ASSOCIATION OF MICROSATELLITE MARKERS AND NRAMP1GENE WITH BOVINE TUBERCULOSIS TRAITS IN ZEBU CATTLE

A. Ali [1,2], H.N. Kadarmideen[1], P. C. Thomson [2], C. Flury[3], B. Müller[4] and J. Zinsstag[4]

[1] CSIRO Livestock Industries, JM Rendel Laboratory, Rockhampton, QLD 4701, Australia.
[2] Faculty of Veterinary Science, University of Sydney, Australia.
[3] Berner Fachhochschule, Scheweizerische Hochschule Für Landwirtschaft, Länggasse 85, Zollikofen , CH-3052, Switzerland. [4] Swiss Tropical Institute, Basel, CH 4002, Switzerland.

SUMMARY

The main objective of this study was to detect association between microsatellite genetic markers and a candidate gene with tuberculosis-related traits in African *zebu* cattle. A total of 249 of Chadian *zebu* cattle was genotyped for 23 microsatellites and for a known candidate gene *NRAMP1* (natural resistance associated macrophage protein 1). These animals were measured for two tuberculosis-related traits, namely, single intra-dermal comparative cervical tuberculin (SICCT) test on live animals and lung lesion (LL) from the same animals at slaughter. A generalised linear mixed model (GLMM) treating both traits as binomially distributed was fitted using *probit* link function. Eleven out of 21 microsatellite markers tested were significantly associated with presence of LL (P-value < 0.001 to P-value <0.01). For SICCT trait, only BM2113 marker was significant (P-value = 0.012). NRAMP 1 gene (chr 1) was significantly associated with LL at P-value = 0.006, but not associated with SICCT test (P-value = 0.488). Reasons for this disagreement are considered. These results show that these genetic markers and *NRAMP1* gene could be potentially used in marker-assisted selection (MAS) strategies in breeding programs to control the spread of *Mycobaterium. Bovis,* which is a causative agent of bovine tuberculosis.

INTRODUCTION

Mycobacterium bovis is a member of the group of *Mycobacterium* genus classified as the *Mycobacterium tuberculosis complex* and the cause of bovine tuberculosis (bTB). *Mycobacterium bovis* (*M. bovis*) has a broad range of hosts including livestock, wildlife and humans. The bTB is a disease of socio-economic and public health importance and constraining international trade of animals and their products. In Africa, the disease is present virtually on the whole continent (Ayele *et al.* 2004), and due to lack of financial resource, very few countries are able to apply a standard control measurement. In previous studies from Chad and Cameroon, significant differences in bTB infection prevalence were observed between Arab and Mbororo breeds in African zebu cattle (Diguimbaye-Djaibe *et al*. 2006). Resistance and susceptibility to infectious diseases is often influenced by the genetics of the host. Based on this consideration, the present study investigates possible association between 23 microsatellite markers and a known candidate gene *NRAMP1* with bTB infection of *zebu* cattle in Chad. This study is first of its kind to investigate association between microsatellite markers and known a candidate gene *NRAMP1* with bTB in African cattle.

MATERIAL AND METHODS

Animals. A total 249 animals of Arab (n=162) and Mbororo (n=87) breeds from African zebu cattle were measured for several phenotypes of bTB-related traits between July and November 2005 at abattoirs in Southern Chad, which included SICCT tests and lung lesions. Animals were raised in a long distance transhumant livestock production system with frequent trans-border movement of herds between the Central African Republic and Chad. These animals are considered a representative sample from a large number of different herds and big area in southern Chad.

Determination of bTB infection. SICCT testing results were available for all animals investigated in this study. To be able to perform SICCT on animals to be slaughtered, an arrangement was made with the slaughterhouse management to maintain animals three days prior to slaughter in the animal confinement area. SICCT testing and reading was carried out according to standard protocols. After slaughter, post-mortem examination was carried out by inspecting lungs and other organs for the presence of TB-like lesion. If a TB like lesion was detected, a specimen was collected for bacteriological culture. *Mycobacterium bovis* was confirmed by detection of acid fast bacilli (AFB) using Ziehl Neelsen method.

