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Systematic Review and Meta-Analysis of Variation in *Toxoplasma gondii* Cyst Burden in the Murine Model

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Abstract

Toxoplasma gondii is an obligate intracellular protozoan parasite that infects approximately 30% of the population of the United States, with worldwide distribution. The chronic (latent) infection, mediated by the bradyzoite parasite life stage, has attracted attention due to possible links to host behavioral alteration and psychomotor effects. Mice are a common model organism for studying the chronic stage, as they are natural hosts of infection. Notably, published studies demonstrate vast ranges of measured cyst burden within the murine brain tissue. The inconsistency of measured cyst burden within and between experiments makes interpretation of statistical significance difficult, potentially confounding studies of experimental anti-parasitic approaches. This review analyzes variation in measured cyst burden in a wide array of experimental mouse infections across published literature. Factors such as parasite infection strain, mouse strain, mode of infection, and infectious dose were all examined. The lowest variation in measured cyst burden occurred with the commonly available Balb/c and CBA mice undergoing infection by the ME49 strain of *T. gondii*. A summary of cyst variation and average cyst counts in *T. gondii* mouse models is presented, which may be useful for designing future experiments.

Keywords

Toxoplasma gondii; Murine Models; Cyst Variability; Method of Infection; Chronic Infection

1. INTRODUCTION

Toxoplasma gondii (*T. gondii*) is an intracellular protozoan parasite capable of causing infection worldwide in nearly all warm-blooded vertebrates, typically through oral transmission of bradyzoite cysts or oocysts [Su, 2003]. Members of the Felidae family are considered the definitive host where *T. gondii* can sexually reproduce, resulting in oocyst production expelled through defecation. Prevalence in humans varies by age of individuals and geographic region due to differences in exposure to cat droppings containing *T. gondii*

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Conflicts of interest: none

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oocysts in contaminated food or water; undercooked meats bearing bradyzoite tissue cysts also serve as a significant reservoir [Gontijo da Silva, 2015].

Once the [oo]cysts are ingested, the parasites transition to the tachyzoite stage in the intestines and disseminate through the hosts' tissue and bloodstream, eventually forming chronic infection in the muscle and brain tissue, characterized by the parasite's bradyzoite stage [Randall, 2011]. Tissue cysts begin forming as early as a week after infection in the murine brain tissue and persist for the remainder of the host's life. Cysts are known to increase diameter during the latent stage of infection in the brains of mice over time, possibly rupturing and reforming cysts in an immunocompetent host [Watts, 2015]. Immunocompromised individuals are at high risk of recrudescent disease due to intracellular brain cysts which can reactivate and potentially cause lethal destruction of neural tissue [Montoya, 2004].

The latent stage is an important parasitic stage for study, as it has been correlated with reduced response time of the host [Flegr, 2007]. Moreover, in rodent species, the fear response has been shown to be attenuated when the host has acquired an infection with *T. gondii*, leading to an increased probability of predation by cats, where the parasite can sexually reproduce [Gatkowska, 2012; Vyas, 2007]. *T. gondii* tissue cysts are commonly studied in the murine model, due to the animal's natural infection in the wild with the majority of tissue cysts present in the brain [Dubey, 1998]. However, current experimental studies attempting to model or reduce cyst burden via treatment in mice frequently report significant variation of cyst counts between infected hosts. Such variability can be a chief limiting factor when interpreting the efficacy of experimental anti-parasitic interventions such as drug or vaccine candidates.

Methods for inducing bradyzoite stage infection in mice also vary considerably between reports in the literature. However, there has not been a body of work which categorizes the various infection models of *T. gondii* and correlates them to cyst load variability. We have compiled and analyzed literature describing latent infection in murine models and noted the corresponding cyst levels and variance. Key variables include: parasite stage during infection, mouse and parasite strain, and method of infection. Such data may prove valuable to future experimental designs to establish greater statistical significance for experimental treatments or neurological evaluations.

2. Materials and Methods

A comprehensive review of literature in PubMed identified papers pertaining to *Toxoplasma gondii* variability of cyst levels in a murine model using keywords: [*Toxoplasma gondii* cyst (variability) and (levels) and (burden) in mice] (October 2017). This search criteria returned 150 papers where 62 met criteria for study inclusion: reported mean cyst burden with standard deviation and written in English. The majority of these papers focus on the ability of drugs or drug-like molecules to reduce cyst burden or the behavioral effects in relation to cyst burden. Literature detailing parasite burden measured by qPCR was not considered, as the focus of this review is cyst number, and not total number of parasites. Literature providing cyst variability with standard error of mean or undefined or non-present error bars

were also excluded. In cases where data was organized non-quantitatively (i.e. figure only) ImageJ (1.8.0_112) measurement tool was used with axial figure units to extrapolate the data of interest (mean and standard deviation). In the case of experimental group sample sizes that were reported in a range, the lowest number was used for analysis. Exotic parasite strains that were not tested in multiple strains of mice were not analyzed in figures but instead only listed in the charts. The coefficient of variation (CV) (standard deviation/mean *100) provides the ratio of standard deviation to mean.

3. CONDITIONS AFFECTING INFECTION

3.1 Parasite Strain

Classical Type II and III *T. gondii* strains are well known to form latent infections and constitute the majority of published works. *T. gondii* strains commonly seen in Europe and North America are either type I, II, or III and will carry genetic variation up to a maximum of 1% [Su, 2012; Saeji, 2005]. *T. gondii* type II strains are most commonly seen in immunocompromised patients in the first world and remain relatively non-lethal most likely due to the production of a less severe parasite burden on the individual compared to Type I/III strains [Saeji, 2005; Xiao, 2015]. Strain virulence is not a constant factor: it is known that continuous passage in animals can increase virulence compared to cell passage, but little research has established the significance or consistency of these changes [Albert, 1941].

3.2 Mouse Strain

It is known that the major histocompatibility complex (MHC) genes capable of triggering an effective Th1 response are primarily responsible for resistance to infection, relative cyst load, and the severity of lethal encephalitis [Resende, 2008; Miller, 2009]. Thus, differing mouse strains are known to present diverse symptoms and severity of disease, regardless of infection administration. Mouse strain responses are briefly summarized below.

Balb/c mice (an inbred strain) are commonly used to study toxoplasmosis due in part to their resistance against the latent infection, suggested to be a result of their MHC haplotype (D) [Resende, 2008; Affymetrix Inc.]. Balb/c are resistant to acute infection through oral inoculation and susceptible via the intraperitoneal route with cysts of the ME49 strain [Subauste, 2012]. However, they generally show resistance to chronic infection, ultimately leading to reduced cyst load [Subauste, 2012]. For instance, in Suzuki et al. (1993), Balb/c mice formed fewer cysts (213 ± 151) in the brain during latent infection compared to CBA/Ca ($3,015 \pm 1,704$) when inoculated with 10 cysts IP.

The inbred strain **C3H** with MHC haplotype H^k , appear to be more resistant to acute challenge when challenged IP as compared to orally with 10 cysts, with nearly all mice surviving up to 21 days post inoculation [Johnson, 1984; Affymetrix Inc.]. In mice receiving the IP injections consisting of cysts, C3H mice were only slightly more resistant than Balb/c and significantly more resistant than Swiss Webster (SW) [Johnson, 1984].

