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<http://dx.doi.org/10.1016/j.exppara.2014.12.008>

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<b>Type</b>	Article
<b>URL</b>	This version is available at:

	<a href="http://usir.salford.ac.uk/id/eprint/33380/">http://usir.salford.ac.uk/id/eprint/33380/</a>
<b>Published Date</b>	2015

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

5

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24 **ABSTRACT**

25 Mouse models differ considerably from humans with regard to clinical symptoms of  
26 toxoplasmosis caused by *Toxoplasma gondii* and, by comparison, the rat model is more  
27 representative of this disease in humans. In the present study, we found that different  
28 strains of adult and newborn rats (Lewis, Wistar, Sprague Dawley, Brown Norway and  
29 Fischer 344) exhibited remarkable variation in the number of brain cysts following  
30 inoculation with the *T. gondii* Prugniaud strain. In adult rats, large numbers of cysts  
31 ( $1231 \pm 165.6$ ) were observed in Fischer 344, but none in the other four. This situation was  
32 different in newborn rats aged from 5 to 20 days old. All Fischer 344 and Brown Norway  
33 newborns were cyst-positive while cyst-positive infection in Sprague Dawley neonates  
34 ranged from 54.5% to 60% depending on their age at infection. In Wistar and Lewis rat  
35 neonates, however, cyst-positivity rates of 0% to 42.9% and 0% to 25% were found  
36 respectively. To investigate whether rat strain differences in infectivity could be related to  
37 inherent strain and genetic differences in the host immune response, we correlated our data  
38 with previously reported strain differences in iNOS/Arginase ratio in adult rats and found  
39 them to be linked. These results show that interactions between host genetic background  
40 and age of rat influence *T. gondii* infection.

41

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

44

45

46 1. **Introduction**

47

48 *Toxoplasma gondii* is an obligatory intracellular apicomplexan parasite that infects  
49 almost all warm-blooded vertebrates, including mammals and birds. It is considered  
50 that *T. gondii* is one of the most successful eukaryotic pathogens based on the wide  
51 range of host species and high prevalence in these species worldwide. Human  
52 infections with *T. gondii* are primarily caused by ingesting undercooked meat  
53 containing viable tissue cysts or by ingestion of food or water contaminated with  
54 oocysts in faeces shed from infected cats. It is widely reported that up to one-third of  
55 the world's population are estimated to be chronically infected (Dubey and Beattie,  
56 1988; Dubey, 2004) and pregnant women are highly at risk in endemic areas due to  
57 the cause of congenital birth defects by toxoplasmosis (Pappas et al. 2009; Gao et al.  
58 2012).

59 Acute parasitic infection with *T. gondii* is usually not found in immunocompetent  
60 individual humans, who can mount an effective immune response to clear most  
61 tachyzoites but not bradyzoites which remain in tissue cysts. Cysts of *T. gondii* can  
62 develop in varied organs and tissues, particularly in the brain or skeletal muscles,  
63 which can later be reactivated if immunosuppression (e. g. AIDS, cancer therapy or  
64 organ transplantation) occurs. This reactivation of latent infection can cause  
65 life-threatening toxoplasmic encephalitis and related diseases (Gianotti et al. 1997;  
66 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.

108 These findings are intriguing, but these older studies (Kempf et al. 1999; Sergent et  
109 al. 2005; Guerrero et al. 1995) concentrate on using a small number of rat strains and  
110 there is very little comparative data on neonatal infection in the same strains.  
111 Consequently, it is difficult to make comparisons between adult strains and neonatal  
112 infection within the same strains. Furthermore, the more recent studies (Li et al. 2012)  
113 concentrate on infection using the virulent (non-cyst forming) *T. gondii* strain which  
114 may not be typical in natural infections. In order to build up a systematic view of a rat  
115 model for understanding the human toxoplasmosis, the aims of our present study are  
116 focused on the resistance/susceptibility to the cyst-forming Prugninaud strain of *T.*  
117 *gondii* infection in newborns and adults of five rat strains. We aim to investigate  
118 whether the differences in resistance/susceptibility are related to innate genetic  
119 mechanisms within the host immune response and specifically to investigate any  
120 correlations, in adult rats, with the previously reported iNOS/Arginase ratios (Li et al.  
121 2012) for those five inbred lines. The impact of the results from this work may  
122 provide very useful data to help to gain a better understanding of human  
123 toxoplasmosis.

