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Polymorphisms of the *SLC11A1* gene and resistance to bovine tuberculosis in African Zebu cattle

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Summary

Bovine tuberculosis (BTB) is a considerable health threat to livestock keepers and general communities in many developing countries. Information on genetic resistance or susceptibility because of polymorphisms of candidate genes could be used in making selection decisions for breeding disease tolerant/resistant animals. Here, we investigated associations between polymorphisms at the *solute carrier family 11 (proton-coupled divalent metal ion transporters), member 1* gene (*SLC11A1*, previously known as *natural resistant associated macrophage protein 1*, *NRAMP1*), with BTB phenotypes in Chadian cattle. Phenotypes were (i) single intradermal comparative cervical tuberculin test (SICCT) outcome, (ii) presence of gross visible lung lesions, (iii) a bacteriological culture test outcome and (iv) a predicted true BTB infection status using a Bayesian model. All traits were recorded as binary (presence or absence) traits. A total of 211 cattle were genotyped for a microsatellite within the *SLC11A1* candidate gene. Standard linear and threshold-liability models regressing BTB traits on copy number of *SLC11A1* alleles revealed statistically significant effects of *SLC11A1* alleles ($P < 0.001$) on most BTB traits. Polymorphisms (alleles 211, 215 and 217) are significantly related to lower incidence of BTB traits in Chadian cattle. This is the first study to report the association of *SLC11A1* gene polymorphisms with BTB traits in Chadian or any other African cattle breeds.

Keywords association, bovine tuberculosis, *Mycobacterium bovis*, *SLC11A1* polymorphism.

Mycobacterium bovis causes bovine tuberculosis (BTB), an important disease of domesticated cattle that has a major economic and health impact, including human deaths through zoonosis. Polymorphisms within candidate genes have been shown to affect phenotypic outcomes in many economically important traits and clinical diseases in animals (e.g. Kadarmideen 2008); this information could be used in making selection decisions for breeding disease tolerant/resistant animals. With this as motivation, we investigated the *solute carrier family 11 (proton-coupled divalent metal ion transporters), member 1* gene (*SLC11A1*, previously known as *natural resistant associated macrophage protein 1*, *NRAMP1*), a known candidate gene associated with natural resistance to infection by *Mycobaterium* spp in cattle (Barthel *et al.* 2000). The *SLC11A1* gene mediates activity of macrophages against intracellular parasites during the early stages of infection.

We investigated polymorphisms of the *SLC11A1* gene and their association with the following four phenotypes: (i) single intra-dermal comparative cervical tuberculin test (SICCT) outcome, (ii) presence of visible lung lesions, (iii) a bacteriological culture test outcome and (iv) predicted true BTB infection by a Bayesian model (BM). A total of 251 animals from Mbororo (87) and Arab (164) breeds were sampled during 2005 at abattoirs in Southern Chad (see Fig. 1a, b). Details of the resource population have been described previously (Ngandolo *et al.* 2009). The SICCT, performed on live animals (details in Müller *et al.* 2009), was recorded as 1 if skin thickness >4 mm or 0 if skin thickness ≤4 mm. After slaughter, all cattle carcasses were subjected to inspection by trained local veterinary officers for the presence of gross visible lung lesions indicative of