C57BL/6, inbred mice with a H^b haplotype, are susceptible to acute infection via the oral route with ME49 cysts but resistant when administered IP [Subauste, 2012]. Unlike Balb/c, these mice are susceptible to chronic infection leading to an increase in cyst burden

[Subauste, 2012]. They are more susceptible to acute toxoplasmosis and weight loss than Balb/c, with a majority expiring after 60 days following acute infection of 5 DX *T. gondii* cysts [Gatkowska, 2006; Affymetrix Inc]. This mouse strain is proven to be more susceptible to lethal acute disease via oral infection compared to IP of 10 cysts, with oral infection causing around a 70% mortality [Johnson, 1984].

The inbred strain **CBA** mice (H^k) typically are sensitive to *T. gondii* infection and die several months after infection from *T. gondii* ME49 cysts through encephalitis [Suzuki, 1993; Affymetrix Inc], potentially due to their relatively high average of 3,000 cysts per brain from IP infection of 20 ME49 cysts [Subauste, 2012].

No significant difference was seen in outbred **Swiss Webster (SW)** mice infected orally or IP with the same infective dose (10 cysts), although a slightly higher mortality rate was seen with IP infection [Johnson, 1984]. Regarding oral acute infection, SW have greater survival than C3H and C57BL strains [Johnson, 1984].

The **Kunming** strain of outbred mice are exclusively used in Asia and present with a susceptibility to the acute stage of *T. gondii* [Wang, 2017]. When Kunming mice were infected with 1,000 or more RH strain (hyper-virulent) tachyzoites they typically died within a week [Li, 2015]. The mouse strain's susceptibility to chronic infection is not clearly stated, mainly due to lack of articles studying Kunming mice.

3.3 Method of Infection Variability

Most infections using mice are delivered with intraperitoneal or oral inoculation, both with tachyzoites grown in tissue culture or bradyzoite cysts harvested from infected tissue. The route of infection determines dissemination of the parasites and the initial symptoms within the murine model. Mouse strains that are relatively more susceptible to oral inoculation are not necessarily susceptible to IP infection; however, IP infection generally leads to a more potent and rapid response compared to peroral. Mice infected via IP injection with 10^6 type II tachyzoites showed that parasites were confined to the abdomen for the first week and seen in the brain by day 12, forming cysts in clustered locations within the brain [Di Cristina, 2008]. There appears to be more inconsistency when mice are orally inoculated, although this may be obfuscated as only a limited number of studies report whether mice were gavaged or fed orally. This clarification is important considering oral gavage leads to inconsistent migration of the parasite compared to the more natural method of feeding: when mice were orally gavaged, within a week many parasites were seen through *in vivo* imaging in the chest cavity, while only a subset initially demonstrated parasites in the intestines. This is starkly contrasted to natural feeding, where all the mice had parasites in their intestines within a week followed by the brain, regardless of the strain [Boyle, 2007].

4. VARIABILITY

4.1 Parasite Strain Variability

The *T. gondii* strain ME49 produces the lowest brain cyst variability in Balb/c, C3H, and CBA mice compared to other *Toxoplasma* strains, either through oral or intraperitoneal inoculation of cysts, or orally with tachyzoites. Kunming, Balb/c, and Swiss Webster mice

display relatively low cyst variability when infected with *T. gondii* PRU strain (Tables 1, 2, and 3). Of note, when Swiss Webster and Balb/c were infected with Pru ku80, mice formed substantially less cysts than when infected with wildtype PRU parasites (Tables 2 and 3). Specifically, Swiss Webster have an average coefficient of variance (CV) of 34.7% when inoculated IP with PRU tachyzoites, compared to PRU cysts infecting Kunming mice orally with a variance of 8.8%. Balb/c mice present with a low variance when infected orally with PRU cysts (15.2%) and IP with PRU tachyzoites (25.8%). In contrast, the highest cyst variance displayed in the CBA strain at 45.6% was caused from IP infection with PRU tachyzoites (Table 4). The 76K *T. gondii* strain was tested only in C57BL (Table 5) and CBA mice, exhibiting the lowest CV in C57BL mice at 23.4%. DX cysts were injected IP produced the highest variance in C3H (Table 6) and C57BL mice. An uncommonly used *T. gondii* strain, Beverly, administered orally with cyst established the largest variance in Balb/c and a relatively low variance (24.5%) in C57BL/6 mice.

Coefficient of Variation utilized the mean and standard deviation seen from cyst variability to determine the ratio of variability, as a percent. Kunming mice were consistently infected with the *T. gondii* PRU strain via oral cysts, with typically low variation. Most variables were kept constant; isolation of brain at four weeks post infection and a three to seven sample size.

The coefficient of variation compared the ratio of the mean and standard deviation, from the cyst variability, and calculated a percentage. A majority of papers infected Swiss Webster mice with ME49 cysts and isolated brains at various weeks post infection.

Studies were ordered based upon parasite strain followed by method of infection. Cyst variability (mean $\pm$ standard deviation) determined the coefficient of variation, shown as a percent. A majority of papers administered ME49 cysts intraperitoneally, with lower variability occurring in smaller inoculation doses.

ME49 is the most common *T. gondii* strain inoculated in C57BL when testing for cyst burden (mean $\pm$ standard deviation). The ratio of standard deviation to the mean is represented in the coefficient of variation values, as a percentage. A few sources did not mention to the sample size, denoted as N.D.

A small portion of papers reviewed tested cyst burden in C3H mice utilizing three strains of *T. gondii*. Cyst variability is listed in terms of mean $\pm$ standard deviation, from which the coefficient of variation ratio is determined, as a percentage. Most of the variation values are similar except when C3H mice were infected with IPB-G, generating a large mean with relatively low standard deviation.

Cyst burden in the Balb/c model is ordered by parasite strain followed by method of inoculation, identifying the number and route of infection. The Coefficient of Variation is determined by the ratio of cyst variability (mean $\pm$ average) and is represented as a percent. Balb/c displayed the lowest cyst variance particularly when infected with ME49 tachyzoites orally and intraperitoneally, along with oral PRU cysts in one study.

4.2 Mouse Strain Variability & Cyst Burden

The lowest cyst variation identified occurred in CBA mice infected IP with 10 ME49 cysts, producing a CV less than 2 (Figure 1). However, when the dose was increased a larger range of variation, 33% - 84%, was observed. In contrast, Kunming mice produced overall the lowest cyst variability compared to other mouse strains studied, generating an average CV value of 8% (Table 7), while also producing the highest cyst burdens. Notably, the Kunming strain is not utilized or available in most countries. Balb/c mice produced the next overall lowest cyst variability compared to other mouse strains studied, generating a CV value of 20% (Figure 2). This value decreases to 15% when mice are infected with the *T. gondii* strain ME49, specially orally with 1,000 tachyzoites (6%) and 20 cysts IP (4%). Swiss Webster mice, an outbred strain, produce the highest variability in CV value at 60%. Mouse strains not commonly utilized are detailed in Table 8.

4.3 Method of Infection Variability

The method of infection does not appear to be a primary determinant in cyst variability. Infecting orally with tachyzoites, a method typically limited to Balb/c, produced the lowest variance in this review. Inbred mouse strains generally experience reduced variability when infected with cysts, either orally or intraperitoneally. The opposite can be seen in the outbred Swiss Webster: tachyzoites injected intraperitoneally exhibited lower variance than cysts administered orally or intraperitoneally.

