Genome association with *Cooperia punctata* resistance in crossbreed cattle in the sub-humid tropics of Mexico

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Abstract:

This study is an evaluation of resistance to natural infection by *Cooperia* spp. in Zebu x Holstein crossbreed calves in the tropics. Fourteen four-month-old calves were dewormed and moved to pastures naturally infested with gastrointestinal nematodes under sub-humid tropical conditions. Fecal samples were collected from each animal every seven days for three months to quantify the number of eggs per gram of feces

(EPG), and nematode species identified with end-point PCR. Hair samples were collected for genotyping using the GeneSeek Genomic Profiler HD-V3 panel, which contains 139,376 SNP markers. Variation in EPG per individual ranged from a minimum of 7+7 to a maximum of 4,657+1,886 EPG. The PCR identified breed differences between the Zebu x Holstein crossbreeds. Genome-wide association studies detected five statistically significant haplotypes ($P<0.001$). The haplotype in chromosome 2 includes four markers, that in chromosome 10 includes three, that in chromosome 15 includes two, that in chromosome 23 includes four and chromosome X includes three. Of these regions only chromosome 23 was found to be associated with parasite resistance, measured as EPG phenotypes. The remaining chromosomes exhibited no association in the studied animals. These regions could be sequenced and tested for gene expression against *Cooperia* and other gastrointestinal nematodes.

Key words: SNP markers, Genomic association studies, Nematode resistance, Crossbreed calves.

Gastrointestinal (GI) nematode infection in young cattle is one of the main health problems affecting livestock under grazing conditions in tropical climates[1]. The *Cooperia* species are GI parasites which negatively affect growth and productivity in cattle by compromising small intestine function[2]. Several mechanisms have been studied to combat *Cooperia* infection, including pharmaceuticals and genetic resistance[3]. Availability of high density DNA marker panels for single nucleotide polymorphisms (SNP) has made it possible to map the loci of traits of commercial interest and thus increase the presence of desirable genes in a population. Haplotypes are regions of the genome (comprising between 100 and 150 base pairs) that are jointly inherited in a specific population and may have significant effects on genes located within a region of the chromosome[4]. Genome-wide association studies (GWAS) allow identification of these regions in genetic material. Recently, genome regions have been identified that indicate resistance to disease and parasites[5].

The present study objective was to identify regions of the genome associated with parasite load (number of eggs per gram of feces) of *Cooperia* species in crossbreed cattle (Zebu x Holstein and vice versa) in a grazing system in the tropics of Mexico.

The study was carried out in the Center for Education, Research and Extension in Tropical Livestock (Centro de Enseñanza, Investigación y Extensión en Ganadería Tropical - CEIEGT), of the Faculty of Veterinary Medicine and Zootechnic, National Autonomous University of Mexico, located in the municipality of Tlapacoyan, in the state of Veracruz, Mexico. Study area altitude is 112 m asl. Its climate is warm humid,

with rains year round and no defined dry season (Af(m) w"(e)[6], an average temperature of 23.5 ± 0.5 °C, and average annual rainfall of 1,991 mm ± 392 mm. Gastrointestinal (GI) nematode prevalence in the area exceeds 75% year round[7]. The experimental animals were fourteen crossbreed calves (¾ Zebu x ¼ Holstein [n = 6] and ¼ Zebu x ¾ Holstein [n = 8]), four months of age in a grazing system. Prior to weaning, both calves and mothers were treated with 7.5 mg Levamisole by intramuscular injection to eliminate any possible GI nematode contamination.

All animals were naturally infected with *Cooperia punctata* (L$_3$) infectious larvae (confirmed by end-point PCR as a dominant genus) by ingestion. The first record of eggs per gram of feces (EPG) was taken 21 days after infection by grazing was initiated, and then continued every seven days for three months. Eggs counts were done following the McMaster technique. Samples were collected directly from the rectum to prevent contamination and processed immediately. Average EPG was calculated during sampling, and the results included in a cases and controls study, considering cases to be animals exhibiting a >200 EPG[8,9]. Infection level was classified by EPG counts for parasitic nematodes (Table 1). Blood samples were taken to measure the percentage of the package cell volume (PCV), using 24% as the level for a non-anemic individual[9]. Levels for PCV were measured using a 22 + 1.6% minimum and a 28 + 3.3% maximum. It is important to mention that the *Cooperia* genus exhibits tissue-feeding habits and thus does not directly affect PCV percentage. This genus was prevalent throughout the three-months study period, meaning that PCV low percentage was a response to animal nutritional status; this was how six individuals were identified as resilient.

