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1 Short Communication: Investigation into the genetic diversity in Toll-like
2 Receptors 2 and 4 in the European badger *Meles meles*.

3

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25

26 **Abstract**

27 The Toll-like receptor (TLR) genes are a conserved family of genes central to the innate immune response to
28 pathogen infection. They encode receptor proteins, recognise pathogen associated molecular patterns
29 (PAMPs) and trigger initial immune responses. In some host-pathogen systems, it is reported that genetic
30 differences, such as single nucleotide polymorphisms (SNPs), associate with disease resistance or
31 susceptibility. Little is known about TLR gene diversity in the European badger (*Meles meles*). We collected
32 DNA from UK badgers, carried out PCR amplification of the badger TLR2 gene and exon 3 of TLR4 and
33 determined DNA sequences for individual badgers for TLR2 (n=61) and TLR4 exon 3 (n=59). No polymorphism
34 was observed in TLR4. Three TLR2 amino acid haplotype variants were found. Ninety five percent of badgers
35 were homozygous for one common haplotype (H1), the remaining three badgers had genotypes H1/H3, H1/H2
36 and H2/H2. By broad comparison with other species, diversity in TLR genes in badgers seems low. This could
37 be due to a relatively localised sampling or inherent low genetic diversity. Further studies are required to
38 assess the generality of the low observed diversity and the relevance to the immunological status of badgers.

39

40 **Keywords**

41 Toll-Like Receptor; Polymorphism; badger; *Meles meles*; TLR2; TLR4

42

43 Toll-like receptors (TLRs) are a family of proteins that target highly conserved molecules essential for parasite
44 and pathogen survival (Takeda and Akira, 2005). Single nucleotide polymorphisms (SNP) in TLR genes have
45 been linked to pathogen susceptibility in some host species. For example, there is a relationship between TLR
46 variation and *Borrelia afzelii* susceptibility (Tschirren et al., (2013)) and TLR polymorphism and susceptibility to
47 bovine tuberculosis (bTB) in both Chinese Holstein cattle (Sun et al. 2012) and water buffalo (Alfano et al., 2014).
48 Other studies have shown links between TLR variation and risks of cancer (Gomaz et al., 2012), diabetes (Liu et
49 al., 2012), asthma (Schwartz and Cook, 2005) and TB (Zhang et al., 2013) in humans.

50 TLR genes are conserved throughout evolution (Lu et al., 2008) and homologues are found across a wide
51 range of species (Vasselon and Detmers, 2002). The proteins that they encode have two broad domains; an
52 extracellular Leucine Rich Repeat (LRR) domain which recognises and binds certain pathogen associated
53 molecular patterns (PAMPs) and an intracellular Toll-Interleukin receptor homology domain (TIR). The LRR
54 displays variability which is thought to be driven by an evolutionary arms race with invading parasites and
55 pathogens (Roach et al., 2005). The TIR domain is highly conserved and functions to deliver intracellular signals
56 triggering an innate immune response. Studies on TLR variation have found a higher degree of variation within
57 the LRR domains, some of which appears to be generated by positive selection on amino acid diversity (Jann et
58 al., 2008, Werling et al., 2009). Polymorphisms in the LRR domain can be associated with enhanced susceptibility
59 to disease. For example, the change from arginine to glutamine at position 753 in the human TLR2 gene
60 increases susceptibility to staphylococcal infection (Lorenz et al., 2000), tuberculosis (Ogus et al., 2005),
61 rheumatic fever (Berdeli et al., 2005) and urinary tract infection (Tabel et al., 2007).

62 TLR population studies are of increasing interest for analysis of broad host responses to disease. This is
63 particularly the case in humans but relevant equivalent data is sparse in wildlife species. To the best of our
64 knowledge, there are no reported studies of TLR variation in European badgers (*Meles meles*) and there are no
65 reported DNA sequences of TLRs from this species. An investigation into badger immune molecules is pertinent
66 in the context of the role of the badger in the perpetuation of bTB in cattle in the UK and Republic of Ireland
67 (Krebs et al., 1997; Bhuachalla et al., 2015). TLR 2 and TLR 4 recognise glycosyl-phosphatidyl-inositol (GPI)
68 anchors, and thus may be relevant to both the pathogenesis of bTB infection and to other pathogens of the

69 badger such as *Toxoplasma gondii* (Anwar et al., 2006; Hide et al., 2009), *Eimeria melis*, *Isospora melis*,
70 (Newman et al., 2001; Cottrell 2011), and *Trypanosoma pestanaei*, (Lizundia et al., 2011; Ideozu et al., 2015). In
71 the present study, we set out to investigate TLR2 and TLR4 genetic diversity in the European badger using a
72 sample of animals from a well-studied population at Woodchester Park, in south-west England, alongside
73 geographically distinct badgers from other UK locations.

