Genetic relationships between indicator traits and parasitic nematode infection in sheep.

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Introduction

Gastrointestinal nematodes cause economic loss to the UK sheep industry. Grazing sheep are exposed to parasitic infection. Parasitic infection is traditionally managed with anthelmintics. However resistance in nematode parasites has developed to anthelmintic compounds and is now a worldwide problem. Alternative control measures are now required to maintain economic sheep production. Possible strategies are grazing management, vaccination, dietary supplementation with protein and selective breeding. Grazing management can be difficult to implement, as pasture requires rest periods or rotational grazing with unaffected species. No vaccines against gastrointestinal nematodes are commercially available. Protein supplementation can enhance immunity and increase the resilience of the animal to infection, however this increases feeding costs. Selective breeding has been a successful strategy for production traits and could be a useful strategy for parasite resistance. *Teladorsagia circumcincta* is the major gastrointestinal parasite of UK sheep. Sub- clinical infection with *T. circumcincta* can cause a 30% decrease in growth rate (Coop *et al.* 1985).

The measurement of relative resistance in grazing animals is difficult and may be achieved with indicator traits that reflect the immune response of the individual or the consequence of infection. There are several possibilities. Immunoglobulin A (IgA) is a secreted antibody which is part of the acquired immune response. It has a major role in gut infections and appears to regulate worm fecundity (Stear *et al.* 1995; Smith *et al.* 1985). Pepsinogen is a precursor of the digestive enzyme pepsin. An increase in pepsinogen concentration is indicative of a rise in the pH of the abomasum. *T. circumcincta* can cause the pH of the abomasum to rise from pH2 to pH7 preventing the formation of pepsin. An increase in pepsinogen therefore indicates the presence of parasites and resultant damage to the gut of the host animal (McKellar *et al.* 1986; Fox *et al.* 1989). Fructosamine concentration reflects average glucose and protein concentrations as well as the rate of protein turnover. *T. circumcincta* can cause a relative protein deficiency as well as an increase in protein turnover. Heath and Connan (1991) observed a decrease in fructosamine concentration following deliberate gastrointestinal infection. Eosinophils are a type of white blood cell and are part of the innate immune response. Eosinophil related responses have been associated with resistance to parasitic infection, however their role is yet to be determined (Doligalska, Moskwa and Stear 1999; Stevenson *et al.* 1994). Faecal egg count is the traditional indicator trait commonly used to assess the level of infection by the number of eggs per gram of faeces. Faecal egg counts indicate the product of the adult nematode numbers and the mean fecundity of resident parasite populations. However faecal egg counts are relatively insensitive to changes in infection intensity (Bishop and Stear 2000).

The aims of this study are firstly to estimate the heritabilities of both parasitic traits and indicator traits; secondly to investigate the relationships between these traits by calculation of environmental, phenotypic, and genetic correlations. From these results the suitability of these traits for inclusion in a selection index will be considered.

Materials and Method

Animals

1000 Scottish Blackface lambs, predominantly twins, were studied over a 5-year period (1992-6). The lambs were kept on a commercial upland farm in southwest Strathclyde and all husbandry procedures followed standard commercial practice. All of the lambs were born outside during the last 2 weeks of April and the 1st week in May, and were continuously exposed to mixed nematode infections by grazing. Anthelmintic treatment (albendazole sulphoxide) was administered, at the dose rate recommended by the manufacturer, and blood samples were taken every 28 days between 4 and 20 weeks of age. Blood samples were not collected in October 1992, October 1993 and August 1995. Faecal egg counts were also collected every 28 days. IgA, fructosamine, eosinophil and pepsinogen concentrations were measured from the blood samples. Approximately half of each cohort was necropsied at 6-7 months of age (6 weeks post final anthelmintic treatment). At necropsy standard parasitological procedures were used to enumerate *T. circumcincta* nematodes in the abomasum

(Armour, Jarrett and Jennings, 1966). Worm length was observed by collecting and measuring a sample of at least 25 female worms from each animal and a mean length recorded for each animal. Fecundity was observed by counting the number of eggs *in utero* for these females and again the mean recorded for each animal.

Data Analysis

Data analysis began with the distribution of the traits; all traits except worm length and fructosamine were skewed and were log transformed prior to further analysis. A restricted maximum likelihood package (ASREML) was used to perform univariate analysis fitting an animal model and heritability estimates were then calculated for each trait. This analysis was repeated fitting a litter effect (c^2); the significance of the litter effect was tested using a likelihood ratio test. Bivariate analysis was also performed using ASREML to enable calculation of environmental, phenotypic and genetic correlations between traits.

