# Genetic markers in the blood of animals: a historical review

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**ABSTRACT**

In this review an attempt is made to list the most important events in the search for genetic markers in the blood of animals. In chronological order, blood groups, biochemical polymorphisms, lymphocyte antigens and DNA markers have been discovered and used in practice. Of all practical uses, parentage verification and exclusion are regarded as the most important, and it can be said with pride that the South African Stud Book is as infallible as any other stud book in the world.

**Key words**: blood groups, biochemical polymorphisms, DNA markers, lymphocyte antigens; major histocompatibility complex.

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## INTRODUCTION

The study of genetic markers in the blood of animals was initiated a century ago when Landsteiner in 1900, observed individual differences in the blood of goats[3]. Without going into too much technical detail, this review will demonstrate what has been achieved, particularly in the last few decades, with regard to genetic markers.

Of all the practical applications, including the diagnosis of twins, early diagnosis of freemartins, studies of population structure, changes in degree of inbreeding, studies of sexual behaviour in herds and related problems, parentage and fatherhood determinations have attracted the greatest interest from farmers and breeders associations. The practical application of these tests was rapidly appreciated, and fully employed by breeders associations. In this way their members are assured that the South African Stud Book is as infallible as possible.

The basic principle of parentage determination relies on Mendel's laws: the mendelian law of dominance excludes progeny not in possession of at least 1 genetic marker in common with one of the parents. The mendelian law of segregation excludes a parent that does not possess a genetic marker that should have been inherited by the progeny.

The study of genetic markers, especially those of red blood cell antigens, progressed rapidly after the second world war. Stormont made a phenomenal contribution in the 1950s by grouping cattle blood into genetic systems, and especially by arranging more than 600 alleles in the B system[9].

The development in other species was also exceptionally rapid, and in 1996 there were no fewer than 102 laboratories worldwide where researchers were busy identifying genetic markers and using them for livestock improvement. Progress was particularly rapid in the USA, where the development of new techniques led to the discovery of large series of immunogenetic differences, and there are currently 11 laboratories, most of which can be considered leaders in the field.

## THE STATUS OF BLOOD GROUPS AS GENETIC MARKERS IN ANIMALS

Red blood cell antigens that are determined by agglutination or haemolysis were the 1st genetic markers to be fully researched. The situation in cattle, sheep, pigs, horses and dogs is shown in Table 1.

The search for new red blood cell antigens and alleles that reached its peak in the 1960s, came to an end for all practical purposes in the 1980s, when the emphasis shifted to other genetic markers.

## BIOCHEMICAL POLYMORPHISMS

The determination of biochemical polymorphisms by electrophoresis led to the discovery of a wide variety of genetic markers. Various media such as starch, agarose or acrylamide gel are used in electrophoresis to visualise genetic differences in the proteins and enzymes of animals. A large number of systems with applicable alleles have been found in all species. For example, the current situation in the research of biochemical polymorphisms in horses, as carried out at the laboratories at Onderstepoort, is shown in Table 2.

Blood group tests and biochemical markers are still internationally accepted as standards for parentage control, but DNA test panels are becoming available worldwide.

## MAJOR HISTOCOMPATIBILITY COMPLEX (MHC)

It is well-known that transplanted organs and tissues are in most cases rejected by the recipient. However, if the donor is related to the recipient, the rejection reaction is significantly decreased, which indicates a genetic basis for the reaction.

The naturally-occurring cell-surface antigens (lymphocyte antigens LA) that

Table 1: **The status of blood groups in production and companion animals.**

| Species[a] | Number of systems | Number of red blood cell antigens | Number of alleles |
|---|---|---|---|
| Cattle | 11 | 121 | 709 |
| Sheep | 7 | 22 | 69 |
| Pigs | 15 | 76 | 76 |
| Horses | 7 | 26 | 36 |
| Dogs | 11 | 12 | 24 |

[a]The inheritance of blood groups in goats[8] and cats[1] has not yet been fully elucidated, so these 2 species are not included in the table.

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are responsible for this, constitute the major histocompatibility complex (MHC). A good description of the MHC systems is given by Newman and Antczek[4]. Table 3 explains the most important MHC systems[6].

