The responses of sheep to an injection with human serum albumin (HSA) and to an infection with the parasite *Haemonchus contortus* were compared in relation to haemoglobin genotype. The subjects were 51 female lambs of the Romanov breed, and were the offspring of 8 sire-families. The 51 animals fell into 3 groups of 17, representing the three haemoglobin genotypes, Hb AA, AB and BB; they were also tested for markers of the major histocompatibility system, OLA. Information was obtained on the development of immunity following exposure to the parasite, and its subsequent partial loss following drenching. The response to HSA did not show any positive correlation with resistance to *H. contortus*. Resistance to *H. contortus* appeared to be independent of Hb genotype. Association with OLA genotype was not excluded.

**KEY WORDS**: sheep, Romanov breed, immune response, human serum albumin, parasitism, *Haemonchus contortus*, resistance, haemoglobin type, OLA system.
INTRODUCTION

During the past twenty years, there have been several reports suggesting that sheep homozygous for the haemoglobin A allele are more resistant to the parasite *Haemonchus contortus* than are homozygotes for haemoglobin B (EVANS et al., 1963; JILEK and BRADLEY, 1969; ALLONBY and URQUHART, 1976; ALTAIF and DARGIE, 1976, 1978a, 1978b; PRESTON and ALLONBY, 1979). It has also been suggested this increased resistance might not only be to *Haemonchus contortus*, but could be linked to a generally improved immune response (CUPERLOVIC et al., 1978).

In the experiments described below, we have looked at the relationship between haemoglobin genotype and resistance to *Haemonchus contortus* on the one hand, and also to the response to immunisation with aggregated human serum albumin (HSA). In addition we were able to study the development of parasite immunity following inoculation, and its loss following oral drenching. The animals in the study were retrospectively tested for antigens of the sheep major histocompatibility system (OLA), and the relevance of these genetic markers was considered in our analysis.

MATERIALS AND METHODS

Animals

The experiments were performed on 51 lambs of the Romanov breed. Only females were used as preliminary studies had shown that females can be more easily immunised than males against *Haemonchus contortus* (LUFFAU et al., 1981a). These animals were the offspring of 8 sires which were of haemoglobin genotype AB; and the 51 lambs fell into three groups of 17, each representing the three haemoglobin genotypes, Hb AA, Hb AB, and Hb BB. The three genotypes were selected evenly from the different sire families to reduce the influence of any sire effect upon the effect of haemoglobin genotype under study. The 51 lambs and most of their dams were typed for antigens of the OLA system; the sires were not typed but their genotype could be inferred and transmission of markers determined in many cases. The animals were maintained on a grass free diet from birth to avoid environmental exposure to *Haemonchus contortus*.

Experimental Protocol

At the age of 6 months all the lambs had been immunised with aggregated human serum albumin (HSA); their serum was tested for haemagglutinating antibodies to HSA after two weeks. Experimental inoculation with *Haemonchus contortus* larvae began at the age of 8 months, a previous study having shown that effective immunisation can be achieved from the age of about 7 months (URQUHART et al., 1966).
Three parasite infection experiments were performed successively on the same group of animals. Three weeks old L_5 larvae artificially incubated were used throughout for infection.

In experiment 1, the 51 animals were divided into three treatment groups:

a) 18 animals were given 5,000 larvae on day 0, followed by 10,000 larvae on day 31 and 20,000 larvae on day 64 (group 1).

b) 18 animals received the above treatment but on days 31 and 64 only (group 2).

c) 15 animals received the day 64 treatment only (group 3). Parasite egg excretion was monitored in all animals.

Experiments 2 and 3 each consisted of an infection of 10,000 larvae on day 0. In each experiment the sheep were divided into two groups; one drenched before day 0, and the other not so treated. Egg excretion was monitored as in experiment 1.

Variables

The 26 animals which produced no detectable antibodies to HSA are scored as zero for this response, while the inverse of the titre detected (1/2 - 1/512) is used to denote the level of response in the remaining 25 animals. Immunity to the parasite is denoted by mean egg count per gramme of faeces (epg) - and log (epg) - at a given time following inoculation (the animals with better immunity being those which excrete the fewer eggs). Figure 1 shows these variables.

