Fine-mapping of BTA 10 and BTA 11 for tick resistance in Gyr x Holstein cross

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Introduction

In tropical regions, the incidence of the bovine tick *Rhipicephalus* (*Boophilus) microplus* deeply impacts dairy cattle production systems, decreasing milk production and affecting reproduction traits and eventually may cause the death of highly susceptible animals. The identification of DNA markers linked to tick resistance would provide a better strategy for selecting resistant animals. Mapping QTL and identification of causative genes that affect tick resistance will allow the use of marker assisted selection (MAS) in breeding programs. MAS could be used to pre-select young animals, shortening generation interval and increasing genetic gain (Beckmann and Soller, 1987). Previous studies by our group (Azevedo *et al.*, 2009) detected six QTL for tick resistance in a Gyr x Holstein F2 population. These QTL were detected with moderate resolution because the distances between markers were relatively large. In order to reduce the confidence intervals, new markers were added in regions where QTL were detected. Typically, confidence intervals for the most likely QTL positions are in the order of 20 to 30 cM. Since most practical implementations of marker information require QTL to be mapped to intervals of 1 to 2 cM, methods of higher precision are needed to refine the region (Olsen *et al.*, 2004). The aim of the present study was refine the position of the detected QTL.

Materials and methods

Experimental Population. The experimental F2 population was produced by crossing four Holstein bulls with 27 Gyr cows to generate 150 F1 (½ Gyr : ½ Holstein) animals, using multiple ovulation with embryo transfer (MOET). Four F1 bulls were mated to 68 F1 females to generate 366 F2 animals, avoiding relationship among sires and cows.

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Phenotype Evaluation. To evaluate tick resistance, each F2 animal was artificially infested with ticks on dry and rainy seasons. Animals were evaluated in contemporary groups with age ranging from 10 to 14 months. Artificial infestation was carried out with approximately 10,000 *Rhipicephalus (Boophilus) microplus* larvae placed in the "dorsal-lumbar" region of each animal. After that, they were kept on pastures for 21 days when the engorged female ticks were counted Additional traits that might interfere with tick resistance were also evaluated, such as coat color, coat thickness, coat length and hair density.

Genotype Evaluation. Blood samples from the parental, F1 and F2 generations were collected using vacuum tubes containing anti-clotting reagents. Genomic DNA was extracted from leukocytes using a modified phenol/chloroform method. A total of eight microsatellite markers were selected to cover the QTL previously mapped on chromosomes 10 and 11. Markers were selected from the bovine genetic linkage map available at MARC/USDA (Meat Animal Research Center/ United States Department of Agriculture). Microsatellite marker alleles were detected by capillary electrophoresis in the MegaBACE 1000 DNA sequencer (GE Healthcare, Buckinghamshire, United Kingdom).

Statistical Analyses. QTL were identified by least square regression analysis using the F2 analysis option of the web-based GridQTL software (Seaton *et al.*, 2006). The F statistic was calculated to test the hypothesis of QTL segregation using a model that included year/group and coat color as fixed effects. Chromosomewise significance 95% and 99% threshold were calculated on the basis of 10,000 permutations.

Results and discussion

Genome wide scan for tick resistance previously detected six QTL regions across the 29 bovine chromosomes (Azevedo *et al.*, 2009). These QTL show high confidence interval complicating implementation of MAS. The confidence interval was 72 cM on BTA 10 QTL and 26 cM on BTA 11 QTL.

We added five new markers on BTA 10 and three new markers on BTA 11. New association analyses were performed including these new markers. We were able to map the QTL at the same previously mapped locations, however the confidence intervals were significantly reduced.

On BTA 10, the confidence interval dropped from 72 cM to 19 cM indicating the existence of two QTL. The wide confidence interval found on the previous results was not able to distinguish the two putative QTL regions. After including five new markers on this region two distinct QTL peaks were identified (Fig 1). The phenotypical variation explained by this QTL was 3.65% and the positive additive effect indicates that Holstein animals harbor alleles that increase susceptibility to tick. This was expected since Holstein breed is extremely susceptibly to ticks.

On BTA 11, the confidence interval dropped from 26 cM to 20 cM (Fig 1). The reduction of the confidence interval was relatively small since the previously mapped QTL showed a defined peak. The phenotypical variation explained by this QTL was 4.75% and the additive effect was positive indicating that tick resistance on this QTL is originated from the Gyr genome.

The remaining tick resistance QTL identified on the previous genome scan are currently being investigated with additional markers. After that, these QTL regions will be validated on commercial Gir x Holstein crossbred populations.

The reduction of confidence interval facilitates validation and identification of candidate genes underlying the QTL

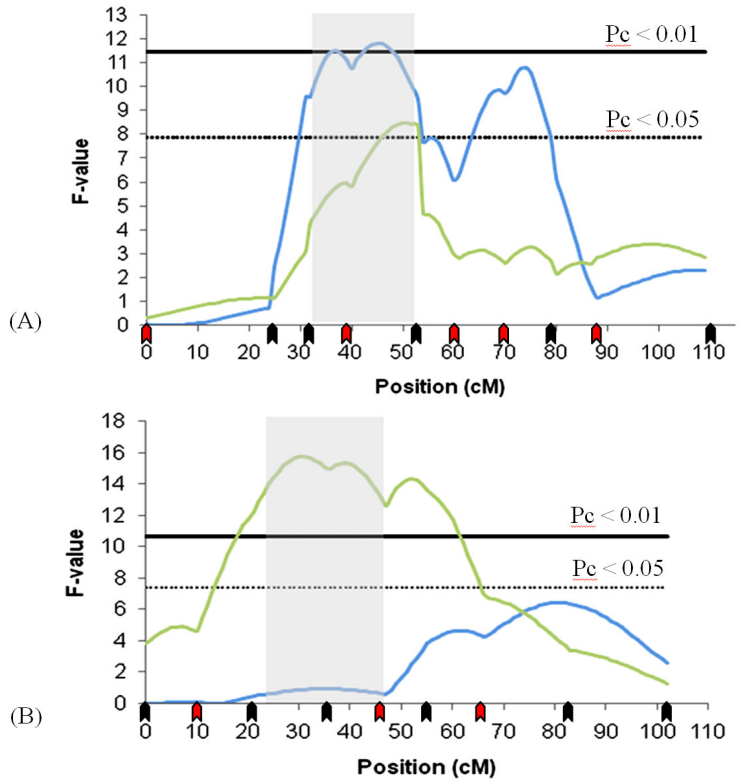

Figure 1: F-statistic profile for tick resistance on BTA10 (A) and BTA 11 (B). The x-axis indicates the relative position in the linkage map. Red arrows indicate marker positions added in fine mapping and black arrows indicate marker position of first genome scan. Green line indicates rainy season and blue line indicates dry season. Gray bar indicates QTL confidence interval. Pc = chromosome wide significance threshold.

Conclusion

The addition of new markers to saturate previously detected bovine tick resistance QTL significantly reduced confidence interval. This would facilitate the identification of candidate genes related to tick resistance.

References

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