Table 1: Selection of resistant, susceptible and resilient calves vis-a-vis *Cooperia punctata* based on eggs per gram of feces (EPG) and package cell volume (PCV) percentage

ID	EPG Mean ± SE	PCV (%) Mean ± SE	C
62	7 ± 7	24 ± 2.2	RR
58	164 ± 89	24 ± 1.1	RR
61	200 ± 107	26 ± 2.2	RR
57	178 ± 154	23 ± 2.8	RR
55	86 ± 291	22 ± 0.7	Rs
56	650 ± 291	28 ± 1.7	Rs
51	557 ± 291	24 ± 3.3	Rs
67	607 ± 165	22 ± 1.5	Rs
65	764 ± 283	23 ± 1.5	Rs
63	1193 ± 636	25 ± 3.3	Rs
59	1221 ± 558	25 ± 0.8	SS
53	1378 ± 291	26 ± 2.7	SS
54	3100 ± 291	22 ± 2.3	SS
64	4657 ± 1886	23 ± 2.1	SS
X ± SE	1055 ± 1155	24 ± 2.0	

ID= individual identification; SE= standard error; C= individual phenotype classification; RR= resistant; Rs= resilient; SS= susceptible.

Hair samples including the follicle were taken from all animals for DNA isolation. These were then genotyped using the GeneSeek Genomic Profiler HD-V3 platform, which contains 139,376 SNP. A genotype quality control analysis was run, eliminating markers with a call rate <0.90, an allele frequency <0.05 and a Hardy-Weinberg equilibrium value of *P*<0.001. After quality control, 120,405 SNP were included in identification of 3,054 haplotypes. As a correction for the effect of source breed, principal component analyses (PCA) were done and included as an adjustment factor in the association analyses. The GWAS were implemented with a logistic regression model, corrected using the Bonferroni test and the false discovery rate. All genomic analyses were run with the SVS-Golden Helix ver. 8.6 software[10].

Molecular identification of the GI nematode genera showed *Cooperia* to be the dominant genus in the region, since no other genera were identified during the three-months follow-up. Individual infection level ranged from a minimum of 7 ± 7 to a maximum of 4,657 ± 1,886 (Table 1). The PCA showed that differences in animal breed composition could be identified (Figure 1), with those having more Zebu genes to the left and those with more Holstein genes to the right.

**Figure 1**: Principal components analysis (PCA) of studied animals and grouping by breed composition
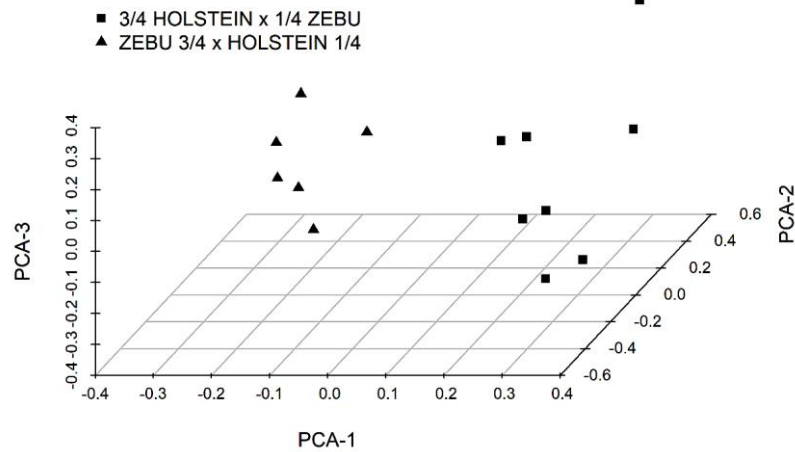
Some breeds, crosses or specific populations exhibit genetic predispositions for some traits and/or diseases[11]. However, no such tendencies were found to be associated with the presence of *Cooperia* cases and controls by crossing (Figure 2).