74 For DNA extraction, blood samples were collected from 54 badgers captured as part of a long-term capture-
75 mark-release study of badgers at Woodchester Park (e.g. Rogers et al., 1998; Delahay et al., 2000),
76 Gloucestershire, England. Ethical approval, ethical practice and appropriate licensing of badger capture and
77 examination were described previously (Ideozu et al., 2015). Additionally, a further 14 DNA extractions were
78 performed on other blood samples provided by Secret World Wildlife Rescue, which were collected from
79 badgers originating from eight geographically distinct locations in a 150 km radius of the Woodchester Park
80 site. DNA was extracted from badger blood samples using a modification of a phenol-chloroform protocol
81 (Morley et al., 2005; 2008; Ideozu et al., 2015). Appropriate measures were taken to prevent cross
82 contamination in DNA extractions and subsequent PCR reactions (Williams et al., 2005; Bajnok et al., 2015).

83 Briefly, 1.0 ml of badger blood was centrifuged at 2500 RPM for 10 minutes and the recovered pellet was then
84 incubated, by the addition of 400 µl lysis buffer (100 mM NaCl, 25 mM EDTA, 0.5% SDS, 20 mM Tris pH 8.0)
85 and 100 µl proteinase K (20 mg/ml), at 56 °C overnight. Then 500 µl Tris buffered phenol-chloroform (pH 8.0)
86 was added, mixed for 10 min before separation of phases by centrifugation for 10 min at 13,000 rpm. The
87 phenol chloroform extraction was repeated twice more. Then 90 µl sodium acetate (3M pH 5.2) and 900 µl of
88 100% ethanol were added to the final supernatant and incubated overnight at -20 °C. Following centrifugation
89 for 20 min at 13000g, the pellet was washed in 70% ethanol, centrifuged again and re-dissolved in 100 µl TE
90 buffer (10 mM Tris pH 8.0, 1 mM EDTA). To design PCR primers, TLR nucleotide sequences were retrieved
91 from PubMed-NCBI and ENSEMBLE, and TLR amino acid sequences from UniProt using database searches.

92 As no badger TLR gene information was available, primers suitable for TLR2 and TLR4 amplification from
93 badgers were predicted from evolutionarily related species (ferret, *Mustela putorius furo*, cat *Felis catus*, dog
94 *Canis lupus familiaris*, giant panda *Ailuropoda melanoleuca* and Pacific walrus *Odobenus rosmarus divergens*).

95 Badger TLR sequences were run through predictive modelling software using the PubMed position-specific
96 iterated basic local alignment search tool (PSI-BLAST) to identify possible protein domains from known
97 sequences.

98 Two overlapping primer sets were designed and optimised to amplify 1909 bp of the 2238 bp third exon of TLR4
99 (TLR44.1F 5'CTGTATCTCTTTTCCCTRTAGGTGTGA3', 5'GTTTAGGAAGCGAGCCAAATG3' nucleotide 1 to
100 1110 (see accession number KR780356), annealing 56°C; TLR44.2F 5'TTACCTTGATATTTCTTATAC3',
101 TLR44.2F 5' GAANCCTTCCTGGATGAT3', nucleotide 1071 to 1909 (see accession number KR780356),
102 annealing 50°C. This fragment covers all of the leucine rich repeat regions of TLR4. Three overlapping primer
103 sets were designed and optimised to amplify the single 2358 bp exon of TLR2 in its entirety (TLR22SF,
104 5'ATGTCACGTGTTYGTGGACA3', TLR22SR 5'GTCRCTGAGATCCAAATATTCTA3', nucleotide 1 to 1110,
105 annealing 60°C; TLR22XF 5'GATGGAAGTTTTAGCGAACTTGTG3', TLR22XR
106 5'TCCCGAGTGAAAGACAGGAAT3', nucleotide 789 to 1643, annealing 56°C; TLR22F
107 5'GCTCCTGTGAATCCTGTCTTTC3', TLR22R 5'GCCGTGTCAGAATAAGCTACCAC3', nucleotide 1613 to
108 2435 (77 bases beyond the stop codon), annealing 64°C. Each PCR Reaction contained 0.1 volume 10x NH4
109 buffer (Bioline, UK), 2 mM MgCl₂, 1 mM dNTP (Bioline UK), 0.5 µM forward primer and 0.5 µM reverse primer
110 and 2.5 units BioTaq Polymerase (Bioline, UK). PCR amplification was carried out using a Stratagene
111 Robocycler, denaturing for 40 secs at 94°C, annealing as specified in Table 1, depending on the primer
112 combination, and extension at 72°C for 1 min 30 secs. The number of PCR cycles also varied for each primer
113 set as shown in Table 1. All PCRs were run with 10 minutes denaturing at 94 °C before cycling and 5 minutes
114 extension at 72 °C at the end of the final cycle. Amplified DNA was examined by gel electrophoresis using 1%
115 agarose then checked for purity and quantity using a nanodrop spectrophotometer (Thermo Fisher Scientific,
116 UK). PCR products were adjusted to 10 ng/µl and sequenced by Sanger sequencing (SourceBioscience,
117 Cambridge) using both forward and reverse primers and a consensus sequence was generated. Sequence
118 graphics were examined by eye, using the program FinchTV (<https://digitalworldbiology.com/FinchTV>), to check
119 correct base calling and identify heterozygous bases (identified as two overlapping peaks of, usually, a smaller
120 amplitude at the same point on the electropherogram). All polymorphisms and heterozygotes were rechecked by

