Genet. Sel. Evol. 35 (Suppl. 1) (2003) S193–S200

DOI: 10.1051/gse:2003027

Original article

Selection assisted by a *BoLA-DR/DQ* haplotype against susceptibility to bovine dermatophilosis

Jean-Charles MAILLARD[a*], David BERTHIER[a], Isabelle CHANTAL[a], Sophie THEVENON[b], Issa SIDIBÉ[b], Frederic STACHURSKI[b], Désiré BELEMSAGA[b], Hanta RAZAFINDRAÏBÉ[c], Jean-Michel ELSEN[d]

[a] Cirad – Département d'Élevage et de Médecine Vétérinaire Tropicale, Animal Health Program, Montpellier, France
[b] Cirdes, Unité de Recherches Biologiques, Bobo-Dioulasso, Burkina Faso
[c] Fofifa – Département de Recherches Zootechniques et Vétérinaires, Antananarivo, Madagascar
[d] Inra – Station d'Amélioration Génétique Animale, Castanet-Tolosan, France

(Accepted 4 February 2003)

Abstract – Bovine dermatophilosis is a severe skin infection of tropical ruminants inducing a severe loss in productivity and a 15% mortality rate. This disease is caused by the actinomycete bacterium *Dermatophilus congolensis* associated with the tick *Amblyomma variegatum*. Currently there are no prospects for a vaccine, and acaricide or antibiotic control is hampered by the development of chemoresistance. Animal breeders have observed that dermatophilosis susceptibility seems to be determined genetically, and we previously identified a *BoLA-DRB3-DQB* class II haplotype marker for high ($R^2 = 0.96$) susceptibility to the disease. With this marker, we developed a successful eugenic selection procedure for zebu Brahman cattle in Martinique (FWI). Over a period of five years, a marked reduction in disease prevalence, from 0.76 to 0.02 was achieved, and this low level has been maintained over the last two years. The selection procedure, based on a genetic marker system targeting the highly polymorphic *BoLA* locus, eliminates only those individuals which are at the highest risk of contracting the disease. In the present work, we discuss the properties of this system, including the "heterozygote advantage" and the "frequency dependence" theories, and examine their involvement in the biological mechanisms at the host/pathogen interface. We speculate on the exact role of the MHC molecules in the control of the disease, how the natural selection pressure imposed by the pathogens selectively maintains MHC diversity, and how our results can be practically applied for integrated control of dermatophilosis in developing countries.

bovine dermatophilosis / *BoLA* / MHC / MAS / Brahman zebu

* Correspondence and reprints
E-mail: maillard@cirad.fr

1. INTRODUCTION

Dermatophilosis is a severe skin infection of tropical ruminants which causes severe production losses and an average mortality rate of 15%. The disease is caused by the filamentous actinomycete bacterium *Dermatophilus congolensis* which is associated with infestation by the tick *Amblyomma variegatum* [2], and there are no imminent prospects for a successful vaccine. Current control methods, using acaricides and antibiotics, are compromised by the development of chemoresistance in the target organisms, but animal breeders have observed that disease susceptibility is genetically determined. Over a period of 10 years we performed an ecopathological survey of 568 zebu Brahman cattle from several herds located on Martinique Island (French West Indies). A functional candidate gene approach was developed, and two groups of animals differing markedly in their innate resistance were compared for DNA polymorphisms among genes coding for molecules implicated in known mechanisms of both non-specific and specific immune responses. The most significant data [6–8] were obtained from the major histocompatibility complex (MHC) locus where the *BoLA-DRB3* and *DQB* genes encode molecules which are involved in pathogen/host interface mechanisms, particularly in antigen presentation to the T cell receptors. In the highly polymorphic *BoLA-DRB3* exon 2, which codes for the antigen binding groove, the occurrence of specific amino acids at certain locations corresponding to four *DRB3.2* alleles is correlated with the highest ($R^2 = 0.96$) observed susceptibility to the disease [6–8]. Secondly, we identified a strong correlation between susceptibility and the *BoLA-DQB**1804 allele [6–8]. Finally, and most interestingly, we found a strong linkage (0.97) of *DRB3.2* alleles and the *DQB* allele in a unique *BoLA* class II haplotype, which constitutes a significant ($P < 0.0001$) marker of susceptibility to bovine dermatophilosis [6]. This haplotype marker of susceptibility has also been found and validated in other bovine populations [6–8]: Brahman zebu of Madagascar, Gudali zebu in Cameroon, zebu (*Bos indicus*) and Baoule (*Bos taurus*) in Burkina Faso, and Criollo cattle in Guadeloupe (FWI). Using this haplotype marker for high susceptibility, we developed an assisted selection procedure in Martinique (FWI).

