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Investigation of infectivity of neonates and adults from different rat strains to *Toxoplasma gondii* Prugniaud shows both variation which correlates with iNOS and Arginase-1 activity and increased susceptibility of neonates to infection

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ABSTRACT

Mouse models differ considerably from humans with regard to clinical symptoms of toxoplasmosis caused by *Toxoplasma gondii* and, by comparison, the rat model is more representative of this disease in humans. In the present study, we found that different strains of adult and newborn rats (Lewis, Wistar, Sprague Dawley, Brown Norway and Fischer 344) exhibited remarkable variation in the number of brain cysts following inoculation with the *T. gondii* Prugniaud strain. In adult rats, large numbers of cysts (1231±165.6) were observed in Fischer 344, but none in the other four. This situation was different in newborn rats aged from 5 to 20 days old. All Fischer 344 and Brown Norway newborns were cyst-positive while cyst-positive infection in Sprague Dawley neonates ranged from 54.5% to 60% depending on their age at infection. In Wistar and Lewis rat neonates, however, cyst-positivity rates of 0% to 42.9% and 0% to 25% were found respectively. To investigate whether rat strain differences in infectivity could be related to inherent strain and genetic differences in the host immune response, we correlated our data with previously reported strain differences in iNOS/Arginase ratio in adult rats and found them to be linked. These results show that interactions between host genetic background and age of rat influence *T. gondii* infection.

Keywords: Toxoplasma gondii Prugniaud strain; cyst; neonate rats; host resistance; iNOS; Arginase-1.

1. **Introduction**

Toxoplasma gondii is an obligatory intracellular apicomplexan parasite that infects almost all warm-blooded vertebrates, including mammals and birds. It is considered that *T. gondii* is one of the most successful eukaryotic pathogens based on the wide range of host species and high prevalence in these species worldwide. Human infections with *T. gondii* are primarily caused by ingesting undercooked meat containing viable tissue cysts or by ingestion of food or water contaminated with oocysts in faeces shed from infected cats. It is widely reported that up to one-third of the world's population are estimated to be chronically infected (Dubey and Beattie, 1988; Dubey, 2004) and pregnant women are highly at risk in endemic areas due to the cause of congenital birth defects by toxoplasmosis (Pappas et al. 2009; Gao et al. 2012).

Acute parasitic infection with *T. gondii* is usually not found in immunocompetent individual humans, who can mount an effective immune response to clear most tachyzoites but not bradyzoites which remain in tissue cysts. Cysts of *T. gondii* can develop in varied organs and tissues, particularly in the brain or skeletal muscles, which can later be reactivated if immunosuppression (e. g. AIDS, cancer therapy or organ transplantation) occurs. This reactivation of latent infection can cause life-threatening toxoplasmic encephalitis and related diseases (Gianotti et al. 1997; Supiot et al. 1997; Dubey et al. 2006). Increasingly evidence indicates that *T. gondii*

67 infection is strongly linked to serious recurrent ocular disease in some regions of
68 Southern Brazil (Jones et al. 2006; Dubey et al. 2012) and to a risk of schizophrenia
69 (Torrey and Yolken, 2003; Torrey et al. 2007).
70 Infection by *T. gondii* differs profoundly between species (Sepulveda-Arias et al.
71 2008). There is evidence that not only the immune status, but also the genetic
72 predisposition of the hosts influence the clinical outcome of *T. gondii* infection
73 (Kempf et al. 1999). Mice, for instance, are susceptible to *T. gondii* infection. All
74 strains of mouse, as far as we know, die from the infection by the virulent type I
75 strains e. g. the RH strain of *T. gondii* (Sibley and Boothroyd, 1992). However, they
76 can also develop chronic infections if they are inoculated with low doses of the less
77 virulent type II strains such as the Prugniaud and ME49 strains or the type III strains
78 such as the VEG strain (Saeij et al. 2005).
79 For many reasons, the majority of our knowledge on the genetic and
80 immunological mechanisms involved in the control of *T. gondii* infection has been
81 obtained by using mouse models, in which, the genetic background, the inoculation
82 route, the inoculum size, the age and the sex of the host may all influence the outcome
83 of infection (Dubey, 1987; Johnson et al. 1995; Liesenfeld et al. 2001; Walker et al.
84 1997). Unfortunately, however, data from the mouse model may not actually mirror
85 the processes involved in human toxoplasmosis since the pathogenesis and the
86 susceptibility in mice are remarkably different from that observed in humans (Kempf
87 et al. 1999).