5. Conclusion

Based on the collected data, our results indicate excessive variation seen in the Swiss Webster mouse strain (as expected with an outbred strain) in contrast to reduced relative variability in the Kunming, CBA, and Balb/c in-bred strains. The coefficient of variation (a ratio of standard deviation to the mean) was lowest in the Kunming strain, although this strain is used almost exclusively in Asia currently. CBA exhibited low variance when infected with 10 cysts of the ME49 strain, but as the dose increased, so also did the CV value. The more widely used mouse strain Balb/c produced low variation when ME49 cysts were infected intraperitoneally (IP). Along with mouse strain variance, parasite strain was also analyzed and ME49 produced the lowest fluctuation in half the mouse strains. This information will aid researchers to construct more optimized protocols with the *T. gondii*-murine model of the latent infection; which will allow improved investigation of interventions to reduce cyst burden or study behavioral changes.

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References

- Abdelbaset AE, Fox BA, Karraam MH, Abd Ellah MR, Bzik DJ, Igarashi M. Lactate dehydrogenase in *Toxoplasma gondii* controls virulence, bradyzoite differentiation, and chronic infection. *PLoS One*. 2017 3 21;12(3):e0173745. doi: 10.1371/journal.pone.0173745. [PubMed: 28323833]

- Affymetrix Inc. (n.d.). Mouse Haplotype Table. Retrieved from http://tools.thermofisher.com/content/sfs/brochures/Mouse_Haplotype_Table.pdf
- Sabin Albert B. Toxoplasmic Encephalitis in Children. *JAMA*. 1941;116(9):801–807. doi:10.1001/jama.1941.02820090001001
- Aldebert D, Hypolite M, Cavailles P, Touquet B, Flori P, Loeuillet C, Cesbron-Delauw MF. Development of high-throughput methods to quantify cysts of *Toxoplasma gondii*. *Cytometry A*. 2011 11;79(11):952–8. doi: 10.1002/cyto.a.21138. [PubMed: 21905211]
- Araujo FG, Huskinson-Mark J, Gutteridge WE, Remington JS. In vitro and in vivo activities of the hydroxynaphthoquinone 566C80 against the cyst form of *Toxoplasma gondii*. *Antimicrob Agents Chemother*. 1992 2;36(2):326–30. [PubMed: 1605598]
- Berenreiterová M, Flegr J, Kub na AA, N mec P. The distribution of *Toxoplasma gondii* cysts in the brain of a mouse with latent toxoplasmosis: implications for the behavioral manipulation hypothesis. *PLoS One*. 2011;6(12):e28925. doi: 10.1371/journal.pone.0028925. [PubMed: 22194951]
- Bernardes ES, Silva NM, Ruas LP, Mineo JR, Loyola AM, Hsu DK, Liu FT, Chammas R, Roque-Barreira MC. *Toxoplasma gondii* infection reveals a novel regulatory role for galectin-3 in the interface of innate and adaptive immunity. *Am J Pathol*. 2006 6;168(6):1910–20. [PubMed: 16723706]
- Bhadra R, Cobb DA, Khan IA. Donor CD8+ T cells prevent *Toxoplasma gondii* de-encystation but fail to rescue the exhausted endogenous CD8+ T cell population. *Infect Immun*. 2013 9;81(9):3414–25. doi: 10.1128/IAI.00784-12. [PubMed: 23817617]
- Bonenfant C, Dimier-Poisson I, Velge-Roussel F, Buzoni-Gatel D, Del Giudice G, Rappuoli R, Bout D. Intranasal immunization with SAG1 and nontoxic mutant heat-labile enterotoxins protects mice against *Toxoplasma gondii*. *Infect Immun*. 2001 3;69(3):1605–12. [PubMed: 11179334]
- Boyle JP, Saeij JP, Boothroyd JC. *Toxoplasma gondii*: inconsistent dissemination patterns following oral infection in mice. *Exp Parasitol*. 2007 7;116(3):302–5. [PubMed: 17335814]
- Brinkmann V, Remington JS, Sharma SD. Protective immunity in toxoplasmosis: correlation between antibody response, brain cyst formation, T-cell activation, and survival in normal and B-cell-deficient mice bearing the H-2k haplotype. *Infect Immun*. 1987 4;55(4):990–4. [PubMed: 3493977]
- Buchholz KR, Bowyer PW, Boothroyd JC. Bradyzoite pseudokinase 1 is crucial for efficient oral infectivity of the *Toxoplasma gondii* tissue cyst. *Eukaryot Cell*. 2013 3;12(3):399–410. doi: 10.1128/EC.00343-12. [PubMed: 23291621]
- Chen J, Li ZY, Petersen E, Huang SY, Zhou DH, Zhu XQ. DNA vaccination with genes encoding *Toxoplasma gondii* antigens ROP5 and GRA15 induces protective immunity against toxoplasmosis in Kunming mice. *Expert Rev Vaccines*. 2015 4;14(4):617–24. doi: 10.1586/14760584.2015.1011133. [PubMed: 25749394]
- Chew WK, Segarra I, Ambu S, Mak JW. Significant reduction of brain cysts caused by *Toxoplasma gondii* after treatment with spiramycin coadministered with metronidazole in a mouse model of chronic toxoplasmosis. *Antimicrob Agents Chemother*. 2012 4;56(4):1762–8. doi: 10.1128/AAC.05183-11. [PubMed: 22271863]
- Chew WK, Wah MJ, Ambu S, Segarra I. *Toxoplasma gondii*: determination of the onset of chronic infection in mice and the in vitro reactivation of brain cysts. *Exp Parasitol*. 2011 1;130(1):22–5. doi: 10.1016/j.exppara.2011.10.004. [PubMed: 22027550]
- Clemente M, Curilovic R, Sassone A, Zelada A, Angel SO, Mentaberry AN. Production of the main surface antigen of *Toxoplasma gondii* in tobacco leaves and analysis of its antigenicity and immunogenicity. *Mol Biotechnol*. 2005 5;30(1):41–50. [PubMed: 15805575]
- Couper KN, Nielsen HV, Petersen E, Roberts F, Roberts CW, Alexander J. DNA vaccination with the immunodominant tachyzoite surface antigen (SAG-1) protects against adult acquired *Toxoplasma gondii* infection but does not prevent maternofetal transmission. *Vaccine*. 2003 6 20;21(21–22):2813–20. [PubMed: 12798622]
- Di Cristina M, Marocco D, Galizi R, Proietti C, Spaccapelo R, Crisanti A. Temporal and spatial distribution of *Toxoplasma gondii* differentiation into Bradyzoites and tissue cyst formation in vivo. *Infect Immun*. 2008 8;76(8):3491–501. doi: 10.1128/IAI.00254-08. [PubMed: 18505811]

