Original article

Resistance to experimental infections with *Haemonchus contortus* in Romanov sheep

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Summary – Responses to immunization with aggregated human serum albumin (HSA) and to repeated experimental infections with *H contortus* were studied in 51 female lambs of the *Romanov* breed, born from 8 sires and 36 dams. The 8 sires were of haemoglobin genotype *Hb AB*; the 51 lambs were distributed into 3 groups of 17 each, corresponding to the 3 genotypes *HbAA*, *HbAB* and *HbBB*. In addition, the experimental lambs were typed for antigens of the major histocompatibility system (*OLA*). The parasitological findings were the following: a repeatability of faecal egg counts between successive infections, a negative correlation between peak faecal egg counts and self-cure intensity, a positive correlation between faecal egg counts and degree of anaemia, an acquisition of immunity to the parasite by previous contact with the parasite and a reduction of this immunity by anthelmintic treatment. According to the genetic investigations, there were significant sire effects on variables reflecting the resistance. The faecal egg counts did not seem to be related to the haemoglobin system, but might be affected by 1 or several genes located in the OLA complex or close to the latter. The humoral response to HSA showed a negative correlation to parasite resistance.

sheep / *Haemonchus contortus* / humoral response / haemoglobin / OLA system

Résumé – **Résistance à des infestations expérimentales par *Haemonchus contortus* en race ovine Romanov.** *Les réponses à une immunisation avec de la sérum albumine humaine agrégée (SAH) et à des infestations expérimentales répétées avec H contortus ont été étudiées chez 51 agnelles de race Romanov, issues de 8 pères et de 36 mères. Les 8 pères étaient hétérozygotes AB pour le système hémoglobine (Hb) et les 51 agnelles*

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étaient réparties en trois groupes de 17 correspondant aux trois génotypes Hb AA, Hb AB et Hb BB. *Par ailleurs, les agnelles expérimentales ont été typées pour le système majeur d'histocompatibilité (OLA). Sur le plan parasitologique, les résultats obtenus mettent en évidence: une répétabilité du taux d'excrétion des œufs entre infestations successives, une corrélation négative entre niveaux des pics d'excrétion et intensité de l'autostérilisation ("self-cure"), une corrélation positive entre taux d'excrétion et degré d'anémie, une acquisition de l'immunité parasitaire par contact préalable avec le parasite et une réduction de cette immunité par vermifugation. Sur le plan génétique, on observe des effets père significatifs sur des variables reflétant la résistance. Le système hémoglobine ne semble pas lié au taux d'excrétion mais pourrait être lié au degré d'anémie consécutif à l'infestation. La résistance à* H contortus *pourrait être influencée par un ou plusieurs gènes situés dans le complexe OLA ou à sa proximité. La réponse humorale à la SAH présente une corrélation négative avec la résistance au parasite.*

ovin / **Haemonchus contortus** / réponse humorale / hémoglobine / système OLA

INTRODUCTION

Since the publications of Warwick *et al* (1949), Whitlock (1955, 1958) and Whitlock and Madsen (1958), the existence of a genetic variability in the resistance to *Haemonchus contortus* has been shown in several studies: the heritability estimates range around 0.25-0.30 (Le Jambre, 1978; Albers *et al*, 1984, 1987; Piper, 1987). As there are almost no genetic correlations between the resistance and various production traits (Alberts *et al*, 1984, 1987; Piper, 1987), selection on resistance to *H contortus* would be possible and economically justified in conditions where this type of parasitism leads to large productivity losses (Holmes, 1986). However, it does not seem to be possible to use the response to an experimental infection as a large-scale selection criterion because of the difficulties of such an experimentation. It would therefore be interesting to identify resistance predictors, either immunological traits or genetic markers (Courtney, 1986; Alberts and Gray, 1987; Cabaret and Gruner, 1988).

Several studies suggest that haemoglobin *A* allele provides a higher resistance to *H contortus* than the haemoglobin *B* allele (Evans *et al*, 1963; Jilek and Bradley, 1969; Radhakrishnan *et al*, 1972; Allonby and Urquhart, 1976; Altaif and Dargie, 1976, 1978a, b; Preston and Allonby, 1979; Dally *et al*, 1980; Luffau *et al*, 1981a, b; Courtney *et al*, 1985). According to Cuperlovic *et al* (1978), this enhanced resistance might be related to a higher humoral immune response.

From a genetic point of view, the main objective of the present experiments was to confirm or invalidate this hypothesis. Because the typing of animals in the major histocompatibility system (*OLA*) was performed retrospectively, a search for relationships between resistance to *H contortus* and the *OLA* marker was also included in this study.

From a parasitological point of view, the experimental goals were to supply additional information on the following phenomena: repeatability of faecal egg counts between successive infections, relationship between egg counts and self-cure, relationship between egg counts and degree of anaemia, acquisition of immunity to the parasite by previous contact with the parasite and effect of anthelmintic treatment on this acquired immunity.

The experiment was designed so as to give responses to questions in the fields of genetics and parasitology.

MATERIALS AND METHODS

Animals

Several studies have shown that females develop stronger immunity against *H contortus* than males (Colglazier *et al*, 1968; Yazwinski *et al*, 1980; Luffau *et al*, 1981a; Courtney *et al*, 1985; Watson, 1986): hence only females were used in the present study, *ie* 51 female lambs of the *Romanov* breed born from 8 sires and 36 dams. The breeding animals were chosen according to their haemoglobin genotype. All sires were *Hb AB* heterozygotes. The dams belonged to genotypes *Hb AA, Hb AB* or *Hb BB*. The 51 lambs fell into 3 groups of 17, each representing 1 of the 3 haemoglobin genotypes. The number of animals in the 3 haemoglobin genotypes was balanced within each sire progeny so as to reduce risks of confusion between a possible haemoglobin genotype effect and a possible sire effect.

Fifty lambs and 24 of their 35 dams were typed for antigens of the *OLA* system. The sires were not typed but their genotypes could be inferred and transmission of markers determined in many cases.

The experimental female lambs were chosen so as to form a group as homogeneous as possible for age, weight, maintenance conditions and health in order to reduce uncontrolled factors of variation. The animals were maintained on a grass free diet from birth to avoid environmental exposure to *H contortus*.

Typing methods for haemoglobin and OLA systems

Haemoglobin types were determined by electrophoresis (Nguyen and Bunch, 1980). Class I antigens of the major histocompatibility system were tested by the microcytotoxicity method on blood lymphocytes; the test was carried out over a period of 2h 30 min (Cullen *et al*, 1985). Lymphocytes of each animal were tested with 120 antisera against 22 provisional specificities, "*OLA*-P". Nine haplotypes, each carrying 1 or 2 specificities, were identified in the tested animals.