88 In contrast, many studies have demonstrated that adult rats are one of the most
89 resistant hosts to *T. gondii* infection with respect to clinical toxoplasmosis and this
90 phenomenon has been known for more than half a century (Lewis and Markell, 1958;
91 Nakayama and Hoshiai, 1960; Fujii et al. 1983; Benedetto et al. 1996; Li et al. 2012;
92 Evans et al. 2014). The similarity between the clinical course in rat and human
93 toxoplasmosis suggests the use of rats as an ideal model to elucidate the mechanism
94 of *Toxoplasma* infection in humans (Santoro et al. 1987; Darcy and Zenner, 1993;
95 Zenner et al. 1998; 1999 a,b).
96 Pioneering work showed that different strains of rat exhibited considerable
97 variation in the brain cyst load following inoculation. For example, the Lewis rat was
98 shown to be highly resistant to cyst formation, in contrast however, Fischer 344 and
99 Brown Norway rats are more susceptible (Kempf et al. 1999; Sergent et al. 2005).
100 Interestingly, Guerrero et al. (1995) found that different age groups of Sprague
101 Dawley rats also presented variance in resistance to *T. gondii* infection.
102 More recently, previous studies (Li et al. 2012) showed that, when comparing
103 resistant and susceptible hosts to *T. gondii* (virulent RH strain) infection, high iNOS
104 and low Arginase levels were correlated with resistant hosts (rats) and high Arginase
105 and low iNOS levels were correlated with sensitive hosts (mice). Furthermore, that
106 study showed that, between the rat inbred lines, there was variation in both resistance
107 to *T. gondii* (RH Strain) and ratios of iNOS/Arginase in the 5 rat strains studied.

These findings are intriguing, but these older studies (Kempf et al. 1999; Sergent et al. 2005; Guerrero et al. 1995) concentrate on using a small number of rat strains and there is very little comparative data on neonatal infection in the same strains. Consequently, it is difficult to make comparisons between adult strains and neonatal infection within the same strains. Furthermore, the more recent studies (Li et al. 2012) concentrate on infection using the virulent (non-cyst forming) *T. gondii* strain which may not be typical in natural infections. In order to build up a systematic view of a rat model for understanding the human toxoplasmosis, the aims of our present study are focused on the resistance/susceptibility to the cyst-forming Prugniaud strain of *T. gondii* infection in newborns and adults of five rat strains. We aim to investigate whether the differences in resistance/susceptibility are related to innate genetic mechanisms within the host immune response and specifically to investigate any correlations, in adult rats, with the previously reported iNOS/Arginase ratios (Li et al. 2012) for those five inbred lines. The impact of the results from this work may provide very useful data to help to gain a better understanding of human toxoplasmosis.

2. **Materials and methods**

2.1. Animals

Brown Norway (BN), Fischer 344 (F344) and Lewis (LEW) rats were purchased from Vital River Laboratories (Beijing, China). Sprague Dawley (SD), Wistar (WST) rats and Swiss Webster mouse were purchased from the Experimental Animal Center of Sun Yat-Sen University. All the adult rats were 8 to 10 weeks old and weighed around 150 to 200 g when used for experiments. They were grouped in cages according to strains and routinely maintained in a special pathogen free room with free access to food and water. Protocols for the use of animals were approved by the Institutional Review Board for Animal Care at Sun Yat-Sen University (973 project, #2010CB530000).

