Comparison of DRB3.2 alleles among false-negative and positive tuberculin skin test cattle with bovine tuberculosis

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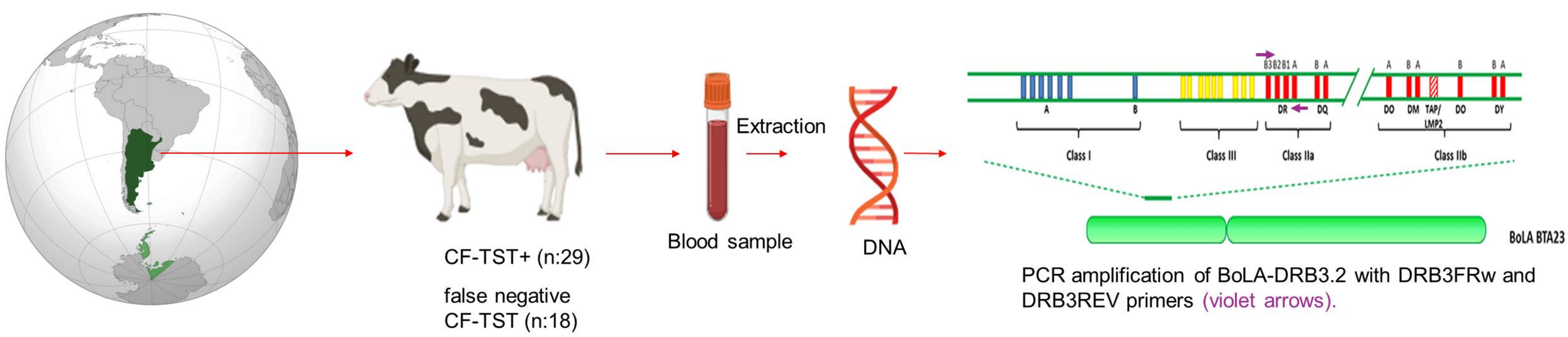
Introduction

M. bovis is worldwide distributed mainly affecting livestock. Caudal Fold-Tuberculin Skin Test (CF-TST) is the primary screening tool that detects the infection in herds. Some tuberculous animals remain undetected (false-negatives) limiting the success of the sanitation programs. Bovine leukocyte antigen (BoLA) DRB3 has been asociated with resistance or susceptibility to several infectious diseases and it is crucial for the antigenic presentation. Characterization of the genetic background among false-negative bovines, related to BoLA DRB3.2, could reveal host aspects associated to this phenotype.

Objetive

To identify BoLA-DRB3.2 polimorphysms in false-negative CF-TST cattle, comparing to CF-TST+ bovines in argentinian herds.

Material and Methods



- Twenty-nine CF-TST+ (controls) and 18 false-negative CF-TST (cases) Hosltein bovines from dairy productive areas were studied.
- DNA was extracted from blood using a commercial kit (PuriPrep-S kit, InbioHigway®).
- PCR amplification of BoLA-DRB3.2 (319bp) was carried out with DRB3FRW and DRB3REV primers.
- PCR products were sequenced by double reading frame (BigDye ® v3.1, Applied Biosystems Inc.).
- DRB3.2 haplotypes were assigned using Haplofinder and allelic frequency was compared using Epidat 3.1.

Results

Considering controls, alleles *0101, *1501 and *1001 were the most frequent followed by *1101, *0902, *0601 and *1701 (Figure 3). Among false-negatives (cases), *1501, *1101, *0101, *4401 and *2703 were the most frequent alleles followed by *1001, *0902, *3701, *0601, *1601, *1701 and *14011 (Figure 2). The *0101 allele was more prevalent in CF-TST+ (p=0.01), while the frequency associated to the other allelles were similar among both, false-negative and CF-TST+ groups (p>0.05). Alelles *1601, *3701, *4401, *14011 and *2703 were only detected in false-negatives, but in a frequency ≤ 8.3% (Figure 1).

Figure 1. Alleles identified in cases and controls groups. Seven alleles were detected in both groups. Five alleles were only identified among cases but in low frecuency (≤ 8.3%).