124

## 125 2. **Materials and methods**

126

### 127 2.1. *Animals*

128



129 Brown Norway (BN), Fischer 344 (F344) and Lewis (LEW) rats were purchased  
130 from Vital River Laboratories (Beijing, China). Sprague Dawley (SD), Wistar (WST)  
131 rats and Swiss Webster mouse were purchased from the Experimental Animal Center  
132 of Sun Yat-Sen University. All the adult rats were 8 to 10 weeks old and weighed  
133 around 150 to 200 g when used for experiments. They were grouped in cages  
134 according to strains and routinely maintained in a special pathogen free room with  
135 free access to food and water. Protocols for the use of animals were approved by the  
136 Institutional Review Board for Animal Care at Sun Yat-Sen University (973 project,  
137 #2010CB530000).

138

### 139 *2.2. Breeding*

140

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

146

### 147 *2.3. Parasites*

148

149 Tissue cysts from the *Toxoplasma gondii* Prugniaud strain were obtained from the  
150 brains of orally infected Swiss Webster mice and prepared as previously described  
151 (Brinkmann et al. 1987; Letscher-Bru et al. 2003). Briefly, mice were anaesthetized  
152 by CO<sub>2</sub> and the brain was removed and homogenized in 1 ml PBS (pH 7.2). The  
153 number of cysts in a 10 µl sample was counted by microscopy with four replicated  
154 samples. The total number of cysts in the brain was calculated using the mean number  
155 of cysts counted in all of the four replicated samples and then scaled up to the total  
156 volume of the homogenate.

157

#### 158 2.4. *Toxoplasma gondii* inoculation

159

160 Brain tissues were collected from Swiss Webster mice chronically infected with the  
161 *T. gondii* Prugniaud strain and were homogenized and diluted in PBS. A suspension  
162 of 0.1 ml containing 50 cysts was intraperitoneally (i. p.) inoculated into each  
163 newborn rat. To mimic natural infection under laboratory conditions, a suspension of  
164 0.2 ml containing 200 cysts was orally administered into each adult rat, according to  
165 Sergent et al. (2005).

166

#### 167 2.5. *Detection of cysts from the brains of inoculated rats*

168

169 Examination of cysts was performed at 60 days post infection. Rats were sacrificed  
170 after being anaesthetized with CO<sub>2</sub> 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

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

198

### 199 **3. Results**

200

#### 201 *3.1. Differences in resistance to T. gondii Prugniaud strain infection among the adult* 202 *individuals of five rat strains*

203

204 We first selected adult rats of five strains to confirm their variance in susceptibility to *T.*  
205 *gondii* infection. Figure 1 shows the resistance/susceptibility of five adult individuals of  
206 each rat strain to the *T. gondii* Prugniaud strain infection. No cysts were detected in the  
207 brains of four rat strains including BN, SD, WST and LEW (Fig. 1), suggesting that these  
208 adult rats are indeed naturally resistant to this parasite. However, a large cyst burden  
209 ( $1231 \pm 165.6$ ; range 820-1800) was found in the brains of F344 rats indicating that this  
210 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

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

256

### 257 3.3. *Toxoplasma gondii* cyst burdens in the brains of newborns from different rat strains

258

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

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

273 significantly different between BN and SD newborns (SD:  $312.1 \pm 74.1$ ,  $p = 0.065$ ).  
274 Consistent with the infection rates described previously, the numbers of cysts counted  
275 in the brains of inoculated LEW and WST rats were significantly lower than those  
276 from the other strains mentioned above (LEW ( $9.68 \pm 7.11$ ) vs F344/BN/SD,  $p <$   
277  $0.001$ ,  $p < 0.001$  and  $p = 0.003$ , respectively; WST ( $46.43 \pm 27.5$ ) vs F344/BN/SD,  $p$   
278  $< 0.001$ ,  $p < 0.001$  and  $p = 0.017$ , respectively).

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

285

#### 286 3.4. *Toxoplasma gondii* brain cyst burdens differ between neonates and adults

287

288 Since the method and size of inocula differs between the newborns and adults, (see  
289 Materials and Methods), it would be far-fetched to make a direct comparison of the  
290 outcomes from them. Despite these factors, we observed that the neonates, taken  
291 together as a general group, showed a greater proportion of cyst-positive animals and  
292 higher cyst burdens than the group of adults. Results from two-way ANOVA tests  
293 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.