2.2. Breeding

Female animals of different strains were placed in the male's bedding of the same strain for 48 h to synchronize estrus and were then caged as described by Letscher-Bru and colleagues (2003). Three females and one male rat were placed in the same cage for five days and were separated to rear their pups (Elsaid et al. 2001). Neonates aged at 5, 10, 15 and 20 days old were used for the experiments.

2.3. Parasites

Tissue cysts from the *Toxoplasma gondii* Prugniaud strain were obtained from the brains of orally infected Swiss Webster mice and prepared as previously described (Brinkmann et al. 1987; Letscher-Bru et al. 2003). Briefly, mice were anaesthetized by CO_2 and the brain was removed and homogenized in 1 ml PBS (pH 7.2). The number of cysts in a 10 µl sample was counted by microscopy with four replicated samples. The total number of cysts in the brain was calculated using the mean number of cysts counted in all of the four replicated samples and then scaled up to the total volume of the homogenate.

2.4. Toxoplasma gondii inoculation

Brain tissues were collected from Swiss Webster mice chronically infected with the *T. gondii* Prugniaud strain and were homogenized and diluted in PBS. A suspension of 0.1 ml containing 50 cysts was intraperitoneally (i. p.) inoculated into each newborn rat. To mimic natural infection under laboratory conditions, a suspension of 0.2 ml containing 200 cysts was orally administered into each adult rat, according to Sergent et al. (2005).

2.5. Detection of cysts from the brains of inoculated rats

169 Examination of cysts was performed at 60 days post infection. Rats were sacrificed
170 after being anaesthetized with CO_2 and brains were collected. Each brain was
171 homogenized with 2 ml of sterile saline. Cysts within 10 µl samples were carefully
172 quantified by microscopy using a cover slip (22 x 22 mm) at 100x magnification. Rats
173 found with brain cysts were considered positive (established infection), otherwise
174 they are recorded as negative. Tissue (brain, heart, liver, spleen, lung, kidney and
175 muscle) and blood from negative rats were further tested by PCR (Filisetti et al, 2003;
176 Homan et al. 2000) to remove any possibility of false negatives.
177
178 *2.6. Statistical analysis*
179
180 Infection rates were analyzed using the Chi-square-test. Cyst counts from each
181 group were also given as Mean ± Standard Error of Mean (SEM), which were
182 analyzed by the two-way-ANOVA test. Levene' Test determined equality of error
183 variances, if $p \leq 0.05$ (in case of inequality), the Dunnett's T3 (not shown) and
184 Tamhane's T2 post-hoc tests were used to confirm which pairs were significant.
185 Otherwise (when $p > 0.05$), Least-significant Difference (LSD) and
186 Student-Neuman-Keuls (SNK) post-hoc tests would be applied. Significant
187 differences were accepted at the level of 95% confidence (i.e., $p < 0.05$). Correlation
188 coefficients were calculated using data on cyst burdens and percentage infection
189 (cyst-positivity) from all neonate groups. Data from previously published studies (Li

et al. 2012) was used to calculate average inducible nitric oxide synthase and arginase-1 (iNOS/Arg-1) protein ratios for peritoneal macrophages from each rat strain. Correlation coefficients were calculated, using EXCEL, for both the rat strain iNOS/Arginase-1 protein ratio vs percentage cyst-positive neonates for each strain and for iNOS/Arg protein ratios vs mean brain cyst burden for the neonates (all age groups) for each strain. *P*-values were derived from statistical tables. Results were presented using GraphPad Prism version 5 and Statistical Package for Social Sciences (SPSS) version 13.0.