Immunization experiments with aggregated human serum albumin

The 51 experimental lambs were immunized at the age of about 6 months with heat aggregated human serum albumin (HSA: 200 mg/animal) by intravenous injection. Their serum was collected before and 14 d after the administration of the antigen, titred by passive haemagglutination using red blood cells tanned and sensitized with HSA (Weir, 1978). The technique used to determine the serum agglutination titre has been described previously (Nguyen, 1984).

Experimental infections with H contortus

According to various studies, sheep develop immunity against *H contortus* from the age of about 7 months (Jarrett *et al*, 1961; Manton *et al*, 1962; Urquhart *et al*, 1966a, b; Knight and Rodgers, 1974; Wilson and Samson, 1974; Benitez-Usher *et al*, 1977; Duncan *et al*, 1978; Riffkin and Dobson, 1979; Smith and Angus, 1980). Our experiments therefore began when the lambs were about 8 months old. During the experimental infections, lambs were kept in well controlled conditions: open

sheepfold fitted with a slatted floor, diets based on compound feed concentrates, hay and straw *ad libitum*. Five infection experiments were conducted successively using 3-week old larvae. Animals were infected with larvae obtained by faecal cultures according to the method of FJS Robert and PJ O'Sullivan and collected with Baerman's apparatus (Luffau *et al*, 1981a, b). The required number of larvae were counted microscopically and suspended in 20 ml of ordinary water. This suspension was administered orally. The strain maintained at the Station of Virology and Immunology was supplied initially by Professors GM Urquhart and EW Allonby (Glasgow).

Experiment 1

In experiment 1, lambs were divided into 3 groups:
– 18 animals were given 3 infections successively: a primary infection on D0 with 5 000 larvae, a secondary one on D32 with 10 000 larvae and a 3rd one on D64 with 20 000 larvae (group 1);
– 18 animals were given 2 infections successively: a primary infection on D32 with 10 000 larvae and a secondary one on D64 with 20 000 larvae (group 2);
– 15 animals were given an infection of 20 000 larvae on D64 (group 3).

The 3 groups were formed so as to obtain a balanced distribution of the various paternal origins and haemoglobin genotypes.

The kinetics of faecal egg counts was established for each animal. Eggs laid by *H contortus* females and eliminated with the faeces were counted using faecal samples of 3g using the Mc Master method. Measurements were made on the following 40 dates: D17, D21, D24, D28, D31, D35, D37, D39, D42, D44, D46, D49, D51, D53, D56, D58, D60, D63, D65, D67, D70, D72, D74, D77, D79, D81, D84, D86, D88, D91, D95, D98, D107, D114, D119, D126, D133, D140, D147 and D156. Each measure (number of eggs per gram) was the mean egg count of 3 different samples. These egg counts were good indicators of the worm burdens of the animals (Roberts and Swan, 1981).

The following 3 haematological parameters were recorded in all animals: number of red blood cells, packed cell volume and haemoglobin content. These measurements were made on the following dates: D9, D16, D23, D30, D39, D45, D53, D58, D67, D74, D88, D95, D102, D109, D116, D123, D130, D137, D144, D151 and D158.

The number of red blood cells (per μl of blood) was determined by measuring the variation in the potential difference (Celloscope 401 – Ljungberg – Stockholm, Sweden) induced by the passage of red blood cells (blood dilution 1/800) in an electric field. The apparatus was periodically checked according to the microscopical method of Malassez.

For measuring haematocrit (packed cell volume), blood was centrifuged in heparinized capillary tubes (inner diameter: 0.55 mm; length: 75 mm) using Janetzki's TH-12 centrifuge at 1 500 r/min for 5 min.

For measuring the haemoglobin content (g/100 ml blood), haemoglobin of the red blood cells lysed by saponin was fixed and transformed into cyanmethaemoglobin. The haemoglobin content was measured by spectrophotometry (absorption at 630 nm).

Experiment 2

The surviving 49 animals were divided into 2 groups, irrespective of the group they were part of in experiment 1:
– the 26 animals of group 1 were not drenched prior to experiment 2; hence they were carriers of a residual *H contortus* population;
– before starting experiment 2 the 23 animals of group 2 were drenched with a highly effective anthelmintic, Thibenzole MSD powder (thiazolyl benzimidazole-thiabendazole ND, Paris, France).

In these 2 groups, each animal was given 10 000 larvae on D0 of experiment 2 (263 days after D0 of experiment 1). Faecal egg counts were made on the following 20 dates: D1, D0, D17, D20, D24, D27, D31, D34, D38, D41, D45, D48, D56, D59, D80, D83, D88, D91, D95 and D98.

Experiment 3

Experiment 3 was a replication of experiment 2. The infection on D0 took place 366 days after D0 of experiment 1. The faecal egg counts were made on the following 18 dates: D5, D8, D9, D12, D15, D19, D22, D26, D29, D33, D36, D40, D43, D47, D50, D54, D57 and D64.

Experiment 4

In experiment 4, each animal was drenched and given 10 000 larvae on D0 (485 days after D0 of experiment 1). The faecal egg counts were made at the following 19 dates: D5, D0, D3, D6, D10, D15, D19, D22, D26, D29, D33, D36, D40, D44, D47, D50, D55, D65 and D72.

Experiment 5

Experiment 5 was a replication of experiments 2 and 3. The animals of each group (drenched and not drenched) were given 10 000 larvae on D0 (560 days after D0 of experiment 1). The faecal egg counts were made on the following 41 dates: D0, D17, D21, D24, D28, D31, D35, D38, D42, D45, D49, D52, D56, D59, D63, D70, D73, D77, D80, D84, D87, D91, D94, D98, D101, D108, D115, D119, D123, D126, D129, D140, D143, D147, D150, D154, D157, D161, D164, D168 and D172.

Statistical analysis

Choice of variables and factors

Variables

The immunological, parasitological and haematological variables used are given in table I. The parasitological variables were defined from decimal logarithms of mean egg counts over certain periods (in order to normalize distributions and obtain more homogeneous variances). The choice of periods was based on the kinetics of faecal eggs counts in the successive infection experiments.

Table I. List of immunological, parasitological and haematological variables in experiments 1, 2, 3, 4 and 5. *HSA: aggregated human serum albumin. **mean log epg from Dn to Dn': variable equal to 0 if the mean log epg from Dn to Dn' is equal to 0; variable equal to the decimal logarithm of the mean epg from Dn to Dn' if not. ***The reference periods used to calculate the means in groups 1, 2 and 3 were the same as for the *RBCPRIM* variable.