Results

Tables 1a and 1b contain summary data for all the traits investigated. Table 1a illustrates the range of infection observed within the flock and the range in worm development traits (worm length and fecundity). Table 1b contains the summary data for the indicator traits; this also shows the number of observations at each time point and also the number of measurements taken for each trait.

Table 1a Summary data for necropsy traits. All traits had 489 observations.

Trait	Mean	Minimum	Maximum	Standard Deviation
Worm Length (cm)	0.87	0.57	1.22	0.13
Worm Fecundity (eggs/worm)	18.93	1.05	67	13.56
L4 Larvae	2593	0	27100	4797
L5 Larvae	173	0	7100	655
Adult Males	1391	0	7850	1365
Adult Females	1905	0	15400	1988
Mature Worm Burden	3291	0	21900	3293
All Worms + Larvae	6073	150	37900	7368

Table 1b Summary data for indicator traits

Trait	Time of Measurement	No. of Observations	Mean	Minimum	Maximum	Standard Deviation
IgA 1	August	772	0.12	0	1.38	0.15
IgA 2	September	962	0.20	0	1.10	0.17
IgA 3	October	562	0.14	0	0.79	0.12
Eosinophil 1	May	389	1.57	0.41	4.2	0.75
Eosinophil 2	June	194	1.47	0	4.17	0.77
Eosinophil 3	July	193	1.75	0	4.73	0.79
Eosinophil 4	August	391	2.32	0	4.53	0.94
Eosinophil 5	September	391	2.39	0.41	5.01	0.78
Fructosamine 1	July	204	165.2	81	205	20.66
Fructosamine 2	August	204	180.8	100	265	25.4
Fructosamine 3	September	592	194.4	96	341	40
Pepsinogen	September	767	30.17	0	281.43	30.7

Heritabilities for necropsy traits are shown in table 2.The worm number traits appear to have very low heritability however the worm development traits, worm length and fecundity, appear to be highly heritable. Heritabilities for indicator traits are shown in table 3.The indicator trait heritabilities

were calculated for every time point that a measurement was taken. The heritability of fructosamine increased over time, from 4 to 6 months of age with the 6-month value suggesting that fructosamine is moderately heritable. All fructosamine measurements also had a significant litter effect. The heritability of IgA remained fairly constant with high heritability values across time. Eosinophil appeared to drop in heritability value across time yet was still moderately heritable at 6 months. The heritability of pepsinogen is also high (Table 3).

Table 2 Necropsy trait heritabilities

Trait	h^2	s.e.
Worm Length	0.53	0.17
Worm Burden	0.13	0.10
Worm Fecundity	0.50	0.16
Adult Females	0.08	0.09
Adult Males	0.12	0.10
L4 Larvae	0.06	0.09
L5 Larvae	0.12	0.09
All Worms	0.12	0.10

Table 3 Indicator trait heritabilities

Trait	h^2	s.e.	c^2	s.e.
Fructosamine 1	0.10	0.17	0.53	0.10
Fructosamine 2	0.05	0.14	0.23	0.11
Fructosamine 3	0.39	0.16	0.21	0.07
Immunoglobulin A 1	0.46	0.13		
Immunoglobulin A 2	0.67	0.11		
Immunoglobulin A 3	0.57	0.15		
Eosinophil 1	0.74	0.25		
Eosinophil 2	0.63	0.33	0.10	0.15
Eosinophil 3	0.31	0.25		
Eosinophil 4	0.43	0.17		
Eosinophil 5	0.35	0.15		
Pepsinogen	0.56	0.16	0.12	0.07

Table 4 illustrates the genetic correlations between the parasitic traits and the indicator traits, at 5-6 months of age. The most significant correlations are between the indicator traits and worm length and fecundity. IgA, eosinophil and pepsinogen all appear to have strong, negative genetic correlations with worm length. In contrast fructosamine and faecal egg count are positively genetically correlated with worm length; fructosamine has a strong correlation and faecal egg count a moderate one. IgA and eosinophil also have strong negative genetic correlations with worm fecundity. With fecundity pepsinogen is again negatively correlated but a weak to moderate correlation. Fructosamine and faecal egg count are both weakly, positively correlated with fecundity. Faecal egg count exhibits a strong positive correlation with worm burden. The correlations between worm burden and all of the traits except faecal egg count have standard errors that are very high and make meaningful interpretation difficult. This is a consequence of the worm burden heritabilities being low.

Table 4 Genetic correlations between indicator and necropsy traits at 5-6 months of age (s.e.)