3. Results

3.1. Differences in resistance to T. gondii Prugniaud strain infection among the adult individuals of five rat strains

We first selected adult rats of five strains to confirm their variance in susceptibility to *T. gondii* infection. Figure 1 shows the resistance/susceptibility of five adult individuals of each rat strain to the *T. gondii* Prugniaud strain infection. No cysts were detected in the brains of four rat strains including BN, SD, WST and LEW (Fig. 1), suggesting that these adult rats are indeed naturally resistant to this parasite. However, a large cyst burden (1231±165.6; range 820-1800) was found in the brains of F344 rats indicating that this strain of rat is susceptible to infection by this parasite. By comparison with the other 4

211 strains of rat, the fact that cyst-positive rats were found in all F344 individuals (100%)
212 suggests that susceptibility in this strain is likely to be due to a genetic predisposition
213 rather than a sporadic event.
214
215 *3.2. Diversity in the infection rate of neonate individuals among the five rat strains*
216
217 We inoculated (i.p.) neonate rats aging 5-20 days from the same five strains to
218 investigate their variance in susceptibility to *T. gondii* infection. The number of cysts in
219 brains was determined at 60 days post-inoculation with *T. gondii* Prugniaud cysts (Table
220 1). Overall, diversity was observed in the proportion of infected neonates in our test strains
221 (Chi-Square Tests, $p < 0.001$). Firstly, taken together as a general group, the neonates
222 showed a greater proportion of cyst-positive animals when compared with the general
223 group of adults (detailed comparisons are shown in the following section 3.4). For
224 example, all the adults of strains SD, BN, WST and LEW had no detectable cysts in any
225 animal while at least some, and in the case of BN all, of the neonates from each of these
226 strains were cyst-positive. Only in strain F344 were all the age groups (including adults),
227 we tested, cyst-positive. All neonates of the F344 and BN rat strains (F344: 42/42; BN:
228 28/28) were found to be cyst-positive with *T. gondii* Prugniaud strain. Only 55.9%
229 (53.8-60%) of the neonates of the SD strain were positive, which was significantly lower
230 than the F344 and BN rat strains (SD vs F344, $p < 0.001$, and SD vs BN, $p < 0.001$) but no

231 significant difference was found when comparing the infection rates in the same rat strain
232 across the 4 age-groups of SD neonates.
233 In the remaining two strains of rat, WST and LEW, both were highly resistant to *T.*
234 *gondii* Prugniaud strain infection even in the neonate individuals. The average infection
235 rate across all time points of these 2 rat strains was found to be significantly lower than
236 F344, BN or SD (WST vs F344/BN/SD, $p < 0.001$, $p < 0.001$ and $p = 0.001$, respectively;
237 LEW vs F344/BN/SD, $p < 0.001$, $p < 0.001$ and $p < 0.001$, respectively). In WST pups,
238 cysts were found only in the 5-day-old individuals (3/7, 42.9%) and 10-day-old individuals
239 (1/6, 16.7%), while cysts were not detected in any older groups. For the LEW strain of rat,
240 cysts were only observed in the 5 day-old individuals (2/8, 25%). The frequency of
241 infected neonates declines with advancing age of infection to zero in the LEW rats and
242 other categories of the WST neonates, indicating the establishment of high resistance
243 against toxoplasmosis in these neonate groups. PCR tests using specific primers were also
244 performed on all the negative rats' brain, heart, liver, spleen, lung, kidney, and muscle
245 tissues, which confirmed the negativity of toxoplasmosis (data not shown).
246 Overall, infection rate results were further analyzed by Chi-Square tests, which showed
247 that the variable of "rat strain" ($p < 0.001$) but not "age" ($p = 0.156$) or "age∗strain"
248 interaction ($p = 0.464$) significantly influenced the sensitivity of neonates to the parasite.
249 When each rat strain was taken individually and analysis conducted to test the hypothesis
250 that increasing age is linked with decreasing infection in neonates, no significant
251 difference in the infection rate was found among different age groups of neonates in any

given rat strain (SD, $p = 0.996$; LEW, $p = 0.105$; no statistics are computed in F344 and BN because all are positively infected). Only in WST neonates does the "age" effect approach significance at $p = 0.066$, suggesting that this strain of rats may have high resistance to cyst formation as adults but show less resistance at a younger age.