Name of variable	Definition of variable
ANTIHSA	Inverse of the anti-HSA* antibody titre (2 for a titre of 1/2, 4 for a titre of 1/4 etc and 0 for absence of antibodies)

PRIMPEAK	Parasitological variable reflecting the primary peak in experiment 1:
	  mean log epg from D24 to D37** in group 1
	  mean log epg from D56 to D67 in group 2
	  mean log epg from D88 to D107 in group 3

RBCPRIM	Mean number of red blood cells per mm^3 during the primary faecal egg count peak;
	  mean from D23 to D39 in group 1
	  mean from D53 to D67 in group 2
	  mean from D88 to D102 in group 3

PCVPRIM	Mean packed cell volume during the primary faecal egg count peak***
HCPRIM	Mean haemoglobin content during the primary faecal egg count peak***

SECPEAK	Parasitological variable reflecting the secondary peak in experiment 1:
	  mean log epg from D46 to D53 in group 1
	  mean log epg from D84 to D88 in group 2

SELFCURE	Parasitological variable reflecting the self-cure in experiment 1:
	  mean log epg from D24 to D37 – mean log epg from D39 to D46 in group 1
	  mean log epg from D56 to D67 – mean log epg from D74 to D81 in group 2

PEAKEXP2	Parasitological variable reflecting the peak in experiment 2: mean log epg from D27 to D48
PEAKEXP3	Parasitological variable reflecting the peak in experiment 3: mean log epg from D26 to D54
PEAKEXP4	Parasitological variable reflecting the peak in experiment 4: mean log epg from D26 to D55
PEAKEXP5	Parasitological variable reflecting the peak in experiment 5: mean log epg from D28 to D52
PEAK235	Parasitological variable equal to the arithmetic mean of the 3 variables, *PEAKEXP2, PEAKEXP3* and *PEAKEXP5* reflecting the peaks in experiment 2 and in its 2 replications (experiments 3 and 5) respectively

Thus, in each of the 3 groups of experiment 1, a peak faecal egg count was observed after the primary infection (fig 1). This peak was located from D24–D37 in group 1, from D56–D57 in group 2 and from D88–D107 in group 3: the *PRIMPEAK* variable reflects this peak.

In groups 1 and 2 of experiment 1, the secondary infection was followed by a very large drop in faecal egg counts (from D39 to D46 in group 1 and from D74 to D81 in
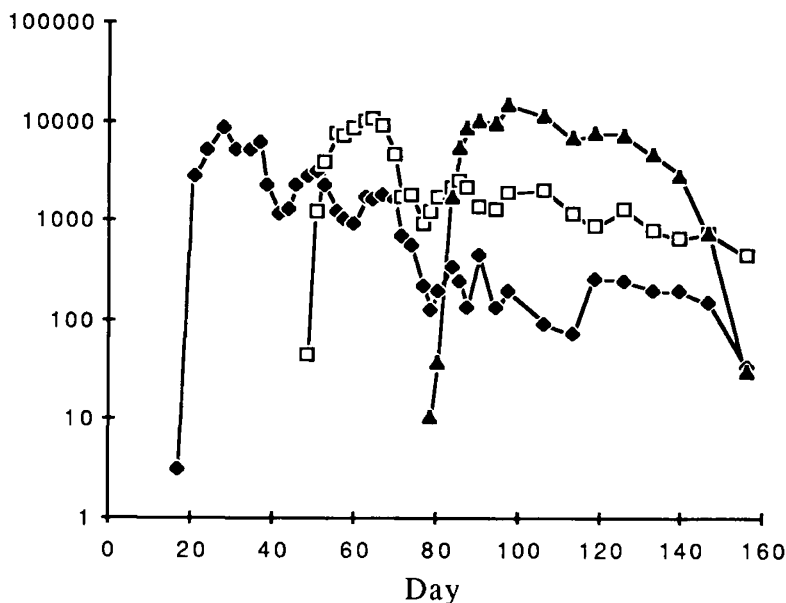
Fig 1. Mean kinetics of faecal egg output in the 3 groups of experiment 1. —◆—Group 1 (3 infections on d D0, D32 and D64 with 5000, 10 000 and 20 000 larvae, respectively); —□—Group 2 (2 infections on d D32 and D64 with 10 000 and 20 000 larvae, respectively); —▲—Group 3 (1 infection on d D64 with 20 000 larvae).

group 2): this was the classical self-cure phenomenon. Variable *SELFCURE* reflects this phenomenon; it is defined as the difference between the primary peak and the depression subsequently to the self-cure. A secondary peak could be observed immediately after this depression (D46 to D53 in group 1 and D84 to D88 in group 2): variable *SECPEAK* reflects this peak.

In experiments 2, 3, 4 and 5, the faecal egg counts increased after the infection (fig 2). Variables *PEAKEXP2*, *PEAKEXP3*, *PEAKEXP4* and *PEAKEXP5* reflect the high egg counts after the infection (from D27-D48 in experiment 2, D26-D54 in experiment 3, D26-D55 in experiment 4 and D28-D52 in experiment 5). The synthetic variable *PEAK235* is the mean of the 3 variables previously defined in expriment 2 and in its 2 replications, *ie* experiments 3 and 5 also involving 2 groups of animals (a group drenched before infection and a non-drenched group). The synthetic variable *PEAK235* does not include experiment 4 in which all animals were drenched prior to infection.

The haematological parameters are defined as means of given measures over certain periods. The choice of periods here is again based on a kinetic examination. The number of red blood cells, the packed cell volume and haemoglobin content decreased during the period corresponding to the primary egg count peak: from D23-D39 in group 1, D53-D67 in group 2 and D88-D102 in group 3 (figs 3a, b, c). Variables *RBCPRIM, PCVPRIM* and *HCPRIM*, respectively account for this decrease in the 3 previously cited parameters.