121 resequencing at least three times. Investigation of predicted structures of TLRs were carried out by sequence
122 alignment with example mammalian species (Program, Clustal Omega www.ebi.ac.uk) and Leucine Rich
123 Repeats (LRRs) were predicted using LRR Finder (<http://www.lrrfinder.com/lrrfinder.php>).

124 From Woodchester Park and other locations, 59 badgers were sequenced (accession number KR780356) across
125 exon 3 of TLR4 (1909 bp of the 2238 bp third exon) which covers the LRR region of TLR4. No polymorphisms
126 were found in the TLR4 gene. Analysis of the predicted protein sequence shows good identity to TLR4 from other
127 species (ferret, 92%; human, 73%; bovine 76%; mouse 62%). Eighteen LRRs are predicted compared to 20 for
128 the other species. The badger sequence is missing LRRs 7,8,19 (relative to the human sequence) but has an
129 additional predicted LRR before LRR1 (human).

130 Sixty-one badgers, from Woodchester Park and other locations, were sequenced across the entire 2355 bp
131 of the single exon TLR2 gene. Low genetic diversity was found. In addition to the wild type sequence, Haplotype
132 1 (H1), two single nucleotide polymorphisms were found in TLR2 (H2 and H3) and both were non-synonymous
133 missense mutations (Table 1) (Accession numbers KR780353 (TLR2-H1), KR780354 (TLR2-H2), KR780355
134 (TLR2-H3)). The three haplotypes generated two homozygous genetic types, one of which (the wild type H1/H1
135 n=58) predominated over the other (H2/H2 n=1). Two heterozygote haplotype combinations were seen (H1/H2,
136 n=1; H1/H3, n=1). One out of 52 (2%) of the badgers from the Woodchester Park study showed variation from
137 the majority consensus TLR2 sequence (heterozygous H1/H3), while 2/9 (22%) badgers from the other regions
138 showed TLR2 sequence variation (H1/H2 or H2/H2).

139 Analysis of the predicted protein sequences showed good identity to TLR2 from other species (ferret, 91%
140 human, 76%; bovine 75%; mouse 67%). Nineteen LRRs (including the LRR C-terminal) are predicted compared
141 to 20 for the other species. The badger sequence is missing LRR 5 (relative to the human sequence).

142 Both SNPs occurred in the extracellular LRR domain. Figure 1 shows the location of SNPs in relation to the
143 overall TLR2 structure. Haplotype 2 (C1007T) causes a missense mutation amino acid change from a threonine
144 to an isoleucine (T336I). Haplotype 3 (C1722G) causes a missense mutation amino acid change from a
145 glutamine to a histidine (Q574H). There was no difference between the predicted LRRs in H1, H2 and H3. The
146 structural or immunological outcomes of these changes have not been elucidated.

