Haemoglobin type and prolificacy in Booroola sheep

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ABSTRACT

Haemoglobin type has been correlated with reproductive traits in several sheep breeds. This study measured haemoglobin type and ovulation rate in over 1300 Booroola Merino cross sheep and 1000 controls of 4 breeds.

Haemoglobin type was measured over several years by 3 different methods; cellulose acetate electrophoresis, starch gel electrophoresis and isoelectric focusing. Though resolution differed between methods, the blood types scored were the same.

In Booroola Merino x Romney ewes there was significant association between the HbB allele and the F gene carriers, as well as between the HbA allele and non-carriers. The Booroola Merino ewes showed the same trend though it was non-significant. In non-Booroola flocks of Romneys, Perendales, Coopworths and Merinos, there was no relation between ovulation rate and haemoglobin type. While the HbB allele was associated with higher ovulation rate in Booroola Merino crosses, it was not an absolute marker for the F gene.

Isoelectric focusing improves haemoglobin resolution and has identified a third, more cathodal allele, at low frequency. Independent tests have confirmed that this is HbC, associated with anaemia in sheep.

Keywords Haemoglobin type; Booroola; ovulation rate; cellulose acetate electrophoresis; starch gel electrophoresis; isoelectric focusing

INTRODUCTION

Biochemical studies over more than 2 decades have found significant associations between sheep haemoglobin (Hb) type and reproductive performance. Two common autosomal alleles are reported in adult sheep haemoglobin, HbA and HbB, as well as rare alleles such as HbC (Tucker, 1971; John and John, 1977). The relationship between a particular allele and prolificacy has not however been consistent across breeds. For example, the HbA allele has been associated with higher lambing percentages in the Scottish Blackface (King *et al.*, 1958), the German Blackheaded Mutton (Meyer *et al.*, 1967) and the Finnish Landrace (Atroshi, 1980) whereas the HbB allele was implicated for the Booroola Merino (Evans and Turner, 1965), the Indian Bikarei (Seth, 1968) and the Florida Native (Olson and Loggins, 1979).

In Booroola sheep, evidence has accumulated over the last 5 years that higher litter sizes are a single gene effect (Piper and Bindon, 1982; Davis *et al.*, 1982; Meyer and Davis, 1983; summarised by Piper *et al.*, 1985). Booroolas are now classified according to their ovulation rate as non-carriers (++), heterozygous carriers (F+) or homozygous carriers (FF).

The minimum of 2 years taken at present from the initial joining to identify an F gene-carrying ram by laparoscopy of his female progeny is valuable time lost in a breeding program. Because early identification of F gene carriers is of commercial importance to the sheep industry, an extensive survey has been undertaken for an F gene marker in the blood of Booroolas. This paper reports the results for haemoglobin.

MATERIALS AND METHODS

In 1985, about 500 Booroola Merino x Romney and Booroola Merino x Merino sheep were tested for haemoglobin type and fecundity genotype. Previously (1978) 800 Booroola Merino x Romney and Booroola Merino x Merinos were tested, as were 1000 New Zealand Merinos, Romneys, Coopworths and Perendales for comparative purposes. Blood samples were collected in 10ml vacuum tubes containing either EDTA or sodium heparin as an anti-coagulant.

Blood type was determined by 3 electrophoretic methods depending on the year of testing and the resolution required. In 1978 electrophoresis was carried out on cellulose acetate strips using a tris-glycine buffer (pH 8.6). The 1985 samples were typed using starch gels and tris-borate buffer (pH 8.5). Some of the 1985 samples were also subjected to thin-layer agarose gel isoelectric focusing (pH range 5 to 8) which confirmed the presence of a third haemoglobin allele. Though the isoelectric focusing technique improved resolution (Sheggeby *et al.*, 1983), all 3 methods yielded the same blood types.

Ovulation rates were determined by laparoscopy of ewes during 2 or more oestrous cycles. Animals carrying none, 1 or 2 copies of the Booroola F gene were classified as ++, F+ or FF respectively (Davis *et al.*, 1982).

Contingency tables classified by fecundity genotype and haemoglobin type were tested for independence using Pearson chi-square contingency tables and log-likelihood ratio tests, the latter preferably in cases where the numbers observed were small in some cells.

(Fienberg, 1980). Where the dependency between blood type and fecundity genotype was highly significant ($P<0.01$), it was further explored using the diagonal effect in a generalised linear model (McCullagh and Nelder, 1983).

RESULTS AND DISCUSSION

Electrophoretic results showed 5 haemoglobin phenotypes in the sheep tested (Fig. 1). The HbC allele occurred in only 15 animals and results from conversion of HbA to HbC in anaemic sheep (Tucker, 1971). Sheep with an AC genotype were therefore reclassified as AA, and BC animals as AB for data analysis.

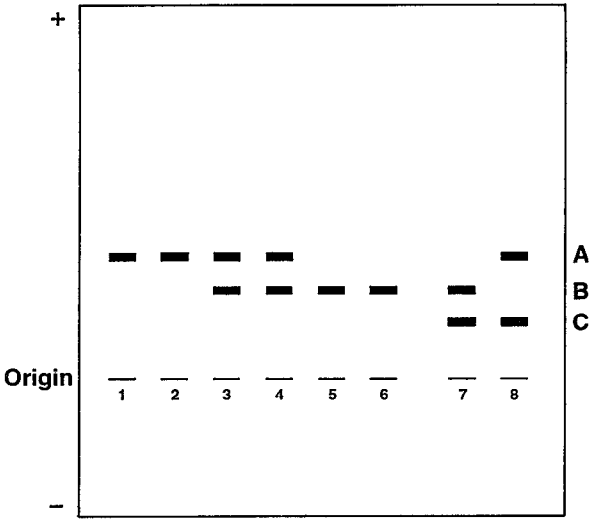

FIG. 1 Haemoglobin phenotypes resulting from starch gel electrophoresis (pH 8.5). Samples scored from left to right; AA, AA, AB, AB, BB, BB, BC and AC.