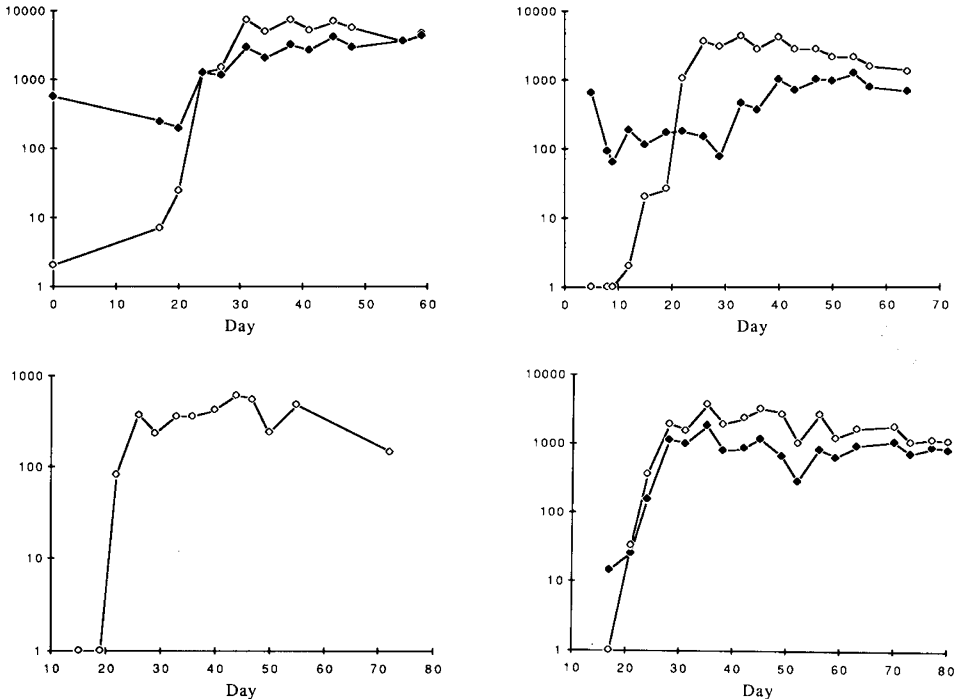**Fig 2.** Mean kinetics of faecal egg output in experiments 2 (fig 2a), 3 (fig 2b), 4 (fig 2c) and 5 (fig 2d). ─◇─ Group drenched prior to infection on day D0 (with 10 000 larvae); ─◆─ Group non-drenched prior to infection on day D0 (with 10 000 larvae).

Factors

The factors of variation considered are given in table II. Two of these factors (*HBALLELE*, the haemoglobin allele received from the sire and *OLALLELE*, the *OLA* haplotype received from the sire) are nested within sire. According to analyses, the response to immunization with HSA was considered as a variable or a factor.

Method of analysis

Analysis of the humoral immune response

Two methods were used for the statistical analysis of the humoral immune response, *ie* χ^2 test and analysis of variance.

Chi-square tests of independence were carried out between the *RESPOND* factor (accounting for the immunization "responder" or "non-responder" character) and various other factors of variation of table II (sire, haemoglobin genotype and *OLA* haplotypes).

Analysis of variance were performed on variable *ANTIHSA*, accounting for the immune response to aggregated human serum albumin (table III). The number of experimental animals was not large enough to make an analysis simultaneously
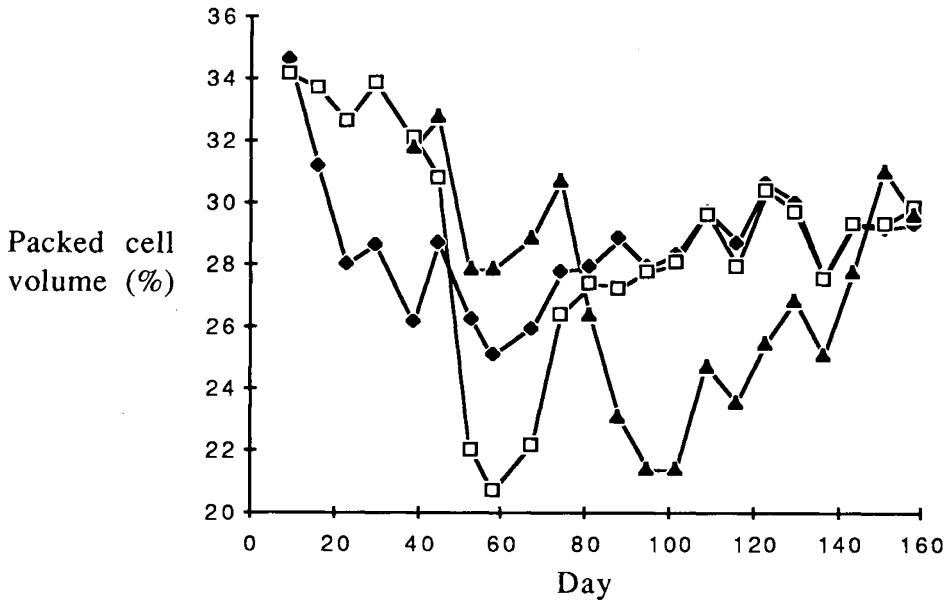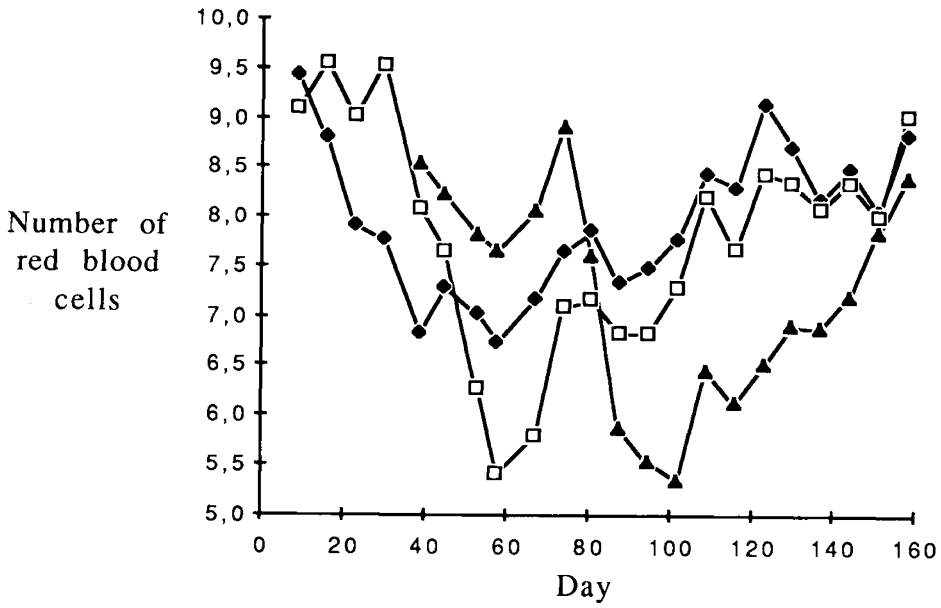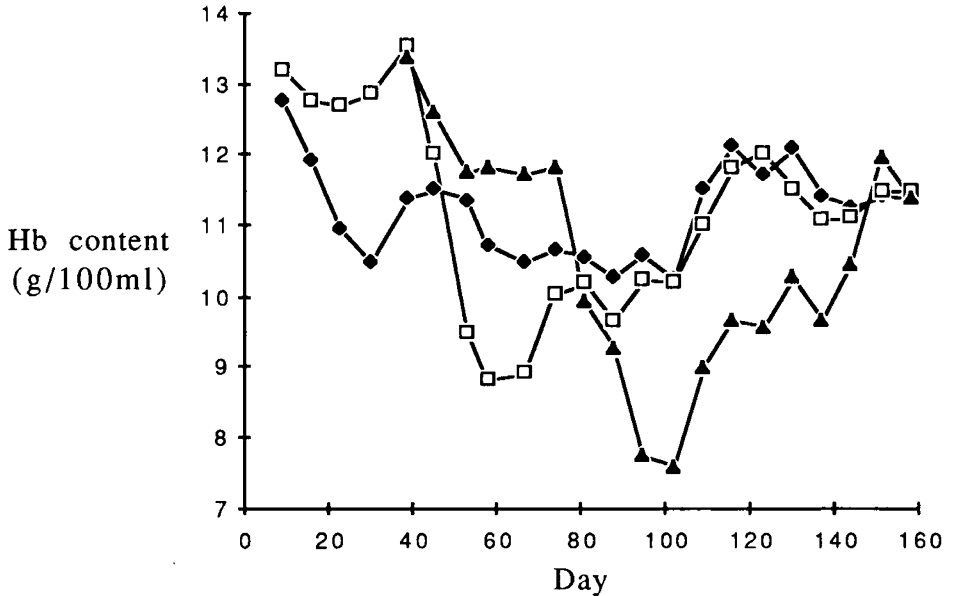
Fig 3 (continued)

