **PubMed:** https://www.ncbi.nlm.nih.gov/pubmed/15094169

68. Wang Z, Zhang DX, Zhao Q. Infection-stimulated anemia results primarily from Interferon Gamma-dependent, signal transducer and activator of transcription 1-independent red cell loss. Chin Med J (Engl). 2015; 128: 948-955. **PubMed:** https://www.ncbi.nlm.nih.gov/pubmed/25836617

69. Petakov M, Stojanovic N, Jovcic G, Bugarski D, Todorovic V, Djurkovic Djakovic O. Hematopoiesis during acute Toxoplasma gondii infection in mice. Haematologia (Budap). 2002; 32: 439-455. **PubMed:** https://www.ncbi.nlm.nih.gov/pubmed/12803118

70. Greene, CE. (1990). Infectious Diseases of the Dog and Cat. W.B. Saunders Company, London, UK

71. Azeem Shahzad, Muhammad Sarwar Khan, Kamran Ashraf, Muhammad Avais, Khalid Pervez Jawaria Ali Khan. Sero-epidemiological and haematological studies on toxoplasmosis in cats, dogs and their owners in Lahore, Pakistan. J. Protozool. Res. 2006; 16: 60-73. https://tinyurl.com/yayfjr2m