Research on animal blood groups and biochemical polymorphisms at Onderstepoort (1956–1990)

D R Osterhoff[a]

ABSTRACT

The introduction and wide use of artificial insemination in cattle in the 1950s led to a need for accurate parentage identification. Blood group determination by means of the newly emerging scientific discipline called immunogenetics provided the answer. A blood group laboratory was consequently established at Onderstepoort in 1956, initially concentrating on the production of blood typing reagents. Once established the technology was also applied to studies on a variety of problems in various animals as summarised in this paper. Investigations include zygosity in cattle twins, blood transfusion in domestic animals, breed relationships, genetic polymorphisms and the identification of useful genetic markers for production and disease parameters in breeding programmes.

Keywords: blood groups, breed relationships, genetic markers, genetic polymorphisms, immunogenetics.

Osterhoff D R **Research on animal blood groups and biochemical polymorphisms at Onderstepoort (1956–1990)**. *Journal of the South African Veterinary Association* (2010) 81(3): 136–138 (En.). 250 Loveday Street, Muckleneuk, Pretoria, 0002 South Africa.

Early studies on animal blood groups were performed in the USA and Europe but the real breakthrough came with the immunisation technique for the detection of antigens on the surface of erythrocytes. Stormont[13,14] demonstrated the occurrence of a large number of antigenic factors, determined by genes, on cattle red cells. At least 9 different loci were identified and the term immunogenetics was introduced for that part of science which utilises immunology as a tool for studying genetic characteristics and variation[3].

There was an obvious interest among European breeders and geneticists in taking up immunogenetic studies. It was expected to open up new possibilities for research in population genetics and to facilitate the search for marker genes influencing production or disease. However, the main reason was a very practical one: a real need had arisen for accurate animal identification after the introduction of artificial insemination (AI) around l950 for solving cases of disputed parentage in cattle. A few years later the storage of frozen semen convinced the AI organisations that accurate methods for pedigree control were essential.

The author was very fortunate to have been at the University at Uppsala when a young graduate, Jan Rendel, returned from the USA to Sweden to start an animal blood group laboratory there. Eventually it led to the planning of a similar laboratory at Onderstepoort, which came into being in l956. With the support of Prof. H Graf, Dean of the Veterinary Faculty, and Dr R A Alexander, Director of the Veterinary Research Institute, Onderstepoort and Director of Veterinary Services, facilities were provided in the Old Hospital of the Institute and experimental animals allocated.

The early work on **cattle** concentrated on the production of blood typing reagents. New types of antibodies were discovered in Afrikaner-cross cattle. Through international cooperation and exchange of test sera the South African laboratory was able to start blood group determinations for different practical purposes in 1960[6]. The antibodies which are used to detect red cell antigens may be either 'normally occurring' or 'immune' in origin. Seasonal variation was observed in normally occurring antibodies, with the highest titres in late summer and the lowest in late winter. The possible influence of light was excluded by keeping animals in complete darkness, proving that the external temperature was responsible for this variation[7]. A corresponding variation in antibodies against *Brucella abortus* to that in normally occurring antibodies could be demonstrated[17]. De Vos recognised normally occurring antibodies against red blood cells in his investigation of immunisation with the Onderspoort babesiosis vaccine, especially after the 2nd inoculation[12].

The value of research on cattle blood groups can be seen in the mushroom-like growth of blood typing laboratories. At a meeting in Uppsala in l954 only 6 persons studying animal blood groups, including the writer, were present. In 1972 there were 180 members of the newly formed European Society for Animal Blood Group Research, representing 48 laboratories. The AI organisations in South Africa were, from the beginning of routine testing, interested in the Onderstepoort activities, and the 1st cases of doubtful parentage were soon resolved. Until 1968 tests on 1068 of these cases were performed and 92.5 % could be solved. In a number of cases it appeared that the registration information was incorrect but breeders' associations tried to assure their members that the South African Studbook is as infallible as possible, using the latest methods available.

The diagnosis of zygosity in cattle twins is of great interest to animal research. If twins have different blood types they are regarded as dizygous and if a pair is found to be identical in all genetic markers, it indicates monozygosity; 671 pairs were tested of which 20 % proved to be monozygous. Twin pairs of different sex are definitely dizygous. If the blood test indicates that no vascular anastomosis has existed between the twin foetuses and that the twins are different in their blood types, results show that l6 % of the females will be potentially fertile while 84 % of the remaining co-twins are sterile (freemartins).