Fig 3. Mean kinetics of the 3 haematological parameters measured in the groups of experiment 1, *ie* number of red blood cells per mm^3 (fig 3a), packed cell volume (fig 3b) and haemoglobin content (fig 3c).—◆—Group 1 (3 infections on d D0, D32 and D64 with 5000, 10 000 and 20 000 larvae, respectively); —□—Group 2 (2 infections on D32 and D64 with 10 000 and 20 000 larvae, respectively).—▲—Group 3 (1 infection on D64 with 20 000 larvae).

including all factors of variation; there would have been numerous either empty or low cells. Accordingly, several analysis of variance models were used each involving a small number of factors. This can also be applied to the analyses of variance of the parasitological and haematological variables, treated in the following paragraphs.

Analysis of the parasitological and haematological variables of experiment 1

Table IV shows the analysis of variance models applied to the parasitological and haematological variables of experiment 1. When the factors did not include *RE-SPOND* (immunization "responder" or "non-responder" trait) or *TITRE* (category of anti-HSA antibody titre), the *ANTIHSA* variable (reflecting the humoral response) was added in order to study its correlation with the parasitological and haematological variables. The same procedure was used for analysis of the variables of experiments 2, 3, 4 and 5.

Analysis of the parasitological variables of experiments 2, 3, 4 and 5

Table V gives the models of the analyses of variance performed on the parasitological variables of experiments 2, 3, 4 and 5. Analyses of variables of experiments 2, 3 and 5 included necessarily factor *GROUP235* corresponding to the group (a group drenched before infection, a non-drenched group). This was not the case for analyses

Table II. List of factors of variation. *OLA-P2** haplotype is an alternative definition of *OLA-P21*) haplotype (all animals possessing *OLA-P21* possess *OLA-P21** but the reverse is not the case). *HSA: aggregated human serum albumin.

Name of factor	Definition of factor
SIRE	Sire
HBGENO	Haemoglobin genotype
HBALLELE	Haemoglobin allele received from the sire (factor nested within sire)
OLA-P3	)
OLA-P3+13	)
OLA-P13	)
OLA-P14	)
OLA-P14+7	) Presence or absence of the 9 detected *OLA* haplotypes
OLA-P14+9	)
OLA-P21	)
OLA-P21*	)
OLA-P22	)
OLALLELE	*OLA* haplotype received from the sire (factor nested within sire)
TITRE	Category of anti-HSA antibody titre* (1/2 to 1/8, 1/16 to 1/64, 1/128 to 1/512 and 0)
RESPOND	HSA immunization "responder" or "non-responder" character
GROUP1	Group in experiment 1
GROUP235	Group in experiments 2, 3 and 5

Table III. Models of analysis of variance of the *ANTIHSA* reflecting the humoral immune response to injection of aggregated human serum albumin. *Models are crossed and additive unless a statement is made to the contrary. Abbreviations of factors are given in table II.

Model No	Analysed variable	Factors in the model*
1	ANTIHSA	SIRE
2	ANTIHSA	HBGENO
3-11	ANTIHSA	OLA-P3 to OLA-P22
12	ANTIHSA	SIRE, HBGENO
13	ANTIHSA	SIRE, HBALLELE (nested within SIRE)
14-22	ANTIHSA	SIRE, OLA-P3 to OLA-P22
23	ANTIHSA	SIRE, OLALLELE (nested within SIRE)

of the variable of experiment 4, since in this experiment all animals were given the anthelmintic treatment before infection.

Analysis of various parasitological variables considered as repeated measures of the same character

A new approach consists of considering that the parasitological variables *PRIM-PEAK, PEAKEXP2, PEAKEXP3, PEAKEXP4* and *PEAKEXP5* (referring to

Table IV. Models of analyses of variance of parasitological and haematological variables in experiment 1. *Models are crossed and additive unless a statement is made to the contrary. **Between brackets: the *ANTIHSA* variable included in the analysis for study of correlations with other variables. ***Factor nested within *SIRE*. Abbreviations of variables and factors are given in tables I and II, respectively.

Model No	Analysed variables	Factors in the model *
24	PRIMPEAK, SECPEAK, SELFCURE, RBCPRIM, PCVPRIM, HCPRIM	GROUP1, RESPOND
25	The same	GROUP1, TITRE
26	The same (+ ANTIHSA**)	GROUP1
27	The same (+ ANTIHSA**)	GROUP1, SIRE
28	The same (+ ANTIHSA**)	GROUP1, HBGENO
29	The same (+ ANTIHSA**)	GROUP1, SIRE, HBALLELE***
30-38	The same (+ ANTIHSA**)	GROUP1, OLA-P3 to OLA-P22
39	The same (+ ANTIHSA**)	GROUP1, SIRE, OLALLELE***

Table V. Models of analyses of variances of parasitological variables in experiments 2, 3, 4 and 5. *Models are crossed and additive unless a statement is made to the contrary. **Between brackets: variables included in the analysis for study of correlations with other variables. ***Factor nested within *SIRE*. Abbreviations of variables and factors are given in tables I and II, respectively.