RESULTS AND DISCUSSION

Experiment 1 demonstrated the effectiveness of immunising by inoculation of larvae (LUFFAU et al., 1981b). The animals which had been exposed to the parasite on day 0 mounted a much greater secondary response when given a second (larger) dose, than did control animals given the same dose as a primary inoculation (figure 2).

In experiment 2 and 3 it was clear that drenching animals with pre-existing immunity to the parasite markedly reduced their resistance to a subsequent inoculation with larvae (figure 2).

With regard to the influence of haemoglobin genotype, no effect was noted in our conditions (namely in Romanov breed) on the development of either parasite immunity or the response to HSA. These results disagree with those of a number of authors (see introduction) but agree with the findings of RIFFKIN and YONG (1984) who found no detectable relationship between Hb genotype and resistance to Haemonchus contortus.
No correlation was found between the intensity of the immune response to the parasite followed in experiment 1, and the antibody titre to HSA. On the other hand, in experiments 2 and 3, those with highest antibody titres were among those least resistant to the parasite. This finding was significant, and while contrary to the hypothesis of CUPERLOVIC et al. (1978), it is in agreement with ALBERS et al. (1984) who found a negative correlation between epg excreted after inoculation and the immune response to chicken red cells. It should be noted that a negative correlation between antibody response and helminth immunity has also been found in the mouse (BLUM and CIOLI, 1978; DEELDER et al., 1978; PERRUDET-BADOUX et al., 1978; WAKELIN, 1978).

Several OLA markers are significantly correlated with the variables in experiments 2 and 3. The transmission of paternal OLA haplotypes within each sire family could be compatible with a hypothesis that there exist, in or near the sheep major histocompatibility complex, genes influencing resistance to Haemonchus contortus. These results would accord with those of OUTTERIDGE et al. (1984) who found an association between OLA markers and the response to vaccination against the parasite Trichostrongylus colubriformis.

Analysis of the variability in genetic resistance to Haemonchus contortus, based on epg measurements at different times considered as the same trait showed a strongly significant "individual" effect; the repeatability, calculated by the coefficient of intra-class correlation, was between 0.35 and 0.43. There were also several indications that a "sire-effect" was operating, although larger numbers of animals would be necessary to confirm such a hypothesis. However, our results would agree with those of ALBERS et al. (1984) who found a heritability of 0.3 for resistance to Haemonchus contortus in Australian Merinos.

CONCLUSION

This study of experimental injection with Haemonchus contortus has demonstrated that immunity can be acquired by deliberate exposure to the parasite but may subsequently be destroyed by drenching. There are several indications of a genetic component in resistance to Haemonchus contortus which could offer a basis for selective breeding. For this purpose it would be useful to have a genetic marker for increased resistance. We have found that haemoglobin genotype and humoral immunity to HSA are not very useful indicators of resistance, but the possibility that OLA markers could be valuable in this respect certainly merits further investigation.
LITERATURE CITED


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Figure 1
Variables describing immunity to *Haemonchus contortus*
in infection experiments 1, 2 and 3

**EXPERIMENT 1**

Group 1

- DO (5000 L3)
- D31 (10000 L3)
- D64 (20000 L3)

- Horizontal axes represent the time (in days).
- Vertical arrows indicate experimental infections (in brackets: number of larvae L3).
- Variables are mean egg counts per gram (epg) of faeces during indicated periods.

Group 2

Group 3

**EXPERIMENT 2**

- DO (10000 L3)

**EXPERIMENT 3**

- DO (10000 L3)

- Horizontal axes represent the time (in days).
- Vertical arrows indicate experimental infections (in brackets: number of larvae L3).
- Variables are mean egg counts per gram (epg) of faeces during indicated periods.
Figure 2
Faecal egg output in infection experiments 1, 2 and 3 with *Haemonchus contortus*

**EXPERIMENT 1**

- Group 1
- Group 2
- Group 3

**EXPERIMENT 2**

- Drenched group
- Not drenched group

**EXPERIMENT 3**

- Drenched group
- Not drenched group