In South Africa, blood transfusions are mainly given to cattle suffering from severe babesiosis or anaplasmosis, when it is often the only means of saving the animal's life. In view of the unknown role of blood groups in producing transfusion reactions, research was undertaken in cooperation with Prof. K van der Walt at Kaalplaas. In single transfusions one need not be concerned about any real danger[15]. However, the incidence of transfusion reactions is significantly higher in pregnant animals. In repeated transfusions no single blood factor or phenogroup appeared to be responsible for transfu-

[a]250 Loveday Street, Muckleneuk, Pretoria, 0002 South Africa.

sion reactions. One or more transfusions can safely be given within the following 5 days. The same donor should, however, be used and pooling of blood from different donors should be avoided[13].

Zone electrophoresis with starch gel as supporting medium gave rise to clear differentiation between proteins according to their molecular size. Genetically controlled polymorphisms in haemoglobin and in serum proteins of cattle – transferrin, albumin and serum amylase – could be demonstrated and also used in parentage determination, resulting in a figure of 98 % of solved parentage cases. These techniques also enhanced the parentage verification of calves born after embryo transfer to ensure that the mother was the genetic and not the uterine dam. The usefulness of blood typing has also been shown in multiple-sire herds when one of the senior bulls is the sire of most of the calves born.

The usefulness of blood group analysis in studies of breed relationship was demonstrated after the collection of about 7000 blood samples from l7 different breeds. The relationship between the Alentejana-cattle in Portugal and the Afrikaner breed was investigated, a relationship between the South Devon and Gelbvieh breed could be proved and genetic similarities between the Swazi and Zulu cattle and other breeds were established[8]. The serum fraction and red cell haemolysates from all the samples were used to study genetic differences in haemoglobin, transferrin and serum albumins. Of special interest were the haemoglobin types A, B, C and D in African cattle, and the frequency of the corresponding genes could be used to study the migration routes of cattle accompanying the movements of human tribes.

With the use of isotope Fe 59 it was shown that the iron-binding in the different electrophoretic bands varies considerably. In another experiment the relationship between transferrin types and adaptability to harsh conditions indicated that animals with the transferrin allele Tf E in homozygous form proved to be better adapted. Milk yield of 1st-lactation cows was compared with transferrin types and it was found that cows homozygous for transferrin Tf D had the highest milk production. The relationship between milk protein types and mastitis in Friesland cows revealed that a correlation existed between the biosynthesis of certain genetic milk protein types and susceptibility or resistance to septic mastitis[2]. Animals heterozygous at the beta-lactoglobulin locus had a significantly superior inheritable relative mastitis resistance (IRMR), indicating that genetic assessments could become a very useful tool in controlling mastitis.

The 1st efforts to produce similar antisera in **horses** began in 1967. Mules and horses from the Virology Section at Onderstepoort were used as donors and horses as recipients. With 1162 immunisations 34 horse blood typing reagents could be produced. Studies on the serum protein types in Equidae (horses, mules, donkeys and zebras) gave interesting results and a number of enzyme type systems were discovered.

Besides all his work on cattle blood groups, Stormont[13,14] also became the expert in horse blood groups. He was invited in 1970 by the Jockey Club, to lecture in Johannesburg to breeders and Jockey Club members. It was easy to convince the horse fraternity to heed the signs of the times and start controlling Thoroughbred breeding by parentage testing. In the resultant routine typing, 6 blood groups, 4 biochemical polymorphisms and 4 different genetic enzyme systems were included. The Jockey Club supported this work very generously (E van Dyk, Faculty of Veterinary Science, University of Pretoria, pers. comm., 2009) and also the study of biochemical genetics and performance ability in horses[11]. The presentation of these results at a conference in Dublin in 1976 led to an interesting television discussion. Genomes became a new study field worldwide and a workshop on the horse genome took place in Skukuza in 2005 revealing that there are still several gaps in the gene mapping of horses.

Blood group studies and the search for genetic polymorphisms in **sheep** showed that marked differences exist between the haemoglobin phenotypes in terms of chemical pathology produced in various experiments with phenylhydrazine, organic selenium and partial exsanguinations[4]. The animals with haemoglobin type BB showed more severe reactions than those with the heterozygous haemoglobin type AB. Differences between geeldikkop and non-geeldikkop flocks suggest an advantage in the possession of the A gene that is responsible for the formation of haemoglobin C .

An attempt to correlate variable transferrins in 2230 Merino rams with the degree of resistance to *Brucella abortus*, *Brucella ovis* and *Actinobacillucs seminis* was unsuccessful. However, differences between the Spanish, Australian and South African Merino produced interesting results[1]. Close relationship between the Döhne and Walrich Merino was established.

Uzbekistan is regarded as the breeding area of the very first Karakuls. Gene frequencies at 10 blood polymorphic loci were used to compare these Russian Karakuls with those from South Africa and Namibia. It could be shown that the differences between the 2 groups was due to a gene flow of 0.28 of Black Head Persian genes into the Southern African flocks, which is also the reason for the marked difference in the well-known fur types[5].