Model No	Analysed variables	Factors in the model*
40	PEAKEXP2, PEAKEXP3, PEAKEXP5, PEAK235 (+PEAKEXP4)**	GROUP235, RESPOND
41	The same	GROUP235, TITRE
42	The same (+ANTIHSA)	GROUP235
43	The same (+ANTIHSA)	GROUP235, SIRE
44	The same (+ANTIHSA)	GROUP235, HBGENO
45	The same (+ANTIHSA)	GROUP235, SIRE, HBALLELE***
46-54	The same (+ANTIHSA)	GROUP235, OLA-P3 to OLA-P22
55	The same (+ANTIHSA)	GROUP235, SIRE, OLALLELE***
56	PEAKEXP4	RESPOND
57	PEAKEXP4	TITRE
58	PEAKEXP4 (+ANTIHSA)	SIRE
59	PEAKEXP4 (+ANTIHSA)	HBGENO
60	PEAKEXP4 (+ANTIHSA)	SIRE, HBALLELE***
61-69	PEAKEXP4 (+ANTIHSA)	OLA-P3 to OLA-P22
70	PEAKEXP5 (+ANTIHSA)	SIRE, OLALLELE***

experiments 1, 2, 3, 4 and 5, respectively) constitute repeated measures of the same parasitological overall variable *OVERALL*. Table VI gives models of analyses of variance on the *OVERALL* variable. Each model includes necessarily:

– the *EXPGROUP* factor corresponding to the experiment and group combination in which the *OVERALL* variable was measured;
– the *ANIMAL* factor corresponding to the experimental animal in which the measure was made.

Table VI. Models of analyses of variance of the overall parasitological *OVERALL* variable. *Models are crossed and additive unless a statement is made to the contrary. Abbreviations are given in text and table II.

Model No	Analysed variable	Factors in the model*
71	*OVERALL*	*EXPGROUP, ANIMAL*
72	*OVERALL*	*EXPGROUP, RESPOND, ANIMAL* (nested within *RESPOND*)
73	*OVERALL*	*EXPGROUP, TITRE, ANIMAL* (nested within *TITRE*)
74	*OVERALL*	*EXPGROUP, SIRE, ANIMAL* (nested within *SIRE*)
75	*OVERALL*	*EXPGROUP, HBGENO, ANIMAL* (nested within *HBGENO*)
76-84	*OVERALL*	*EXPGROUP, OLA-P3* to *OLA-P22, ANIMAL* (nested within *OLA-P3* to *OLA-P22*)

RESULTS

Analysis of the humoral immune response

Chi-square tests of independence between the "responder" character and various other factors (sire, haemoglobin genotype and *OLA* haplotypes)

No χ^2 was significant at the 0.05 level, with the exception of the test of independence between the "responder" character and the *OLA* $-P14+9$ haplotype ($\chi^2_{1df} = 5.953$, significant at the 0.02 level) : this χ^2 resulted from a preferential association between the "responder" character and the *OLA* $-P14+9$ haplotype.

Analyses of variance of the *ANTIHSA* variable reflecting the humoral immune response

Among the 23 analyses of variance described in table III, only analyses no 8 and 19 showed a significant effect at the 0.05 level; in both cases, it was the effect of the *OLA* $-P14+9$ haplotype whose presence in the studied sample was related to an increase in the level of anti-HSA antibodies.

Analysis of the parasitological and haematological variables of experiment 1

Table VII summarizes the results of the analyses of variance whose models are described in table IV. Table VIII gives the residual correlations calculated on the parasitological and haematological variables relative on the primary peak of experiment 1 and on the immunological *ANTIHSA* variable. Table IX gives the residual correlations calculated on all parasitological variables of experiment 1 (relative to the primary peak, secondary peak and to the self-cure) as well as on the immunological *ANTIHSA* variable.

Table VII. Statistically significant effects evidenced by analyses of variance of the variables in experiment 1. Lines : factors, Columns: variables. +, *, **; ***: significant effects at the 0.10, 0.05, 0.01 and 0.001 levels, respectively. The numbers correspond to the model Nos of the analyses of variance resulting in significant effects; these Nos refer to table IV. In each factor *x* variable cell, only the highest significant effects are indicated (among all those encountered). Abbreviations of variables and factors are given in tables I and II, respectively.

	PRIMPEAK	SECPEAK	SELFCURE	RBCPRIM	PCVPRIM	HCPRIM
GROUP1	*25	*30, 33	**24, 26 28, 32- 34, 36, 37	***24, 25, 27, 28, 39 -38	***24 25, 27, 28, 30 -38	***24, 25, 27, 28, 30 -38
RESPOND						
TITRE		*25	*25			
SIRE	*27, 39					+27
HBGENO						
HBALLELE					*29	
OLA-P3						*30
OLA-P3+13				+31	*31	**31
OLA-P13			**32			
OLA-P14		*33				
OLA-P14+7						
OLA-P14+9						
OLA-P21	*36				+36	*36
*OLA-P21**	*37			+37	*37	**37
OLA-P22						
OLALLELE						

Analysis of the parasitological variables of experiments 2, 3, 4 and 5

Table X summarizes the results of the analyses of variance whose models are described in table V. Table XI gives the residual correlations calculated on the parasitological variables of experiments 2, 3, 4 and 5 as well as on the immunological *ANTIHSA* variable.

Table VIII. Residual correlations calculated from the parasitological and haematological variables relative to the primary peak in experiment 1 as well as from the immunological *ANTIHSA* variable using model No 26 with the group *(GROUP1)* as the sole factor of variation. $^+$, *, **, ***: coefficient of correlation significantly different from zero at the levels 0.10, 0.05, 0.01 and 0.001, respectively. Abbreviations of variables are given in table I.

	PREMPEAK	RBCPRIM	PCVPRIM	HCPRIM
RBCPRIM	− 0.68***			
PCVPRIM	− 0.62***	0.88***		
HCPRIM	− 0.61***	0.86***	0.89***	
ANTIHSA	0.01	0.16	0.24$^+$	0.16

Table IX. Residual correlations calculated from the parasitological variables relative to the primary peak, secondary peak and self-cure in experiment 1 as well as from the immunological *ANTIHSA* variable using model No 26 with the group *(GROUP1)* as the sole factor of variation. ***: coefficient of correlation significantly different from zero at the level 0.001. Abbreviations of variables are given in table I.

	PRIMPEAK	SECPEAK	SELFCURE
SECPEAK	0.55***		
SELFCURE	− 0.22	− 0.71***	
ANTIHSA	0.05	0.19	− 0.17

Analysis of the various parasitological variables considered as repeated measures of the same character

Table XII summarizes the results of the analyses of variance whose models are described in table VI.

The repeatability of the character measured by the *OVERALL* variable was estimated in model No 71 (including the crossed *EXPGROUP* and *ANIMAL* factors) by the intra-class coefficient of correlation (ratio of "individual" variance to the sum of "individual" variance and residual variance): the estimated repeatability was 0.26.

Figure 4 illustrates the effect of the *TITRE* factor (anti-HSA antibody titre) on the overall parasitological *OVERALL* variable.

