

DETECTION OF A QTL ASSOCIATED WITH TICK RESISTANCE ON BOVINE CHROMOSSOME 7 (BTA7) USING A F₂ EXPERIMENTAL POPULATION

G.Gasparin¹, M.Miyata¹, L.L. Coutinho², M.L. Martinez³, M.A. Machado³, M.V.G.B. Silva³, A.L. Campos³, T.S. Sonstegard⁴, L.C.A. Regitano⁵

¹UFSCar São Carlos Federal University Rodovia Washington Luiz Km 235
São Carlos SP 13.565-905, Brazil

²ESALQ/USP Agricultural Graduate School/University of São Paulo Avenida Pádua Dias 11
Piracicaba SP 13.418-900, Brazil

³Embrapa Dairy Cattle Reasearch Center Rua Eugênio do Nascimento 610
Juiz de Fora MG 36.038-330, Brazil

⁴Beltsville Agriculture Center Research Center EastBeltsville MD207075, USA

⁵Embrapa Southeast Cattle Research Center Rodovia Washington Luiz Km 234
São Carlos SP 13.560-970, Brazil

INTRODUCTION

In Brazil and other tropical countries, the *Boophilus microplus* tick has become one of the most nocive bovine ectoparasites, causing great losses in the economic system of these regions. According to Kashino *et al.* (2005), this number is in the order of billions of dollars annually on these regions. Frisch *et al.* (2000) estimated that the mean weight loss of an animal infested with 40 ticks/day is around 20 Kg/year. Usually, tick infestations are treated with the use of accaricides. This is not a good approach, since these chemical products are not totally efficient, and contaminate meat, milk and environment (Vercruisse *et al.*, 2000). Other approach to combat ticks is the development of vaccines, an attractive strategy since it is safe for the environment, for the host, and for consumers. Nevertheless this method is still facing problems to be adopted due to the great ability of the ectoparasite to modulate the host immune system (Wikel and Alarcon-Chaidez, 2001; Brossard and Wikel, 2004).

Another approach is to use the genetic variability for resistance to ticks between *Bos taurus* and *Bos indicus* cattle (Oliveira and Alencar, 1990; Teodoro *et al.*, 1994). Generally, *Bos indicus* cattle are more resistant to tick infestations than *Bos taurus* (Wanbura *et al.*, 1998). With the advance of the molecular genetics, the use of genetic markers, such as microsatellites, associated with economic traits (Quantitative Trait Loci – QTL) has become a powerful tool in molecular breeding programs. The idea to use a molecular approach to study cattle tick resistance is not new. In 1968, Asthon and co-workers found an association between serum amylase phenotype and tick infestation in cattle ($P < 0.01$ in some populations). Similar result ($P < 0.05$) was found by Panepucci *et al.* (1989) in a study with Canchim cattle (3/8 Zebu, 5/8 Charolais). More recently, few papers have been published about this subject. Acosta-Rodrigués *et al.* (2005), analyzing microsatellite markes in the bovine class II BoLA complex, found association among two of these microsatellites and tick infestation. Martinez *et al.* (2005) found a QTL ($P < 0.05$) for resistance to tick on chromosome 18 in the same F₂ population we used in our research.

It has been shown that tick can modulate the host immune system (Brossard and Wikel, 2004). The mechanisms are not well established yet, but is well known that a common strategy adopted by parasites to attack their hosts is through interaction with the host cytokines (Wikel and Alarcon-Chaidez, 2001). Cytokines are messenger molecules that orchestrate the complex cross-talk and actions of the immune system. Cytokines produced by leukocytes to act on others leukocytes are called interleukins (IL). Some interleukins have been associated with tick infestations. Salivary gland extracts of *Dermacentor andersoni* suppressed production of the Th1-lymphocyte cytokines interleukin (IL-1 β) and interferon gamma (IFN- γ) by lymphocytes

from uninfested mice (Ramachandra and Wikel, 1992). Ferreira and Silva (1999), studying the levels of several interleukins in mice infested with the tick *Rhipicephalus sanguineus*, found that lymphatic cells of the host, in contact with tick saliva, increased the levels of IL-4 and IL-10, and inhibited the production of IL-2, IL-12 and IFN- γ

Since the gene encoding IL-4 is located at bovine chromosome 7 (BTA7), the objective of this paper was to identify QTL for resistance/susceptibility to the bovine tick *Boophilus microplus* in an experimental F₂ population (Holstein x Gyr), using an interval mapping approach.