2. MATERIALS AND METHODS

2.1. Animals

The study was developed in Martinique from a 10 year long survey on a male and female zebu (*Bos indicus*) Brahman cattle population ($n = 568$) of all ages from the same Trois Rivières farm in the south of the island. All animals were reared in the same location, under the same environmental conditions (tropical climate, extensive grazing with constant and similar levels of tick challenge),

and they were routinely given acaricide treatment in a dipping tank. The severity of the disease was scored visually on a scale going from benign (some spots and scabs) to most severe (death). During the whole survey, the animals infected by dermatophilosis were treated with penicillin (Duphapen-LA®, IM 1 mL per 15 kg live weight).

2.2. Methods

2.2.1. Marker typing

The BoLA-*DRB3.2**09/*45 and *DQB**1804 alleles are strongly associated in a unique marker haplotype for susceptibility (0.97) [6–8]. PCR-RFLP genotyping of this *BoLA* class II haplotype of susceptibility classically involves three successive restriction digestions (*Rsa*I, *BstY*I and *Hae*III) as described by the official *BoLA* nomenclature reference group[1]. To improve the efficiency of such a genotyping, we developed a PCR amplification of specific alleles (*PASA*) to quickly and specifically identify this marker, using a forward *PASA* inner primer (5′-*GCG GGC GGG G*TT CCT GGA GAG ATC-34) located in the exon 2 region spanning the E (28) and S (30) marker amino acid positions (Fig. 1).

The reaction is specific when three primers are incorporated: the two classical *Bod* 31 and *Bod* 32 outer primers[1], and the *PASA* inner primer. The reactions were performed in a final volume of 50 μL which contained: *Bod* 31 and *Bod* 32 primers (3 pmoles); *PASA* primer (2 pmoles); dNTPs (200 μM); $MgCL_2$ (1.5 mM); Tris-HCl (10 mM); 50 ng of DNA; and *Amplitaq Gold* (PE Applied Biosystems, Fosters City, CA, USA, 0.5 U). The amplification was carried out in a Perkin Elmer 2400 thermal cycler using the following conditions: initial denaturation 94 °C, 12 min; followed by 30 cycles of denaturation (94 °C, 30 s), annealing (59 °C, 30 s) and extension (72 °C, 30 s); ending with a final extension (72 °C, 5 min). The *Bod* 31/*Bod* 32 primers amplify a 304 bp segment of *BoLA-DRB3* exon 2, while the *PASA*/*Bod* 32 primers amplify a 235 bp segment only in the *BoLA-DRB3**09 allele marker of susceptibility (Fig. 2).

2.2.2. Field selection

In May 1994, there was a total of 429 individuals in the Trois Rivières herd, and the disease prevalence was 0.20. The prevalence was on the increase, however, reaching 0.50 by the end of 1995. As a result of the death of the most severely affected animals, the size of the herd had dramatically decreased to less than 200 individuals by May 1996, with a disease prevalence of 0.76 (Fig. 3). At that time, extrapolation of the evolution of the population size suggested

[1] [http://www.projects.roslin.ac.uk/bola/classii.html]