DISCUSSION

Phenotypic relationships between the various parasitological variables

A highly significant ($P < 0.001$) positive residual correlation was observed in experiment 1 between the variables reflecting the primary and secondary peak faecal egg counts. In contrast, these 2 variables were negatively correlated with

Table X. Statistically significant effects evidenced by analyses of variance of the parasitological variables in experiments 2, 3, 4 and 5. Lines: factors, Columns: variables. $^+$, *, **, ***: significant effects at the 0.10, 0.05, 0.01 and 0.001 levels, respectively. The numbers correspond to the model Nos of the analyses of variance resulting in significant effects; these Nos refer to table V. In each factor x variable cell, only the highest significant effects are indicated (among all those encountered). Abbreviations of variables and factors are given in tables I and II, respectively.

	PEAKEXP2	PEAKEXP3	PEAKEXP5	PEAK235	PEAKEXP4
GROUP235	*42, 46, 47, 49-51, 53	***42, 44, 46-53		**49	
RESPOND	$^+$40	$^+$40	$^+$40	$^+$40	*56
TITRE		$^+$41			
SIRE					
HBGENO					
HBALLELE	$^+$45				
OLA-P3					
OLA-P3+13	*47				
OLA-P13					
OLA-P14	**49	**49		**49	$^+$64
OLA-P14+7	$^+$50		*50	$^+$50	
OLA-P14+9		$^+$51			$^+$66
OLA-P21					
OLA-P21*					
OLA-P22					
OLALLELE					

Table XI. Residual correlations calculated from the parasitological variables of experiments 2, 3, 4 and 5 as well as from the immunological *ANTIHSA* variable in analysis of variance No 42 with the group *(GROUP235)* as the sole factor of variation. $^+$, *, **, ***: coefficient of correlation significantly different from zero at the levels 0.10, 0.05, 0.01 and 0.001, respectively. Abbreviations of variables are given in table I.

	PEAKEXP2	PEAKEXP3	PEAKEXP5	PEAK235	PEAKEXP4
PEAKEXP3	0.49***				
PEAKEXP5	0.34*	0.35*			
PEAK235	0.82***	0.70***	0.60***		
PEAKEXP4	0.46***	0.38**	0.14	0.46***	
ANTIHSA	0.26$^+$	0.36*	0.17	0.31*	0.26$^+$

the variable reflecting the self-cure, the correlation being only highly significant ($P < 0.001$) between the self-cure and the secondary peak: in other words, the animals with the lowest faecal egg counts were also those that best expelled their parasites.

Positive (generally significant) residual correlations were observed in the successive experiments 2, 3, 4 and 5 (table XI) between the variables accounting for
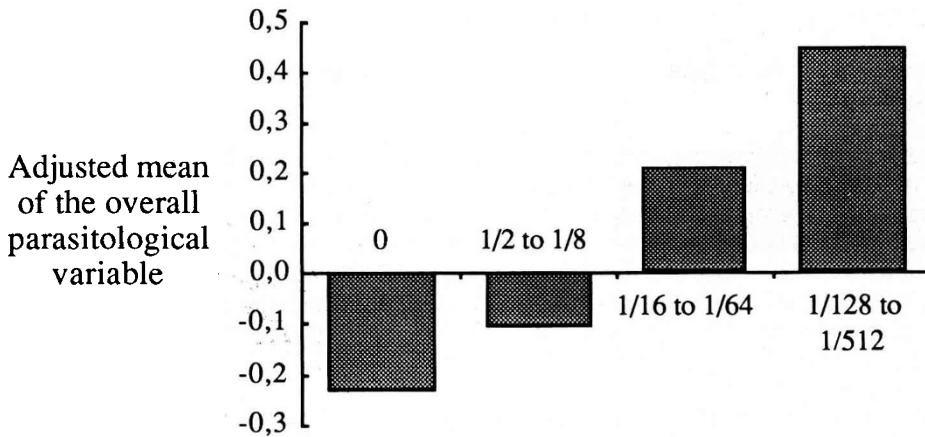
Fig 4. Adjusted means (expressed as a deviation from the general mean) of the overall parasitological *OVERALL* variable for the factor *TITRE* (anti-HSA antibody titre) in analysis of variance model No 73 including the factors *EXPGROUP* (experiment-group combination), *TITRE* and *ANIMAL* (experimental animal), nested in the previous one.

Table XII. Statistically significant effects evidenced by analyses of variance of the overall parasitological *OVERALL* variable. Lines: factors. Columns: variables. [+], [*], [**], [***]: significant effects at the 0.10, 0.05, 0.01 and 0.001 levels, respectively. The numbers correspond to the model Nos of the analyses of variance resulting in significant effects; these Nos refer to table VI. In each factor x variable cell, only the highest significant effects are indicated (among all those encountered). Abbreviation factors are given in table II.

	OVERALL
EXPGROUP	[***]71-84
ANIMAL	[***]71-84
RESPOND	[*]72
TITRE	[*]73
SIRE	[+]74
HBGENO	
OLA-P3	
OLA-P3+13	
OLA-P13	
OLA-P14	[**]79
OLA-P14+7	[+]80
OLA-P14+9	[+]81
OLA-P21	
*OLA-P21**	
OLA-P22	

the egg counts. The highly significant effect of the *ANIMAL* factor on the overall parasitological *OVERALL* variable (table XII) illustrates the repeatability of the mean egg output during the peaks of the 5 successive infection experiments.

Phenotypic relationships between faecal egg counts and degree of anaemia

There were highly significant ($P < 0.001$) correlations between the mean number of red blood cells per mm^3 of blood, the average packed cell volume and the mean level of haemoglobin during the primary peak of eggs passed in experiment 1 (table VIII). The *PRIMPEAK* variable, reflecting the peak faecal egg counts during primary infection was negatively correlated with the 3 haematological variables. This corresponds to the phenomenon of anaemia classically associated to large faecal egg counts (Whitlock, 1955, 1958; Evan *et al*, 1963; Pradhan and Johnstone, 1972; Altaif and Dargie, 1978a, b; Roberts and Swan, 1982; Albers *et al*, 1984).

Effect of primary dose of infective larvae of faecal egg counts and anaemia

The factor *GROUP1* (group in experiment 1) had a significant effect on all parasitological and haematological variables measured during the primary peak of experiment 1 (table VII): as expected, the larger the larval intake, the higher the faecal egg counts and the degree of anaemia (figures 1 and 3).

Effect of vaccination on immunity to the parasite

Considering the kinetics of faecal egg output in group 1 of experiment 1 (figure 1): the peak faecal egg counts after the lst infection (with 5000 larvae) was higher than the peak after the 2nd infection (with 10 000 larvae) which was higher than the peak after the 3rd infection (with 20 000 larvae). Likewise, in group 2 of experiment 1 (figure 1), the primary peak exceeded the secondary peak although the 2nd dose of infective larvae was 2-fold higher than the lst one (20 000 larvae instead of 10 000).

   With the same dose of infective larvae (10 000 larvae on D32 or 20 000 larvae on D64), animals which had previously experienced infection with *H contortus* reacted by eliminating fewer eggs than those infected with the parasite for the first time.