In mice, most of the research has been completed and more than 30 genetic loci have been identified[6]. In the 1980s, studies on lymphocyte antigens advanced rapidly. Research directed towards a possible connection between MHC and disease resistance is conducted particularly in cattle (BoLA) but also in pigs (SLA) and horses (ELA).

## DNA MARKERS

The breakthrough in the study of deoxyribose nucleic acid (DNA) occurred in the 1990s, and DNA markers will in due course replace red cell antigens, biochemical polymorphisms and the major histocompatibility complex.

The great advantage of DNA determinations is that genetic material is investigated directly, and any source of DNA, *e.g.* blood and hair roots, is suitable. The fact that DNA testing does not depend upon fresh blood samples has permitted it to be performed in numerous laboratories.

Comparisons are made between multiple locus fingerprints (= mini-satellites), restriction fragment length polymorphism (RFLP), diallelic systems or single nucleotide polymorphism (SNP), and microsatellites, also known as short tandem repeats (STR)[2].

At present the microsatellite technique appears to be most popular in the search for genetic differences. Genetic variation in microsatellites is determined by using the polymerase chain reaction (PCR) with electrophoresis. The DNA primer that surrounds the microsatellite indicates the direction of the composition of a specific chromosome fragment. Starting with a small amount, as little as a single gene copy, the polymerase chain reaction can duplicate the DNA sequence a millionfold in a few hours. The tandem repeats are visualised by analysis of the fragment size after electrophoresis of the PCR products.

During the XXV International Conference on Animal Genetics in Tours, France (1996), a decision was taken to standardise the nomenclature of DNA microsatellites internationally. The letters A to Z will be used from smaller to larger alleles, with M denoting the middle allele.

The development of research on genetic markers in animal blood is shown in Table 4.

Large shifts from studies on blood groups and biochemical polymorphisms to molecular genetic studies (DNA) over a period of 30 years have obviously occurred. This trend was noted during the XXV International Conference on Animal Genetics in 1996 (Table 5).

A further shift took place, from investigations of polymorphisms that included blood groups and DNA markers, to gene mapping and associations between genetic markers and qualitative and quantitative traits in animals.

## APPLICABILITY OF GENETIC MARKERS

The applicability of genetic markers was also appreciated in South Africa, and paternity determinations have been carried out in cattle since 1957.

In the 1960s, blood factors were used

Table 2: **Biochemical polymorphisms in horses (electrophoretic systems)**[a].

| System | Locus symbol | Recognised alleles |
|---|---|---|
| Albumin | ALB | A B 1 |
| Acid phosphatase | AP | F S |
| Carbonic anhydrase | CA | E F I L O S |
| Catalase | Cat | F S |
| NADH-diaphorase | Dia | F S |
| Esterase | Es | F G H I L M O R S |
| Peptidase | Pep A | F S |
| Vitamin A binding protein | Gc | F S |
| Haemoglobin | Hb | A AII BI BII N V |
| 6-phosphogluconate dehydrogenase | PGD | D F S |
| Phosphoglucomutase | PGM | F S V |
| Phosphohexose-isomerase | PHI | F I S |
| Protease inhibitor | Pi | F G H I J K $L_1$ $L_2$ N O P Q R S T U V W Z |
| Transferrin | Tf | D $D_2$ E $F_1$ $F_2$ $F_3$ G $H_1$ $H_2$ J M O R |
| A1B glycoprotein | A1B | F K S |
| Glucophospho-isomerase | GPI | F I L S |
| Plasminogen | PLG | 1 2 |

[a]Bowling[2] and E van Dyk (Blood Group Laboratory, Faculty of Veterinary Science, University of Pretoria, 1997, pers. comm.).

