Genotypic and phenotypic resistance and resilience of sheep to gastro-intestinal parasitism
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Introduction
Selection for genetic resistance and resilience to gastro-intestinal nematodes (GIN) is established practice in the Australian sheep industry. Genetic resistance is mediated by host immune response[1], which is a significant contributor to reduced productivity[2,3,4,5] in scourworm-infected sheep. Scourworms (the non-blood sucking worms of the abomasum and small intestine – *Teladorsagia circumcincta* and *Trichostrongylus* spp.) are responsible for substantial losses to industry in Australia, New Zealand and other temperate winter rainfall areas worldwide and it would seem logical to select for an accepted indicator of resistance such as reduced worm egg count (WEC). However, concern has arisen around the possibility that genetic selection of sheep for resistance to worms may be associated with unfavourable production responses, particularly in the specifications–orientated meat sheep (prime lamb) industry, where re-partitioning of resources towards immune response in exposed lambs may adversely affect the main profit driver of the industry, namely growth rate. This paper will broadly review and critique for the veterinarian, rather than for the specialist immunologist or geneticist, the operation of the genetic selection process, currently identified outcomes for industry, and some recent structured experimentation in lambs of known genetic backround. The objective is not to provide immediate miraculous solutions to vexing issues, nor to review the subject in depth, but to draw the relevant information together, define the indisputable boundaries of our current general knowledge, identify questionable assumptions, and assemble some ideas on the information needed to underpin ongoing efficient selection for resistance and resilience to worm infections in sheep.

The concept of resistance to gastrointestinal parasites
Resistance to worm infections in sheep is complex and includes innate resistance, a genetic characteristic of every individual, which provides the first line of immune defence by responding rapidly to a foreign stimulus with increased blood flow, vascular permeability and cell infiltration. Primary cell types involved in this innate response travel to the draining lymph nodes to influence the development of the adaptive immune response. The adaptive immune response is characterized by specific receptors on T-cells and B- cells that are generated through gene rearrangement to recognise an unlimited number of antigens. Depending on the pathogen the adaptive immune response can develop in many different directions. In worm infections the response is generally type 2, in which eosinophil and mast cell immunity is promoted. The innate immune system is the main driver of the adaptive immune response. "The genes involved in the acquired immune response are not the determinants of genetic variability but a read-out of the innate response genes activated at the start of an infection"[6]. Current understanding is that the most important genes influencing the recognition of parasite molecules lie in the Class II region of the major histocompatibility complex. It is not known how many genes are involved in immune defence in sheep, but in humans there are more than 1500, representing 7% of the human genome.

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Manifestations of resistance to adult worms can include expulsion of the primary population (rare) or loss of adults due to acquired immunity as a consequence of repeated infection (common), changes in morphology, and reduction in fecundity of female worms. Larval worms can suffer developmental arrest (hypobiosis) or fail to establish in the host due to multiple expulsion patterns[1]. Loss of adults, reduced fecundity in female worms and immune assault on larvae arriving at the site are the main manifestations, but the genetics driving these defence patterns or combinations thereof in individuals have not been elucidated.

Another way of expressing this line of thought is that significantly different expressions of resistance to GIN have been identified and it is likely that separate immunological mechanisms are involved in these processes. This applies particularly to the immune responses generated by larval and adult infections and the various effector mechanisms responsible for their rejection. The kinetics of rejection identified for one particular parasite stage of any of the important species may reflect a unique immune response profile which may not necessarily be consistent between hosts[1].

The most accurate measure of the level of resistance to worm infection of an individual is the number of worms that establish from a single infection or that accumulate over time from repeated infection, the total worm count (TWC), which should include both adult and immature worms. Establishment of the value is clearly impractical for selection programs because it involves the destruction of the animal. An indirect measure of estimating TWC is the number of worm eggs passed in a given mass of faeces (WEC), which although reasonably correlated with TWC in young animals is not so in older sheep. Reservations are expressed in the literature concerning its use for this purpose in established scourworm infections or in those with a high proportion of immature worms. Furthermore, WEC can be dramatically influenced by many factors, including diet quality, proportional representation of parasite species in the total worm burden, seasonal variations in consistency of faeces and patterns of natural infection, flock management around the time of sample collection and inconsistency in laboratory processes. Nevertheless, by default, mainly for its ease of application and simplified conceptual presentation, WEC has become the accepted measure of worm resistance internationally. Ironically, the crudest of laboratory tools (a "bucket science" faecal worm egg count, WEC, with high error) is universally, successfully, employed as the yardstick for resistance measurement. Using this process worm-resistant and susceptible lines of sheep were rapidly developed with only a few generations of selection in Australia, New Zealand, United Kingdom and France and research using these resource flocks has led to significant diverse advances in knowledge.