3.3. Toxoplasma gondii cyst burdens in the brains of newborns from different rat strains

With the same set of newborn rats we describe in Table 1, we further investigated the cyst-forming capability of *T. gondii* Prugniaud strain in the brains of different strains of rats. The results are shown in Fig. 2. Consideration of the data showed that the equal variance assumption was rejected by Levene´s test ($p < 0.001$) and the appropriate statistical protocol was applied (see Methods). No significant difference in the cyst number was found among different age groups in any given rat strain and results from two-way ANOVA tests showed that the variable of "rat strain" ($p < 0.001$) but not "age" ($p = 0.196$) significantly influenced the cyst burden. However, the "age∗strain" interaction approached significance at $p = 0.056$, suggesting that strains of rats that have high resistance to cyst formation as adults may show less resistance at a younger age.

In detail, all pups in both F344 and BN rat strains were positive with *T. gondii* cysts. The cyst loads in the brains were observably more in F344 rats than in BN rats (1941 ± 141 versus 608.6 ± 75.0, $p < 0.001$). The difference in cyst loads were not

significantly different between BN and SD newborns (SD: 312.1 ± 74.1, $p = 0.065$). Consistent with the infection rates described previously, the numbers of cysts counted in the brains of inoculated LEW and WST rats were significantly lower than those from the other strains mentioned above (LEW (9.68 ± 7.11) vs F344/BN/SD, $p < 0.001$, $p < 0.001$ and $p = 0.003$, respectively; WST (46.43 ± 27.5) vs F344/BN/SD, $p < 0.001$, $p < 0.001$ and $p = 0.017$, respectively).

Taken together, data from the current work provide evidence that there are marked differences in the resistance to cyst formation of the *T. gondii* Prugniaud strain in the brains of newborn individuals among the five different rat strains. In brief, the F344 newborns are the most susceptible to *T. gondii* infection, followed by the BN strain with moderate susceptibility. The LEW and the WST strains, on the other hand, show high resistance while the SD has mild resistance to the *T. gondii* Prugniaud strain.

3.4. Toxoplasma gondii brain cyst burdens differ between neonates and adults

Since the method and size of inocula differs between the newborns and adults, (see Materials and Methods), it would be far-fetched to make a direct comparison of the outcomes from them. Despite these factors, we observed that the neonates, taken together as a general group, showed a greater proportion of cyst-positive animals and higher cyst burdens than the group of adults. Results from two-way ANOVA tests showed that the variable of "rat strain" ($p < 0.001$) and "age" ($p = 0.003$) and the

294 "age∗strain" interaction (p = 0.016) significantly influenced the cyst burden. The
295 significance by variable of "age" was not observed if adults' data was excluded (see
296 section 3.2), as the main differences were found only between the adult groups and
297 neonate groups.
298 However, a closer look at the situation of each strain, "age" does not always
299 significantly influence the cyst burden either when the adult groups are included
300 (LEW, p = 0.164; SD, p = 0.283; WST, p = 0.352) or excluded (LEW, p = 0.147; SD,
301 p = 0.500; WST, p = 0.341), except F344 which approaches significance
302 (with/without the adult group, p = 0.053/0.109) and BN which is significant
303 (with/without $p < 0.001$/ 0.002).
304 Taken together, we have observed a limited "age" effect in our data, which may be
305 due to the narrow age windows (day 5 to day 20 and adult) tested.
306
307 *3.5. Investigation into the relationship of susceptibility of rat strains and expression*
308 *of the iNOS and Arginase-1 genes*
309
310 Previous published studies (Li et al. 2012) established that differences in iNOS and
311 Arginase expression levels were linked with susceptible and resistant hosts (mice and
312 rats respectively). Furthermore, they demonstrated differences in the balance of iNOS
313 and Arginase in different rat strains. Comparison of the infection data from adults and
314 neonates, obtained in this study, with the protein expression data of inducible nitric

