DEER BLOODTYPING : DEVELOPMENTS AND APPLICATIONS

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ABSTRACT

In three years of bloodtyping deer in New Zealand, protein markers have been developed which differentiate red deer from wapiti, sika and Pere David's deer. An enzymatic marker has also been found which discriminates the two fallow deer species, European and Mesopotamian, and there is the potential to also differentiate the different sub-species of rusa deer by similar methods.

While the European fallow deer is very low in electrophoretic variation, the European red deer is highly polymorphic. This protein variation in red deer means that electrophoresis can often be used to resolve questions when calf maternity or paternity are uncertain.

INTRODUCTION

During three years of bloodtyping deer at Invermay we have made some significant strides toward differentiating individual animals and species. The purpose of this paper is to describe the variation in deer which has been found and to discuss how that polymorphism can be used by veterinarians to help with particular problems of deer breeding.

Electroporesis is the method used in deer bloodtyping and there have been at least 90 published reports of electrophoretic studies on deer in the last 25 years. Although many of these studies have been surveys of wild populations, they are still pertinent because the same electrophoretic variants can often be found in red or white cells from blood samples of farmed deer.

The emphasis of this paper is on results rather than methods which are detailed elsewhere (Manlove et al, 1975; Gyllensten et al, 1983; Dratch and Gyllensten, 1985). It is important, however, for veterinarians to know the methods used in taking, processing and storing blood samples in order to achieve the best electrophoretic results.

METHODS

Deer blood samples are best taken in heparinised vacutainer tubes, cooled but never frozen, and processed at the laboratory within 48 hours of sampling. Samples are washed in saline, centrifuged and divided into plasma, white blood cell and red blood cell fractions. Processing blood samples is time consuming, and it is far more efficient to process several samples at one time than one or two. After processing, blood fractions are frozen until electrophoresis is carried out.

Different fractions are applied to electrophoretic gels of starch, agarose or polyacrylamide depending on the analysis required. After electrophoresis, gels are stained for specific enzymes or general proteins, the latter using Coomassic blue or silver nitrate depending on the resolution required.

The staining is scored independently by two scientists in the laboratory. When resolution is unclear the samples are run again. From the time blood samples are received we seek to have the bloodtyping completed within two weeks. All blood samples analysed are stored at -70°C for future comparative reference.

RESULTS

In discriminating red deer and wapiti, four proteins have different predominant alleles (Table 1). The differences are absolute for haemoglobin and haptoglobin; all red deer show one electrophoretic pattern all imported wapiti show a second pattern and all F_1 hybrids show a third

combination pattern. For transferrin and superoxide dismutase, the differences are not absolute but the allele frequencies differ markedly between red deer and wapiti. All four protein markers are used in testing for hybridisation among Fiordland wapiti or other animals of unknown lineage.

Table 1: Predominant phenotypes of four protein markers for New Zealand red deer, wapiti imported from Canada within the last six years and F_1 hybrids from imported wapiti bulls over red deer hinds.

	Red deer (n= 296)	Canadian wapiti (n= 98)	F_1 Red X Wapiti (n= 50)
Haemoglobin	AA	BB	AB
Haptoglobin	22	11	12
Superoxide dismutase	SS	FF	SF
Transferrin	AA,BB	BB	AB,AA

Two of the same proteins, haptoglobin and superoxide dismutase also discriminate red and sika deer. Transferrin and albumin are absolute markers differentiating red and Pere David's deer. These markers were used to confirm hybridisation of the Red X Pere David's calves produced in New Zealand during the past year (Table 2).

Table 2: Predominant phenotypes for two protein markers differentiating red deer, Pere David's deer and F_1 hybrid calves born in 1986.

	Red deer (n= 296)	Pere David's deer (n= 27)	F_1 Red X Pere David's (n= 5)
Albumin	FF	SS	SF
Transferrin	AA,AB	PP	AP,BP

In fallow deer, different alleles of the enzyme glucose phosphate isomerase discriminate the European and Mesopotamian subspecies. This marker too has been used to verify hybridisation as the recently produced F_1 fawns show a combination heterozygous pattern. We are in the final stages of developing similar blood markers for distinguishing Javan and Molluccan rusa.

In addition to the markers which discriminate deer species and detect hybrids, there is considerable protein polymorphism within red deer. These variants include the serum proteins transferrin and post-albumin, haemoglobin from red blood cells and the following enzymatic proteins from either red or white blood cells: acid phosphatase, glucose phosphate isomerase, isocitrate dehydrogenase, malate dehydrogenase, malic enzyme, mannose phosphate isomerase, phosphoglucomutase and superoxide dismutase.

DISCUSSION

Bloodtyping of deer has a great deal of similarity to that done on race-horses, cattle and other domestic animals. Not only does it employ many of the same electrophoretic techniques, but as with other bloodtyping, it requires a laboratory with trained staff, expensive equipment and bio-chemicals, and particularly the means of storing samples at low temperature (-70°C) for later comparison.

The abundant variation found in red deer at Invermay and at laboratories in Europe (Gyllensten et al, 1983) make it increasingly possible to use bloodtyping in cases where either the paternity or maternity of red deer calves is in doubt. This biochemical tool could prove very useful in group breeding schemes or other plans for intensive selection where it is critical to know the genetic potential of progeny with certainty. Due to much lower levels of electrophoretic variation in European fallow deer, there is little potential for parentage verification in fallow by the present methods.

In the last year we have used the electrophoretic markers previously described to assist farmers and veterinarians in determining whether

particular animals are hybrids. While we have identified several hybrids by electroporesis, purity is much more difficult to determine absolutely. Because bloodtypes are inherited as discrete units at each protein locus, evidence of hybridisation can be lost over many generations.

Much more important than testing for purity in the long term of the industry, is the possibility that such genetic markers as developed at Invermay could be used as predictors of performance. This is where deer bloodtyping differs from that in racehorses where it is used solely to verify parentage. The day may not be too distant when a calf bloodtyped at birth may be analysed for its potential growth and its genetic potential.

As with all bloodtyping in farmed animals, veterinarians are the vital link between the genetic laboratory and the animal owner. While it is not necessary to understand all of the laboratory procedures, it is important the veterinarians fully understand the results, and that they know both the capabilities and limitations of the blood tests performed at Invermay.

REFERENCES

Dratch, P A (1987). A marker for red deer-wapiti hybrids. Proceedings of the New Zealand Society of Animal Production 46:

Dratch, P and Gyllensten, V (1985). Genetic differentiation of red deer and North American elk (wapiti). In: The Biology of Deer Production. The Royal Society of New Zealand 22:37-40.

Gyllensten, V; Reutenwell, C; Ryman, N and Dratch, P (1983). Genetic differentiation in four European subspecies of red deer (Cervus elaphus L.) Heredity 51:561-80.

Manlove, M N; Price, J C; Hillstead, H O; Ramsey, S E; Smith, M H and Straney, D O (1975). Starch gel electrophoris for the study of population structure in deer. Proceedings of the 29th Annual Conference of the Southeast Game and Fish Commission 29:392-403.