PARENTAGE CONTROL IN CATTLE USING BLOOD TYPES

By

V. MARY JOHN

THESIS

Submitted in partial fulfilment of the requirement for the degree

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Faculty of Veterinary and Animal Sciences
Kerala Agricultural University

Department of Animal Breeding and Genetics
COLLEGE OF VETERINARY AND ANIMAL SCIENCES
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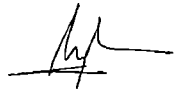
DECLARATION

I hereby declare that the thesis entitled **PARENTAGE CONTROL IN CATTLE USING BLOOD TYPES** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship fellowship or other similar title, of any other University or Society



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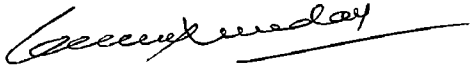
Dr B Nandakumaran,
(Chairman, Advisory Board),
Associate Professor,
Department of Animal Breeding & Genetics, College of Veterinary & Animal Sciences, Mannuthy

Mannuthy,
17 7 '92

CERTIFICATE

We, the undersigned members of the Advisory Committee of Dr V Mary John, a candidate for the degree of Master of Veterinary Science in Animal Breeding and Genetics, agree that the thesis entitled **PARENTAGE CONTROL IN CATTLE USING BLOOD TYPES** may be submitted by Dr V Mary John in partial fulfilment of the requirement for the degree

Dr B Nandakumaran,
Associate Professor,
Department of Animal Breeding & Genetics
(Chairman, Advisory Committee)



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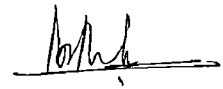
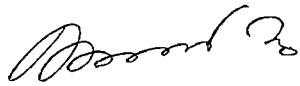
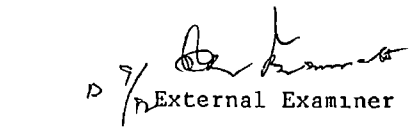
Director, CASAGB,
Mannuthy



Dr P A Devassia,
Professor,
University Livestock Farm,
Mannuthy



Dr V Jayaprakasan,
Associate Professor,
Department of Microbiology



External Examiner

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The task would not have been completed successfully but for the patience and love of my parents, my brothers and sister My gratitude to them for bearing with all the inconveniences

Dedicated to
my beloved parents

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INTRODUCTION

In advanced countries, genetics has played a major role in the development of dairy cattle As a result of the very rapid spread of artificial insemination, a need has arisen for an objective method of parentage determination The probability of inseminating cows to different bulls during the same or consecutive heat period in short intervals has increased Hence the frequency of unknown or doubtful parentage has increased enormously The doubt on paternity may originate from different sources, which include (a) Incomplete note on insemination cards, (b) the owners claim of wrong insemination, (c) two inseminations with different bulls in the same heat and, (d) insemination with different bulls in successive heat periods

Considering that progeny testing is an important programme in the evaluation of bulls, the importance of strict parentage control cannot be over emphasised It is said that bias that occurs in sire evaluation is due to inclusion of mis-identified records in sire group averages The bias increases with fraction of mis identified cows that have been in-advertently included in the programme

In large scale field recording programme like progeny testing where thousands of animals are recorded, there are bound to be errors in recording the parentage even if the field workers are motivated It is for this purpose that parentage determination either by blood typing or DNA finger printing is mandatory in progeny testing This ensures that the genetic estimates and the evaluation of the sires is very accurate and breeding strategies based on these estimates prove to be effective in improving the productivity of the animals Many developed countries in the world realised the importance of blood typing and made it statutory that every bull to be used in artificial insemination scheme should be blood typed

Blood typing is done to characterise the structures on blood cells and a determination of differences among soluble macro-molecules of the blood and can be considered as a kind of finger print The structures or configurations being characterised or differentiated are under genetic control, they are referred to as genetic markers Genetic markers on erythrocytes are mainly blood group factors The first major step in the study of blood groups of livestock was taken by Ferguson (1941) in cattle, when he developed a technique for producing individual iso-immune reagents

against different antigenic factors and also a procedure for carrying out haemolytic test During the following decades, large number of blood antigenic factors were reported by many workers in cattle The factors were designated in order of discovery by letters of the alphabet and utilizing the symbol

A real expansion of genetic typing took place in early fifties as a result of the introduction of Smithies s starch gel electrophoresis Existance of genetically controlled biochemical variants in large number of proteins/enzymes were detected by this technique Such studies are usually known as Biochemical polymorphism studies Polymorphism has been reported in red cell proteins like haemoglobin and carbonic anhydrase, serum proteins like albumin, transferrin, ceruloplasmin, amylase and alkaline phosphatase and in milk proteins like alpha-lactalbumin, beta lactoglobulin and the caseins

The studies based on blood groups and biochemical polymorphism made rapid progress and proved quite useful in solving problems connected with the livestock breeding and improvement The application of blood groups and biochemical polymorphism in animals is mainly directed towards breeding and genetics

The chance that two animals selected at random will have exactly the same blood type is very rare or zero Ever since 1924 when Schiff and Adelsbery (see Rendel 1957) first applied blood groups to solve the disputed parentage cases in human beings, this method has become widely used in most developed countries By far, the most important application of blood grouping and biochemical typing is in the establishment of paternity to confirm the pedigree

The use of blood types as genetic markers has assured a bright future for the dairy industry and without these tests, the artificial breeding of cattle in developed countries would not have flourished so much as it is today It can rightfully claim some credit for the progress made in disseminating superior germ plasm

From the above, it is imperative to check the rate of mis identification and now it is well established that blood groups and biochemical polymorphism is the reliable, economic and most effective method for identification and parentage control

Sire evaluation through progeny testing scheme is being carried out in Kerala State since 1980 Often errors erupt

in while the sons of the proven bulls are chosen as young bulls every year Blood typing of bulls or identification of bulls with gene markers was not in vogue in Kerala or in India In order to fill up the gap a study was undertaken with the objectives of

1 preparation of univalent blood group reagents to blood type the cattle

2 to find out the haemoglobin variants of the cross-bred cattle

3 to check the recorded parentage and estimate the error in farm records if any, and