Sheep lines which have been selected on the basis of high resistance to a particular gastrointestinal species are also more resistant to other species compared to sheep selected for low resistance[7], indicating that the genotype of individual sheep largely determines potential resistance status across all gastrointestinal nematode infections. But the level of expression of resistance, i.e., the proportional level of reduction of WEC, is by no means equal between parasite species, so that the rate of genetic advancement for a given level of selection may not necessarily be consistent across geographical areas with different parasite populations.

The concept of resilience to gastrointestinal parasites
In its simplest definition resilience is the ability to minimize the adverse consequences of infection, which can be viewed from the perspective of many traits, including greater weight gain or less weight loss, fleece production, and in the case of haematophagous parasites, haematocrit. This paper will concentrate on aspects relating to the

scourworms, *Teladorsagia circumcincta* and *Trichostrongylus* spp. Essentially there are three main approaches to selection for resilience on an industry platform. The first and simplest is to use a post weaning weight (adjusted) as the measure of gain, assuming, of course, that the animal, or rather the population under observation, is or has recently been naturally exposed to worms. The second (very difficult on an industry scale) is to measure loss of productivity over time in known exposed animals, the third is to base selection on the number and timing of drenches necessary in the early life of the lamb. The latter, alone or in combination with selection based on body weight has successfully evolved in New Zealand to produce elite selection flocks which it could be argued might be as close to 'truly resilient' as can be achieved. The first approach, which is the yardstick for productivity in the Australian prime lamb industry may in many cases simply represent the capacity of an individual to grow, and cannot be separated from true resilience without experimentation. There is great difficulty in distinguishing production from depression in production. In effect, the distinction between the two is probably of no practical consequence.

Australian Sheep Breeding Values (ASBVs)

Australian Sheep Breeding Values (ASBVs) are an estimate of an animal's true breeding value based on pedigree and performance recorded information. Data are constantly updated on the Sheep Genetics LAMBPLAN database (www.sheepgenetics.org.au) presenting ASBVs for numerous traits in terms of deviation from the mean of all recorded 1990 progeny. Traits include the value of individual sire's genes for carrying, (or not carrying) worm burdens at 7-10 months (PWEC) and their genetic merit for growth at 7-10 months (PWT). As described above these two traits are equated for practical purposes with resistance and resilience respectively. A high ASBV for PWT means that an animal is genetically faster growing, a positive ASBV for WEC implies that is more likely to have higher worm burdens than those with a lower PWEC value.

Genetic and phenotypic correlations of resistance and resilience with production traits

Blackburn (unpublished PhD thesis, 2016) has summarised extensive research (33 papers in sound refereed journals from 1987-2007) relating to genetic and phenotypic correlations of growth rate/bodyweight and WEC from Australia, Ethiopia, France, New Zealand, Poland, South Africa and the United Kingdom. There is great variability in the values, with both positive and negative correlations reported between WEC and body weight/weight gain in wool and meat sheep. The difficulty in assembling the available information for the purpose of extracting practical recommendations for commercial production is that some correlations have large standard errors, and there are many confounding disparities in age, breed and strain of host, and worm species and levels present.

The most relevant (and comprehensive) research from our regional perspective of breeds, production systems and environment is that from Australia and New Zealand. Ranked on production alone the top 50 of 1299 Coopworth rams registered in the 2004 New Zealand Sheep Improvement Limited database had much reduced ability to limit WEC[8]. Based on field studies a very relevant review applicable to grazing sheep in the Australian and New Zealand environments has been provided by Morris *et al.*,(2005)[9]. "In general, genetic correlations of growth or fleece traits with log FEC (WEC) in New Zealand Perendale, Romney and Coopworth populations have tended to be close to zero or positive (unfavourable), in Australian Merinos about zero for growth and slightly unfavourable for fleece weight, and elsewhere zero to negative. ….the relative

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importance of breed, climate/management and nematode species in determining these apparently inconsistent findings…. *is unknown.*" (Our emphasis).