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

343

#### 344 **4. Discussion**

345

346 A good deal of evidence demonstrates that rats are naturally resistant to *T. gondii*  
347 infection (Guerrero et al. 1995; Li et al., 2012). Our data presented here show that rats  
348 such as LEW are highly resistant to *T. gondii* type II strain infection. In addition,  
349 these resistant characteristics of the LEW rat to *Toxoplasma* infection are not parasite  
350 strain-specific as it has been observed with three different cyst-forming strains  
351 (Prugniaud, NED, CT1) and the virulent non-cyst forming RH strain (Kempf et al.  
352 1999; Sergent et al. 2005; Li et al. 2012).

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

357 the ability of macrophages to impede the proliferation of the parasite within the  
358 parasitophorous vacuole and to control the spread of the parasitic infection (Cavaillès  
359 et al. 2006). However, the true identity of *Toxo1* has not been confirmed. A large  
360 number of reports have demonstrated that nitric oxide (NO) is a major effector  
361 molecule for macrophage-mediated cytotoxicity in mouse macrophages and is a key  
362 anti-pathogen factor used by the infected host to control progression of intracellular  
363 pathogens including *Toxoplasma* (Adams et al. 1990; James, 1995; Davis et al. 2007;  
364 EI Kasmi et al. 2008; Von Bargen et al. 2011). Recent evidence indicates that the high  
365 expression of inducible nitric oxide synthase (iNOS), which is also located on  
366 chromosome 10, and low expression of Arg-1 in the macrophages of rats are strongly  
367 linked to this resistance when *T. gondii* RH strain was used to infect the cells (Li et al.  
368 2012; Zhao et al. 2013). These data suggest that, at least for *T. gondii* type I strain,  
369 iNOS is a key effector to control the parasite infection. Our present data here show  
370 that such resistance is also applied to the cyst-forming type II/III strains of *T. gondii*.  
371 Based on our results, all neonates of the 5 strains of rat showed a degree of  
372 susceptibility to the *T. gondii* Prugniaud infection. The rat strain with an  
373 iNOS/Arg-1 protein ratio close to 1:1 (eg BN and F344) conferred a higher degree of  
374 susceptibility to the cyst forming *Toxoplasma* strain than those with a higher ratio  
375 such as LEW, WST and SD. These results were further corroborated by another study  
376 in our laboratory (Wang et al., 2014). Wang and colleagues demonstrated that the  
377 treatment with glucocorticoids could significantly increase the cyst formation of *T.*

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

394 Interestingly, in our study, the BN and F344 strains both had 100% infection rates  
395 in their respective neonates (irrespective of age group – apart from adults) when  
396 inoculated with the Prugniaud strain of *T. gondii*. However, in the case of the infection  
397 of adult rats, F344 was highly susceptible, but BN resistant despite both having a  
398 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.

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

432

433 **Acknowledgments:** The authors would like to thank all members in the authors'  
434 laboratories who provided great help when the work was carried out and the data were  
435 analysed. This work was supported by the National Basic Research Program of China (973  
436 Program; NO:2010CB530000). We would like to thank suggestions and the critical  
437 comments from anonymous reviewers, who helped us to significantly improve our  
438 manuscript.

439

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632

633

634 **Figure legends:**

635 **Fig. 1.** Development of *T. gondii* cysts in adult rats. Five strains of rat aged at 8 to  
636 10 weeks were orally infected with 200 *T. gondii* Prugniaud cysts and the brain cysts  
637 of infected animals were detected by microscopy 60 days post inoculation. Fischer  
638 344 (F344; n=5), Brown Norway (BN; n=5), Sprague Dawley (SD; n=5), Wistar  
639 (WST; n=5) and Lewis (LEW; n=5) rats were used. The mean and standard deviation  
640 of each group is indicated.

641

642 **Fig. 2.** Differences in cyst numbers in the brains of different ages of newborn rats  
643 inoculated with *T. gondii* Prugniaud strain. 5-day-old, 10-day-old, 15-day-old and  
644 20-day-old rats were injected intraperitoneally with 50 cysts respectively. The cyst  
645 numbers in the brains of all rats were detected 60 days later. The Fischer 344 (F344;  
646 n=9, 8, 11 and 14), Brown Norway (BN; n=8, 5, 5 and 10), Sprague Dawley (SD;  
647 n=11, 9, 7 and 5), Wistar (WST; n=7, 6, 10 and 5) and Lewis (LEW; n=8, 10, 8, and 5)  
648 strain rats were used. The mean and standard deviation of each group were indicated.

649

650 **Fig. 3A.** Relationship between the iNOS protein/Arginase protein ratio in peritoneal  
651 macrophages in each rat strain and proportion of cyst-positive pups taken overall from  
652 all age groups of rats. A correlation coefficient of -0.88 shows that there is a strong  
653 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-1 protein  
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).