4 to study the breed structure of the cross-bred cattle in terms of gene frequencies of blood group factors and haemoglobin variants

Review of Literature

REVIEW OF LITERATURE

Blood groups

Studies of animal blood groups were initiated during the turn of this century when Ehrlich and Morgenroth (1900), demonstrated individual differences in the blood of goats These workers introduced the immunisation technique, which has ever since been utilized in many experimental studies on animal blood groups

Von Dungern and Hirszfeld (1910) adopted the method of immunisation in dog and were able to demonstrate that the blood groups were inherited in a Mendelian manner

Todd and White (1910) were the first to take up blood group studies in cattle by iso immunisations and antibody absorption techniques They suggested that there were serological differences between the red blood cells of the individual animals

The pioneer work in the cattle blood group study was carried out by Ferguson (1941) In his extensive work by iso immunisation and haemolytic techniques, he developed nine blood group reagents viz A, B, C, D, E, F, G, H and I

Seven of these reagents were used in a study on 104 offsprings and from the pattern of inheritance he suggested that each of these factors were inherited as if controlled by a single gene He reported that the antigens C and E are closely related and presumably are controlled by allelomorphic genes He also noted that the cells of an individual contained a particular antigen only if one or both parents possessed it and suggested that blood typing can be used in cases of disputed parentage in cattle

Ferguson et al (1942) reported additional antigens in the erythrocytes of cattle They suggeted that blood factors B G O, Y_2 and E_1 represent discrete genetic characters in themselves and each one is controlled by a single gene non-allelic to others Thirty antigens were detected by this study and these antigens were assigned symbols (A, B C, E, G H I J K, M, Z A C H) in the order of their discovery No relationship between A and A , C and C etc was implied in this system

Stormont et al (1945) observed that certain cattle transmit factors B and G as a unit Accordingly a minimum of four reactive groups, symbolised B_B, B_G, B_{BG}, B_{BGK} could explain the distribution of these three factors Antigen K

was observed only in combination with both of the antigen B and G in a so called complex BGK

Six new blood antigens viz F, I J', K , L and Z were added to the previous list of 30 by Stormont (1950) Evidence was also presented for additional associations among bovine blood factors In accordance with this evidence, serological subtypes of each of the antigenic factors, T, U, X O and E' were proposed

The most remarkable progress in the genetics of cattle blood groups was made when Stormont et al (1951) after elaborate studies of large sire families, were able to demonstrate that no less than 21 of the 38 antigenic factors studied were governed by multiple alleles at one locus, the B locus Seven factors belonged to another complex system, the C system Of the remaining ten factors, all except the very rare Z could be shown not to belong to the B and C system The alleles at these two loci determined antigens which are characterised by a varying number of antigenic specificities They detected a minimum of 80 alleles in the B system and 22 in the C system

Stone and Miller (1953) reported that certain normal antibodies of cattle serum were reacting with cells of U_2

factor only and not with cells possessing U_1 and U_2 or U_1 alone

Stormont (1955) critically reviewed the theory of closely or absolutely linked genes and of pseudo allelic genic elements as applied to blood groups The complex B blood group system of cattle was taken as the model throughout the discussion He concluded that the hypothesis of multiple alleles proposed to explain the more complex systems of blood groups holds good

Allocation of the D blood factor to the A system based on the results of blood typing in American bison was made by Stormont and Suzuki (1956)

Rendel (1958 a) carried out extensive work on the techniques of developing blood group reagents in cattle He stressed the value of re-immunisation technique and reported that no untoward reactions could be noticed while immunising pregnant cows

The notations and symbols commonly used in cattle blood groups were listed by Neimann-Sørensen (1958) On the basis of genetic studies, these factors were classified in 11 different blood group systems, viz the A, B, C, FV, J, L,

M, SU, Z H and Z system The gene frequencies for different blood group factors were estimated for the complex B system Detailed description on the various methods and procedures used for production of cattle blood typing antisera (reagents) including iso or hetero-immunisations and absorptions were given

Stormont (1959) stated that the number of loci controlling the serologic properties of red blood cells do not exceed 12 in any one species The number of alleles per locus was reported to range from 2-160 The method of phenogrouping and its application in the determination of parentage was also described He suggested that the blood factors M or Z or both belong to the D or SU systems though they were assigned separate systems previously The factor H was found to be a subgroup of SU system rather than a separate one Studies on native African breeds revealed that a third allele F° occurs at the FV locus which in homozygous state can give rise to a condition wherein both F and V were absent The frequency of this allele was particularly high (about 0 6) in Africander cattle

Chet Ram and Khanna (1961) utilized the haemolytic technique of blood groups to study the breed diffeences in

Hariana and Kumaoni breeds Their studies revealed that though many blood factors were distributed in both the breeds, certain factors like J , V, J, E and U were predominant in Hariana and the frequencies of factors like K, Q, G W and B were significantly lower in Kumaoni cattle The latter breed showed similarity to Guernsey and Holstein with reference to the incidence of G and B factors They also reported that factors R and M occurred rarely in both the breeds The procedure for preparing anti J blood typing reagent was also described

Many new blood factors were discovered and assigned to different blood group systems by Stormont (1962) Sub-type D_2 of blood factor D in the A system, factors E_2 and NF_{12} in the C system M in the M system and R and S factors of the new R S system were detected He observed that a subtype of F factor called as F_2 reacted with the cells possessing the factor V_2 More than 300 alleles were recognised in the B system and believed that over 200 phenogroups could be distinguished in the C system Stormont defined the blood group systems, as those blood factors which were controlled by alleles at one locus The resultant products of an allele a group of blood antigens when

inherited together was referred to as a phenogroup He also listed the frequency of each factor in different breeds

Using ten blood group reagents, prepared by iso-immunisation and hetero immunisation, Naik et al (1963) carried out blood group studies on imported Jersey cattle Five of these reagents were comparable with those internationally accepted The factors B',I_1, G, X_1 and V were present in 28 1, 3 4, 18 8, 17 9 and 4 6 percentage, respectively