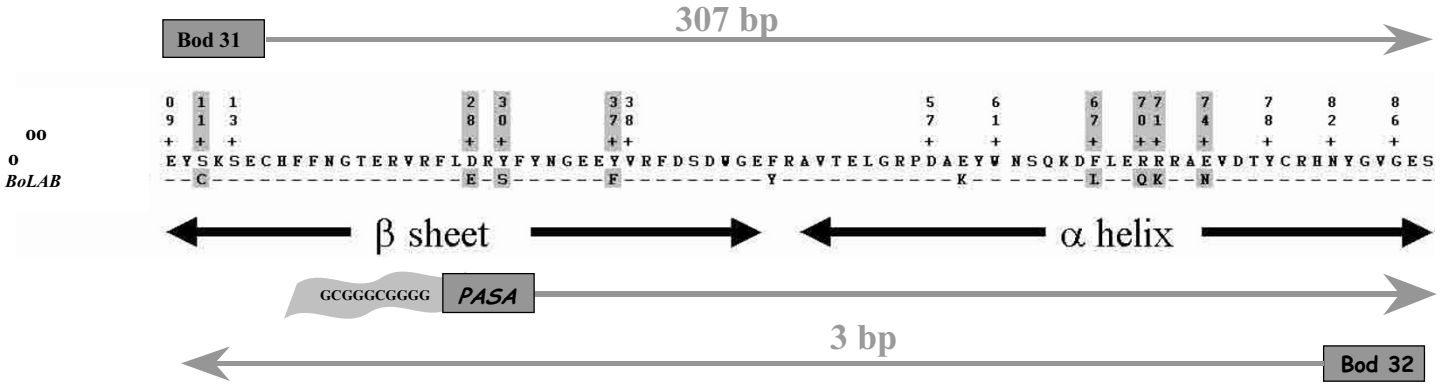

Figure 1. Amino acid residues constituting the CESFLQKN susceptibility marker in exon 2 of the *BoLA-DRB3**09 allele.

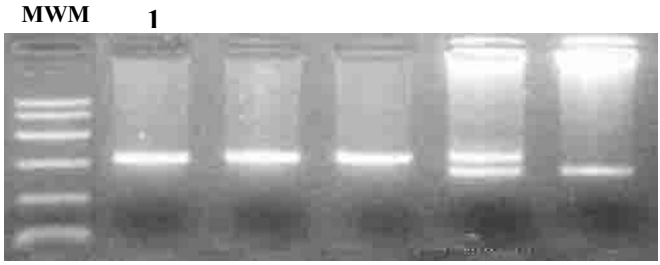

Figure 2. Electrophoretic gel pattern of the PCR amplification of specific alleles (PASA). Lane 1: molecular weight marker (MWM); lanes 1–3: positive amplification of any *BoLA-DRB3* exon 2 alleles (307 bp); lane 4: heterozygous profile including one *PASA* susceptibility allele (235 bp); and lane 5: homozygous profile of *PASA* alleles.

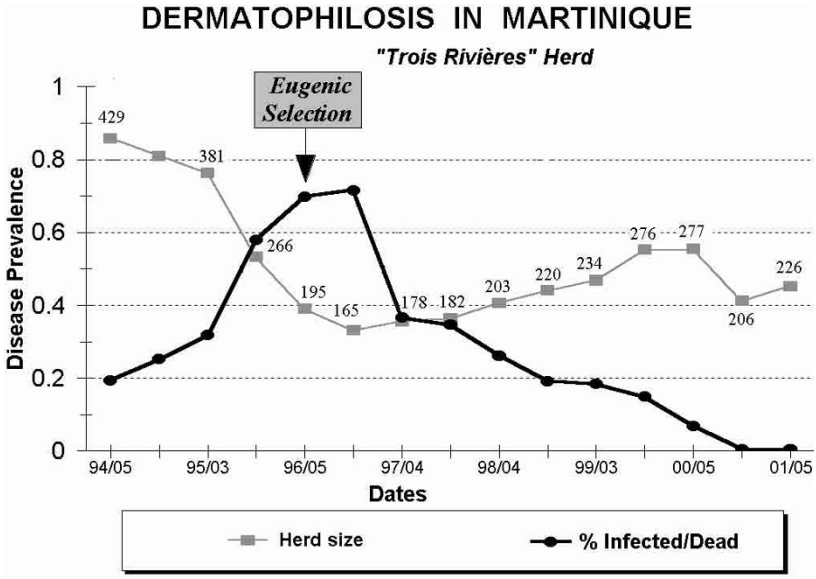

Figure 3. Evolution of the clinical status of dermatophilosis after selection assisted by a *BoLA* haplotype marker of susceptibility.

that, without intervention or reduction of the dermatophilosis challenge, the entire herd would probably die out in a short time. In the summer of 1996, after consulting the breeder, we decided to systematically cull the animals with the *BoLA-DRB3**09 or *45 prediction markers of susceptibility (positive *PASA*), without taking into consideration the score of severity. The frequency of animals with this haplotype was 0.34 in May 1994 [8], and the haplotype of high susceptibility had completely disappeared from this herd in May 1996, after selection was completed.

3. RESULTS

Figure 3 shows the development of clinical dermatophilosis in the herd. Six months after initiating the selection procedure, the disease prevalence started to decrease rapidly, getting back to the initial 1994 level of 0.2 by the end of 1998, and falling eventually to 0.02 by the end of 2000. During the same 5 years of the survey, the animals reproduced and the herd size increased slowly. During the same period, we observed no change in the ecopathological factors related to dermatophilosis. In 2000, the herd was serologically monitored using a dermatophilosis-specific ELISA test, and the average antibody prevalence was 0.98. This confirmed that there was a continual disease challenge, and that the surviving animals had developed efficient protective immunity.