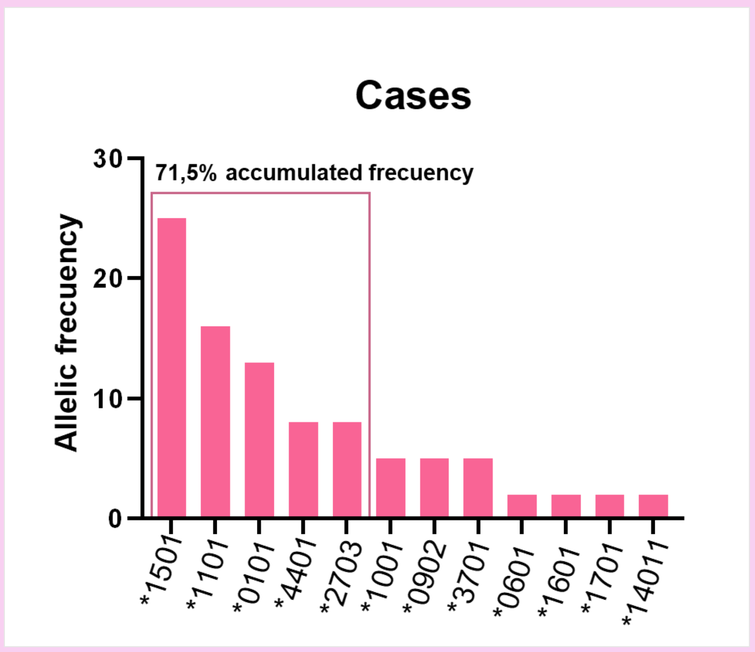


Figure 2. Allelic frecuency observed among cases.

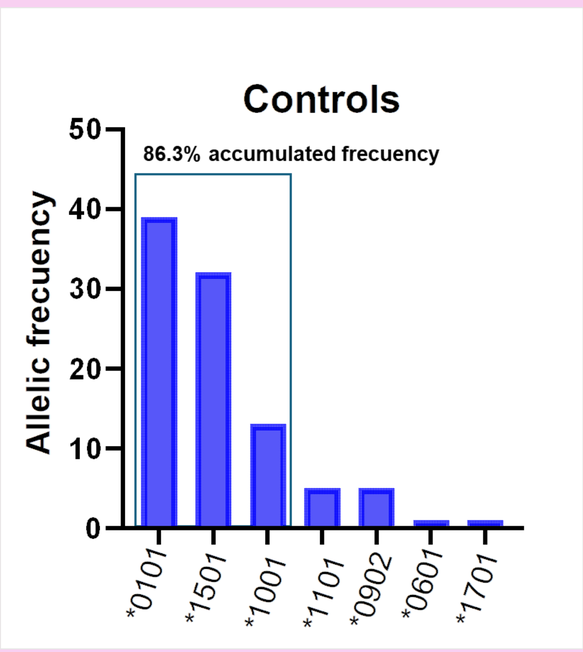


Figure 3. Allelic frecuency observed among controls.

Conclusions

A higher diversity of BoLA-DRB3.2 alleles was observed in false-negative CF-TST comparing to CF-TST+ cattle; with some alleles exclusively identified, in a low proportion, among false-negatives bovines. To note, some of these alleles (*2703 and *1601) were associated to mastitis resistance (2) and to a high bovine leukemia virus proviral load in cattle (3). In addition, all the alleles exclusively detected among cases were described in cattle with confirmed bTB infection, however, the CF-TST status was not unknown. Further studies are needed to really comprenhend the implicancies of the DRB3 diversity and a possible association between DRB3.2 alleles and the false negative CF-TST phenotype.

1.Yoshida et al;2012. Association of BoLA-DRB3 alleles with mastitis resistance and susceptibility in Japanese Holstein cows

2. Miyasaka et al;2013. Identification of bovine leukocyte antigen class II haplotypes associated with variations in bovine leukemia virus proviral load in Japanese Black cattle

3.Eirin et al., 2020. BoLA-DRB3 exon2 polymorphisms among tuberculous cattle: Nucleotide and functional variability and their association with bovine tuberculosis pathology.