Naik et al (1965) identified three new blood group reagents belonging to B & S-U systems by iso-immunisation and hetero-immunisation The frequencies and pattern of inheritance of these factors were studied in Malvi, Khillari, Kankrej, Dangi and Gir breeds of cattle The two new factors in the SU system were found to have sub type relationship The factors showed interesting variations among breeds but none showed total absence

Miller (1966) evidenced two new systems of blood groups in a study conducted on several breeds of cattle and bison The new systems were named N and R -S N system was a two allele three phenotype system and the frequency was zero in

bison while it varied from 0 12 0 63 in cattle breeds R -S system was a two allele three phenotype closed system with a gene frequency ranging from 0 03-0 49 in cattle breeds All the 72 bisons tested had the phenotype R S suggestive of a third gene controlling a phenogroup which cross-reacts with both R' and S reagents

Khanna et al (1969) studied the FV blood group system in ten Indian cattle breeds They concluded that a cold climate favoured F allele, while a hot climate favoured the V allele They observed considerable breed differences between Kankrej, Kangayam, Rath, Tharparkar and Hallikar The frequency of allele V was lowest in Kankrej (0 0114) and was highest in Rath (0 367)

Naik (1970) made an attempt to find out the association between the known and unknown blood factors to assign the new factors to different blood group systems Factors IND 3, IND 6 and IND 7 showed association with either of B G or Y factor Since the latter factors belonged to B system, the new factors IND 3, 6 and 7 were also suggested to belong to B system The factors IND 9 10 and 11 were assigned to the A system and IND_8 to the J system None of the blood group factors showed association with the haemoglobin

variants which led to the conclusion that the genes determining the synthesis of haemoglobin are situated on different chromosomes from those which determine blood factors

Singh et al (1970) studied the variations in gene frequencies of blood group factors in Hariana and Jersey Sindhi cross breds Out of the fifteen reagents used eleven were comparable with those internationally recognised (including naturally occurring J from Kumaoni cattle) and four reagents probably contained antibodies against some new factors present in Hariana cattle Significant differences were noted between the Hariana and cross bred cattle in respect of the frequencies of C, R, W, J M and I^{Z}_{33} antigens

Khanna and Singh (1971) estimated the gene frequencies of L and M alleles in some Indian cattle breeds The frequencies for L system ranged from 0 352 (Sahiwal) to 0 878 (Tharparkar) and for M system for 0 010 (Red Sindhi) to 0 141 (Hariana) From their study, it was revealed that the frequencies were higher for L and M genes in drought and dual purpose breeds in comparison to milch breeds

Genetic analysis of the FV blood group system in three Indian grey cattle breeds was carried out by Mishra and Prabhu (1972) The frequencies of F gene were 0 767, 0 787 and 0 800 in Hariana, Ongole and Tharparkar breeds, respectively The locus was at equilibrium in all the Tharparkar and Hariana herds except the one at Izatnagar It was not in equilibrium in Ongole herds FF genotype was found to be more suitable under Indian conditions

Frequencies of occurrence of blood antigenic factors E, R, F, V, J, S and H in two cross breds (Jersey Sindhi and Sahiwal-Holstein) were calculated and compared by Bhagi et al (1972) The relative frequency of occurrence indicated that factor R_1 was higher at Military farm, Bareilly and the H was generally high in all the breeds The two breeds differed significantly in respect of blood antigenic factors R_1 and H but did not differ in respect of factors E, S , J, F and V Gene frequencies of blood factors F_1, V and J in Jersey x Sindhi crosses were 0 833 0 167 and 0 182 respectively, while the corresponding figures for Sahiwal x Holstein crosses were 0 685, 0 315 and 0 127 respectively The two herds were not in equilibrium in respect of this system

Prabhu and Mishra (1972) scrutinised the estimates of incidence of blood factors in the different cattle breeds of India Their study revealed that wide variation occurred in the incidence of a given factor in different breeds, the incidence of different blood factors in a given breed differed from factor to factor and the variation found in one breed was different from that seen in another They listed out, the phenotypic frequencies of blood factors, and the inheritance of 18 cellular antigens in different breeds of cattle in India

Studies on blood group antigenic factors in ten Indian cattle breeds by Khanna et al (1972 b) revealed that no antigenic factor occurred exclusively in one breed Among the 47 reagents used all the factors except IZ_{30} and IZ_{37} had significantly different breed distributions Several breeds were found to have close relationships in blood group factors

Rao et al (1973) blood typed 230 cattle of Ongole breed and 99 Zebu-Jersey cross-breds They found that factor R_1 occured with a frequency of 0 1348 (Ongole) and 0 1717 (cross-breds) The frequency of factor J was 0 2569 and 0 2160 in Ongole and cross breds, respectively

Significant difference was noted between the two breeds with respect to factor E The frequencies were 0 6652 in Ongole and 0 8484 in cross breds The cause was attributed to increased use of a popular bull with homozygous E locus

Hines et al (1977) estimated the gene frequencies at ten blood group loci in a herd of Holstein cattle They had listed the gene frequencies in the codominant and simple dominant system and phenogroup frequencies at the more complex B and C systems The B alleles B, G_2, Y_2, E_1, Q occurred more frequently than any other B phenogroup

Genetic map of the B blood group system was drawn by Grosclaude et al (1979) This was based on the irregularities noted in the inheritance of B phenogroups In addition to single crossing over, double crossing over or gene conversion and deletion were also attributed as the cause for irregularities in inheritance The results supported the hypothesis that the genetic structure of the B system of cattle blood groups is basically the same in all taurine breeds The genetic distance between terminal factors Q and I was calculated to be 0 7 centimorgan

Stur et al (1979) carried out detailed investigations on the factors responsible for variations in the immune response of recipient cattle to donor erythrocytic antigens According to them, best results were obtained when there were high degree of heterozygosity in the recipient, 3-4 factor difference between donor and recipient animals and in cases of re-immunisation with the same donor

Attempts were made for the first time by Duniec et al (1979) to isolate blood typing antibodies from colostrum of immunised animals Antibodies were found in the colostrum and serum of 16 immunised cows and the titre in the colostrum on the day of calving was higher than that in the serum in all the cases But no anti erythrocytic antibodies could be detected in the milk of any of the cows examined on the 10th day after calving The reagents so produced were stored for two years at about -18°C with only a slight decrease in the titre of antibodies