In addition to these data reviews, comprehensive analyses of the Australian sheep genetics (SG) database for relationships between resistance and resilience to internal parasites and production were undertaken in November 2013 by Daniel Brown (SG) and Paul Blackburn under the oversight of Dr. Robert Banks (SG). Data for Poll Dorset, White Suffolk and Merino breeds dating back to 1990 were used. Full details of the analyses (unpublished) appear in Amended Milestone Report No 4 (14 March 2014) for Project B.AHE.0054 "Estimating cost of immunity to gastro-intestinal nematodes in meat sheep differing genetically in resistance and resilience to infection". Meat and Livestock Australia Limited. In summary, genetic correlations between post weaning or yearling body weight and post weaning or yearling worm egg count for White Suffolk, Poll Dorset and Merino breeds were generally not significantly different to zero. Phenotypic correlations were small and slightly negative. However, there was a cost in body weight of resistance (low WEC) of nearly 1kg at 9 months of age, which was greater when growth potential of progeny was lower. It was also confirmed that PWEC ASBV's are producing consistent differences in the PWEC of their progeny (both in high and low growth animals). Overall, this is not discouraging news for the prime lamb and wool industries in Australia.

A move forward from correlation data
Correlations, although extremely informative and valuable, are simply measures of overall relationships that can guide selection decisions, but there are many underlying factors that require structured experimentation to elucidate. An experimental model based on comparisons between infected lambs and infected immune-suppressed lambs[2] has provided a basis for partitioning the penalty associated with worm infections in meat breed lambs into two components, namely that due to host immune response and that due to direct damage by the parasite[2,3,4,5]. These studies, using identical immune suppression regimens, have demonstrated that in *Trichostrongylus colubriformis*[2] and *Teladorsagia circumcincta*[3] infections the cost of the host immune response is the principal cause of loss, whereas in *Trichostrongylus vitrinus* infection a significant component of the loss is due to direct effects of the parasite on the host[,4,5].

Relevant recent Australian research
The key methods and outcomes of this work[4,5] are described here in some detail, because it provides the first estimate of the cost of immune response in grazing meat production lambs differing in genotype for resistance and resilience to infection with GIN. The initial report[5] presented estimates for the cost of host immune response (CI) in terms of bodyweight growth penalty (g/day) which because of their mode of derivation were not appropriate for statistical analysis. That difficulty has been rectified in novel alternative modifications of the earlier method which provide for statistical analysis of outcomes (Blackburn and others, in preparation) and confirm the trends and order of differences found earlier. To avoid confusion, however, only currently published results are included in this paper.

Five or six Poll Dorset rams (total 22) selected from each of four quadrants reflecting industry extremes for resistance and resilience each contributed 8 second cross lambs by Border Leicester/Merino ewes to the experiment. Genetic resistance was based on ASBV for post-weaning worm egg count (PWEC), and genetic resilience on ASBV for post-weaning weight (PWT). To avoid over-representation of specific genetic lines, a maximum of one sire from any stud was included within each quadrant. Quadrants were

