DETECTION OF QTL AFFECTING PARASITE RESISTANCE IN A SELECTED HERD OF ANGUS CATTLE

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INTRODUCTION

Economic impact of nematode parasite infection of cattle. Gastrointestinal (GI) nematodes severely reduce the efficiency of raising cattle on pasture throughout the world. Even well managed dairy and beef herds that exhibit no outward signs of infection can show reduced milk production (Thomas *et al.,* 1984; Gross *et al.,* 1999) and retarded growth in young animals (Hawkins 1993 ; Ploeger *et al.,* 1990). As a result, producers have become more reliant on drugs to control economic losses, and this has led to the appearance of anthelmintic resistant nematodes in cattle in New Zealand (Vermunt *et al.,* 1995 ; Hosking *et al.,* 1996) and Great Britain (Stafford and Coles, 1999). Producers adopting "organic" husbandry programs have few options for parasite control, and as such nematode infections are one of the most economically important diseases for these producers.

Immunological basis for resistance to nematodes of cattle. Resistance to infection with nematodes in cattle is manifested in several ways. Most parasite species stimulate effective levels of protective immunity in most herd members within several months on pasture, and subsequent infection of these animals results in a significant reduction in the number of parasites becoming established (Weber and Lucker, 1959 ; Gasbarre and Canals, 1989). In contrast, establishment of immunity to reinfection by *Ostertagia ostertagi* requires months or years, and thus *O. ostertagi* remains the most economically important nematode of cattle in temperate regions.

Genetics of resistance. Early studies in our laboratory using the Wye Angus herd demonstrated that the number of nematode eggs/gram (EPG) in feces of pastured cattle has a heritability of approximately 0.30 (Leighton *et al.,* 1989; Gasbarre *et al.,* 1990). Also, EPG values are not distributed normally; a small percentage of the herd is responsible for the majority of parasite transmission (Gasbarre *et al.,* 1990). This "overdispersion"of EPG values first described by Crofton (1971a, 1971b) has been reported in other cattle populations (Genchi *et al.,* 1989). This pattern strongly suggests that genetic management of a small percentage, estimated to be between 15 and 25% of the herd (Anderson and May, 1985), could considerably reduce overall parasite transmission. Results also indicate that the odds of certain bulls producing high EPG calves are 20 times greater than other bulls (Gasbarre *et al.*, 1995).

Thus an attractive alternative for control of parasite transmission in cattle populations is DNA marker-based selection, relying on the identification of economic trait loci (ETL) influencing acquired or innate parasite resistance. Because different parasite species may not be susceptible to the same immune responses, identification of ETL affecting resistance to specific parasite species requires extensive phenotypic analyses in a population structure amenable to statistical analyses ; preferably with pedigree connections to popular sires used in commercial populations.

MATERIALS AND METHODS

Resource population. A divergent selection program was initiated in our laboratory using parental stock originating from the Wye Angus herd at the University of Maryland. Early studies indicated that the bovine major histocompatibility complex (BoLA) had minor effects upon EPG phenotype (Gasbarre, unpublished), so animals were bred to be homozygous at this primarily to provide flexibility for immunological assays. Once initial breeding females were identified, semen from high and low bulls was used to produce calves of the desired phenotypes. To date, nearly 350 progeny have been tested in parasite challenge studies. Complete pedigree records for this population have been assembled that trace back to the original founding animals of the Wye Angus herd. Initial pedigree analysis of the resource population reveals that >90% of the animals are paternally descended from a Wye bull born in 1944. This extreme relationship to a single bull may have resulted from the selection for a single MHC haplotype. DNA for genetic analysis has been acquired from all the animals in the resource population and over 70 sires in the historic pedigree.