- Dias RR, Carvalho EC, Leite CC, Tedesco RC, Calabrese Kda S, Silva AC, DaMatta RA, de Fatima Sarro-Silva M. *Toxoplasma gondii* oral infection induces intestinal inflammation and retinochoroiditis in mice genetically selected for immune oral tolerance resistance. *PLoS One*. 2014 12 1;9(12):e113374. doi: 10.1371/journal.pone.0113374. [PubMed: 25437299]
- Djurkovi -Djakovi O, Milenkovi V, Nikoli A, Bobi B, Gruji J. Efficacy of atovaquone combined with clindamycin against murine infection with a cystogenic (Me49) strain of *Toxoplasma gondii*. *J Antimicrob Chemother*. 2002 12;50(6):981–7. [PubMed: 12461021]
- Doggett JS, Nilsen A, Forquer I, Wegmann KW, Jones-Brando L, Yolken RH, Bordón C, Charman SA, Katneni K, Schultz T, Burrows JN, Hinrichs DJ, Meunier B, Carruthers VB, Riscoe MK. Endochin-like quinolones are highly efficacious against acute and latent experimental toxoplasmosis. *Proc Natl Acad Sci U S A*. 2012 9 25;109(39):15936–41. [PubMed: 23019377]
- Dubey JP, Lindsay DS, Speer CA. Structures of *Toxoplasma gondii* tachyzoites, bradyzoites, and sporozoites and biology and development of tissue cysts. *Clin Microbiol Rev*. 1998 4;11(2):267–99. [PubMed: 9564564]
- Dziadek B, Gatkowska J, Brzostek A, Dziadek J, Dzitko K, Grzybowski M, Dlugonska H. Evaluation of three recombinant multi-antigenic vaccines composed of surface and secretory antigens of *Toxoplasma gondii* in murine models of experimental toxoplasmosis. *Vaccine*. 2011 1 17;29(4):821–30. doi: 10.1016/j.vaccine.2010.11.002. [PubMed: 21087690]
- Dziadek B, Gatkowska J, Grzybowski M, Dziadek J, Dzitko K, Dlugonska H. *Toxoplasma gondii*: the vaccine potential of three trivalent antigen-cocktails composed of recombinant ROP2, ROP4, GRA4 and SAG1 proteins against chronic toxoplasmosis in BALB/c mice. *Exp Parasitol*. 2012 5;131(1):133–8. doi: 10.1016/j.exppara.2012.02.026. [PubMed: 22445587]
- Eissa MM, Barakat AM, Amer EI, Younis LK. Could miltefosine be used as a therapy for toxoplasmosis? *Exp Parasitol*. 2015 10;157:12–22. doi: 10.1016/j.exppara.2015.06.005. [PubMed: 26112396]
- EL-Malky MA, Al-Harhi SA, Mohamed RT, EL Bali MA, Saudy NS. Vaccination with *Toxoplasma* lysate antigen and CpG oligodeoxynucleotides: comparison of immune responses in intranasal versus intramuscular administrations. *Parasitol Res*. 2014 6;113(6):2277–84. doi: 10.1007/s00436-014-3882-0. [PubMed: 24728558]
- El-Malky M, Shaohong L, Kumagai T, Yabu Y, Noureldin MS, Saudy N, Maruyama H, Ohta N. Protective effect of vaccination with *Toxoplasma* lysate antigen and CpG as an adjuvant against *Toxoplasma gondii* in susceptible C57BL/6 mice. *Microbiol Immunol*. 2005;49(7):639–46. [PubMed: 16034207]
- El-Sayed NM, Ismail KA, Badawy AF, Elhasanein KF. In vivo effect of anti-TNF agent (etanercept) in reactivation of latent toxoplasmosis. *J Parasit Dis*. 2016 12;40(4):1459–1465. [PubMed: 27876967]
- El-Zawawy LA, El-Said D, Mossallam SF, Ramadan HS, Younis SS. Preventive prospective of triclosan and triclosan-liposomal nanoparticles against experimental infection with a cystogenic ME49 strain of *Toxoplasma gondii*. *Acta Trop*. 2015 1;141(Pt A):103–11. doi: 10.1016/j.actatropica.2014.09.020. [PubMed: 25305510]
- Flegr J Effects of toxoplasma on human behavior. *Schizophr Bull* 2007 5;33(3):757–60. [PubMed: 17218612]
- Gatkowska J, Hyszczynska-Sawicka E, Kur J, Holec L, Dlugonska H. *Toxoplasma gondii*: an evaluation of diagnostic value of recombinant antigens in a murine model. *Exp Parasitol*. 2006 11;114(3):220–7. [PubMed: 16707125]
- Gatkowska J, Wiczorek M, Dziadek B, Dzitko K, Dlugonska H. Behavioral changes in mice caused by *Toxoplasma gondii* invasion of brain. *Parasitol Res*. 2012 7;111(1):53–8. doi: 10.1007/s00436-011-2800-y. [PubMed: 22223035]
- Gontijo da Silva M, Clare Vinaud M, de Castro AM. Prevalence of toxoplasmosis in pregnant women and vertical transmission of *Toxoplasma gondii* in patients from basic units of health from Gurupi, Tocantins, Brazil, from 2012 to 2014. *PLoS One*. 2015 11 11;10(11):e0141700. doi: 10.1371/journal.pone.0141700. [PubMed: 26558622]
- Han Y, Zhou A, Lu G, Zhao G, Wang L, Guo J, Song P, Zhou J, Zhou H, Cong H, He S. Protection via a ROM4 DNA vaccine and peptide against *Toxoplasma gondii* in BALB/c mice. *BMC Infect Dis*. 2017 1 11;17(1):59. doi: 10.1186/s12879-016-2104-z. [PubMed: 28077075]

- Hrdá S, Votýpka J, Kodým P, Flegr J. Transient nature of *Toxoplasma gondii*-induced behavioral changes in mice. *J Parasitol.* 2000 8;86(4):657–63. [PubMed: 10958436]
- Huang S, Holmes MJ, Radke JB, Hong DP, Liu TK, White MW, Sullivan WJ, Jr. *Toxoplasma gondii* AP2IX-4 Regulates Gene Expression during Bradyzoite Development. *mSphere.* 2017 3 15;2(2). pii: e00054–17. doi: 10.1128/mSphere.00054-17. [PubMed: 28317026]
- Jebbari H, Roberts CW, Ferguson DJ, Bluethmann H, Alexander J. A protective role for IL-6 during early infection with *Toxoplasma gondii*. *Parasite Immunol.* 1998 5;20(5):231–9. [PubMed: 9651924]
- Johnson AM. Strain-dependent, route of challenge-dependent, murine susceptibility to toxoplasmosis. *Z Parasitenkd.* 1984;70(3):303–9. [PubMed: 6741220]
- Jones NG, Wang Q, Sibley LD. Secreted protein kinases regulate cyst burden during chronic toxoplasmosis. *Cell Microbiol.* 2017 2;19(2). doi: 10.1111/cmi.12651.
- Kim JH, Lee J, Bae SJ, Kim Y, Park BJ, Choi JW, Kwon J, Cha GH, Yoo HJ, Jo EK, Bae YS, Lee YH, Yuk JM. NADPH oxidase 4 is required for the generation of macrophage migration inhibitory factor and host defense against *Toxoplasma gondii* infection. *Sci Rep.* 2017 7 25;7(1):6361. doi: 10.1038/s41598-017-06610-4. [PubMed: 28743960]
- Lee DH, Kim AR, Lee SH, Quan FS. Cross-protection induced by *Toxoplasma gondii* virus-like particle vaccine upon intraperitoneal route challenge. *Acta Trop.* 2016 12;164:77–83. doi: 10.1016/j.actatropica.2016.08.025. [PubMed: 27586039]
- Li XZ, Wang XH, Xia LJ, Weng YB, Hernandez JA, Tu LQ, Li LT, Li SJ, Yuan ZG. Protective efficacy of recombinant canine adenovirus type-2 expressing TgROP18 (CAV-2-ROP18) against acute and chronic *Toxoplasma gondii* infection in mice. *BMC Infect Dis.* 2015 3 4;15:114. doi: 10.1186/s12879-015-0815-1. [PubMed: 25886737]
- Lu G, Zhou J, Zhou A, Han Y, Guo J, Song P, Zhou H, Cong H, Hou M, Wang L, He S. SAG5B and SAG5C combined vaccine protects mice against *Toxoplasma gondii* infection. *Parasitol Int.* 2017 10;66(5):596–602. doi: 10.1016/j.parint.2017.06.002. [PubMed: 28602862]
- Luo F, Zheng L, Hu Y, Liu S, Wang Y, Xiong Z, Hu X, Tan F. Induction of Protective Immunity against *Toxoplasma gondii* in Mice by Nucleoside Triphosphate Hydrolase-II (NTPase-II) Self-amplifying RNA Vaccine Encapsulated in Lipid Nanoparticle (LNP). *Front Microbiol.* 2017 4 5;8:605. doi: 10.3389/fmicb.2017.00605. [PubMed: 28424680]
- Makino M, Uemura N, Moroda M, Kikumura A, Piao LX, Mohamed RM, Aosai F. Innate immunity in DNA vaccine with *Toxoplasma gondii*-heat shock protein 70 gene that induces DC activation and Th1 polarization. *Vaccine.* 2011 2 24;29(10):1899–905. doi: 10.1016/j.vaccine.2010.12.118. [PubMed: 21236236]
- Martínez-Gómez F, García-González LF, Mondragón-Flores R, Bautista-Garfias CR. Protection against *Toxoplasma gondii* brain cyst formation in mice immunized with *Toxoplasma gondii* cytoskeleton proteins and *Lactobacillus casei* as adjuvant. *Vet Parasitol.* 2009 3 23;160(3–4):311–5. doi: 10.1016/j.vetpar.2008.11.017. [PubMed: 19111993]
- Martins-Duarte ES, Lemgruber L, de Souza W, Vommario RC. *Toxoplasma gondii*: fluconazole and itraconazole activity against toxoplasmosis in a murine model. *Exp Parasitol.* 2010 4;124(4):466–9. doi: 10.1016/j.exppara.2009.12.011. [PubMed: 20045696]
- Miller CM, Smith NC, Ikin RJ, Boulter NR, Dalton JP, Donnelly S. Immunological interactions between 2 common pathogens, Th1-inducing protozoan *Toxoplasma gondii* and the Th2-inducing helminth *Fasciola hepatica*. *PLoS One.* 2009 5 25;4(5):e5692. doi: 10.1371/journal.pone.0005692. [PubMed: 19478853]
- Moiré N, Dion S, Lebrun M, Dubremetz JF, Dimier-Poisson I. Mic1–3KO tachyzoite a live attenuated vaccine candidate against toxoplasmosis derived from a type I strain shows features of type II strain. *Exp Parasitol.* 2009 10;123(2):111–7. doi: 10.1016/j.exppara.2009.06.003. [PubMed: 19508866]
- Mokua Mose J, Muchina Kamau D, Kagira JM, Maina N, Ngotho M, Njuguna A, Karanja SM. Development of Neurological Mouse Model for Toxoplasmosis Using *Toxoplasma gondii* Isolated from Chicken in Kenya. *Patholog Res Int.* 2017;2017:4302459. doi: 10.1155/2017/4302459. [PubMed: 28630781]
- Montoya JG, Liesenfeld O. (2004) Toxoplasmosis. *The Lancet* 262:1965–1976.