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defined as high resistance/high resilience genotype (H/H), high resistance/low resilience genotype (H/L), low resistance/high resilience genotype (L/H) and low resistance/low resilience genotype (L/L). PWEC and PWT ASBVs of each sire immediately prior to the experiment (September 2012) are presented in Fig.1. The absence of sires with negative ASBVs for PWT reflects the intensity and success of industry selection post-1990. The relative ease with which sires having low resistance against worms were sourced indicates slower progress in selection by industry for this trait over the intervening 22 years.
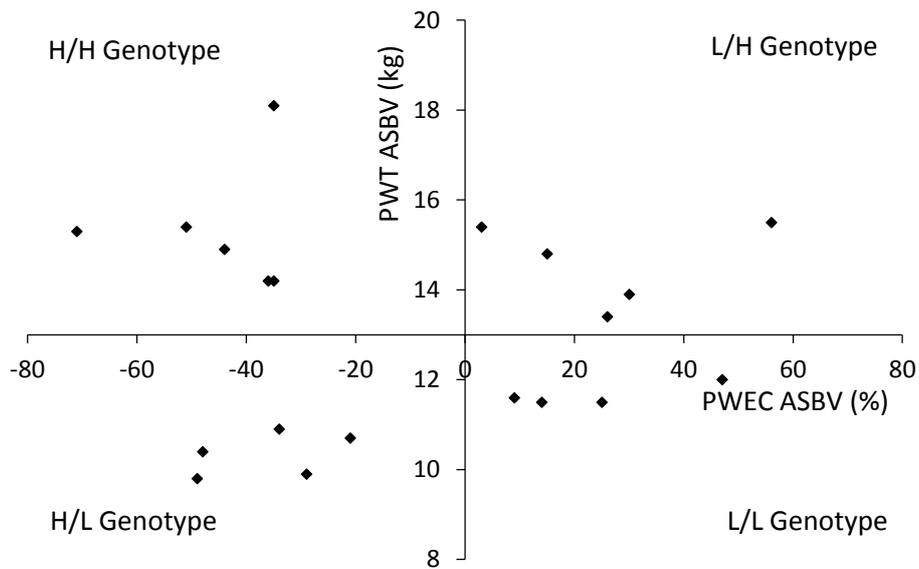
Fig. 1. Distribution of sires contributing to the four quadrants of resistance and resilience genotype*
Two additional low resilience sires fall below the scale of this graphic and are excluded for clarity; H/L Genotype PWEC = -41, PWT = 1.5; L/L Genotype PWEC = 29, PWT=4.4.

Lambs grazed concurrently with their dams receiving natural exposure to worm infection until weaning at 12 weeks of age. From weaning for a period of 112 days 176 experimental lambs were immune suppressed with methylprednisolone, and/or trickle infected with *T. vitrinus* in a 2x2 factorial design within genotype quadrant and rotated through a "worm free" cell-based grazing system to prevent reinfection with worms. Comparison of weight gain between treatment groups enabled calculation of the cost of immune response in grams per day reduced growth rate for each of the genotype quadrants. Results are given in Tables 1 and 2.

Table 1 Cost of immune response (g/day) by genotype

Genotype	High	Low
Resistance	26.76 (0.17)*	21.92 (0.14)*
Resilience	13.92 (0.08)*	34.76 (0.23)*

*Proportion of total 'control' (non-infected/non-immune suppressed) growth rate lost due to immune response within each genotype

According to these values selection for high genetic resistance to internal parasites was approximately 5g/day more costly than selecting for low resistance. However, the calculated values are very similar and error may well account for the difference (Table 1). Conversely, low resilience animals had an immune response cost 21g/day higher than their high resilience counterparts, which is unlikely to be explained by error.

Table 2. Cost of immune response (g/day) by genotype quadrant

Genotype	High Resistance	Low Resistance
High Resilience	39.24 (0.23)*	-8.7 (N/A)*
Low Resilience	16.96 (0.12)*	52.55 (0.33)*

*Proportion of total 'control' (non-infected/non-immune suppressed) growth rate lost due to immune response within each quadrant.

The cost of host immune response in ascending order was lowest in animals selected for low resistance/high resilience, followed by high resistance/low resilience, high resistance/high resilience and low resistance/low resilience respectively. These differences in cost of immune response across the quadrants of resistance and resilience genotypes are difficult to explain because no statistical analysis between genotype quadrants was possible. Nevertheless, a simple proposition might be that disparity between quadrants may result from different mechanisms of immune response operating in each. The apparent variation in cost of immune response between genotypes and quadrants may support reservations that selection for animals with high genetic resistance to internal parasites might lead to an inadvertent reduction in productivity. But they might also suggest that failure to select for animals with high resilience to infection may lead to an inadvertent reduction in productivity as a result of increased cost of immune response.