Genotype data. Blood samples were collected and genomic DNA was extracted using the QIAamp® DNA Blood Kit (QIAGEN, Cat. No. 51106).A total of 249 animals were available for genotyping. Twenty three markers:BM1818, BM1824, BM2113, CSRM60, CSSM66, ETH10, ETH225, ETH3, ETH152, ETH185, TGLA122, TGLA126, TGLA227, TGLA53, ILSTS005, ILSTS006, HEL5, HAUT27, SPS115, INRA32, INRA35, INRA23, MM12 and a known candidate *NRAMP1* gene was investigated in this study. Markers were selected from genetic diversity markers of African zebu cattle recommended by Domestic Animal Diversity (DAD) Information System of the Food and Agriculture Organisation (http://dad.fao.org/). All of the loci under investigation were polymorphic. The ranges of genotypes and alleles were 10 to 51 and 4 to 16 respectively. Markers with missing fractions of more than 20% were excluded from the analysis (see table 1).

Table 1. Number of genotypes and percent of missing genotypes for the Arab and Mbororo breeds of African zebu cattle.

Arab breed (n=162)			Mbororo breed (n=87)		
Marker	# genotypes	% missing	Marker	# genotypes	% missing
BM1818	35	1.5	BM1818	29	6.8
BM1824	14	0.0	BM1824	14	0.0
BM2113	32	0.0	BM2113	31	0.0
CSRM60	30	0.0	CSRM60	30	0.0
CSSM66	42	0.0	CSSM66	42	0.0
ETH10	30	1.5	ETH10	30	0.0
ETH225	23	1.5	ETH225	23	0.0
ETH3	20	0.0	ETH3	20	0.0
ETH152	10	0.0	ETH152	9	1.4
ETH185	45	1.4	ETH185	41	0.0
HAUT27	19	13.7	HAUT27	17	10.8
HE5	22	26.7	HE5	22	29.7
ILSTS006	30	3.8	ILSTS006	30	5.4
ILSTS005	18	7.6	ILSTS005	15	4.1
INRA23	32	0.8	INRA23	32	0.0
INRA32	32	3.8	INRA32	32	2.7
INRA35	21	2.3	INRA34	21	4.1
SPS115	14	0.0	SPS115	16	0.0
TGLA122	37	3.8	TGLA122	37	2.7
TGLA126	27	0.0	TGLA126	28	0.0
TGLA227	31	0.0	TGLA227	31	0.0
TGLA53	50	29.0	TGLA53	51	19.9
MM12	51	0.0	MM12	51	0.0
NRAMP1	10	0.0	NRAMP1	10	0.0

Statistical analysis. Association analyses were restricted to SICCT and LL. These response variables were recorded as discrete data (1 recorded as positive and 0 as negative). A generalized linear mixed models (GLMM) treating both traits as binomially distributed was fitted using the probi*t* link function and dispersion parameter was fixed at 1 in GenStat 11.1 package, terms included in the model were sex, age and breed and genotypic effects of each marker and NRAMP1 gene as fixed effects. The marker-trait association analysis included one marker at a time, hence there were 22 analyses. A marker effect was considered significance if a P-value ≤ 0.01.

Results and discussion. Of the 249 animals tested, the proportions of visible lesion detected and positive SICCT reaction observed were 6.8% and 28.0% respectively, more Mbororo breed was effected by lung lesion and positive SICCT reaction than Arab breed (data not shown). Microsatellite markers, chromosomal location and P-value for association with lung lesion and SICCT traits are shown in Table 2. Overall, eleven out of 21 microsatellite markers tested were significantly associated with presence of lung lesion. For SICCT, only BM2113 marker was significance associated with positive skin tuberculin test (P-value =0.012). For lung lesion trait, BM1824 (chr 1), ETH10 (chr 5), ETH185 (chr 17) and INRA35 (chr 16) markers show high significance association (table 2).

