TOWARDS MOLECULAR GENETIC CHARACTERISATION OF HIGH RESISTANCE TO INTERNAL PARASITES IN INDONESIAN THIN TAIL SHEEP

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INTRODUCTION

Internal parasitism is a significant impediment to livestock production throughout the world. Many of these are low cost production systems in developing countries where access to chemo-prophylactic compounds is limited. A long term, low cost and sustainable approach could include increasing genetic resistance of the target spp, and or immuno-regulatory control.

Our previous work identified and confirmed Indonesian Thin Tail (ITT) sheep exhibit superior resistance to *Haemonchus contortus* (Subandriyo *et al*., 1996) and express a very high level of innate and acquired resistance to *Fasciola gigantica* infection (compared with Indonesian Fat Tail and Merino sheep), and that this resistance is immunologically mediated (Wiedosari and Copeman, 1990 ; Estuningsih *et al*., 1996 ; Roberts *et al*., 1996 ; 1997). Preliminary evidence suggested that resistance is heritable and results from the expression of a major (perhaps single) gene (Roberts *et al*., 1997), based on the mode of inheritance in F2 and F3 ITTxStCroix progeny. Of all evidence available to date, the Merino appears a breed most susceptible to *F. gigantica* and *H. contortus*, where as the ITT can be regarded as highly resistant.

In this paper we report on the design and preliminary findings of a programme of research on the genetic and immunological characterisation of the resistance in ITT sheep with the long-term aim of identifying the gene(s) determining resistance. The principal strategy was to use the high resistance in the ITT in a genome analysis to identify the putative major gene for *Fasciola gigantica* (FG^R) resistance, provide evidence for major gene(s) in resistance to (HC^R) through a positional candidate cloning approach, and identify the homologues in the main target species-cattle and buffalo through comparative mapping. The strategy for the immunological programme to be developed in parallel with the genome screen, is to provide function/identity of putative candidate genes through understanding the mechanisms of resistance determined by the FG^R gene. This approach is now used in contemporary genome biology as a "functional genomics" approach for gene identification and isolation to open new avenues in genetic control or identification of new regulatory factors for control of internal parasites in ruminants.

MAJOR GENE ANALYSIS

Segregation analysis. The presence of a putative major gene for resistance to internal parasites can be confirmed by several approaches. A relatively simple approach is to use segregation

analysis of phenotype records in appropriate resource families (LeRoy and Elsen, 1992). Our gene mapping approach uses 10 resource families, which are a highly heterozygous cross between resistant (ITT) and susceptible (Merino) genotypes. These families are used to create 900 backcross progeny to obtain gene expression in a susceptible (Merino) background. We aimed at a minimum target of 225 experimental animals for testing each year, repeated over 4 rounds (years). Sire families showing putative segregation for a major gene, are being expanded for higher resolution linkage mapping by repeat mating the sires in round 3 and 4. All progeny are being phenotyped for expression of resistance to *Haemonchus contortus* and *Fasciola gigantica* dy direct challenge with immature parasites. In case of *H contortus* challenge all sheep are challenged twice, approximately 12 weeks apart. Each challenge consists of a weekly dose *per os* with 2000 L3 *H contortus* larvae for the first 3 weeks of the challenge period. This is followed by weekly Faecal Egg Counts (FEC) and Packed Cell Volume (PCV) measurements up to 8 weeks weeks post challenge when all animals are drenched with Ivermectin. Inter-challenge interval is 4 weeks. Resistance to *Fasciola gigantica* is measured following a single challenge with 300 viable *Fasciola gigantica* metacercaria and slaughter at 16 weeks post challenge for assessment of parasite burden. In our design, we used a comparison of within sire family heterogeneity of phenotype distribution. We compared the goodness of fit of phenotypes to a bi-modal distribution as a composition (mixture) of two sub-distributions of equal proportion and variance (segregating major gene) to the fit of data under a normal (non-mixture, no major gene) distribution using a maximum likelihood analysis approach. A likelihood ratio test was used to establish critical values for the test statistic.

Linkage approach. A weakness with segregation analyses is the inability to confirm the presence of a major gene in the presence of major background genes and or non-genetic factors. A design with significantly greater power to detect the presence of major genes is through linkage analyses. The segregation of a major gene can be confirmed through significant linkage with polymorphic DNA markers located in close proximity to the major gene. In the case where the chromosomal location of the major gene is unknown, a large number of polymorphic markers can be selected to give coverage across the genome. The same design used in segregation analysis can be used, with the added complexity that animals across the three generations require genotyping with a panel of polymorphic markers, thus increasing the cost and possibly experimental timelines. The major additional advantage is that this approach provides evidence for a possible chromosomal location, and can be used as a foundation in a positional cloning strategy to evaluate positional candidate genes in a chromosomal region containing the major gene. The location of a Quantitative Trait Locus (QTL) for resistance to *H. contortus* and *F. gigantica* is unknown, and the design required a full genome scan with polymorphic markers. Our full genome scan used a panel of 85 highly polymorphic micro-satellite markers to obtain a chromosomal location of the putative major gene. QTL analysis was conducted using QTL express http://qtl.cap.ed.ac.uk/, based on the interval regression approach detailed by Haley and Knott (1992).