The procedure for production of blood group reagents was made easier by Ikemoto et al (1979) They tested the agglutinin activity of the extracts of seeds, leaves and roots of about 300 different species of plants against animal red cells Lectin from Feijao chumbinho was found to have affinity for F_c system in cattle, the estimated

phenotypic frequency being 40 per cent for F_c type and 60 per cent for f_c type The gene frequencies were F_c 0 2294 and f_c 0 7706

Partial genetic map of the C system was deduced by Guerin et al (1981) using the inheritance pattern of phenogroups in C system The operational length of the DNA sequence coding for the C system was estimated to be 0 3 centimorgan (almost half of that for B system) It was conluded that the phenogroups of the C system, like those of B system, were controlled by a cluster of loci

An additional factor Epsilon was reported in the FV system of British Friesian cattle by Hall nd Ross (1981) The factor existed in genetic association with F and V or independently

Kumar and Prasad (1982) carried out immunogenetic studies on erythrocytic antigens of Indian cross-bred cattle using 21 reagents The reagents KS_1, KS_2 and KS_3 differed from the known antigens and were transmitted in combination with J and T_1 antigens They noticed lower frequencies for the C and R_1 antigens in the Karan-Swiss breed than those reported for other Indian breeds The F and V alleles showed

an incidence of 77 63 per cent and 22 37 per cent respectively Blood typing data on the sire, dam and progeny indicated the existance of a third allele at the FV locus

Larsen (1982) studied the F system of three cattle breeds using four specific antisera In Jersey, six phenotypes and three alleles were recognised but in Danish Red and Danish Black Pied, only three phenotypes and two alleles were recognised, with no indication of a null allele He suggested that from the phenotypes previously observed in zebu cattle, at least six alleles may be present in the bovine F system He also concluded that the factors V_1 and V_2 did not appear to form a linear sub type system in all cattle breeds

Andersson (1985) developed a new allocation method for the estimation of gene frequencies According to him, unbiased estimates can be made for a given phenotypic class when the genotypes of a fraction of individuals are unknown, ignoring the family information The sampled individuals were divided into classes according to their own and their parents phenotypes The expected proportion of possible genotypes in such classes were then calculated on the

assumption that parental genotypes occur in Hardy-Weinburg proportions and that the alleles segregate in Mendelian ratio

Georges et al (1990) proved the linkage between two pairs of blood group systems B-Z and S-F/V, respectively Evidence for linkage between S and F/V was demonstrated by adding one locus, the most likely order being Piz S F/V with maximum likelyhood recombination rates of 0 208 and 0 211 Five pedigrees were informative for both B and Z simultaneously A maximum lodscore value of 5 7 at M 0 24 was obtained, clearly demonstrating linkage

Naturally occurring antibodies

Among other blood group factors, Ferguson et al (1942) discovered the J factor which occurred in the serum of cattle and some times on the erythrocytes This was identified by antibodies that occurred naturally in the serum of certain cattle which lacked the J factor in their serum or erythrocytes

Stormont (1949) studied the acquisition of J factor by the bovine erythrocytes and found that this substance

existed in the serum of J positive animals in a soluble form early in life and that the substance was acquired by the erythrocytes on their surfaces only later The J substance was reported to be produced by some other tissues and not by the haemopoetic tissues He found phenotypically different cattle twins, which were genetically different for J, even if they were otherwise mosaic A definite serological relationship could also be established between cattle J and human A blood groups

Docton et al (1952) reported the presence of J antigen in various body fluids including semen

Several studies were carried out to elucidate the cross-reaction pattern of inter species erythrocytes Naturally occuring haemolysins were detected in a Hereford bull and three of his daughters, that caused lysis of erythrocytes of group O sheep (Stormont, 1953)

Strong relationships were reported between anti J sera of cattle, anti R of sheep and anti-A of human beings, in a comparative study by Neimann Sorensen et al (1954)

Developmental and immunogenetic studies on the J system by Stone and Irwin (1954) concluded that the J system was

controlled by a triple allelic series of causative genes designated as J^{cs}, J^{s} and J^{a} in the descending order of dominance Accordingly, they classified cattle in to three groups, viz , J^{cs} - those with J substance on cells and in serum, J^{s} - those with J substance only in serum, and J^{a} - those without J substance but whose sera may contain anti-J

All the J negative animals were not reported to have anti-J in their serum

Many reasons were attributed to the fluctuations in the titre of anti J in the serum of cattle Seasonal variations to this effect was studied by Stone (1956) who observed a peak in the titre of iso-antibodies for the J in autumn (August to October)

Sprague (1958) studied the inheritance of natural antibody in cattle He assumed that the antibody was a product of single dominant gene designated as anti O The phenotypic expression was influenced by cattle O and cattle J substances Accordingly, cattle whose serum was positive for O and or J substance, lacked the anti-O But the O and or J negative offspring born to positive parents might have Anti-O in its serum He reported that the gene showed

hypostatic dominant condition with regular autosomal segregation and that four phenotypes could be detected in cattle viz J, J^{oc}, 0^{c} and --

Many workers attempted to produce anti J by immunisation, but all in vain J substance obtained from many sources were utilized for this purpose in cattle by Hayashi et al (1958) Blood group specific substance was prepared from bovine gastric mucosa and this was reported to have behaved similarly in cross reactions within other blood group systems But they failed to produce specific precipitating antisera against the J-substance by immunising rabbits cattle or chickens

High frequency of naturally occurring iso-antibodies and its significant role in blood transfusion reactions was reported in cattle by Otte (1959)

Stone and Miller (1961) reported naturally occuring antibodies in cattle sera, reactive to U_1 and U_2 factors of S system Using these sera they detected a new specificity called U , possessed by the U_2 and not by U_1 cells The naturally occuring antibodies were reported to exhibit the phenomenon of prozone which was a distinctive feature that

contributed to the failure to detect this specificity before.