Table 2. Association of microsatellite markers with lung lesion and tuberculin tests (SICCT) from cattle screened for bovine tuberculosis (P-values indicate significance of marker effects).

Lung lesion trait (n=249)			SICCT trait (n=246)		
Marker	Chr.	P-value	Marker	Chr.	P-value
BM1818	23	0.005	BM1818	23	0.085
BM1824	1	<0.001	BM1824	1	0.182
BM2113	2	0.001	BM2113	2	0.012
ETH3	19	0.002	ETH3	19	0.707
ETH10	5	<0.001	ETH10	5	0.308
ETH152	5	0.002	ETH152	5	0.357
ETH185	17	<0.001	ETH185	17	0.343
ILST005	10	0.008	ILST005	10	0.133
ILST006	7	0.003	ILST006	7	0.063
INRA35	16	<0.001	INRA35	16	0.102
TGLA126	20	0.003	TGLA126	20	0.147
NRAMP1	1	0.006	NRAMP1	1	0.488

SICCT = single intra-dermal comparative cervical tuberculin test Chr = chromosomal location of markers

At a genotypic level of within each marker, we observed some genotypes whose effects were either positive or negative on SICCT and LL (data not shown here); these genotypes are of interest to geneticists to implement marker assisted selection (MAS) of animals based on their genotypes. *NRAMP 1* gene (chr 1) was significantly associated with LL (P=0.006), but not associated with SICCT test (P=0.488). This may be due to some false negative results involved in the SICCT test as it can only detects an early stage of bTB infection. A considerable number of animals that were sampled had an advanced stage of bTB as previously described (Ngandolo *et al*. 2009), consequently, some SICCT tests gave false negative results., It is important to know that SICCT detects only a cell-mediated immune (CMI) response which predominates in the early stage of the disease, but this response declines and is eventually superseded by antibody-mediated response as

the disease progresses. Hence, incidence of positive SICCT tests may have been underestimated and subsequently also the marker association effects. Genetic variation in cattle hosts is an important determinant of the manifestation of infection with *Mycobacterium spp*. In previous study, an allelic variant of several genes have been implicated in the genetic susceptibility to tuberculosis infection both in human and mouse model, but little is known in cattle. These results show that these genetic markers and *NRAMP1* gene could be potentially used in MAS strategies in breeding programs to control the spread of *M. bovis* infection by increasing the frequency of desirable genes in the herds. A whole genome scan study using high density single nucleotide polymorphisms (SNPs) on a large number of genetically structured populations (i.e. backcross, F2, half-sibs and full-sibs of large families) and well recorded animals would be an ideal situation, but this may not be realistic in Africa. Although, microsatellite markers used here were for genetic diversity study, we included them in the association analyses with an expectation that some of the markers might turn out to be significant. Results showed that this is true and could be used in MAS or gene assisted selection (GAS); these MAS / GAS strategies would be more economical to apply in less developed countries where there are no comprehensive phenotypic and/or pedigree recording as in developed countries. A statistical model that fits allele substitution effects instead of genotypic effects at markers will reduce the number of parameters to be estimated. Such a model might allow fitting all 24 markers at once in multiple-marker regression. However, the peculiarity of this dataset was that traits were non-normal or binary and sample sizes were too small; both pose statistical challenges in terms of fitting threshold-liability models for data with small sample sizes (Kadarmideen *et al*. 2004). These approaches or problems are currently being investigated.

CONCLUSIONS

This study provides evidence for genetic variation between African cattle at DNA marker level for susceptibility to tuberculosis infection. Although microsatellite markers used here were for study of genetic diversity, we found that some markers and *NRAMP1* gene are significantly associated with bTB related traits of SICCT and LL. Those markers and *NRAMP1* could be potentially used to implement MAS/GAS strategies for breeding program to control the spread of *M. bovis* by increasing the frequency of a favourable genotype(s) in the herds.

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