Figure 2: *Cooperia* cases and controls distribution in studied animals
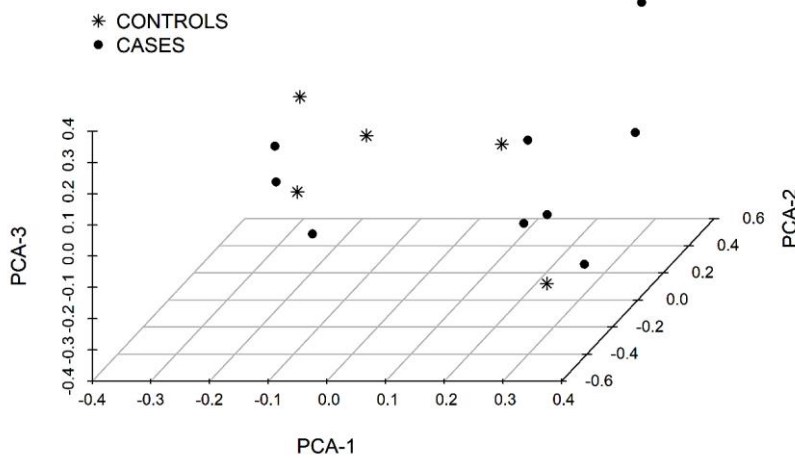
The GWAS identified five significant haplotypes ($P \leq 0.001$; Figure 3). Chromosome 2 includes four markers (BTA-55603-no-rs, BovineHD4100001149, BovineHD0200010866 and BovineHD0200010867); chromosome 10 includes three (BovineHD1000009272, ARS-BFGL-NGS-44351 and BovineHD10000 30584); chromosome 15 includes two (ARS-BFGL-NGS-18481 and ARS-BFGL-NGS-103542); chromosome 23 includes four (BovineHD2300011368, BovineHD2300011369, BovineHD2300011370 and BovineHD2300011371); and chromosome X includes three (BovineHD3000031659, BovineHD3000031667 and BovineHD3000031676).

Figure 3: Manhattan graph of association analysis of haplotypes identified in *Cooperia* cases and controls study. Chromosome X is indicated as chromosome 30
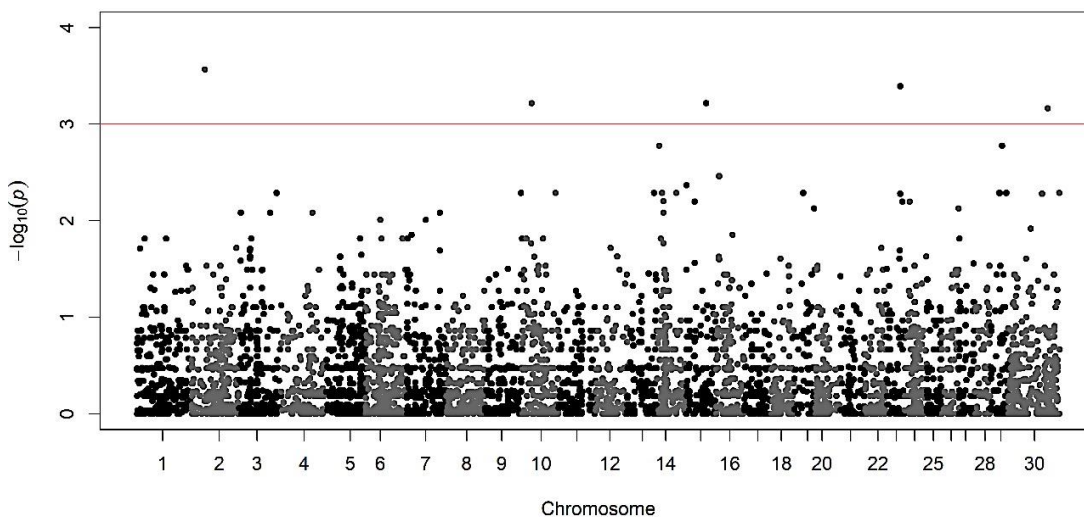
Due to the limited number of genomic association studies to disease resistance, there are few annotations in the genome for references. Of the significant regions found in the present study for chromosomes 2, 10, 15 and X, no genes were identified that could be related to the found regions (results verified in NCBI database)[12]. Only the significant region of chromosome 23 has a previous report associated with the infection rate for trypanosome in N'Dama and Boran cattle in West a protozoan parasite in Africa[13]. Since most of the identified regions are not associated with EPG traits, nor specifically with *Cooperia*, they might be candidates for sequencing and testing the existence of genes for resistance to this nematode and other GI parasites.

In conclusion, despite of the low number of calves included in the present study, the results suggest existence of SNP associated with nematode resistance, mainly on chromosome 23. However, more data is required to define this relationship. Study of the host/parasite interaction implies greater knowledge of the diversity of immune evasion mechanisms, and anthelmintic detoxification in nematodes. The present contribution to

understanding the factors associated with the host genome and *Cooperia punctata* infection processes suggests that in the future, mechanisms may be found that make feasible the use of markers associated with nematodes in cattle under grazing conditions in the tropics.

Acknowledgements

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