Table 3: **Nomenclature of the major histocompatibility complex in various species**[6].

| Species | Name | Symbol |
|---|---|---|
| Mouse | H-2 | H-2 |
| Rat | RT1 | RT1 |
| Dog | Dog lymphocyte antigen | DLA |
| Pig | Pig lymphocyte antigen | SLA |
| Goat | Goat lymphocyte antigen | GLA |
| Sheep | Sheep lymphocyte antigen | OLA |
| Ox | Bovine lymphocyte antigen | BoLA |
| Horse | Horse lymphocyte antigen | ELA |
| Rhesus monkey | Rhesus lymphocyte antigen | RhLA |
| Human | Human lymphocyte antigen | HLA |

Table 4: **Research contributions during various conferences** (%)[a].

| Subjects | 1964 | 1974 | 1984 | 1994 |
|---|---|---|---|---|
| Blood groups | 58 | 42 | 18 | 5 |
| Biochemical polymorphisms | 34 | 50 | 48 | 18 |
| Major histocompatibility complex | 8 | 8 | 33 | 17 |
| DNA and gene mapping | 0 | 0 | 1 | 60 |

[a]Proceedings of the XXIV International Conference on Animal Genetics, Prague, Czech Republic, 1994.

Table 5: **Research contributions during the XXV International Conference on Animal Genetics in Tours, France, 1996.**

| Subject | Number of contributions | Percentage |
|---|---|---|
| Polymorphisms and biodiversity | 105 | 26 |
| Major histocompatibility complex | 47 | 12 |
| Gene mapping | 118 | 30 |
| Molecular genetics | 51 | 13 |
| Association between markers and traits | 76 | 19 |

exclusively in South Africa, and 92.5 % of all parentage cases could be solved[7]. (Factors common to parents and progeny exist in some cases that do not permit resolution.) In later years, protein and enzyme markers were increasingly used, and the percentage of resolved cases rose to 98 %. Current work in South Africa is directed at including DNA markers to achieve close to 100 % resolution.

Embryo transplants in cattle have made parentage determination essential and increased the applied value of genetic markers in cattle. The number of parentage errors is at present 3 % (S J du Plessis, Blood Group Laboratory (cattle), ARC – Animal Improvement Institute, Irene, 1996, pers. comm.).

The importance of genetic markers in sheep has become evident with *in vitro* fertilisation and cloning. Population studies are also of importance, as demonstrated by a single comparative study on karakul sheep[5]. Performance and progeny schemes are particularly important in pigs, but are costly. The identification of pigs that can participate in these schemes is essential.

In horses, the horse 'passport', which includes all genetic markers, has become more important because since 1997. South African horses can once again compete on international race courses. During 1996/97, 99.5 % of all parentage cases were resolved. As in the case of cattle, parentage errors were detected in 3 % of cases.

Identification of breeds and monitoring of parentage is still expanding in dogs, as the movement of dogs across international boundaries becomes more important.

## GENETIC MARKERS IN CURRENT AND FUTURE RESEARCH

As indicated in Table 5, gene mapping is already in full swing. During the 1996 International Conference on Animal Genetics in Tours, France, no fewer than 118 papers on gene mapping in all the most important livestock species were presented. This research has no direct benefit for animal breeders, but will be of use in correlating genes and disease resistance in animals. The information will lead to better understanding of genetic trends, *e.g.* the BLUP analysis (best linear unbiased prediction).

MAS (marker-assisted selection) is a new departure in the use of genetic markers.

Since the number of known markers in various species has increased, tracing of loci of markers and quantitative trait loci (QTL) followed. The proliferation of research worldwide is reflected in 76 contributions that attempted to clarify the association between markers and mainly quantitative traits (Table 5).

Complete gene mapping is the cornerstone for the search for quantitative trait loci (QTL) that are applied in the marker-associated selection (MAS) of animals. Great breakthroughs are expected to result from molecular genetic methods and their application in breeding.

The complexity of the searches for correlation and associations between markers and qualitative and quantitative traits increases with the number of markers found. The following heritable diseases can be diagnosed by molecular genetic methods: cardiomyopathy (CMP), spinal muscular atrophy (SMA), uridine monophosphate synthesis deficiency (DUMPS), bovine progressive degenerative myeloencephalopathy (BPDME) and bovine lymphocyte antigen deficiency (BLAD). The potential for developing the highly topical 'mad cow disease' (bovine spongiform encephalopathy, BSE) is also diagnosed by molecular methods[10]. Molecular genetic techniques are also applicable to infectious diseases such as bovine leukosis, bovine rhinotracheitis, brucellosis, foot-and-mouth disease, and others.

However, the great challenge lies in the realm of MAS. If markers are found that can be linked to disease or that have a direct connection with fertility, growth or production characteristics, it will all have been worthwhile.

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