315 oxide synthase (iNOS) and arginase-1 (Arg-1) of adults of each rat strain (derived
316 from Li et al. 2012) provides interesting observations. The protein abundance ratios of
317 iNOS/Arg-1 in adult rats (taken from Li et al. 2012) were high in three out of the five
318 rat strains compared here – LEW (ratio 8.31), WST (ratio 4.42) and SD (ratio 4.27)
319 and this was associated with lack of infection with the Prugniaud strain in adult rats.
320 The remaining two strains had ratios that were close to 1:1 – BN (ratio 0.54) and F344
321 (ratio 1.48) (Li et al. 2012). In these two cases, only adult F344 rats, surprisingly with
322 the higher ratio, were susceptible to Prugniaud infection.

323 There was a highly significant negative correlation (-0.88; $p < 0.01$) between rat
324 strain iNOS/Arg-1 ratio in adults obtained from the previous studies (Li et al. 2012)
325 and percentage of cyst-positive neonates reported in this study. Furthermore, there
326 was a highly significant negative correlation (-0.65; $p < 0.01$) between rat strain
327 iNOS/Arg-1 ratios, previously reported, and means of brain cyst burden for all age
328 groups in same strain found in this study. Fig. 3a shows the relationship between total
329 percentage of neonates capable of establishing infection for each rat strain and the
330 peritoneal macrophage iNOS/Arg-1 protein ratio previously reported for each strain.
331 Rat strains BN and F344 with ratios close to 1:1 both show 100% infection in
332 neonates, while increasing iNOS/Arg-1 ratios are associated with a decreasing
333 proportion of cyst-positive pups in SD, WST and LEW respectively. Interestingly, the
334 LEW rat strain has a very high ratio of iNOS/Arg-1 protein (8.31) and this seems to
335 be associated with strong resistance to infection in the LEW neonates.

There appears to be differences in age-related susceptibility between the rat strains. Fig. 3b shows the relationship between age-related cyst positivity and rat strain (and iNOS/Arg-1 protein ratio). There is a striking reduction in infection rate of neonates associated with those rat strains with high iNOS/Arg-1 protein ratios. The age related decline in infection observed in the LEW and WST neonates appears to be mirrored by higher iNOS/Arg-1 ratios. A comparison of cyst burden in neonates and iNOS/Arg-1 protein ratios also produces a similar pattern (data not shown).

4. Discussion

A good deal of evidence demonstrates that rats are naturally resistant to *T. gondii* infection (Guerrero et al. 1995; Li et al., 2012). Our data presented here show that rats such as LEW are highly resistant to *T. gondii* type II strain infection. In addition, these resistant characteristics of the LEW rat to *Toxoplasma* infection are not parasite strain-specific as it has been observed with three different cyst-forming strains (Prugniaud, NED, CT1) and the virulent non-cyst forming RH strain (Kempf et al. 1999; Sergent et al. 2005; Li et al. 2012).

However, it is not well understood yet why some rats are naturally resistant to *T. gondii* infection. It was suggested that *Toxo1*, a large piece of chromosome 10 in the rat genome, directs toxoplasmosis outcome. It has been further proposed that *Toxo1*-mediated refractoriness of the LEW rat to *T. gondii* infection is associated with