147 In this study, we report the first TLR sequences from European badgers. There is low gene variation in both
148 TLR2 and TLR4 with the latter being invariant in the study cohort. However, population level data on the variation
149 in either TLR2 or TLR4 genes in wildlife species is scarce. In comparison to other studies conducted, haplotype
150 diversity in the badger was lower than that in other species, for example, TLR 2 from bank voles (Tschirren et
151 al., 2013), TLR4 from grey partridges (Vinkler et al., 2015), pigs (Palermo et al., 2009) and both TLRs from water
152 buffalo (Alfano et al., 2014), humans (Mukherjee et al., 2014). Furthermore, studies on other TLRs have also
153 shown greater diversity in other species than in the badger genes reported here (e.g. TLR11 and TLR12,
154 Woodmice (Morger et al., 2014); TLR 1 and TLR9, cattle (Sun et al., 2012)). There is a growing body of evidence
155 to suggest that TLR variation, may have some influence on resistance or susceptibility to infection, for example
156 in *Borrelia* infection in wild rodents (Tschirren et al., 2013, Tschirren 2015) and bTB susceptibility in cattle (Sun
157 et al., 2012). Studies show that populations with low genetic diversity are less adaptable to exposure to pathogens
158 (e.g. Kaslow et al., 2008; Chapman et al., 2012). The restricted genotype range in the two badger genes,
159 examined here, raises questions about susceptibility to infection.

160 However, lack of variation could be explained by restricted sample size and geographical spread. Indeed,
161 TLR2 variation is heavily influenced by geographic dispersion (Tschirren, 2015). The TLR data presented here
162 are consistent with a study based on a wider set of data (microsatellite loci; mitochondrial genes) that shows
163 lower genetic diversity amongst European badgers in western regions of Europe (e.g. Britain) (Frantz et al.,
164 2014). Future studies should be aimed at gaining a broader perspective of the role of innate immune genes in
165 infection in badgers and investigation of the variability of TLRs over a wider geographical range.

166

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168

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176

177 **Conflict of Interest**

178 The authors declared no conflict of interest.

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183 **References**

184

185 Alfano F, Peletto S, Lucibelli MG, Borriello G, Urciuolo G, Maniaci MG, Desiato R, Tarantino M, Barone A,

186 Pasquali P, Acutis PL, Galiero G (2014) Identification of single nucleotide polymorphisms in Toll-like receptor

187 candidate genes associated with tuberculosis infection in water buffalo (*Bubalus bubalis*). BMC Genetics

188 15:139-147

189 Anwar A, Knaggs J, Service KM, McLaren GW, Riordan P, Newman C, Delahay RJ, Cheeseman C, Macdonald

190 DW (2006) Antibodies to *Toxoplasma gondii* in Eurasian badgers. Journal of Wildlife Diseases 42:179-181

191 Bajnok J, Boyce K, Rogan MT, Craig PS, Lun ZR, Hide G (2015) Prevalence of *Toxoplasma gondii* in localised

192 populations of *Apodemus sylvaticus* is linked to population genotype not to population location. Parasitology

193 143:680-690

194 Berdeli A, Celik HA, Ozyürek R, Dogrusoz B, Aydin HH (2005) TLR-2 gene Arg753Gln polymorphism is

195 strongly associated with acute rheumatic fever in children. Journal of Molecular Medicine 83:535-541

196 Bhuachalla DN, Corner LAL, More SJ, Gormley E (2015) The role of badgers in the epidemiology of

197 *Mycobacterium bovis* infection (tuberculosis) in cattle in the United Kingdom and the Republic of Ireland:

198 current perspectives on control strategies. Veterinary Medicine: Research and Reports 6:27-38

199 Chapman SJ, Hill AVS (2012) Human genetic susceptibility to infectious disease. Nature Reviews Genetics

200 13:175-188

201 Cottrell ERA (2011) Variation of parasite burden within the European badger (*Meles meles*): the effect of

202 season, habitat, body condition, gender and age on the prevalence of *Eimeria melis* and *Capillaria*. Managing

203 Wildlife Disease. MSc Dissertation, Exeter University, UK.

204 Delahay R, Frolich K 2000 Absence of antibodies against canine distemper virus in free-ranging populations of

205 the Eurasian badger in Great Britain. Journal of Wildlife Diseases 36:576-579