Trait	Worm Length	Worm Fecundity	Worm Burden	Adult Males	Adult Females	L5 Larvae
IgA	-0.53 (0.24)	-0.62 (0.26)	-0.36 (0.46)	-0.64 (0.51)	-0.32 (0.57)	-0.18 (0.44)
Eosinophil	-0.58 (0.27)	-0.69 (0.27)	-0.61 (0.50)	-0.73 (0.67)	-0.49 (0.57)	0.07 (0.55)
Pepsinogen	-0.48 (0.21)	-0.26 (0.22)	0.17 (0.38)	0.28 (0.40)	0.11 (0.48)	0.43 (0.34)
Fructosamine	0.67 (0.27)	0.31 (0.24)	0.03 (0.32)	0.10 (0.50)	-0.45 (0.46)	-0.14 (0.36)
Faecal Egg Count	0.32 (0.25)	0.21 (0.26)	0.65 (0.28)	0.62 (0.38)	0.70 (0.31)	-0.01 (0.36)

An interesting phenomenon demonstrated with these results is that the genetic and environmental correlations are opposite in sign, with the consequence that the phenotypic correlations are low. An example of this is shown for IgA (Table 5). Table 5 illustrates the environmental, genetic and phenotypic correlations between IgA and worm length, fecundity and burden.

Table 5 Correlations between IgA and Necropsy traits at 5-6 months of age.

Trait	Re (se)	Rp (se)	Rg (se)
IgA – Worm Length	0.27 (0.27)	-0.15 (0.07)	-0.53 (0.24)
IgA – Worm Fecundity	0.42 (0.26)	-0.07 (0.07)	-0.62 (0.26)
IgA – Worm Burden	0.03 (0.14)	-0.06 (0.06)	-0.36 (0.46)
IgA – Faecal Egg Count	0.30 (0.16)	-0.13 (0.05)	-0.78 (0.18)

Discussion

This study has enabled us to estimate the heritability of parasitic traits and indicator traits. It has also facilitated the investigation of relationships between parasitic and indicator traits by the calculation of correlations which indicate the extent to which easily measurable traits represent the infection status of the host. These results have aided the assessment of the suitability of these traits as selection criteria to breed for increased resistance to gastrointestinal nematodes

The heritability values calculated provide good evidence that one or more of IgA, pepsinogen, fructosamine or eosinophil may be useful for inclusion in a selection index. The parasite development traits appeared to be significantly more heritable than the parasite number traits (Table 2 and 3).

The results illustrated in table 4 provide an informative description of the relationships between the indicator traits and the parasite development traits. The strong negative correlations between IgA and both worm length and fecundity illustrate that families which have high levels of IgA have shorter, less fecund worms than those with a lower level of IgA. This compares favourably to previous research which linked IgA with the regulation of worm growth and fecundity (Stear *et al.* 1995; Smith *et al*. 1985). These results also imply that IgA would be a very useful trait for selection purposes. The genetic correlations between eosinophil and both worm length and fecundity were also strong and negative. This, as with IgA, indicates that families with high eosinophil counts will have shorter less fecund worms. It is interesting that the traits that provide a comparison between the acquired and innate immune response produce very similar correlations with the parasite development traits. The correlations between fructosamine and worm length and fecundity were both positive, however the correlation with worm length was much stronger than that with worm fecundity. This indicates that families with a higher level of fructosamine have longer and slightly more fecund worms. This could reflect either an increase in both protein and/or glucose concentrations or an increase in protein turnover, or possibly density-dependent interactions between worm growth and size.

The IgA correlations (Table 5) illustrate a very interesting phenomenon. When considered that the genetic correlation indicates the underlying biological relationship, by utilising the family information, and that the environmental correlation reflects the infection status of an animal at a single point in time. Then, the difference in sign observed between these correlations is informative and helps to explain the observation that the phenotypic correlations are very low. An interpretation of this result could be that the genetic correlation indicates the underlying biological relationship and hence suggests that the ability to produce IgA is linked to the resistance mechanism in that the better the ability of the animal to produce IgA then the lesser the worm problem. But the environmental correlation suggests that after stripping out genetic effects, higher IgA levels may indicate infection status. A similar phenomenon has been observed for correlations between faecal egg count and anti-nematode antibodies in cattle (Morris *et al*. 2003) and also between faecal egg count and IgG in sheep (Shaw *et al.* 1999).

In conclusion worm length and fecundity were found to be highly heritable. The indicator traits were found to be moderately to highly heritable. The indicator traits appeared to be moderately to highly genetically correlated to the worm development traits. The environmental and genetic correlations were found to be opposite in sign. These indicator traits could be used to form a selection index to aid selection for enhanced resistance, as they are heritable and correlated with the worm development traits.

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