   These observations (based on figure 1 and confirmed statistically by analyses of variances not shown here) illustrate the phenomenon of immunity to the parasite (*ie* protection) acquired by "vaccination", *ie* by previous infection with the parasite (Clunies Ross, 1932; Luffau, 1975; Luffau *et al*, 1981a, b).

Effect of anthelmintic treatment on immunity to the parasite

In experiments 2 and 3, the group drenched before infection eliminated significantly more eggs than the non-drenched group (figure 2 and first line of table X); the anthelmintic treatment substantially reduced the immunity acquired previously by contact with the parasite. The phenomenon was more marked in experiment 3 which was a replication of experiment 2. In experiment 5, the same trend was observed (figure 2), but the difference between the 2 groups was not significant (table X). Thus, total elimination of residual worms by anthelmintic treatment prior to D0

of the previous experiment (experiment 4) seemed to have reduced the difference between the 2 groups.

All these results, which show the reduction of immunity to the parasite after anthelmintic treatment, confirm those obtained by Benitez-Usher *et al* (1977) according to whom application of such a treatment after vaccination with irradiated larvae lowered the immunity to the parasite.

Phenotypic relationships between resistance to parasitism and humoral immunity

Factors relating to the anti-HSA antibody titre (*RESPOND* and *TITRE*) had significant effects on various parasitological variables of experiments 1 (table VII), 2, 3, 4 and 5 (table X) as well as the overall parasitological *OVERALL* variable (table XII). The higher the production of anti-HSA antibodies the larger the faecal egg counts, as shown by adjusted means in figure 4. This positive relation between faecal egg count and humoral immunity is also illustrated by the positive coefficients of correlation between various parasitological variables reflecting the faecal egg output and the variable *ANTIHSA* reflecting the humoral immunity (tables IX and XI).

Contrary to the hypothesis put forward by Cuperlovic *et al* (1978), response to an immunization with aggregated human serum albumin is not a predictor of resistance to *H contortus*. This negative correlation between resistance to helminths and response to an immunization has already been observed in mice (Blum and Cioli, 1978; Deelder *et al*, 1978; Perrudet-Badoux *et al*, 1978; Wakelin, 1978). In sheep, Albers *et al* (1984) did not find any significant correlation between resistance to *H contortus* and response to an immunization with chicken red blood cells.

Sire effect on resistance to H contortus

Significant sire effects were evidenced on the *PRIMPEAK* variable accounting for the primary peak faecal egg counts in experiment 1 (table VII) and on the overall parasitological *OVERALL* variable pertaining to all experiments (table XII): these effects were significant at the 0.05 and 0.10 level, respectively. The number of experimental animals (51 offspring of 8 sires) was too small to make a heritability estimation. However, our results are in favour of a sire effect on resistance; they are in keeping with those of Le Jambre (1978), Albers *et al* (1984, 1987) and Piper (1987) who found a heritability ranging from 0.25-0.30 for resistance to *H contortus*.

Relationships between haemoglobin system and immunological, parasitological and haematological variables

Neither the *HBGENO* factor (haemoglobin genotype) or the *HBALLELE* factor (haemoglobin allele received from the sire) had any significant effect at the 0.05 level on the immunological or parasitological variables, although the experiment was designed to verify the existence of such effects.

These findings do not agree with those of other authors (Evans *et al*, 1963; Jilek and Bradley, 1969; Radhakrishnan *et al*, 1972; Allonby and Urquhart, 1976; Altaif

and Dargie, 1976, 1978a, b; Preston and Allonby, 1979; Dally *et al*, 1980; Luffau *et al*, 1981a, b; Courtney *et al*, 1985), but they are in keeping with the results of Le Jambre (1978), Riffkin and Dobson (1979), Courtney *et al* (1984), Riffkin and Yong (1984) and Albers and Burgess reported by Piper (1987), who did not find any relationship between resistance to *H contortus* and haemoglobin system.

Thus, although our data do not lead to detection of any relationship of humoral responsiveness (to human serum albumin) or of resistance to *H contortus* with the haemoglobin system, there is evidence of a statistically significant effect ($P < 0.05$) of the *HBALLELE* factor (haemoglobin allele received from the sire) on the mean packed cell volume (table VII). Hence a relationship between haemoglobin system and post-infection degree of anaemia cannot be excluded. According to the adjusted means, it seems that animals carrying the *HbA* allele were less anaemic than the others. These results are in agreement with those of Evans and Whitlock (1964), Radhakrishnan *et al* (1972), Altaif and Dargie (1976, 1978a, b) and Albers and Burgess reported by Piper (1987). The post-infection differences observed between animals of various haemoglobin genotypes might simply be due to differences existing in non-infected animals (Agar *et al*, 1972). These differences might arise from oxygen affinity differences between haemoglobins *A* and *B*. Haemoglobin *A* has a higher oxygen affinity: at equal pressure, it releases less oxygen, which might cause the creation of compensatory mechanisms in haemoglobin *A* carriers (Agar *et al*, 1972).

Relationships between the *OLA* system with immunological, parasitological and haematological variables

The results obtained show statistically significant effects of various *OLA* haplotypes on the humoral response as well as on the faecal egg counts and the degree of anaemia after parasite infections (tables VII, X and XII). Thus, we cannot exclude the existence, within or close to the *OLA* system, of genes affecting these various phenomena. These results disagree with those of Cooper *et al* reported by Piper (1987), who did not find any association between *OLA* system and resistance to *H contortus*. But they do agree with those of Outteridge *et al* (1984, 1985, 1986, 1987 and 1988) who found an association between the *OLA* system and the response to a vaccination against *Trichostrongylus colubriformis*. A relationship between the major histocompatibility complex and resistance to nematode parasites has also been demonstrated in the case of the guinea pig-*Trichostrongylus colubriformis* system (Geczy and Rothwell, 1981) and the mouse-*Trichostrongylus spiralis* system (Wassom *et al*, 1979).

CONCLUSION

In terms of parasitology, the results obtained lead to a more accurate determination of a certain number of phenomena such as repeatability of faecal egg counts between infections, negative relationship between faecal egg count peaks and self-cure intensity, positive relationship between faecal egg counts and degree of anaemia, acquisition of immunity by previous contact with the parasite and reduction of this immunity by anthelmintic treatment.

In terms of genetics, the results invalidate the hypothesis that homozygous sheep carriers of haemoglobin *A* have lower faecal egg counts than the others as well as the hypothesis that animals with the best humoral immune response are the most resistant to parasitism. On the other hand, they do not exclude the hypothesis that genes within or close to the *OLA* system might affect the resistance to *H contortus*. The latter conclusion, in keeping with those of other studies, emphasizes the role of the *OLA* system as a potential marker of resistance to parasitism.

ACKNOWLEDGMENTS

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