the ability of macrophages to impede the proliferation of the parasite within the parasitophorous vacuole and to control the spread of the parasitic infection (Cavaillès et al. 2006). However, the true identity of *Toxo1* has not been confirmed. A large number of reports have demonstrated that nitric oxide (NO) is a major effector molecule for macrophage-mediated cytotoxicity in mouse macrophages and is a key anti-pathogen factor used by the infected host to control progression of intracellular pathogens including *Toxoplasma* (Adams et al. 1990; James, 1995; Davis et al. 2007; EI Kasmi et al. 2008; Von Bargen et al. 2011). Recent evidence indicates that the high expression of inducible nitric oxide synthase (iNOS), which is also located on chromosome 10, and low expression of Arg-1 in the macrophages of rats are strongly linked to this resistance when *T. gondii* RH strain was used to infect the cells (Li et al. 2012; Zhao et al. 2013). These data suggest that, at least for *T. gondii* type I strain, iNOS is a key effector to control the parasite infection. Our present data here show that such resistance is also applied to the cyst-forming type II/III strains of *T. gondii*. Based on our results, all neonates of the 5 strains of rat showed a degree of susceptibility to the *T. gondii* Prugniaud infection. The rat strain with an iNOS/Arg-1protein ratio close to 1:1 (eg BN and F344) conferred a higher degree of susceptibility to the cyst forming *Toxoplasma* strain than those with a higher ratio such as LEW, WST and SD. These results were further corroborated by another study in our laboratory (Wang et al., 2014). Wang and colleagues demonstrated that the treatment with glucocorticoids could significantly increase the cyst formation of *T.*

gondii Prugniaud strain in F344 and data indicated that this treatment was linked to lower iNOS/Arg-1 ratios (Wang et al. 2014). This suggests that the resistance to *Toxoplasma* infection in different strains of rat is a significant genetic trait that is variable between strains which can be further modified by drug treatment. This variability in these inbred rat lines may be indicative of a wider diversity of resistance/susceptibility in naturally occurring outbred individuals. Unfortunately, we were unable to assay the iNOS and Arg-1 expression levels of the peritoneal macrophages from the neonates due to the limitation on animal numbers in our licenses. Thus, we cannot comment on whether there is a difference in iNOS and Arg-1 expression levels during the development of rat neonates and therefore, also, cannot comment on the iNOS/Arg-1 ratios. Nevertheless, our findings are consistent with the previous reports which indicated that LEW rats are totally resistant to *T. gondii* CT1, NED and Prugniaud strain infections resulting in no trace of parasite infection as determined by negative serology and microscopic examination of brain cysts and other organs, unlike the susceptible BN and F344 rats (Kempf et al. 1999; Sergent et al. 2005).

Interestingly, in our study, the BN and F344 strains both had 100% infection rates in their respective neonates (irrespective of age group – apart from adults) when inoculated with the Prugniaud strain of *T. gondii*. However, in the case of the infection of adult rats, F344 was highly susceptible, but BN resistant despite both having a similarly low iNOS/Arg-1 protein ratio. It is possible, therefore, that factors other than

399 iNOS and Arg-1 also contribute to the host susceptibility/resistance phenotype. In a
400 recent study, Woods et al. (2013) have proposed a role for MAP kinase phosphatase-2
401 as a modulator of Arg-1 expression in mice. Perhaps variation in other rat genetic loci,
402 such as this, may also influence resistance/susceptibility to the parasite.
403 Interestingly, our results demonstrated that the neonates of rats, at least for the SD,
404 WST and LEW strains showed their native resistance to *T. gondii* Prugniaud infection
405 at a very early developmental stage. This conclusion was supported by Chinchilla et al.
406 (1981) who found that the natural resistance to *T. gondii* RH strain infection in SD
407 rats occurred at an early age (5 days old) and at least 10^7 to 10^8 tachyzoites were
408 required to kill a newborn animal whereas only a few tachyzoites of the same strain
409 could cause death in an adult mouse.
410 Neonatal rats have long been considered to be more susceptible to *T. gondii*
411 infection than the adults (Lewis and Markell, 1958). Lewis and Markell (1958)
412 demonstrated that the newborn WST rats showed a greater degree of susceptibility to
413 *Toxoplasma* tachyzoite infection than 3-week-old individuals. Results from Guerrero
414 et al. (1995) also indicated these similar phenotypes in SD rats. They found more
415 brain cysts in the 10-day-old SD neonates than in the 15-day-old neonates when they
416 were perorally administered with *T. gondii* oocysts from an avirulent strain. We also
417 observed mild trends in BN rats and F344, but not in the other three strains, which
418 contributed more to our primary concept that host strain differences determine
419 Prugniaud infection outcome.

