A QTL FOR RESISTANCE TO *Boophilus microplus* MAPS TO BOVINE CHROMOSOME 14

L.C.A. Regitano[1,] M. Miyata[2], G. Gasparin[2], L.L. Coutinho[3], M.L. Martinez[4], M.A. Machado[4], M.V.G.B. Silva[4], A.L. Campos[4], T. Sonstegard[5],

[1]Embrapa Southeast Cattle Research Center, CP 339, São Carlos, SP, 13560-970, Brazil
[2]UFSCar São Carlos Federal University, Rodovia Wasghington Luiz Km 235, São Carlos, SP 13565-905, Brazil
[3]USP ESALQ São Paulo University, Av. Pádua Dias, 11, Piracicaba, SP, 13418-900, Brazil
[4]Embrapa Dairy Cattle, Rua Eugênio do Nascimento, 610, Juiz de Fora, MG, 36038-330, Brazil
[5]USDA, ARS Bdlg. 200 Rm 217, Beltsville Agricultural Rsearch Center, East Beltsville, MD 20705, USA

INTRODUCTION

In countries of tropical climate like Brazil, the tick *Boophilus microplus* causes damages to cattle through hematophagism, injuries in skin and transmission of tick-born diseases. In Australia, Frisch *et al.* (2000) estimated that the average loss of weight per year, of an animal between 12 to 18 months and with 40 parasites per day could be of 20 kg and that an animal could die if it remains infested with more than 200 ticks for six weeks. The overall losses caused by *B. microplus* were estimated to be 100 million dollars per year in Australia and one billion dollars per year in Central and South America (Sabatini *et al.*, 2001).

The main used strategy in tick control or erradication is the use of carrapaticides. Besides the development of resistance from parasites to the carrapaticides, several factors should be considered: the costs of chemicals, labor, equipment; the losses of milk or meat production associated with treatment and market restrictions due to chemical residues; the effects on human health and environment (Jonsson, 1997). Tick control alternatives are being developed in research institutions around the world. Among the developed strategies are the selection of resistant bovines, biological control using pathogens or ticks predators, use of knowledge of the ecology of the tick and development of vaccines. Vaccines against tick antigens produce injuries in the intestine of ticks fed on immunized animals, impairing their growth and reproduction (Kemp *et al.,* 1989). Although vaccines have great potential in tick control, at the moment they only confer partial protection to the *Boophilus microplus* species. The improvement of vaccines efficiency is possible with integration between the use of vaccines and animals of high resistance, mainly in grazzing systems (Martinez *et al.*, 2004).

The higher resistance of *Bos indicus* if compared to the *Bos taurus* subspecies must have arisen from thousand of years of coexistence with the tick *Boophilus microplus*, that resulted in a natural elimination of the most sensitive animals, in benefit of those that were genetically more resistant (Lemos, 1986). Wambura (1998), Scholtz *et al.* (1991) and Utech *et al.* (1978) demonstrated that the zebu cattle have fewer ticks than its crosses with *B. taurus*. Currently marker assisted selection (MAS) is one of the tools suggested to exploit the information of genetic resistance to parasites. The exploration of genes with large effect on resistance to ticks is the best way to prevent losses of productivity (Martinez *et al.,* 2004). Analysis by Frisch (1994) suggested the presence of a gene with great effect on the number of ticks per animal in a crossbred Shorthorn x Hereford population, where each copy of the gene reduced the number of ticks in 75%. The results presented by Frisch (1999) indicate that it is possible to develop *Bos taurus* animals with high resistance to ticks with the introduction of this gene in the population. In previous studies, Acosta-Rodrigués *et al.* (2005) found some MHC BoLA class II alleles that control at least partially the susceptibility to tick infestation in two different

breeds in Mexico. The present work belongs to a major project that aims to scan all the bovine chromosomes for traits of economic relevance. The objective of this paper was to identify Quantitative Trait Loci (QTL) for resistance to the tick *Boophilus microplus* in an experimental Holstein-Gyr F$_2$ population using microsatellite markers on BTA14.

MATERIAL AND METHODS

Population. An experimental F2 population is under development from crosses of Holstein-Gyr F1 animals, from which about 400 F2 animals were produced. The crosses are being carried in the Santa Maria Farm, belonging to EMBRAPA, in the state of Rio de Janeiro.

DNA extraction. Blood samples were collected from all animals, including parental, F1 and F2 for DNA extraction. The DNA of bulls was extracted from semen. The DNA was extracted by protocol of Hallerman *et al*. (1988). All the samples were quantified in spectrophotometer (HITACHI U-2000 model), diluted at 40ηg/μL and stored at -25º C.