- Mouveaux T, Oria G, Werkmeister E, Slomianny C, Fox BA, Bzik DJ, Tomavo S. Nuclear glycolytic enzyme enolase of *Toxoplasma gondii* functions as a transcriptional regulator. *PLoS One*. 2014 8 25;9(8):e105820. doi: 10.1371/journal.pone.0105820. [PubMed: 25153525]
- Pinzan CF, Sardinha-Silva A, Almeida F, Lai L, Lopes CD, Lourenço EV, Panunto-Castelo A, Matthews S, Roque-Barreira MC. Vaccination with Recombinant Microneme Proteins Confers Protection against Experimental Toxoplasmosis in Mice. *PLoS One*. 2015 11 17;10(11):e0143087. doi: 10.1371/journal.pone.0143087. [PubMed: 26575028]
- Randall LM, Hunter CA. Parasite dissemination and the pathogenesis of toxoplasmosis. *Eur J Microbiol Immunol (Bp)*. 2011 3;1(1):3–9. doi: 10.1556/EuJMI.1.2011.1.3. [PubMed: 24466431]
- Resende MG, Fux B, Caetano BC, Mendes EA, Silva NM, Ferreira AM, Melo MN, Vitor RW, Gazzinelli RT. The role of MHC haplotypes H2d/H2b in mouse resistance/susceptibility to cyst formation is influenced by the lineage of infective *Toxoplasma gondii* strain. *An Acad Bras Cienc*. 2008 3;80(1):85–99. [PubMed: 18345378]
- Roberts CW, Alexander J. Studies on a murine model of congenital toxoplasmosis: vertical disease transmission only occurs in BALB/c mice infected for the first time during pregnancy. *Parasitology*. 1992 2;104 Pt 1:19–23. [PubMed: 1614736]
- Saeij JP, Boyle JP, Boothroyd JC. Differences among the three major strains of *Toxoplasma gondii* and their specific interactions with the infected host. *Trends Parasitol*. 2005 10;21(10):476–81. [PubMed: 16098810]
- Schultz TL, Hencken CP, Woodard LE, Posner GH, Yolken RH, Jones-Brando L, Carruthers VB. A thiazole derivative of artemisinin moderately reduces *Toxoplasma gondii* cyst burden in infected mice. *J Parasitol*. 2014 8;100(4):516–21. doi: 10.1645/13-451.1. [PubMed: 24524228]
- Scorza T, D'Souza S, Laloup M, Dewit J, De Braekeleer J, Verschueren H, Vercammen M, Huygen K, Jongert E. A GRA1 DNA vaccine primes cytolytic CD8(+) T cells to control acute *Toxoplasma gondii* infection. *Infect Immun*. 2003 1;71(1):309–16. [PubMed: 12496180]
- Singh U, Brewer JL, Boothroyd JC. Genetic analysis of tachyzoite to bradyzoite differentiation mutants in *Toxoplasma gondii* reveals a hierarchy of gene induction. *Mol Microbiol*. 2002 5;44(3):721–33. [PubMed: 11994153]
- Su C, Evans D, Cole RH, Kissinger JC, Ajioka JW, Sibley LD. Recent expansion of *Toxoplasma* through enhanced oral transmission. *Science*. 2003 1 17;299(5605):414–6. [PubMed: 12532022]
- Su C, Khan A, Zhou P, Majumdar D, Ajzenberg D, Dardé ML, Zhu XQ, Ajioka JW, Rosenthal BM, Dubey JP, Sibley LD. Globally diverse *Toxoplasma gondii* isolates comprise six major clades originating from a small number of distinct ancestral lineages. *Proc Natl Acad Sci U S A*. 2012 4 10;109(15):5844–9. doi: 10.1073/pnas.1203190109. [PubMed: 22431627]
- Subauste C Animal models for *Toxoplasma gondii* infection. *Curr Protoc Immunol*. 2012 2;Chapter 19:Unit 19.3.1–23. doi: 10.1002/0471142735.im1903s96.
- Sugi T, Ma YF, Tomita T, Murakoshi F, Eaton MS, Yakubu R, Han B, Tu V, Kato K, Kawazu S, Gupta N, Suvorova ES, White MW, Kim K, Weiss LM. *Toxoplasma gondii* Cyclic AMP-Dependent Protein Kinase Subunit 3 Is Involved in the Switch from Tachyzoite to Bradyzoite Development. *MBio*. 2016 5 31;7(3). pii: e00755–16. doi: 10.1128/mBio.00755-16.
- Suzuki Y, Orellana MA, Wong SY, Conley FK, Remington JS. Susceptibility to chronic infection with *Toxoplasma gondii* does not correlate with susceptibility to acute infection in mice. *Infect Immun*. 1993 6;61(6):2284–8. [PubMed: 8500870]
- Velge-Roussel F, Moretto M, Buzoni-Gatel D, Dimier-Poisson I, Ferrer M, Hoebeke J, Bout D. Differences in immunological response to a *T. gondii* protein (SAG1) derived peptide between two strains of mice: effect on protection in *T. gondii* infection. *Mol Immunol*. 1997 10;34(15):1045–53. [PubMed: 9519763]
- Vyas A, Kim SK, Giacomini N, Boothroyd JC, Sapolsky RM. Behavioral changes induced by *Toxoplasma* infection of rodents are highly specific to aversion of cat odors. *Proc Natl Acad Sci U S A*. 2007 4 10;104(15):6442–7. [PubMed: 17404235]
- Walker R, Gissot M, Croken MM, Huot L, Hot D, Kim K, Tomavo S. The *Toxoplasma* nuclear factor TgAP2XI-4 controls bradyzoite gene expression and cyst formation. *Mol Microbiol*. 2013 2;87(3):641–55. doi: 10.1111/mmi.12121. [PubMed: 23240624]