To overcome haemoglobin gene frequency differences between breeds, all 1985 Booroola Merino crosses were pooled in the initial analysis. There were large differences between observed and expected values in several cells (Table 1) and the difference between blood types was highly significant ($P<0.01$).

When the data were broken down by breed, it became clear that the significant differences came from the Booroola Merino x Romneys (Table 1). There are more AA/++, AB/F+, BB/FF and BB/F+ animals than expected by chance, fewer AA/F+, AB/++ and BB/++ animals. The association between the HbB allelle and the F gene resulted in the highly significant difference of observed and expected values.

In the 1985 Booroola Merino x Merinos (Table 1), though there were more AA/++ animals than expected, there was no significant dependence between blood type and F gene classification.

Blood typing work done early in the Booroola breeding program was subjected to the same analysis. There were no homozygous carriers (FF) among the ewes tested in 1978, as these were the progeny of carrier rams over non-Booroola ewes. Romney and Merino controls were included in the 1978 test.

The 1978 results (Table 2) show the same pattern. There were more AA/++ and AB/F+ animals than expected, and many fewer AA/F+ and AB/++ ewes. Again the differences were significant in the Booroola Merino x Romneys and not in the Booroola Merino x Merinos.

The rare cathodal allele was confirmed to be HbC in blood samples sent to Britain for independent analysis (E.M. Tucker, personal communication). HbC, described first by van Vliet and Huisman (1964), results from the conversion of a single HbA allele to HbC in anaemic sheep. It is therefore found only in the heterozygous form, either as phenotypes AC or CB, from AA and AB animals respectively. Sheep which are homozygous for the HbB allele cannot produce HbC.

The relationship between fecundity genotype and blood type showed a consistent pattern both across breeds and across years. The marked diagonal effect in the data, with more AA/++, AB/F+ and BB/++ animals than expected and fewer AA/FF, AB/++ and BB/++, was tested using a generalised linear model and assuming a relationship between diagonal cells as well as rows and columns. Assuming a relationship between diagonal cells at 3 levels (Table 3), the expected values closely match those observed (Table 4). It should be noted that it is a diagonal effect that we would expect to find in a marker for the action of a single gene (Dratch, 1986).

Lastly, the dependence between blood type and fecundity genotype was significant in the Booroola Merino x Romneys in both years, though not in the Booroola Merino x Merinos. One explanation for this is the background gene frequencies of HbA and HbB in the 2 breeds (Table 5). Because HbB is at a higher frequency in New Zealand Merinos, the relationship between that allele and the F gene is obscured. The table also shows that HbB is at a still higher frequency in Coopworths and Perendales, which does not bode well for using haemoglobin as a general F gene marker.

TABLE 1 Contingency tables relating haemoglobin genotypes (AA, AB, BB) to fecundity genotypes (++, F+, FF) within groups of ewes in 1985. Observed numbers shown **80**; expected numbers shown 80.0.

	AA	AB	BB
Booroola Romney and Booroola Merino			
++	**80**	**64**	**21**
	60.0	82.0	22.8
F+	**58**	**90**	**23**
	62.4	85.0	23.6
FF	**42**	**91**	**24**
	57.3	78.0	21.7
Probability[a]		<0.01	
Booroola Merino x Romney			
++	**41**	**27**	**16**
	26.4	32.1	25.5
F+	**14**	**38**	**33**
	26.7	32.5	25.8
Probability[b]		<0.001	
Booroola Merino x Merino			
++	**39**	**31**	**26**
	33.2	31.4	31.4
F+	**50**	**52**	**58**
	55.4	52.3	52.3
FF	**20**	**20**	**19**
	20.4	19.3	19.3
Probability[a]		>0.1	

[a] from chi-square
[b] log-likelihood ratio = 25.1

TABLE 2 Contingency tables relating haemoglobin genotypes (AA, AB, BB) to fecundity genotypes (++, F+) within groups of ewes in 1978. Observed numbers shown **80**; expected numbers shown 80.0.

	AA	AB	BB
Booroola Merino x Romney			
Romney	**171**	**147**	**41**
controls	168.9	152.2	37.8
++	**85**	**53**	**17**
	72.9	65.7	16.3
F+	**88**	**110**	**19**
	102.1	92.0	22.9
Probability[a]		<0.05	
Booroola Merino x Merino			
Merino	**57**	**81**	**45**
controls	50.7	85.8	46.5
++	**54**	**79**	**52**
	51.2	86.7	47.0
F+	**71**	**148**	**52**
	80.1	135.5	73.5
Probability[a]		>0.1	

[a] from chi-square

TABLE 3 Three level diagonal effect in the generalised linear model.

	AA	AB	BB
++	1	2	3
F+	2	1	2
FF	3	2	1

TABLE 4 1985 Booroola Romney contingency table using diagonal effect in a generalised linear model. Observed numbers shown **80**; expected numbers shown 80.0. (Log-likelihood ratio = 1.1, $P>0.1$)

	AA	AB	BB
++	**41**	**27**	**16**
	39.8	27.8	16.4
F+	**14**	**38**	**33**
	15.6	38.0	31.4
FF	**1**	**3**	**5**
	0.6	2.2	6.2

TABLE 5 Gene frequencies for the HbA and HbB alleles in the breeds currently crossbred with Booroolas.

	n	A	B
Romneys	359	0.65	0.35
Merinos	288	0.53	0.47
Coopworths	180	0.39	0.61
Perendales	195	0.40	0.60

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