206 Frantz AC, McDevitt AD, Pope LC, Kochan J, Davison J, Clements CF, Elmeros M, Molina-Vacas G, Ruiz-

207 Gonzalez A, Balestrieri A, Van Den Berge K, Breyne P, Do Linh San E, Agren EO, Suchentrunk F, Schley L,

208 Kowalczyk R, Kostka BI, Cirović D, Sprem N, Colyn M, Ghirardi M, Racheva V, Braun C, Oliveira R, Lanszki J,
209 Stubbe A, Stubbe M, Stier N, Burke T (2014) Revisiting the phylogeography and demography of European
210 badgers (*Meles meles*) based on broad sampling, multiple markers and simulations. *Heredity* 113:443-453
211 Gomaz A, Pavelic J, Glavan TM (2012) The polymorphisms in Toll-like receptor genes and cancer risk.
212 *Periodicum Biologorum* 114:461-469
213 Hide G, Morley EK, Hughes JM, Gerwash O, Elmahaishi MS, Elmahaishi KH, Thomasson D, Wright EA,
214 Williams RH, Murphy RG, Smith JE (2009) Evidence for high levels of vertical transmission in *Toxoplasma*
215 *gondii*. *Parasitology* 136:1877-1885
216 Ideozu EJ, Whiteoak AM, Tomlinson AJ, Robertson A, Delahay RJ, Hide G (2015) High prevalence of
217 trypanosomes in European badgers detected using ITS-PCR. *Parasites & Vectors* 8:480
218 Jann OC, Werling D, Chang JS, Haig D, Glass EJ (2008) Molecular evolution of bovine Toll-like receptor 2
219 suggests substitutions of functional relevance. *BMC Evolutionary Biology* 8:288
220 Kaslow RA, McNicholl, J, Hill AVS (2008) Genetic susceptibility to infectious diseases. Oxford University Press,
221 New York.
222 Krebs JR, Anderson R, Clutton-Brock T, Morrison I, Young D, Donnelly C, Frost S, Woodroffe R (1997) Bovine
223 Tuberculosis in Cattle and Badgers: Report to the Rt Hon Dr Jack Cunningham MP. Her Majesty's Stationary
224 Office, London.
225 Liu F, Lu W, Qian Q, Qi W, Hu J, Feng B (2012) Frequency of TLR 2, 4, and 9 gene polymorphisms in Chinese
226 population and their susceptibility to Type 2 diabetes and coronary artery disease. *Journal of Biomedicine and*
227 *Biotechnology* doi:10.1155/2012/373945
228 Lizundia R, Newman C, Buesching CD, Ngugi D, Blake D, Sin YW, Macdonald DW, Wilson A, McKeever D
229 (2011) Evidence for a role of the host-specific flea (*Paraceras melis*) in the transmission of *Trypanosoma*
230 *pestanai* to the European badger. *PLoS ONE* 6:e16977doi:10.1371/journal.pone0016977
231 Lorenz E, Mira JP, Cornish KL, Arbour NC, Schwartz DA (2000) A novel polymorphism in the toll-like receptor 2
232 gene and its potential association with staphylococcal infection. *Infection and Immunity* 68:6398-6401
233 Lu Y, Yeh W, Ohashi PS (2008) LPS/TLR4 signal transduction pathway *Cytokine* 42:145-151

234 Morger J, Bajnok J, Boyce K, Craig PS, Rogan MT, Lun ZR, Hide G, Tschirren B (2014) Naturally occurring Toll-
235 like receptor 11 (TLR11) and Toll-like receptor 12 (TLR12) polymorphisms are not associated with *Toxoplasma*
236 *gondii* infection in wild wood mice. Infection Genetics and Evolution 26:180-184

237 Morley EK, Williams RH, Hughes JM, Thomasson D, Terry RS, Duncanson P, Smith JE, Hide G (2008)
238 Evidence that primary infection of charollais sheep with *Toxoplasma gondii* may not prevent foetal infection and
239 abortion in subsequent lambings. Parasitology 135:169-173

240 Morley EK, Williams RH, Hughes JM, Terry RS, Duncanson P, Smith JE, Hide G (2005) Significant familial
241 differences in the frequency of abortion and *Toxoplasma gondii* infection within a flock of Charollais sheep.
242 Parasitology 131:181-185

243 Mukherjee S, Ganguli D, Majumder PP (2014) Global footprints of purifying selection on Toll-like receptor
244 genes primarily associated with response to bacterial infections in humans. Genome Biology and Evolution
245 6:551-558

246 Newman C, Macdonald DW, Anwar A (2001) Coccidiosis in the European badger, *Meles meles*, in Wytham
247 Woods: infection and consequences for growth and survival. Parasitology 123:133-142

248 Ogun AC, Yoldas B, Ozdemir T, Uguz A, Olcen S, Keser I, Coskun M, Cilli A, Yegin O (2004) The Arg753GLn
249 polymorphism of the human toll-like receptor 2 gene in tuberculosis disease. European Respiratory Journal
250 23:219-223