- Wang JL, Elsheitkha HM, Zhu WN, Chen K, Li TT, Yue DM, Zhang XX, Huang SY, Zhu XQ. Immunization with *Toxoplasma gondii* GRA17 Deletion Mutant Induces Partial Protection and Survival in Challenged Mice. *Front Immunol* 2017 6 29;8:730. doi: 10.3389/fimmu.2017.00730. [PubMed: 28706518]
- Wang M, Yang XY, Zhang NZ, Zhang DL, Zhu XQ. Evaluation of Protective Immune Responses Induced by Recombinant TrxLp and ENO2 Proteins against *Toxoplasma gondii* Infection in BALB/c Mice. *Biomed Res Int*. 2016;2016:3571962. [PubMed: 27803923]
- Wang T, Yin H, Li Y, Zhao L, Sun X, Cong H. Vaccination with recombinant adenovirus expressing multi-stage antigens of *Toxoplasma gondii* by the mucosal route induces higher systemic cellular and local mucosal immune responses than with other vaccination routes. *Parasite*. 2017;24:12. doi: 10.1051/parasite/2017013. [PubMed: 28367800]
- Watts E, Zhao Y, Dhara A, Eller B, Patwardhan A, Sinai AP. Novel Approaches Reveal that *Toxoplasma gondii* Bradyzoites within Tissue Cysts Are Dynamic and Replicating Entities In Vivo. *MBio*. 2015 9 8;6(5):e01155–15. doi: 10.1128/mBio.01155-15. [PubMed: 26350965]
- Witting PA. Learning capacity and memory of normal and *Toxoplasma*-infected laboratory rats and mice. *Z Parasitenkd*. 1979;61(1):29–51. [PubMed: 543216]
- Xiao J, Li Y, Prandovszky E, Kannan G, Viscidi RP, Pletnikov MV, Yolken RH. Behavioral Abnormalities in a Mouse Model of Chronic Toxoplasmosis Are Associated with MAG1 Antibody Levels and Cyst Burden. *PLoS Negl Trop Dis*. 2016 4 28;10(4):e0004674. doi: 10.1371/journal.pntd.0004674. [PubMed: 27124472]
- Xiao J, Yolken RH. Strain hypothesis of *Toxoplasma gondii* infection on the outcome of human diseases. *Acta Physiol (Oxf)*. 2015 4;213(4):828–45. doi: 10.1111/apha.12458. [PubMed: 25600911]
- Xu Y, Zhang NZ, Tan QD, Chen J, Lu J, Xu QM, Zhu XQ. Evaluation of immuno-efficacy of a novel DNA vaccine encoding *Toxoplasma gondii* rhoptry protein 38 (TgROP38) against chronic toxoplasmosis in a murine model. *BMC Infect Dis*. 2014 9 30;14:525. doi: 10.1186/1471-2334-14-525. [PubMed: 25267356]
- Yang WB, Zhou DH, Zou Y, Chen K, Liu Q, Wang JL, Zhu XQ, Zhao GH. Vaccination with a DNA vaccine encoding *Toxoplasma gondii* ROP54 induces protective immunity against toxoplasmosis in mice. *Acta Trop*. 2017 12;176:427–432. doi: 10.1016/j.actatropica.2017.09.007. [PubMed: 28935555]
- Yin H, Zhao L, Wang T, Zhou H, He S, Cong H. A *Toxoplasma gondii* vaccine encoding multistage antigens in conjunction with ubiquitin confers protective immunity to BALB/c mice against parasite infection. *Parasit Vectors*. 2015 9 30;8:498. doi: 10.1186/s13071-015-1108-7. [PubMed: 26420606]
- Zenner L, Darcy F, Capron A, Cesbron-Delauw MF. *Toxoplasma gondii*: kinetics of the dissemination in the host tissues during the acute phase of infection of mice and rats. *Exp Parasitol*. 1998 9;90(1):86–94. [PubMed: 9709034]
- Zhang G, Huong VT, Battur B, Zhou J, Zhang H, Liao M, Kawase O, Lee EG, Dautu G, Igarashi M, Nishikawa Y, Xuan X. A heterologous prime-boost vaccination regime using DNA and a vaccinia virus, both expressing GRA4, induced protective immunity against *Toxoplasma gondii* infection in mice. *Parasitology*. 2007 9;134(Pt 10):1339–46. [PubMed: 17506929]
- Zhang NZ, Huang SY, Zhou DH, Chen J, Xu Y, Tian WP, Lu J, Zhu XQ. Protective immunity against *Toxoplasma gondii* induced by DNA immunization with the gene encoding a novel vaccine candidate: calcium-dependent protein kinase 3. *BMC Infect Dis*. 2013 10 31;13:512. doi: 10.1186/1471-2334-13-512. [PubMed: 24176018]
- Zhang NZ, Xu Y, Wang M, Petersen E, Chen J, Huang SY, Zhu XQ. Protective efficacy of two novel DNA vaccines expressing *Toxoplasma gondii* rhomboid 4 and rhomboid 5 proteins against acute and chronic toxoplasmosis in mice. *Expert Rev Vaccines*. 2015;14(9):1289–97. doi: 10.1586/14760584.2015.1061938. [PubMed: 26111968]

Highlights

- *Toxoplasma gondii* chronic infection displays varied cyst numbers between mouse strains.
- The parasite strain, ME49, produces the lowest cyst variation in 3 of the 6 mouse strains evaluated.
- No method of infection was identified to substantially reduce the diverse cyst load amount in the brain.
- The Kunming mouse strain established the most consistent cyst burden but is rarely used outside Asia.

Figure 1. CBA Cyst Variability.

As an illustration, cyst burden is displayed in CBA mice and the coefficient of variation was determined for each parasite strain and mode of inoculation tested. Sample size is denoted as the thickness of the circles. Mean coefficient of variation values are denoted beside each variable, with larger values expressing a greater variance of standard deviation from the mean. CV values for each sample set were averaged to form representative means for each variable (e.g. parasite strain, mode of inoculation). In the CBA strain high variance was seen in all conditions even when a large sample size infected ME49 cysts intraperitoneal.

Figure 2. Balb/c Cyst Variability.

As an illustration, cyst burden is displayed in Balb/c mice and the coefficient of variation was determined for each parasite strain and mode of inoculation tested. Sample size is denoted as the thickness of the circles. Mean coefficient of variation values are denoted beside each variable, with larger values expressing a greater variance of standard deviation from the mean. CV values for each sample set were averaged to form representative means for each variable (e.g. parasite strain, mode of inoculation). In the Balb/c strain high variance was seen in all conditions except when mice were infected with ME49 cysts intraperitoneal or orally with ME49 tachyzoites.