MATERIAL AND METHODS

Population and phenotypic data. 150 F₁ generation animals were obtained from crosses using the semen of four Holstein sires and 28 Gyr dams. Of these, 4 F₁ sires and 68 F₁ dams were chosen to be the parents of the F₂ generation, avoiding inbreeding in the progeny. A total of 258 F₂ animals were evaluated to the incidence of ticks. Artificial infestations were made in these animals, with 10.000 larvae per animal. The number of female ticks, ranging from 4.5 to 8 mm diameter, was counted on one side of the animal, and then this number was multiplied by 2 to obtain the total number of ticks per animal. These countings were done around the 21st day after infestation.

Molecular genetics. Microsatellite marker information, such as position on the chromosome and annealing temperature of the primers, were obtained from the MARC-ARS-USA (Meat Animal Research Center – Agriculture Research Service – United States Department of Agriculture) database at <http://www.marc.usda.gov>. We chose eight markers on chromosome 7 (BTA7), with an average space between them of approximately 20 centiMorgans (cM), trying to cover the entire chromosome. The Polymerase Chain Reactions (PCRs), the analysis of the amplified products, and the genotyping of the animals were performed according to Gasparin *et al.* (2005).

Statistical analysis. The effects of sex and the year in which the animal was evaluated were assumed as fixed effects, and the age of the animal at evaluation was considered as a covariable. Tick countings were normalized using a Box-Cox transformation. The QTL analysis was performed by the method of multiple interval mapping for F₂ families (Haley *et al.*, 1994) using the *QTL Express* software (Seaton *et al.*, 2002). We applied permutation tests for the threshold determination, and the bootstrap technique to estimate the confidence interval (CI) of a possible QTL.

RESULTS AND DISCUSSION

A suggestive QTL ($P < 0.05$) for tick resistance/susceptibility was detected in chromosome 7. The highest F-statistics value ($F = 9.88$) was at 56cM from the most centromeric marker (figure 1), which does not correspond to the position of the gene for IL-4 (32 cM). Discarding the involvement of this gene on part of the QTL effect is not possible in the present data, since bootstrap analysis determined a confidence interval (CI) of 105cM, between 11 and 116cM. Such high CI value must be due to the small population used in this analysis, or even due to a QTL with small effect (Vissher, 1996). The trait average was 22.75 ± 3.25 ticks/animal, while the additive effect of the QTL was of 3.55 ± 1.13 ticks. The dams were, in general, more resistant to tick than the sires (-1.86 tick/animal).

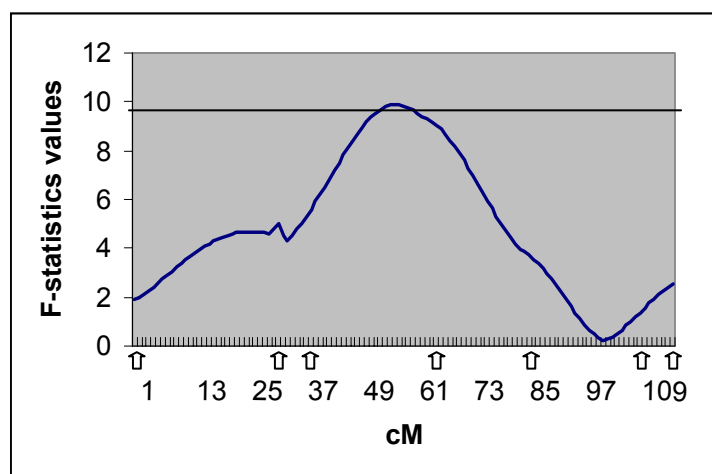


Figure 1: F-statistics for resistance/susceptibility to ticks on BTA7. The arrows indicate the marker positions on the chromosome in centiMorgans (cM): BM7160 (0.0), BM2706 (29.61), IL4 and BOBT24 (32), BM6117 (62.25), INRA192 (82.47), BM9065 (101.11) and ILSTS006 (116.63).

Only 258 animals were phenotypically evaluated to the trait analyzed. Martinez *et al.* (2005) affirm that, which more data being incorporated in the interval mapping analysis, higher values of F-statistic can be reached, increasing the significance of the QTL.