251 Palermo S, Capra E, Torremorell M, Dolzan M, Davoli R, Haley CS, Giuffra E (2009) Toll-like receptor 4 genetic
252 diversity among pig populations. Animal Genetics 40:289-299

253 Roach JC, Glusman G, Rowen L, Kaur A, Purcell MK, Smith KD, Hood LE, Aderem A (2005) The evolution of
254 vertebrate Toll-like receptors. Proceedings of the National Academy of Sciences, USA 102:9577-9582

255 Rogers LM, Delahay RJ, Cheeseman CL, Langton S, Smith GC, Clifton-Hadley R (1998) Movement of badgers
256 (*Meles meles*) in a high density population: individual, population and disease effects. Proceedings of the Royal
257 Society Series B 265:1269-1276

258 Schwartz DA, Cook DN (2005) Polymorphisms of the Toll-like receptors and human disease. Clinical Infectious
259 Diseases 41:403-407

260 Sun L, Song Y, Riaza H, Yang H, Hua G, Guoc A, Yanga L (2012) Polymorphisms in toll-like receptor 1 and 9
261 genes and their association with tuberculosis susceptibility in Chinese Holstein cattle. *Veterinary Immunology*
262 and *Immunopathology* 147:195-201

263 Tabel Y, Berdeli A, Mir S (2007) Association of TLR2 gene Arg753Gln polymorphism with urinary tract infection
264 in children. *International Journal of Immunogenetics* 34:399-405

265 Takeda K, Akira S (2005) Toll-like receptors in innate immunity. *International Immunology* 17:1-14

266 Tschirren B, Andersson A, Scherman K, Westerdahl H, Mittl P, Råberg L (2013) Polymorphisms at the innate
267 immune receptor TLR2 are associated with *Borrelia* infection in a wild rodent population. *Proceedings of the*
268 *Royal Society of London Series B* 280:doi:101098/rspb20130364

269 Tschirren B (2015) *Borrelia burgdorferi sensu lato* infection pressure shapes innate immune gene evolution in
270 natural rodent populations across Europe. *Biological Letters* 11:20150263

271 Vasselon T, Detmers PA (2002) Toll receptors: a central element in innate immune responses. *Infection and*
272 *Immunity* 70:1033-1043

273 Vinkler M, Bainová H, Bryjová A, Tomášek O, Albrecht T, Bryja J (2015) Characterisation of Toll-like receptors
274 4, 5 and 7 and their genetic variation in the grey partridge. *Genetica* 143:101-112

275 Williams RH, Morley EK, Hughes JM, Duncanson P, Terry RS, Smith JE, Hide G (2005) High levels of
276 congenital transmission of *Toxoplasma gondii* in longitudinal and cross-sectional studies on sheep farms
277 provides evidence of vertical transmission in ovine hosts. *Parasitology* 130:301-307

278 Werling D, Jann OC, Offord V, Glass EJ, Coffey TJ (2009) Variation matters: TLR structure and species-
279 specific pathogen recognition. *Trends in Immunology* 30:124-130

280 Zhang Y, Jiang T, Yang X, Xue Y, Wang C, Liu J, Zhang X, Chen Z, Zhao M, Li J (2013) Toll-like receptor -1, -
281 2, and -6 polymorphisms and pulmonary tuberculosis susceptibility: A systematic review and meta-analysis
282 *PLoS ONE* 8:e63357

283

284 **Figure Legends**

285

286 **Fig. 1: Schematic diagram of the amino acid structure of the badger TLR2 gene showing SNP locations.**

287 Spotted area = LRR domain; Hatched region = TIR signalling domain; The positions of variant amino acids T336I

288 (C1007T) and Q574H (G1722C) are marked at the base of the gene image by upward pointing arrows (▲). A

289 300 base-pair scale is marked horizontally along the top for reference.

290

291 **Table 1: DNA sequence polymorphisms in badger TLR2**

292 This table shows the three haplotypes found, indicating position of changes and types of mutation.

293

294

295 Table 1. DNA sequence polymorphisms in Badger TLR2

296

Haplotype	Nucleotide Variation	SNP type	Amino Acid Variation	Mutation
H1 (Wild Type)	Position 1007 ACA Position 1722 CAG			
H2	Position 1007 ACA > ATA	Transition	Position 336; Threonine (T) >Isoleucine (I)	Missense
H3	Position 1722 CAG > CAC	Transversion	Position 574; Glutamine (Q) > Histidine (H)	Missense

297

298

299

Figure 1