 Modelled analyses of the same data by Blackburn and others are complicated by failure in the experiment of sire genotypes for resistance (ie.,ASBVs) to generate corresponding differences in the progeny for both WEC and TWC values. For analysis, phenotypic resistance based on WEC and TWC and resilience based on growth rate were determined from the experimental data. These analyses will be published shortly. In

addition, we have another full data set for a companion study with an identical protocol, replacing *T. vitrinus* with *Tel. circumcincta*; preliminary results show that host immune response accounts for a much larger proportion of the overall cost of infection in *Tel. circumcincta* infection, supporting findings in New Zealand[3]. There is strong putative evidence that the immune responses to the two parasites are not identical.

The immune suppression model – an update
There are some interpretational difficulties inherent in the immune suppression model using methylprednisolone acetate because the drug certainly does not "abrogate" the immune response as advanced in some publications. For example, in chronic infections with *T.vitrinus* there was no effect on certain subjective estimates of pathology in infected animals such as mucosal type (villous atrophy), mucosal score and mononuclear cell infiltration, or measures such as mitotic index or mucosal thickness. There was an inconsistent influence of circulating anti-*T. vitrinus* IgA levels, partial reduction in goblet cell numbers but not on goblet cell size and profound reductions in mucosal mast cell and globule leucocyte concentrations (Carmichael and Blackburn, unpublished data). Similarly, in chronic *Tel. circumcincta* infections methylprednisolone acetate had no influence on subjective estimates of macroscopic mucosal pathology and inflammation score or measures such as mucosal thickness and parietal cell area, but markedly reduced eosinophil, mucosal mast cell and globule leucocyte concentrations in infected animals, although to a lesser degree than in *T. vitrinus* infections (Carmichael and Blackburn, unpublished data). Technically, only the terms "suppression" or "partial suppression" should be used in reference to immune system influence of methylprednisolone in sheep and then only on the broad understanding that significant unidentified components of immune function are retained in treated animals.

Nevertheless, this model may still provide a basis for further developments by enabling, in carefully structured field experiments, the generation of comparative information on costs of immune response (partial or otherwise) in selected flocks of resistant and susceptible sheep which can be relatively easily bred, so that in each line genotype and phenotype align. It is accepted that selected lines are artificial constructs that do not represent the genetic diversity in the overall population, but they have served industry well. Their value could be in detecting genetic differences in the efficiency of mounting immune responses to parasites and from this an opportunity through the application of phenotypic and genetic markers to measure resistance more accurately or to identify, independently of infection, genetically resistant animals or those which incur lower costs for resistance to GIN. Allowing for advances in gene technology, identification of such animals and characterizing their inherent immune advantage could be something more than wishful thinking.

Some important relevant points and questions for consideration
This short list, is in lieu of a summary, which would likely develop into a literature review, and is not the point of the presentation. It represents an abbreviated "wish list" to provoke thought and some simple observations presented with no order of priority.

- Colditz[10] points out that as resistance to internal parasites is a highly polymorphic trait selection for resistance in various populations may have been for defence mechanisms that differ both in their costs and in their efficacy in controlling parasites. For traits with additive genetic variation positive genetic progress can be made despite negative genetic correlations between the traits, provided an appropriate weight is applied to the traits in a breeding objective. The question

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arises, is the status quo in the prime lamb industry acceptable and progress in resistance selection sufficiently rapid or should there be a re-think?

- It is known that resistant animals reduce pasture contamination but high WEC lambs grazed concurrently with low WEC lambs in New Zealand have done better than their resistant counterparts. However, when grazed separately for three years, the previously observed unfavourable relationship between WEC and body weight was removed, presumably from the epidemiological benefit of reduced pasture contamination. As such, whilst potential exists for a higher 'cost' of immune response in resistant animals, reduced exposure over time may potentially offset any observed reduction in productivity.

- Industry has a workable tool for selection for genetic resistance and flocks including selected non-resistant and resistant animals have been created to considerable benefit, yet science remains none the wiser concerning an explanation for the complexities of genetic interaction that determine the placement of any one animal or group within a particular line. Surely there is certainty that resistant lines could be selected to be *even more resistant* but that development would bring with it an obligation to ensure that there were no adverse associations, including the development of sheep resistant parasites.

- Genetic resistance is most likely controlled by many loci with moderate to small effects which means that resistance in the parasites is unlikely to develop in the short term.

- What proportion of high resilience is related to diet in grazing animals and is there a relationship with animals that are simply more efficient at targeting a higher quality diet?

Acknowledgements

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