Bednekoff et al. (1962) could succeed in the preparation of strong and specific antisera for J substance from a heat stable fraction of cattle serum. The reagent was prepared in rabbits by injecting erythrocytes intramuscularly along with Freunds adjuvant. But the preparations from urine, abomasal mucosa and untreated serum showing high J activity were antigenically weak.

Stone (1962) opined that at least four alleles involved in each of the two J positive classes (J^{cs}, J^{s}) and that the effects of these genes were quantitative. In vitro experiments revealed that the acquisition of J-substance by the red-cells purely depended on the concentration of J-substance in the serum and the cells played no role in their absorption on to the surface. He observed differences in the antigenicity of the J substance obtained from different sources of the same animals in that, substance from gastric mucosa failed to produce antibodies in the rabbit while that from serum and saliva did. Chemical composition of the same from gastric mucosa was reported to have carbohydrate only, while that of serum and saliva contained carbohydrate and protein.

Conneally et al (1962) carried out detailed study on the mode of inheritance of the J character and suggested at least 8 alleles at this locus They observed negligible effect on the variations found within the J^s and J^{cs} alleles due to interactions or genetic modifiers to J^a allele

Several infertility cases were studied on the grounds of erythrocyte antigenic incompatability Matousek (1964) recognised J antigen activity in ovarian follicular fluids also According to Matousek (1964 a) J antigen was absent on the sperms but it was present in the seminal plasma

Similar observations were also made by Prakash (1965) while studying the breeding efficiency of cattle He noted that the number of services increased with the increase in titre of anti-J in cattle serum The highest titre in zebu cattle was reported in early winter (October to December)

Nagarajachar et al (1988) studied the incidence of naturally occuring iso-antibodies in 250 serum samples About 46 per cent gave positive reactions and variations from previous reports were attributed to the breed, season, place, pregnancy, intercurrent disease method of testing and variations in J antibody titre among and within the same individuals from time to time

Haemoglobin

Haemoglobin - the respiratory protein belongs to the class of heme proteins They are conjugates of proteins with heme an iron-porphyrin compound Various species differ in their haemoglobin structure These differences are related to the variations in the amino acids of the globin part of the molecule Each molecule contains four heme groups and in general have a molecular weight of 65,000 The four globin peptide chains, in a molecule of haemoglobin each combined with a heme group, are held together in definite arrangement or conformation by hydrogen bonds primarily, though weak salt linkages and Van der Waals forces are also involved The individual peptide chains may be separated by first removing the heme groups by acid treatment and then subjecting the chains to procedures such as column chromatography, and electrophoresis

The studies on haemoglobin and other protein variants were initiated by Pauling et al (1949), employing paper electrophoresis They found that, in man, patients with sickle-cell anaemia, possessed haemoglobin with varied electrophoretic properties

Haemoglobin variation in cattle was first described by Cabannes and Serain (1955) with the use of paper electrophoresis These workers found three haemoglobin phenotypes in Algerian cattle Of the 80 Algerian cattle typed, 64 had a single band, 15 had a second faster migrating component also and one possessed the faster component alone

Bangham (1957) reported two haemoglobin types controlled genetically by a pair of alleles at a single locus with co dominance The individuals heterozygous at this locus exhibited both the haemoglobin variants He described them as bovine A and bovine B haemoglobins bovine B being the fast moving type

Salisbury and Shreffler (1957) supported the theory of Bangham and designated the adult bovine haemoglobin types as Hb^A and Hb^B, the slow and fast moving types respectively The variant which was found only in animals less than 80 days of age with a wide range of mobil ty and diffuse band was designated as foetal haemoglobin or Hb^F

The occurrence of foetal haemoglobin in the foetus and new born was also reported by Grimes et al (1957, 1958)

They studied the post natal persistence and relationship of foetal haemoglobin with that of adult haemoglobin and could find that the variant Hb^F was replaced by Hb^A in Holstein-Friesian, Brown-Swiss and Ayrshire breeds but in Guernseys and Jerseys the disappearance was obscured by Hb^B The Hb^F was found to have the same electrophoretic mobility as that of Hb^B

Bangham and Blumberg (1958) reported that bovine Hb^B occured only in Jersey Guernsey and South Devon breeds of Britain and this was consistent with one of the suggested ancestral line of the Jersey breed ie from African breeds of cattle

According to the observations of Lehmann and Rollinson (1958), Hb^A was relatively less frequent in the pure-bred zebu cattle in Africa than in other breeds

A fourth type of haemoglobin variant, Hb^C was reported by Vella (1958) in cattle of Bos indicus origin The mobility of Hb^C was found to be in between Hb^A and Hb^B

Shreffler and Salisbury (1959) studied the distribution and inheritance of haemoglobin variants in American cattle

The gene frequency estimated for Hb^B was 0 33 for Jersey as compared to 0 116 and 0 117 for Guernsey and Brown swiss, respectively They pointed out the practical application of electrophoretic studies of the haemoglobin variants viz Parentage determination, tracing of breed origins and adaptation of phenotypes to climatic conditions

The association of Hb^B gene with Bos indicus led Lehmann (1959) to examine the haemoglobin of Indian Zebu cattle He found the gene frequencies of Hb^A and Hb^B to be equal in the Gir cattle and there was also an excess of heterozygous phenotypes He also suggested that in Gir cattle natural selection favoured the heterozygotes

In a study on adaptation of zebu and British breeds of cattle to subtropical environments in relation to erythrocyte characters Evans (1963) found that Hb^B was associated with tolerance to tropical climate

The fifth variant Hb^D was observed by Efremov and Braend (1965) in cattle of Africa origin This variant had a mobility slower than that of Hb^A and occurred either independently or in combination with Hb^A or Hb^B

Work carried out in several breeds of Indian cattle by Naik and Sanghvi (1965) led to the discovery of a new but very rare haemoglobin type Hb Khillari in the Khillari breeds of India (Malvi, Kankrej and Dangi)

Srivastava (1965) reported that Hb^A occurred only in Holstein crosses and was absent in Jersey, Brown swiss and Sindhi cross-breds The gene frequency for Hb^B was greater in cattle of Sindhi lineage than European crosses