Information on **goats** was obtained from breeds in South Africa and Switzerland. The relationship between Boer goats and local goats was established by known genetic markers, and it could be postulated that a major gene is responsible for the typical body and head colouring of the Boer goat. Research on the qualitative liver enzyme patterns in aborting and non-aborting Angora goats could not establish any significant difference[10].

In **pigs** a seasonal variation of naturally occurring blood group antibodies was also demonstrated. More than 300 kidneys from a local bacon factory were investigated and it was shown that the enzymes sorbitol dehydrogenase and 6-phosphogluconate dehydrogenase exhibit genetically determined polymorphism.

There is no doubt that in future the restriction fragment length polymorphism will replace the older techniques, but I believe that polymorphism determination and DNA fingerprinting will be used to supplement each other for some time to come[9].

With the help of many veterinarians in different places in South Africa blood samples were collected from **other species** (impala, buffalo, zebra, elephants, giraffe, lion, blesbok, wild duck, gerbils and springbok), the results being summarised elsewhere[9]. One could speculate that species with greater genetic variation should be able to adapt better to changing circumstances. The search for possible correlations between genetic markers and production and disease parameters in domestic species as well as investigating the reasons for population changes in wild animals will continue. South Africa, with so many livestock breeds, a variety of infectious diseases and a large number of wild animal species, could be an ideal outdoor laboratory where, using the most modern laboratory techniques available, a significant contribution to Veterinary and Production Animal Science worldwide could be made.

REFERENCES

1. Clarke S W, Osterhoff D R, Tucker E M 1985 Genetic polymorphism in South African Merino Sheep (Abstract). *Animal Blood Groups, Biochemistry and Genetics* 16: 66–67
2. Giesecke W H G, Osterhoff D R l975 Milk

protein phenotyping as a practical genetical means of augmenting conventional methods of mastitis control and prevention. *IDF Proceedings of the Seminar on Mastitis Control, Reading, England*: 223–230
3. Irwin M R 1956 Blood grouping and its utilisation in animal husbandry. *Proceedings of the 7th Congress on Animal Husbandry,* Madrid, Spain 2: 7–35
4. Neethling L P, Brown J M, Osterhoff D R, de Wet P J, Ward-Cox I S, l969. The functional advantage of hemoglobin type A in haemolytic syndromes in sheep. Phenylhydrazine, organic selenium and partial exsanguinaton as external agents in the production of anaemias. *Journal of the South African Veterinary Medical Association* 40: 121–126
5. Nguyen T C, Osterhoff D R, 1992 Comparison between Russian and South African Karakul sheep based on blood group markers. *Journal of the South African Veterinary Association* 63: 20–22
6. Osterhoff D R 1960 Blood groups in bovines. I. Production of cattle iso-immune sera and blood group reagents. *Onderstepoort Journal of Veterinary Research* 28: 479–491
7. Osterhoff D R 1962 Blood groups in bovines. II. Normally occurring iso-antibodies in cattle blood. *Onderstepoort Journal of Veterinary Research* 29: 89–106
8. Osterhoff D R 1968 Immunogenetical studies in South African cattle breeds. DSc thesis, Genetics Department, Faculty of Agriculture, University of Pretoria
9. Osterhoff D R 1980 Two decades of immunogenetical research in the Republic of South Africa. *Zeitschrift für Tierzücht und Züchtungsbiologie* 97: 197–209
10. Osterhoff D R 1995 Studies on blood groups and biochemical polymorphisms in goats. *Archiv für Tierzüchtung, Dummersdorf* 38: 553–561
11. Osterhoff D R, Azzie M A J, Op't Hof J 1975 Biochemical genetics and performance ability in horses. *Journal of the South African Veterinary Association* 45: 311–316
12. Osterhoff D R, de Vos A J 1977 Isoimmune blood group antibodies in cattle after the use of a blood vaccine. *Journal of the South African Veterinary Association* 49: 15–18
13. Stormont C 1949 Acquisition of the J factor by the bovine erythrocyte. *Proceedings of the National Academy of Sciences* 35: 232–237
14. Stormont C 1950 Additional gene-controlled antigenic factors in the bovine erythrocyte. *Genetics* 35: 76–94
15. Van der Walt, K, Osterhoff D R l969 Blood transfusion in cattle with special reference to the influence of blood groups. I. Single transfusions into young animals and pregnant cows. *Journal of the South African Veterinary Medical Association* 40: 107–117
16. Van der Walt K, Osterhoff D R 1969 Blood transfusion in cattle with special reference to the influence of blood groups. II. Repeated blood transfusions. *Journal of the South African Veterinary Medical Association* 40: 265–275
17. Van Drimmelen l953 A herd with unexplained serological reaction to *Brucella abortus. Journal of the South African Veterinary Medical Association* 24: 13–24