Phenotypic data. Resistance of the F2 animals to the tick *Boophilus microplus* was evaluated through the counting of female ticks that had completed their cycle following artificial infestations with 10,000 larvae per animal. For the infestation, tick larvae were placed in two bottles attached to the cervical region of the animal, so that the larvae could reach the two sides of the body. The infestations occurred at two times of the year, in the rainy season and in the dry season. Tick countings were carried in the 21st day after challenge in only one side of the animal. The observed tick number was multiplied by two to obtain the total number of ticks per animal. The countings were generally made in the morning, before mature ticks detached from animals to hatch in the soil. A total of 258 F2 animals had tick counting data available for QTL analysis.

Molecular markers. The molecular markers were chosen based on their map position, in order to have one marker every 20 cM, number of alleles and heterozygosity higher than 50%, according to the available map produced by the Meat Animal Research Center (MARC) at http://www.marc.usda.gov. A total of seven microsatellite markers were used in this work. Polymerase chain reactions (PCR) were done on termocycler *Mastercycler Gradient* (Eppendorf) and the amplified products were analyzed on an ABI Prism 3100 Avant (Applied Biosystems) sequencer using the softwares GeneScan and Genotyper (Applied Biosystems).

Linkage map. The linkage map was built from genotypes of the three generation pedigrees using the software Crimap (Green *et al.*, 1990). Through the options prepare, build, all and fixed, it was possible to build the map of BTA14, determining the distance between markers from recombination fractions with Kosambi's mapping function.

Statistical analysis. QTL statistical analysis used the linear regression method (Haley & Knott, 1992) for F2 reference families through the software QTL Express (Seaton *et al.*, 2002) at http://qtl.cap.ed.ac.uk. The F-statistic was calculated to test the hypothesis of QTL segregation at 1 cM intervals using a model that included the fixed effect of year. The effect of age was used as covariable on tick counting. Permutation tests were applied for the threshold determination adopting 1,000 permutations for the value of α = 0.01 to obtain stable estimates (Churchill and Doerge, 1994). Bootstrap method was applied for determination of the confidence interval (CI) for the presence of a possible QTL (Visscher *et al.,* 1996).

RESULTS AND DISCUSSION

The linkage map presented a higher length if compared to the map built by MARC. This fact can be explained by the small number of animals per family used in this work, small number of markers and large number of recombinations. Through the linear regression analysis, a significant QTL (P<0.01) was found on BTA 14 for tick resistance (Figure 1). The QTL was

localized at 47 cM from the centromere with F-ratio value of 10.79. The average tick counting was 21.52 ± 3.15 ticks and the additive effect attributed to the QTL was of 4.10 ± 1.25 ticks. Bootstrap analysis determined a confidence interval of 184.5 cM, between 8.0 to 192.5 cM. The width of the confidence interval depends on the size of the population and on the QTL effect, although variation in marker spacing does not result in very different confidence intervals (Visscher *et al.*, 1996).
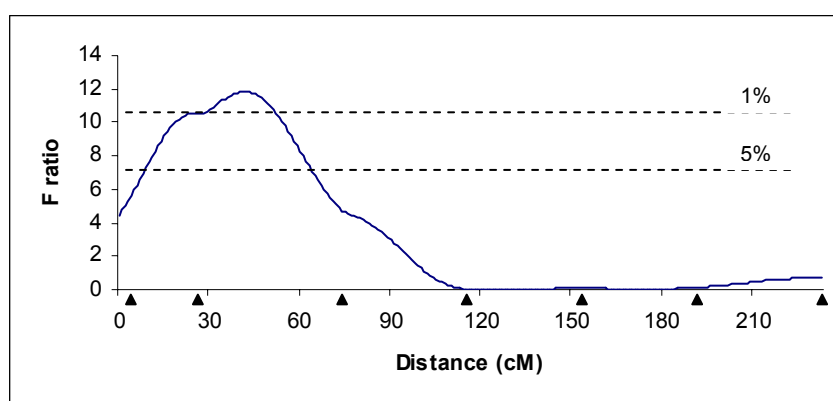
Figure 1: F-statistic distribution for tick resistance on BTA14. The arrows under the X axis indicate marker positions in chromosome from the centromere: CSSM066 (5.0 cM), ILSTS011 (27.6 cM), BMC1207 (74.3 cM), BMS740 (118.5 cM), BMS1899 (157.3 cM), BL1036 (192.8 cM) and BMS2055 (233.6 cM). The upper and lower horizontal lines represent the threshold levels at 1% (F = 10.57) and at 5% (7.86). The maximum probability for the QTL was located at 47 cM from the centromere (F = 10.79).

CONCLUSION

The Holstein-Gyr F2 population proved to be adequate for the detection of QTL for tick resistance. Given the difficulty of tick resistance evaluation, and the impact of this parasite on both cattle production and on the environment, once confirmed in other populations, application of this QTL information on MAS would be of great benefit.

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