Balakrishnan and Nair (1966) conducted electrophoretic studies of haemoglobin in agar gel tubes in Sindhi, Sahiwal and Tharparkar breeds Though some breed differences could be noticed in the gene frequency of Hb^A and Hb^B alleles, they could f nd no significant difference with sex

In a study on some African breeds of cattle like Muturu (West African dwarf short horn) and N Dama, Braend et al (1966) reported a new allele Hb^D in addition to Hb^A and Hb^B The gene frequency of Hb^D varied from 0 13 to 0 26 in different breeds while all the N Dama animals were of Hb^{AA} type

Studies at the amino acid level of the two haemoglobin types were first carried out by Schroeder et al (1967)

Beta chains of Hb^A and Hb^B were found to differ at 15th, 18th and 19th of the 145 amino acid residue and the beta chain of Hb^A had two more tryptic cleavage sites than the beta chain of Hb^B

Braend and Khanna (1968) found genes indistinguishable from Hb^B and Hb^C in two zebu breeds of Africa viz Gudali and Red Bororo cattle

Naik et al (1969) reported a rare haemoglobin variant Hb^X which was similar to Hb^C, with a low frequency in some Indian zebu cattle viz Khillari, Rathi and Kumaoni Hill cattle

Khanna et al (1970) reported a new haemoglobin phenotype Hb BC in Hariana cattle in addition to BB, BA, CA and AA Age and sex of the animals were found to have no effect on the distribution of haemoglobin types in cattle

Braend (1971) carried out a comparative study on all cattle haemoglobin variants reported till then He concluded that the Hb^C reported by different workers in different breeds could be classified into three groups depending upon their electrophoretic mobility He found a

new haemoglobin variant, Hb^G in three of the 101 East African zebu cattle with a gene frequency of 0 01 Its migration was slowest than any cattle haemoglobin variant previously reported

Khanna et al (1972 a) discovered a rare haemoglobin variant tentatively designated as Hb^E Muk, in four pure bred Afghan cattle, six Afghan x Kumaoni cross breds and one Afghan x Jersey cross bred adult lactating cow The new variant was not observed in pure Kumaoni cattle and in Red Sindhi and Sahiwal breeds

Singh and Khanna (1973) opined that the different Hb^C types reported elsewhere should be tested simultaneously when they found the variant C in relatively high frequency in Kumaoni Hill cattle

Schwellnus and Guerin (1977) compared the Hb^C variant in Brahman and indigenous south African cattle breeds They suggested that the faster moving variant in Brahman cattle be called Hb^C and slower migrating type of South African breed be called as Hb^I They confirmed the theory that genetic variation was restricted to the non-alpha chain of bovine haemoglobin

Singh and Bhat (1979) found a rare variant Hb^A Cuttack in an aged Red-Sindhi cow This phenotype had a faintly staining Hb^A like band in combination with Hb^B band.

Nandakumaran et al (1979) observed three haemoglobin phenotypes HbAA, HbAB and HbBB controlled by two alleles HbA and HbB in Hariana cross-breds No significant differences could be noticed between different populations with respect to gene frequencies They also reported that the population was in agreement with the observed and expected phenotype frequencies

Studies on haemoglobin polymorphism among 23 different herds belonging to pure bred and cross-bred Indian cattle by Singh and Bhat (1980 a, 1980 b) revealed a trend to increase in heterozygosity in cross-bred cattle over the respective parental population

Starch gel electrophoresis for haemoglobin polymorphism in three grey cattle breeds of India carried out by Singh and Bhagi (1981) revealed good agreement between observed and expected values of genotype frequencies The results obtained were comparable to that of earlier reports in Hariana breed They could observe a very high frequency or

heterozygote phenotype which was attributed to the adaptation of heterozygotes towards some environmental factors

Studies by Singh et al (1981) on the average heterozygosity at haemoglobin locus for pure-breds and cross-breds revealed that the same was only 19 6 - 33 6 per cent in pure breds as compared to 35 3 - 42 7 per cent in cross-breds

Nandakumaran et al (1982) estimated the genetic variability in four cross bred populations using gene frequencies at six polymorphic loci namely haemoglobin, amylase, transferrin, albumin, ceruloplasmin and alkaline phosphatase systems The heterozygosity observed in the four populations at the haemoglobin locus was 0 3956 (Holstein x Hariana), 0 4401 (Brown-Swiss x Hariana) 0 4873 (Jersey x Hariana) and 0 3787 (pooled cross breds having 3/4 exotic blood)

Shanker and Bhatia (1982) observed three haemolgobin alleles (Hb^A, Hb^B and Hb^C) with five different genotypes viz Hb AA, Hb AB, Hb BB Hb AC and Hb BC in Sahiwal and Jersey cattle The genotype frequency in most of the breeds

was highest for Hb AA followed by Hb AB and Hb BB Tharparkar, Red Sindhi and Holstein Friesian breeds had only two alleles viz Hb^A and Hb^B

Queval and Petit (1982) reported a relatively high frequency of Hb^A allele and low frequency of Hb^B allele in trypano-tolerant cattle of west Africa viz N'Dama and Baoule The susceptible population had a relatively high frequency of Hb^{AB}

Singh et al (1983) studied the genotypic plasticity of Friesian herds in India Relatively higher frequency of Hb^{AB} genotype was noticed in all the herds The high incidence of Hb^A allele was consistent with earlier reports on Friesian herds The low incidence of Hb AB observed in the Military farm, Meerut was attributed to the low diffusion of zebu genes among Friesians

Significant differences between breeds and between herds within breeds for the gene frequencies of alleles at haemoglobin locus was reported by Singh and Bhat (1983) in breeds such as Gir, Haryana, Kangayam, Kankrej, Ongole, Red Sindhi, Sahiwal and Tharparkar

Braend (1988) showed the usefulness of immobiline method for the characterisation of molecules which were non separable by various methods of electrophoresis He reported the occurrence of three haemoglobin phenotypes in Norwegian Red cattle by this method at pH 7 1 7 7 (which was otherwise considered homozygous for Hb^A allele) viz two single band phenotype designated as Hb A_4 and Hb A_6 and one two band phenotype designated as Hb A_4A_6 phenotype These were considred as sub divisions of original Hb^A and cathodal A_6 was always the stronger band than A_4 The distribution of phenotypes was in agreement with codominant single gene inheritance, the gene frequencies being 0 94 for Hb A_4 and 0 6 for Hb A_6