4. DISCUSSION

The present work confirms the existence of correlations between susceptibility to disease and a specific MHC haplotype reported in animal and man [1, 5, 10].

One member of our group (BD) has built a three dimensional model [4] which gives a possible explanation of the present observations. The antigen presentation site (APS) of the class II MHC protein is a groove formed between β sheets on one side and two α helices on the other. This APS region is encoded by exon 2 of the *DRB3* or *DQB* genes, and it contains 16 polymorphic amino acid residues implicated in antigenic peptide binding (Fig. 1). The CESFLQKN marker sequence which we observed could induce spatial modifications in the binding groove pockets leading to alterations in the binding efficiency of some peptides [1, 10]. It is possible that these peptides are exposed to pathogen proteases and are either not recognized or poorly recognized by the T cell receptor. The efficacy of the immune response may then be specifically and/or quantitatively modified by this kind of escapement mechanism. The high level of polymorphism in the *BoLA* system may provide new alleles in the host population which bind antigenic peptides of slightly variant pathogens (Fig. 4), some of which might otherwise escape the protection mechanism [3].

A selection procedure focused on retaining one specific resistance allele might undermine the advantages brought about by *BoLA* heterogeneity since the pathogen could readily mutate, thereby escaping the resistance conferred by the single allele, with serious consequences at the population level. In contrast, a "eugenic" selection which eliminates the most susceptible alleles from the global population, but retains other alleles, could be more effective. This approach is consistent with the "heterozygote advantage" and "frequency dependence" theories described by Apanius *et al.* [3] for other man and animal diseases, and it was validated by the results presented in this paper.

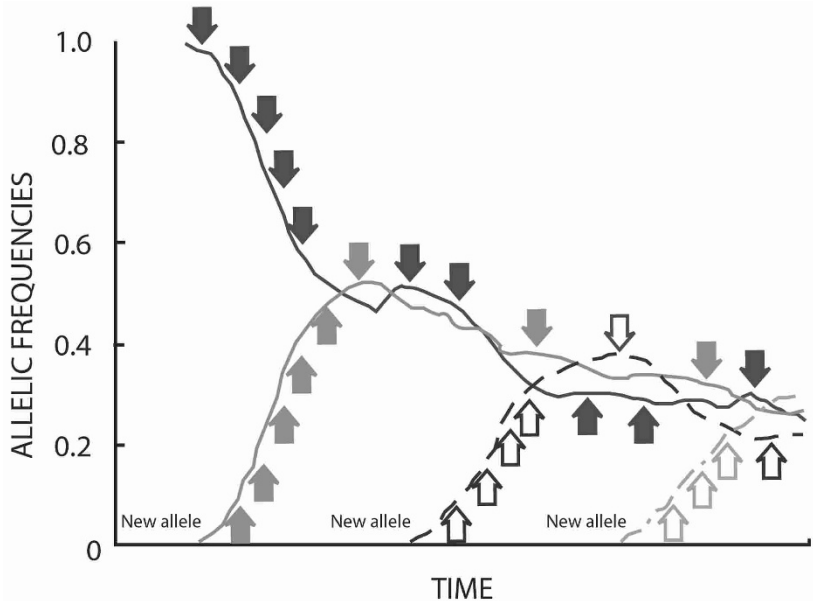

Figure 4. A theoretical graph of the MHC allelic turnover at the host population level to control the pathogen variability and its escapement mechanisms (source Apanius *et al.* [3]).

Other genes are probably also involved in the susceptibility to the disease but focusing on the *DRB3/DQ* haplotype is sufficient to eliminate the acute clinical cases in the herd, and to decrease the epidemiological prevalence of dermatophilosis. At the population level, maintaining a small number of chronic cases of the disease maintains a dermatophilosis-specific genetic "memory" in the herd, particularly with respect to MHC antigen presentation molecules.

Selection assisted by markers of susceptibility might be particularly valuable in developing countries under livestock management systems with a high constant pathological pressure in the field, even in the absence of pedigree information. This rapid and specific genotyping technique with its subsequent culling of carriers also makes it applicable in transhumant cattle populations.

ACKNOWLEDGEMENTS

We acknowledge the European Community for its financial support (INCO-DC, IC18-CT98-0334) and Dr. B.A. Allsopp from the Onderstepoort Veterinary Institute (OVI)-South Africa for reading the first draft of this paper.

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