Table 1.

Kunming Variability in Cyst Load.

Kunming						
Cyst Count (+SD)	Coefficient of Variation (Percentage)	Infection Method	Parasite Strain	Brain Isolation in Weeks Post Infection	Sample Size	Reference
18080 ± 642	4	Oral 5 cysts	PRU	4	5	Li, 2015
3963 ± 37	1	Oral 10 cysts	PRU	4	6	Yang, 2017
3876 ± 37	1	Oral 10 cysts	PRU	4	6	Yang, 2017
3167 ± 672	21	Oral 10 cysts	PRU	4	6	Xu, 2014
3232 ± 534	17	Oral 10 cysts	PRU	4	6	Xu, 2014
3167 ± 259	8	Oral 10 cysts	PRU	4	3	Zhang, 2013
3233 ± 197	6	Oral 10 cysts	PRU	4	3	Zhang, 2013
1956 ± 44	2	Oral 10 cysts	PRU	4	3	Chen, 2015
1933 ± 67	4	Oral 10 cysts	PRU	4	3	Chen, 2015
3337 ± 183	6	Oral 10 cysts	PRU	4	6	Zhang, 2015
3276 ± 231	7	Oral 10 cysts	PRU	4	6	Zhang, 2015
4296 ± 687	16	Oral 20 cysts	PRU	5	6	Wang, 2017

Table 2.

Balb/c Strain Cyst Variability.

Balb/c						
Cyst Count (+SD)	Coefficient of Variation (Percentage)	Infection Method	Parasite Strain	Brain Isolation in Weeks Post Infection	Sample Size	Reference
193 ± 58	30	Oral 20 cysts	Beverly	8	N.D.	Roberts, 1992
122 ± 79	65	10/20 cysts oral/IP	Beverly	4	10	Couper, 2003
192 ± 67	35	IP 5 cysts	DX	4	10	Dziadek, 2012
63.5 ± 6.758	11	IP 15 cysts	Kenya chx	2	4	Mokua, 2017
115.8 ± 12.53	11	IP 15 cysts	Kenya chx	3	4	Mokua, 2017
192.8 ± 47.23	25	IP 15 cysts	Kenya chx	4	4	Mokua, 2017
345 ± 56.42	16	IP 15 cysts	Kenya chx	5	4	Mokua, 2017
416.3 ± 32.2	8	IP 15 cysts	Kenya chx	6	4	Mokua, 2017
216 ± 8	4	IP 20 cysts	ME49	4	6	Lee, 2016
250 ± 107	43	Oral 20 cysts	ME49	6	N.D.	Resende, 2008
237 ± 106	45	Oral 4 cysts	ME49	6	N.D.	Resende, 2008
1198 ± 153	13	Oral 1000 tachys	ME49	4	9	Chew, 2012
885 ± 70	8	Oral 1000 tachys	ME49	2	10	Chew, 2011
1489 ± 35	2	Oral 1000 tachys	ME49	4	10	Chew, 2011
1587 ± 46	3	Oral 1000 tachys	ME49	8	10	Chew, 2011
1621 ± 63	4	Oral 1000 tachys	ME49	16	10	Chew, 2011
640 ± 72	11	IP 10 ³ tachys	Pru Ku80	N.D.	4	Abdelbaset, 2017
231 ± 116	50	IP 10 ⁴ tachys	Pru Ku80	N.D.	6	Abdelbaset, 2017
169 ± 27	16	IP 10 ⁶ tachy	Pru Ku80	5	4	Abdelbaset, 2017
260 ± 124	48	N.D. 5×10 ² tachys	Pru Ku80	4	9	Mouveaux, 2014
721 ± 257	36	Oral 10 cysts	PRU	4	6	Wang, 2016
1340 ± 225	17	Oral 20 cysts	PRU	4	12	Lu, 2017
1860 ± 60	3	Oral 20 cysts	PRU	4	6	Wang, 2017
1747 ± 133	8	Oral 20 cysts	PRU	4	6	Wang, 2017
1924 ± 82	4	Oral 20 cysts	PRU	4	6	Wang, 2017
2050 ± 57	3	Oral 20 cysts	PRU	4	6	Wang, 2017
1955 ± 120	6	Oral 20 cysts	PRU	4	6	Wang, 2017
1283 ± 193	15	Oral 20 cysts	PRU	4	10	Han, 2017
2350 ± 757	32	Oral 20 cysts	PRU	6	6	Luo, 2017
1735 ± 495	29	Oral 20 cysts	PRU	4	6	Yin, 2015

Table 3.

Cyst Variability in Swiss Webster Mice.

Swiss Webster						
Cyst Count (+SD)	Coefficient of Variation (Percentage)	Infection Method	Parasite Strain	Brain Isolation in Weeks Post Infection	Sample Size	Reference
560 ± 57	10	IP 10 cyst	ME49	4	10	El-Sayad, 2016
9914 ± 6209	63	IP 20 cysts	ME49	4	7	Martins-Duarte, 2010
13611 ± 21260	156	IP 20 cysts	ME49	4	7	Martins-Duarte, 2010
4966 ± 4095	82	IP 20 cysts	ME49	4	11	Martins-Duarte, 2010
14090 ± 19767	140	IP 20 cysts	ME49	4	9	Martins-Duarte, 2010
2000 ± 500	25	IP 20 cysts	ME49	14	5	Araujo, 1992
750 ± 100	13	IP 20 cysts	ME49	16	5	Araujo, 1992
750 ± 100	13	IP 20 cysts	ME49	26	5	Araujo, 1992
450 ± 100	22	IP 20 cysts	ME49	28	5	Araujo, 1992
14.4 ± 2.9	20	Oral 10 cysts	ME49	8	20	Eissa, 2015
12.48 ± 5.72	46	Oral 10 cysts	ME49	8	10	El-Zawawy, 2015
11.37 ± 6.24	55	Oral 10 cysts	ME49	8	10	El-Zawawy, 2015
1289 ± 1579	123	Oral 10 cysts	ME49	4	15	Djurkovi -Djakovi , 2002
1315 ± 1315	100	Oral 20 cysts	ME49	7	15	Djurkovi -Djakovi , 2002
1782 ± 1695	95	Oral 20 cysts	ME49	7	15	Djurkovi -Djakovi , 2002
95 ± 33	35	IP 500 tachys	Pru Ku80	3	4	Singh, 2002
1083 ± 609	56	Oral N.D.	PRU	8	4	Aldebert, 2011
1143 ± 553	48	Oral N.D.	PRU	8	5	Aldebert, 2011
912 ± 371	41	IP 30 cysts	Weiss	6	30	Witting, 1979

Table 4.

Cyst Variability in the CBA Strain.