Polymorphic studies in Bali cattle by Bell et al (1990) revealed a second variant Hb^C Bali in addition to the B variant This new variant occurred in Bali cattle either as homozygotes or heterozygotes, the mobility of which was intermediate between those of the common A and B variants but closer to B This appeared to be similar to the variant C of Khillari and C of Asian cattle, differing from those of Kenyan cattle, Rhodesian cattle and Mithun

Several workers carried out electrophoretic studies on haemoglobin locus in different breeds of Indian and exotic

cattle and could find differences in gene frequencies of haemoglobin alleles between herds and between breeds of cattle Table 1 shows the gene frequencies of haemoglobin alleles in different cattle breeds

Table 1 Haemoglobin gene frequencies in different cattle breeds

Author/(s)	Breeds of cattle	Hb^A	Hb^B	Hb^C	Other variants
Naik et al (1963)	Jersey	0 556	0 444		
Sen et al (1966)	Hariana	0 578	0 422		
	Deshi	0 705	0 295		
	Sahiwal	0 625	0 375		
	Gir	0 569	0 431		
	Tharparkar	0 700	0 300		
	Red Sindhi	0 824	0 176		
Balakrishnan and Nair (1966)	Red Sindhi	0 700	0 300		
		0 625	0 375		
	Sahiwal	0 707	0 293		
		0 671	0 329		
	Tharparkar	0 897	0 103		
		0 894	0 106		

Author/(s)	Breeds of cattle	Hb^A	Hb^B	Hb^C	Other variants
Naik, et al (1969)	Malvi	0 543	0 454	0 003	
	Khillari	0 518	0 479	0 002	Hb Khillari 0 001
	Dangi	0 512	0 485	0 003	
	Gir	0 509	0 491		
	Kankrej	0 587	0 410	0 003	
	Rath	0 581	0 412	0 007	
	Kumaoni	0 735	0 244	0 021	
Khanna, et al (1970)	Hariana	0 430	0 570		
Singh and Khanna (1971)	Hariana	0 538	0 462		
	Hariana x HF	0 796	0 204		
	Hariana x Jersey F1	0 586	0 414		
	Hariana x Jersey F2	0 658	0 342		
Singh, et al (1972)	Ongole	0 780	0 220		
	Hariana	0 380	0 620		
		0 270	0 730		
		0 540	0 460		
		0 430	0 570		

Author/(s)	Breeds of cattle	Hb^A	Hb^B	Hb^C	Other variants
	Kankrej	0 440	0 560		
		0 540	0 460		
		0 660	0 340		
	Gir	0 450	0 550		
		0 470	0 520		
	Sahiwal	0 570	0 430		
	Rath	0 730	0 270		
Singh and Khanna (1973)	Kumaoni	0 708	0 276	0 016	
Singh and Bhat (1979)	Indian Zebu cattle	0 625	0 375		
	½ Friesian Crossbred	0 787	0 213		
	3/4 Friesian crossbred	0 886	0 114		
	Friesian	1 000			
Nandakumaran et al (1979)	Hariana crossbred	0 580-0 746	0 254-0 420		
Singh and Bhat (1980)	Hariana	0 429	0 571		
	Sahiwal	0 807	0 193		

Author/(s)	Breeds of cattle	Hb^A	Hb^B	Hb^C	Other variants
	Friesian	1 000			
	Kankrej	0 619	0 381		
	Ongole	0 750	0 250		
	Red Sindhi	0 557	0 443		
	Kangayam	0 645	0 355		
	Gir	0 530	0 470		
	Tharparkar	0 731	0 269		
Singh and Bhagi (1981)	Hariana	0 479	0 521		
	Malvi	0 690	0 310		
	Nagauri	0 521	0 479		
Shankar and Bhatia (1982)	Sahiwal	0 748	0 249	0 003	
	Tharparkar	0 859	0 141		
	Red Sindhi	0 702	0 298		
	Holstein-Friesian	0 900	0 100		
	Jersey	0 548	0 404	0 048	
Han and Lee (1982)	Korean cattle	0 895	0 071	0 014	
	Hostein-Friesian	1 000			

Author/(s)	Breeds of cattle	Hb^A	Hb^B	Hb^C	Other variants
Singh, et al (1983)	Friesian	1 000	0 000		
		1 000	0 000		
		0 989	0 011		
Khanna and Tandon (1987)	Mithun	-	0 040	0 960	
	Mithun hybrid with cattle	0 400	0 130	0 470	
	Local Zebu cattle	0 810	0 190		
	Local Zebu x Exotic (Crossbred cattle)	0 750	0 250		
	Pooled crossbred (Hariana x Exotic)	0 710	0 290		
Al-Timemi and Al-Murrani (1990)	Sha-abi (Iraq)	0 640	0 460		
	Holstein	1 000	-		

Parentage

The large number of blood factors detected in cattle, and the many ways in which genes determining these factors can combine, make the chances very small indeed that two animals chosen at random, will have exactly the same blood type In large farm animal species particularly in cattle, blood typing is routinely used for parentage control in all developed countries In farm animals the use of blood type in solving the paternity cases was first used on horses by Kaempffer in 1935

The importance of blood group studies in solving disputed parentage in cattle was soon recognised by Ferguson (1941) He showed that an animal inherited an antigen only when one or both of the parents possessed it

Stone and Palm (1952) opined that parentage tests in cattle must be interpreted with caution when twins are involved Calves born to a dam sired by different bulls were found to possess certain blood factors, which were not detected in the sire s or dam s blood It was discovered later that the dam was a twin to a female which was not available for testing and subsequent tests revealed weak

reactions involving unexplained blood groups of calves, in the dam s blood

In an analysis of 114 solved paternity cases where calving had occurred few days earlier in relation to second matings, Humble (1952) reported that 78 per cent of the cases had resulted from second mating and only 22 per cent of calves from first mating