Resistance is a trait that has a medium level of heritability. Andrade (2001), studying Caracu cattle, found a value of 0.22 for tick counting heritability which considerable genetic additive variance to be used on breeding programs. Davis (1993) found an average heritability of 0.34, similar to the heritability of milk production in temperate regions. Conceição Jr (1997) estimated in 0.49 the heritability for resistance to ticks in a population of dairy cattle.

The results reported on this paper are preliminary, and constitute a part of a major project. As we get more phenotypic data, we hope to increase the estimates of the F-statistic for the identified QTL for resistance/susceptibility to tick. Since it is an important economic trait, and shows good level of heritability, once we found molecular markers associated to this trait they could be applied on a marker assisted selection (MAS) breeding program.

CONCLUSION

The use of microsatellite markers in an interval mapping analysis, using a bovine F₂ Holstein x Gyr population, has shown to be an appropriated strategy for mapping tick resistance associated QTLs. With only 258 animals it was possible to detect a QTL ($P < 0.05$, $F = 9.88$) associated to resistance/susceptibility to *Boophilus microplus* tick, at 56cM from the most centromeric marker of the chromosome 7.

REFERENCES

- Acosta-Rodrigués, R., Alonso-Morales, R., Balladares, S., Flores-Aguilar, H., García-Vasquez, Z. and Gorodezky, C. (2005) *Vet Parasitol* **127(3-4)**:313-321.
- Andrade, A.B.F. (2001) PhD Thesis, Universidade Estadual de São Paulo, Jaboticabal, Brazil.
- Ashton, G.C., Seifert, G.W. and Francis, J. (1968) *Aust J Biol Sci* **21**:303-308.
- Brossard, M. and Wikel, S.K. (2004) *Parasitol* **129**:161-171.
- Conceição Jr., V. (1997) PhD Thesis, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil.

- Davis, G.P. (1993) *Aust J Agric Res* **44**:179-198.
- Ferreira, B.R. and Silva, J.S. (1999) *Immunology* **96(3)**:25-32.
- Frisch, J.E., O'Neill, C.J. and Kelly, M.J. (2000) *Int J Parasitol* **30**:253-264.
- Gasparin, G., Miyata, M., Coutinho, L.L., Martinez, M.L., Machado, M.A., Silva, M.V.G.B., Campos, A.L. and Regitano, L.C.A. (2005) *Genet Mol Biol* **28(4)**:670-676.
- Haley, C.S., Knott, S.A. and Elsen, J.M. (1994) *Genome Res* **14**:1987-1998.
- Kashino, S.S., Resende, J., Sacco, A.M.S., Rocha, C., Proença, L., Carvalho, W.A., Firmino, A.A., Queiroz, R., Benavides, M., Gershwin, L.J. and De Miranda Santos, I.K.F. (2005) *Exp Parasitol* **110** : 12-21.
- Martinez, M.L., Azevedo, A.L.S., Machado, M.A., Guimarães, M.F.M., Silva, M.V.G.B., Campos, A.L., Nascimento, C.S., Prata, M.C.A., Furlong, J. and Teodoro, R.L. (2005) *Annals XLII Reun. An. Soc Brás. Zootec, 2005*.
- Oliveira, G.P., Alencar, M.M. and Freitas, A.R. (1990) *Pesq Agropec Bras* **24(10)**:1267-1270.
- Panepucci, L.L.L., Alencar, M.M. and Oliveira, G.P. (1989) *Rev Soc Bras Zoot* **18(2)**:172-178.
- Ramachandra, R.N. and Wikel, S.K. (1992) *J Med Entomol* **29(5)**:818-826.
- Seaton, G., Haley, C.S., Knott, S.A., Kearsey, M. and Vissher, P.M. (2002) *Bioinformatics* **18(2)**:339-340.
- Teodoro, R.L., Lemos, A.M. and Madalena, F.E. (1994) *Ver Soc Brás Zoot* **23(2)**:223-228.
- Vercruisse, J., Donny, P., Clarebout, E., Demeulenaere, D., Smets, K. and Agneessens, J. (2000) *Vet Parasitol* **89(1-2)**:63-69.
- Vissher, P.M., Thompson, R and Haley, C.S. (1996) *Genetics* **143**:1013-1020.
- Wanbura, P.N., Gwakisa, P.S., Silayo, R.S. and Rugaimukamu, E.A. (1998) *Vet Parasitol* **77**: 3-70.
- Wikel, S.K. and Alarcon-Chaidez, J. (2001) *Vet Parasitol* **110**:275-287.