Phenotypic characterization. Calves are kept with their dams on pastures with extremely low numbers of parasites until weaning. When the median age of the contemporary group is 205 days, calves are weaned and placed on pastures infected with the 2 most common nematode parasites of US cattle, *O. ostertagi* and *Cooperia oncophora*. Calves are monitored weekly for the following : fecal EPG, serum pepsinogen level, serum antibodies of the IgG1, IgG2, IgA and IgM subclasses to *Ostertagia* and *Cooperia*, blood eosinophil levels, hematocrit, hemoglobin, red blood cell count, white blood cell count, mean cell volume, body weight, hip height, and scrotal circumference of the bull calves. Calves are kept on pasture for at least 120 days, and are then selected for replacement breeders, for re-challenge experiments, or for immediate kill. Data collected at kill includes : parasite species and numbers recovered, sex and length of worms, enumeration of *Ostertagia*- and *Cooperia*-specific T cells in the regional lymph nodes, weight of abomasal lymph nodes, enumeration by of CD3, CD4, CD8, IL2-receptor, B-cell marker, surface IgM, and γδ T cells, and semi-quantitative competitive PCR measure of mRNA expression of Interleukin 2 (IL2), IL4, IL10, IL13, IL15, IL18, gamma-interferon(γ-IFN), Tumor Necrosis Factor-α, and Transforming Growth Factor-β in the regional lymph nodes.

Genotyping. Microsatellite markers for genetic analysis were selected from the USDA MARC reference linkage map (http://www.marc.usda.gov/genome/cattle/cattle.html). A total of 216 markers were tested, and complete sets of marker genotypes were generated from 200 markers spaced at regular intervals (~20 cM) across the entire genome (3,000 cM). Preliminary analysis (N=103 markers) resulted in an expected heterozygosity index of 50% and polymorphic

information content of 45. Markers associated (<1 cM) with 12 candidate cytokine genes involved in growth and differentiation of bovine T-cells were genotyped to enhance detection of ETL associated with acquired immunity, including markers associated with IFN-γ, a gene previously found near QTL for resistance to GI nematodes in sheep (Crawford, 1998).

RESULTS AND DISCUSSION

Extensive phenotypic characterization has allowed evaluation of indicator variables based on correlations between phenotypic measurements, parasite load, and immune response. The best indicator of the level of *Cooperia* infection is EPG value (r = 0.6), while *Ostertagia* is best measured by serum pepsinogen levels (r = 0.7), weight gain (r = -0.5) and measures of anemia (r = 0.5). Other phenotypic measures are poor predictors of parasite numbers (Gasbarre, 1997). These relationships agree with studies in similar environments (Eysker and Ploeger, 2000 ; Claerebout and Vercruysse, 2000), but differ from tropical areas where significant correlations between fecal EPG values and total worm numbers are reported (Bryan and Kerr, 1989).

Based on our studies, calves can be assigned to one of three types: 1) Type I- never demonstrate high EPG values, 2) Type II - have increased EPG values through the first 2 months of test then levels fall to those of the Type I calves, and 3) Type III - maintain high EPG levels throughout the test. The respective percentages of these calves from the first generation was approximately 25:50:25. A reinfection of calves showed that EPG values for Type I and II calves remain low, while Type III calves shed high numbers of eggs. We feel that calves can be categorized as innately immune (Type I), acquired immune (Type II), and immunologically non-responsive (TypeIII), and that control of parasite transmission and parasite-induced loss should target the Type III animals.

Results also indicate that selection based on expected progeny difference (EPD) values for EPG has effectively increased the fraction of Type I and Type III calves. In addition, the range of EPD values has been reduced to 0.5 of the mean EPG value for calves tested to date, further supporting the role of host genetics in parasite transmission.

The marker genotypes needed to initiate ETL analysis of this population on a genome-wide basis have been generated (n >95,000). Statistical power of the genome-wide scan has been strengthened by inclusion of genotypic data from historic animals. The complex pedigree analysis increases statistical power compared to within family analyses, especially when family size is small. In our herd, paternal half-sib families range from 5-13 progeny/sire. Complex pedigrees, especially those containing loops, have posed a challenge for data analysis to detect ETL, and have caused us to use a multiple locus allelic peeling algorithm (Thallman *et al.,* 1999a ; Thallman *et al.,* 1999b). This algorithm allows reconstruction of genotypes of founding sires where DNA is unavailable, and aids detection of genotyping errors. We are currently completing this analyses.

CONCLUSIONS

As ETL are identified, ETL locations will be refined to identify the genes underlying disease phenotypes. Characterization of the genes affecting resistance offers producers alternatives for disease control. Non-organic producers can target susceptible animals for drug administration, which will reduce both the cost of anthelmintics used and the likelihood of selection for drug resistance. A second option is to target susceptible animals for immuno-therapy. A final option is selection to remove susceptible animals from the herd. Producers at high risk for parasite-

induced production losses, such as organic producers or producers in areas of high parasite transmission, will benefit from this strategy. Producers at low risk could select against susceptibility as needed.

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