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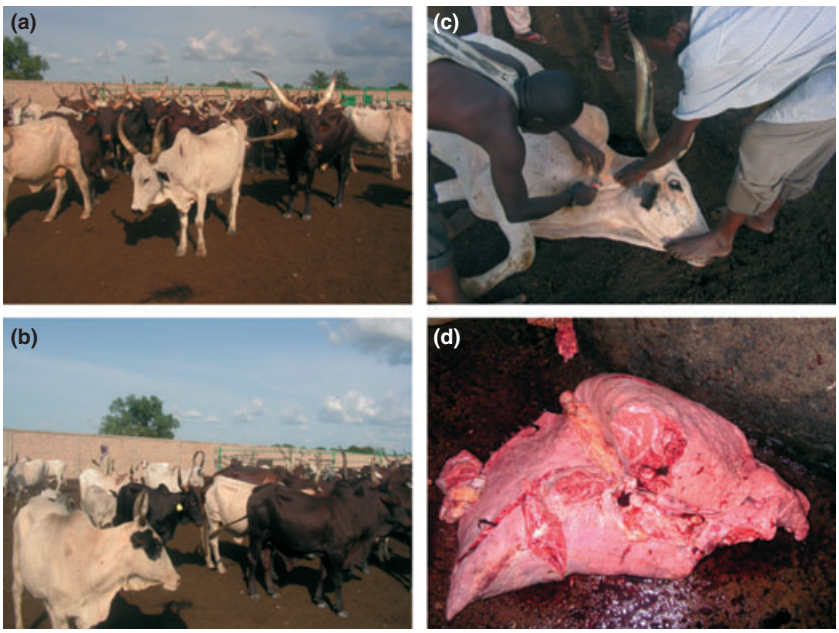

Figure 1 (a) Mbororo cattle and (b) Arab cattle at the holding facility of abattoirs in Southern Chad; (c) single intra-dermal comparative cervical tuberculin (SICCT) test being performed on Mbororo cattle and (d) presence of gross visible lung lesions in the post-mortem examination.

infection by *M. bovis*. Lesions were recorded as 1 = visible lesions present and 0 = no visible lesions present. Figure 1 shows SICCT and lesion phenotyping. Tissue samples were collected from 102 animals that exhibited gross visible lesion. The presence of acid fast bacilli (AFB), an indicator of *Mycobacterium* spp, was recorded as 1 = AFB present or 0 = AFB absent. BM prediction of true *M. bovis* infection status of all animals (yes = 1 and no = 0) sampled in Chad was taken from Müller *et al.* (2009).

Using extracted DNA, a genomic region of the 3′UTR with respect to cDNA sequence of the cattle *SLC11A1* gene was amplified using primer pair SLCA1-F: GTGGAATGAGTGGGCACAGT and SLC11A1-R: TCTCCGTCTGCTGTGCAT. Only 211 of 251 animals were successfully genotyped at *SLC11A1*. BTB traits (SICCT, lesion and BM score) were available for all 211 animals, and culture records were available for 86 of these 211 animals.

Standard linear and threshold-liability models were fitted to BTB binary phenotypes to investigate the allele substitution effects of the *SLC11A1* gene on BTB traits in ASReml 3.0 (Gilmour *et al.* 2009). A standard linear allele substitution model was

$$y_i = \alpha_0 + \mathrm{A}_i + \mathrm{S}_i + \mathrm{B}_i + \sum_{J=1}^{4} \alpha_J . cSLC11A1_J + \varepsilon_i,$$

where α_0 is the intercept, A is age in years (up to 12 years), S is sex (male or female), B is breed (Arab or Mbororo) and ϵ is residual. Term $cSLC11A1_j$ is the number of copies of *SLC11A1* allele (0, 1 or 2 copies) for alleles J = 211, 213, 215 and 217 (with frequencies 0.476, 0.211, 0.252 and 0.061, respectively). The least common allele, 217 was set as a reference level (=α_0). A threshold-liability model was fitted using the probit link function as $\pi_i = \Phi(\eta_i)$, where π_i is the probability of observing a BTB trait, and

$$\eta_i = \alpha_0 + \mathrm{A}_i + \mathrm{S}_i + \mathrm{B}_i + \sum_{J=1}^{4} \alpha_J . cSLC11A1_J.$$

An example of applications of different liability models to ordinal and binary disease data can be found in the study by Kadarmideen *et al.* (2004), and applications of allele substitution models can be found in the study by Kadarmideen (2008).