In conclusion, the infectivity of neonates among the five strains of rat indicates that their resistance to *T. gondii* infection occurs at an early age and this resistance is linked to the genetic background of the rat. The resistance to *T. gondii* infection in rats is not only found against the virulent RH strain (Li et al. 2012) but also observed in the less virulent cyst-forming type II (Prugniaud) strain. Differences in the levels of resistance of neonatal rats to Prugniaud infection are linked to rat strain inherent and genetic differences. Degree of expression of iNOS and Arg-1 in the peritoneal macrophages in rat strains are strongly linked with resistance/susceptibility to the Prugniaud strain of *T. gondii* in adult rats suggesting that this could be one of the critical mechanisms used to control this parasite infection. Further research is necessary to establish whether the iNOS/Arg-1 balance is associated with resistance/susceptibility of neonates to *T. gondii* infection.

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Figure legends:

Fig. 1. Development of *T. gondii* cysts in adult rats. Five strains of rat aged at 8 to 10 weeks were orally infected with 200 *T. gondii* Prugniaud cysts and the brain cysts of infected animals were detected by microscopy 60 days post inoculation. Fischer 344 (F344; n=5), Brown Norway (BN; n=5), Sprague Dawley (SD; n=5), Wistar (WST; n=5) and Lewis (LEW; n=5) rats were used. The mean and standard deviation of each group is indicated.

Fig. 2. Differences in cyst numbers in the brains of different ages of newborn rats inoculated with *T. gondii* Prugniaud strain. 5-day-old, 10-day-old, 15-day-old and 20-day-old rats were injected intraperitoneally with 50 cysts respectively. The cyst numbers in the brains of all rats were detected 60 days later. The Fischer 344 (F344; n=9, 8, 11 and 14), Brown Norway (BN; n=8, 5, 5 and 10), Sprague Dawley (SD; n=11, 9, 7 and 5), Wistar (WST; n=7, 6, 10 and 5) and Lewis (LEW; n=8, 10, 8, and 5) strain rats were used. The mean and standard deviation of each group were indicated.

Fig. 3A. Relationship between the iNOS protein/Arginase protein ratio in peritoneal macrophages in each rat strain and proportion of cyst-positive pups taken overall from all age groups of rats. A correlation coefficient of -0.88 shows that there is a strong significant negative correlation ($P<0.01$). iNOS/Arg-1 protein ratios were calculated

654 for peritoneal macrophage data collected from each rat strain from the studies by Li et
655 al. (2012).
656
657 **Fig. 3B.** Relationship between the proportion of cyst-positive pups inoculated at
658 different ages and the iNOS/Arg-1 protein ratio of peritoneal macrophages of each rat
659 strain. (iNOS/Arginase protein ratios are given in brackets after the name of the rat
660 strain). Rat strains with iNOS/Arg-1 ratios in peritoneal macrophages that are close to
661 1:1 (BN, 0.54; F344, 1.48) appear to support parasite growth when inoculated at all
662 time points after birth. Neonates from rat strains showing higher iNOS/Arg-1protein
663 ratios in peritoneal macrophages (SD 4.27; WST, 4.42; LEW 8.31) have an
664 increasingly diminishing susceptibility to infection after birth which correlates with
665 increasing iNOS/Arg-1 protein ratio. iNOS/Arginase protein ratios were calculated for
666 peritoneal macrophage data collected from each rat strain from the studies by Li et al.
667 (2012).