CBA						
Cyst Count (+SD)	Coefficient of Variation (Percentage)	Infection Method	Parasite Strain	Brain Isolation in Weeks Post Infection	Sample Size	Reference
10250 ± 3140	31	Oral 70 cysts	76K	4	10	Bonenfant, 2001
2309 ± 825	36	Oral 100 cysts	76K	4	40	Velge-Roussel, 1997
1451 ± 16	1	IP 10 cysts	ME49	5	3	Araujo, 1992
1373 ± 26	2	IP 10 cysts	ME49	6	3	Araujo, 1992
1342 ± 37	3	IP 10 cysts	ME49	7	3	Araujo, 1992
1353 ± 68	5	IP 10 cysts	ME49	9	3	Araujo, 1992
1451 ± 41	3	IP 10 cysts	ME49	11	3	Araujo, 1992
1306 ± 42	3	IP 10 cysts	ME49	13	3	Araujo, 1992
1368 ± 42	3	IP 10 cysts	ME49	15	3	Araujo, 1992
1150 ± 52	5	IP 10 cysts	ME49	17	3	Araujo, 1992
1400 ± 737	53	IP 18 cysts	ME49	7	7	Schultz, 2014
1673 ± 854	51	IP 18 cysts	ME49	7	6	Schultz, 2014
1251 ± 419	34	IP 18 cysts	ME49	9	10	Schultz, 2014
2523 ± 894	35	IP 18 cysts	ME49	7	19	Doggett, 2012
3888 ± 3268	84	IP 18–20 cysts	ME49	3	29	Watts, 2015
3072 ± 2170	71	IP 18–20 cysts	ME49	4	29	Watts, 2015
3342 ± 1499	45	IP 18–20 cysts	ME49	5	12	Watts, 2015
3086 ± 1829	59	IP 18–20 cysts	ME49	6	15	Watts, 2015
2540 ± 1567	62	IP 18–20 cysts	ME49	8	14	Watts, 2015
112 ± 73	65	IP 20 cysts	ME49	6	6	Brinkmann, 1987
29.5 ± 10	34	IP 20 cysts	ME49	6	7	Brinkmann, 1987
14 ± 13	93	IP 10 ⁴ tachys	Mic1–3KO	6	3	Moiré, 2009
5 ± 7	140	IP 10 ³ tachys	Mic1–3KO	6	4	Moiré, 2009
2 ± 1	50	IP 10 ² tachys	Mic1–3KO	6	8	Moiré, 2009
369 ± 224	61	IP 200 tachys	Pru Ku80	4	15	Jones, 2017
164 ± 63	38	IP 2.5×10 ⁴ tachys	Pru Ku80	4	3	Buchholz, 2013
435 ± 230	53	IP 2.5×10 ⁴ tachys	Pru Ku80	8	6	Buchholz, 2013

Table 5.

Cyst Variability in C57BL Mice.

C57BL/6						
Cyst Count (+SD)	Coefficient of Variation (Percentage)	Infection Method	Parasite Strain	Brain Isolation in Weeks Post Infection	Sample Size	Reference
2448 ± 573	23	Oral 10 cysts	76K	4	20	Velge-Roussel, 1997
13433 ± 3306	25	Oral 85 cysts	Beverly	5	N.D.	El-Malky, 2005
13295 ± 3237	24	Oral 85 cysts	Beverly	5	14	El-Malky, 2014
2273 ± 1361	60	IP 5 cysts	DX	4	7	Dziadek, 2011
1142 ± 320	28	Oral 10 cysts	Fukaya	6	3	Makino, 2011
1100 ± 260	24	Oral 10 cysts	Fukaya	6	3	Makino, 2011
391 ± 188	48	IP 40 cysts	ME49	2	7	Kim, 2017
3193 ± 412	13	Oral 10 cysts	ME49	3	6	Bhadra, 2013
5609 ± 693	12	Oral 10 cysts	ME49	5	6	Bhadra, 2013
1083 ± 402	37	Oral 10 cysts	ME49	7	6	Bhadra, 2013
3471 ± 1559	45	Oral 10 cysts	ME49	10	6	Bhadra, 2013
1002 ± 111	11	Oral 40 cysts	ME49	4	N.D.	Pinzan, 2015
500 ± 100	20	IP 20,000 tachys	PLK	6	2	Zhang, 2007
210 ± 91	43	IP 10,000 tachys	Pru Ku80	7	4	Sugi, 2016
248 ± 63	25	IP 200 tachys	Pru Ku80	5	5	Walker, 2013
142 ± 92	65	IP 200 tachys	Pru Ku80	4	18	Jones, 2017
139 ± 91	66	IP 200 tachys	Pru Ku80	4	18	Jones, 2017

Table 6.

C3H Cyst Variability.

C3H						
Cyst Count (+SD)	Coefficient of Variation (Percentage)	Infection Method	Parasite Strain	Brain Isolation in Weeks Post Infection	Sample Size	Reference
2055 ± 723	35	IP 5 cysts	DX	4	7	Dziadek, 2011
5793 ± 347	6	IP 40 cysts	IPB-G	6	4	Scorza, 2003
25 ± 8	32	IP 20 cysts	ME49	6	5	Brinkmann, 1987
4550 ± 1400	31	Oral 20 cysts	ME49	4	5	Clemente, 2005

Table 7.

Cyst Variability Summary

	Average	Median	Range	Maximum	Minimum
Balb/c					
Cyst Count	913	681	2287	2350	64
CV	20	14	62	65	2
C3H					
Cyst Count	3106	3303	5768	5793	25
CV	26	31	29	35	6
C57BL/6					
Cyst Count	2922	1100	13294	13433	139
CV	34	25	55	66	11
CBA					
Cyst Count	1750	1368	10248	10250	2
CV	41	38	139	140	1
Kunming					
Cyst Count	4460	3255	16147	18080	1933
CV	8	6	20	21	1
Swiss Webster					
Cyst Count	2881	1083	14079	14090	11
CV	60	48	146	156	10

Cyst count and CV numbers were analyzed for their average, median, range, maximum, and minimum values.

Table 8

Various mouse stain cyst variability

Mouse Strain	Cyst Count (+SD)	Coefficient of Variation (Percentage)	Infection Method	Parasite Strain	Brain Isolation in Weeks Post Infection	Sample Size	Reference
Balb/k	3120 ± 766	25	oral 20 cysts	Beverly	8	N.D.	Roberts (1992)
CD-1	111 ± 72	65	IP 500 tachys	GT1	20	11	Xiao (2016)
CD-1	611 ± 402	66	oral 10 cysts	HIF	18	7	Berenreiterová (2011)
CD-1	883 ± 938	106	oral 10 cysts	HIF	18	7	Berenreiterová (2011)
F1 cross	100 ± 66	66	oral 1 cyst	01529/38	3	6	Hrdá (2000)
F1 cross	124 ± 42	34	oral 1 cyst	01529/38	6	10	Hrdá (2000)
F1 cross	97 ± 39	40	oral 1 cyst	01529/38	12	7	Hrdá (2000)
Gal3+/+	840 ± 145	17	oral 20 cysts	ME49	2	5	Bemardes (2006)
Gal3-/-	4408 ± 1266	29	oral 20 cysts	ME49	2	5	Bemardes (2006)
IL-6 +/+	2080 ± 960	46	oral 20 cyst	Beverly	4	4	Jebbari (1998)
IL-6 -/-	10079 ± 6079	60	oral 20 cyst	Beverly	4	4	Jebbari (1998)
NIH	3181 ± 89	3	oral 20 cysts	ME49	8	21	Martínez-Gómez (2009)
TR	122 ± 50	45	oral 50 cysts	ME49	12	6	Dias (2014)
TR	258 ± 55	21	oral 100 cysts	ME49	12	6	Dias (2014)
TS	249 ± 59	24	oral 50 cysts	ME49	12	6	Dias (2014)
TS	449 ± 72	16	oral 100 cysts	ME49	12	6	Dias (2014)

Several mouse strains were not commonly mentioned throughout literature and are seen in this table. The coefficient of variation took the mean and standard deviation, seen under cyst variability, to produce a ratio indicating percent variability in cyst burden.