The incidences of various binary BTB phenotypes were calculated as the proportion of animals that were positive (score = 1). Of 211 animals used in the analysis, there were 27.4%, 41.5%, 48.3% and 25.9% of animals positive for SICCT, lesion, culture and Bayesian score, respectively. Bishop & Woolliams (2010) argue that incomplete exposure to infection can reduce disease incidences and accuracy of disease heritability estimates, which in this case translates to estimable gene/marker effects. In both linear and threshold models, sex effects were statistically significant for all BTB traits investigated ($P < 0.01$); however, age and breed effects were only marginally significant (in the range $0.01 < P < 0.10$). Compared to male cattle, cows had a significantly greater incidence of lung lesions (11.6%) and BM predicted TB (8.2%), with a minor increase in incidence of skin reactions (2.2%). However, with the culture test, cows had a significantly lesser incidence of presence of AFB (28% less from the mean of 48%) than males. Older animals had an increased probability of developing BTB for all BTB traits analysed. The Mbororo breed was likely to have

Table 1 Allele substitution effects on linear and liability scales for tests of association of *SLC11A1* alleles with SICCT, lesion and BM outcomes (both model *SLC11A1* effects were significant at $P < 0.001$).

SLC11A1 allele	SICCT	Lesion	Culture[1]	BM[2]
Linear model				
Intercept (0/1)	0.14 (0.09)	0.43 (0.28)	0.48 (0.68)	0.14 (0.11)
211	0.05 (0.03)	0.03 (0.01)	0.08 (0.19)	0.05 (0.06)
213	0.15 (0.09)	0.04 (0.01)	0.07 (0.16)	0.15 (0.08)
215	0.03 (0.02)	0.03 (0.01)	0.07 (0.19)	0.03 (0.02)
217[3]	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Threshold model[4]				
Intercept (liability)	−1.08 (0.98)	−0.19 (0.12)	−5.04 (7.90)	−1.05 (0.87)
211	0.17 (0.18)	−0.11 (0.10)	0.87 (1.03)	0.17 (0.12)
213	0.48 (0.30)	−0.19 (0.12)	0.48 (0.76)	0.48 (0.20)
215	0.06 (0.05)	−0.17 (0.16)	0.53 (0.81)	0.07 (0.08)
217[3]	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)

[1]All tests of associations with culture outcomes were not significant ($P < 0.05$).

[2]Bayesian model (BM) prediction for true bovine tuberculosis status.

[3]Allele 217 is the reference level allele.

[4]The estimated liabilities (η) can be transformed to incidences on [0,1] interval using cumulative density function as: $\Phi(\eta)$.

11.2% more lesions, 12.6% more SICCT positive outcomes, 26.3% more culture positives and 18% more predicted TB status, compared to the Arab breed.

Allele substitution effects presented in Table 1 are estimated regression coefficients with (s.e.), which gives the expected linear increase in substituting 0 or 1 or 2 copies of an allele against the reference allele (217). Alleles 211, 213 and 215 generally increased the incidence of skin reactions, lung lesions and BM predicted infectious state compared to reference allele 217. However, allele 215 was favourable in these traits, as it contributed to smaller increases in skin, lesion and infection state incidences and liabilities than did alleles 213 and 211. In most cases, allele 213 increased the incidence and liability to disease much more strongly than the other alleles. Results for association with culture tests were non-significant (because of the small sample size of 86 animals). We recommend that greater emphasis be placed on BM predicted infection status, because it combines several different diagnostic tests. Comparing results from linear models with liability models, the ranking of which alleles are favourable did not change much. In conclusion, alleles 211 and 215 (and the reference allele 217) at *SLC11A1* are associated with reduced incidence of BTB. Biologically, polymorphisms exert their effects on incidence via *SLC11A1* gene expression differences between animals carrying favourable versus unfavourable allelic sequences. This is the first study to report on the strength of association between the *SLC11A1* gene and BTB diagnostic traits in African cattle.

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