THE GENETIC BASIS OF RESISTANCE TO *H. CONTORTUS* IN ITT SHEEP

Segregation Analysis. All FEC data were transformed with a cube root transformation prior to segregation analysis. Within each challenge an average of FEC at 3, 5, 7, and 8 weeks were

calculated as response to primary and secondary challenge respectively. Based on our first set of available data from three families (n = 165), all progeny showed significant segregation in FEC on primary challenge. This was not evident for FEC on secondary challenge.

Linkage Analysis. In a combined linkage analysis of all three families, evidence (P < 0.01) was obtained for a putative QTL in mean FEC on primary challenge (figure 1), or for the combined FEC of primary and secondary FEC data. There was no evidence for the presence of a QTL in FEC on secondary challenge (figure 1).

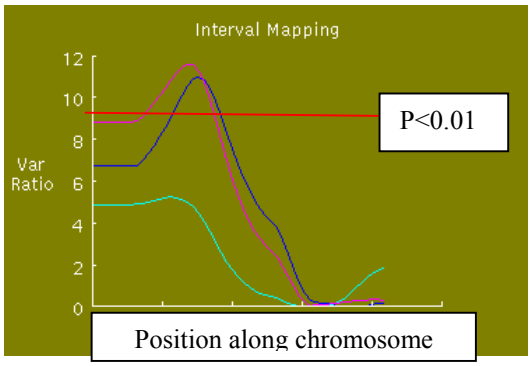

Figure 1. Interval regression analysis showing the chromosomal position for a putative QTL in mean FEC on primary (----) ,secondary (- - -), and combined primary and secondary (——) FEC following challenge with *H. contortus* L3 larvae. Critical threshold value (P<0.01) is derived from a permutation test of 1000 iterations of the data.

THE GENETIC BASIS OF RESISTANCE TO *F. GIGANTICA* IN ITT SHEEP

Segregation Analysis. Preliminary segregation analysis in 400 progeny from 7 sires, supports the presence of a gene with large effect for resistance to *F gigantica* in this population based on departure from normality and goodness of fit of data to a bi-modal (mixture) distribution in five families (figure 2). Further analysis identified a strong sex x major gene interaction, in which males expressed the putative gene, whereas females did not in 4 out of the 5 families in which segregation was evident. The major gene effect was consistent for male progeny groups with approximately a 50 % reduction in fluke burden (20-28 flukes).

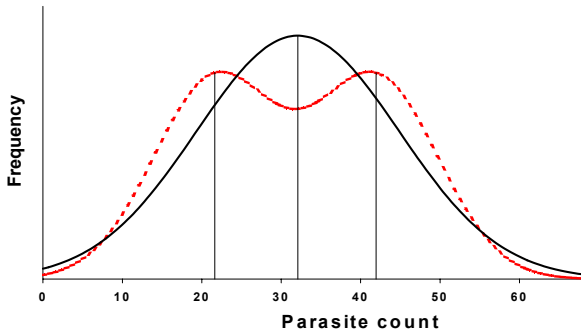

Figure 2. Example of significant segregation of resistance to *F. gigantica* based on fitted distribution of parasite counts among ITT X Merino backcross in a single family. Goodness of fit of the observed frequency of data (not shown) to a mixture distribution (---) vs normal distribution (___).

Linkage Analysis. Preliminary interval regression analyses of the genome scan in progeny from our first three families, provided support (P < 0.05) for two putative QTL. Once again

these QTL were identified in male progeny only, and absent in half-sib female counterparts. The two putative QTL for *F. gigantica* were located on different chromosomes to the QTL for resistance to *H. contortus*, suggesting resistance to these parasitic diseases is under separate genetic control in this resource population. To our knowledge this report also provides the first evidence for a chromosomal location of major genes for resistance to both *H. contortus* and *F. gigantica*.

FURTHER CHARCTERISATION OF RESISTANCE

This report provides additional support for evidence of major genes in resistance to internal parasites in sheep. It provides an avenue to map and isolate major genes regulating the impact of *F. gigantica* and *H. contortus* and apply this in other susceptible livestock *spp*.

Positional cloning approach. Based on a functional assessment of the genetic mechanism for resistance, a fine mapping approach in extended families, and detailed comparative mapping to man, mouse and other ruminant systems, we intend to obtain a positional clone for all major candidate genes as potential FG^R and HC^R resistance genes. Based on a novel in-vitro killing assay of juvenile Fasciola parasites, our *in vitro* studies have demonstrated the effector cells mediating killing of *F. gigantica* immature parasites include ITT macrophages and eosinophils and the molecular mechanism of killing involves superoxide radicals. Furthermore IgG_2 responses correlated with susceptibility to infection. Results from our immunological approach, using *in vitro* and *in vivo* experimental approaches, collectively suggests Th1-like responses are associated with the susceptibility of Merino sheep to *F. gigantica* infection while, conversely, Th2-like responses are associated with the resistance of ITT sheep to *F. gigantica* infection.

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