Based on the knowledge of inheritance of the blood group factors within the known blood group systems B, C, FV etc Braend (1956) could solve few disputed parentage cases in Norwegian cattle

Rendel (1956, a), reported that 7 9 per cent of the stated parentage in a sample of 394 complete sire dam offspring families were wrong

Studies on Swedish Red and White and Swedish low land (Friesian) cattle by Rendel (1956,b) revealed that paternity was sufficiently doubtful to warrant a blood group test when a cow was served by two bulls within an interval of twelve days and a cow was served by two bulls at an interval of 13 30 days and calves 5 16 days earlier, in relation to

the second service, than would normally be expected One hundred and thirteen cases of doubtful paternity were investigated of which 81 were definitely solved

Neimann Sorensen et al (1956) could not eliminate 14 per cent of the suspected bulls in parentage studies

The success in solving wrong parentage depended upon the amount of variation present in different blood group loci within a breed (Rendel 1957) He observed that the percentage of solved cases of paternity was between 70 and 90 percentage

Rendel (1958 b) discussed the principles involved in parentage tests using blood groups The investigations of 260 parentage cases revealed that all bulls except one were excluded as sire in 78 4 per cent of the 167 cases and all bulls were excluded as sire in 4 8 per cent of cases while in the remaining 16 8 per cent no exclusions could be made Exclusion of one sire was possible in 40 per cent of the cases of incomplete paternity Further the accuracy of breeding records was investigated by the immunogenetic technique in 814 sire-dam offspring combinations About four per cent of the records in both types of herds were found to be erroneous

Parentage tests were carried out by Rendel and Gahne (1961) in Swedish cattle breeds using 39 erythrocyte antigenic factors In the complete parentage cases with two possible sires, one of the two sires was excluded in about 80 per cent In 1 2 per cent of the cases both the given sires were excluded while no exclusion was possible in 18 per cent of the cases They obtained excellent results in solving paternity cases when tests for transferrin and cellular antigens were combined and used simultaneously In Swedish Red and White Breed the combined use of tests for cellular antigens and transferrin gave solutions in about 84 per cent of the complete paternity cases with two possible sires Of the 26 cases of suspected interchange of animals, each comprising 2 calves all but one case were solved ie one of the calves could be assigned to one pair of parents while the other was assigned to the remaining parental combination The probability of making exclusion with the aid of various blood grouping systems was also estimated B system was much more efficient than any of the other cellular antigen systems The transferrin system was found to be as efficient as the B locus in Swedish Red and White Breed

Schmid (1962) using blood type alone reported 86 per cent success in solving disputed parentage

Rendel et al (1962) critically analysed the results of parentage studies carried out at various centres in Germany Netherlands and Sweden and reported that 20 per cent of cows inseminated twice within a short interval (1-11 days) became pregnant due to first service In cases where service interval was 18-24 days six per cent of them were conceived by first service

Using A, B, C, FV, J, L M, S and Z blood group systems and transferrin and post-albumin types, Salerno (1964) could find 18 animals to be incorrect in their presumed parentage He concluded that the blood type at the FV and Z loci and transferrin type were the most useful systems for detecting wrong parentage

Kovacs Gy (1965) advocated blood testing of animals before progeny testing He could not confirm the supposed parentage for 22 per cent of the progeny supposedly sired by 10 bulls

Later workers, used various systems to increase the efficiency of parentage determination Rausch et al (1966) in a study of 32 parentage disputes, made use of transferrin studies along with blood types

Schleger and Soos (1967) pointed out that the combined use of blood proteins and blood groups greatly increased the efficiency of parentage tests while the blood proteins alone could exclude false parentage only upto 25 per cent.

Allocation of wrong parentage due to different blood group systems by Osterhoff (1968) in South African cattle revealed that the complex blood group systems (B, C, S and A) allowed most of the parentage exclusions possible. He could solve 92.5 per cent of the disputed parentage by blood tests. Both transferrin and haemoglobin types were also utilized for the study.

Determination of the reliability of parentage recording in Russian cattle by Slepcanko (1970), using 56 immune sera revealed that paternity records could not be confirmed in 23 per cent of cases. He could find disagreement even for dams in 1.4 per cent of cases.

Grancin and Curen (1971) reported 20.4 per cent of false entries in pedigrees in Pinzgau cattle using blood types.

Investigations of parents and offsprings in certain organised Indian herds revealed five per cent error in the recordings (Mishra and Prabhu, 1971).

Vsyakikh et al (1973) reported 23 5 to 49 5 per cent error in registered parentage in four breeding stations

Singh and Nair (1980) stressed the need for blood typing in sire evaluation programmes, especially when small number of sire mates were used They observed 20 per cent error in the stated parentages in the NDRI herd at Karnal

Stefanescue et al (1982) could confirm the paternity in 15 8 per cent of the Romanian cattle in which 95 per cent of the cases were confirmed by blood groups alone and the remaining 5 per cent of the cases using transferrin and hemoglobin typing

Kaup (1983) in his study among female progeny of German black pied bulls recorded an incidence of wrongly attributed parentage ranging from 0 40 per cent among registrations of different inseminators The incidence was 16 7 per cent where multiple inseminations of the same cow with semen from different sires were carried out In 8 2 per cent of the cases, the bulls registered as the male parent were excluded as the sire

Using data on seven biochemical polymorphism systems, in two Spanish breeds Altarriba et al (1983) reported that

parentage error can be detected in 62 20 and 67 78 per cent of the Friesian bulls and Fighting bulls respectively

Meyer et al (1985) stated that blood typing of the calf and ovum and semen donors in embryo transfer technology was must for the verification of parentage Of the 707 cases tested 1 6 per cent (11 cases) proved to be incorrect probably because the recipient cows were already in calf at the time of transfer

Lazareva and Sukhova (1985) reported 28 5 - 38 1 per cent of incorrect parentage registration on three breeding farms

According to Bagrii and Mechacheryakov (1987) blood typing was a must in USSR under the Ministry of Agriculture regulation, for all sires and replacement female breeding stock (cattle, sheep, pigs and horses) as a check on the accuracy of parentage records However, in the Russian Soviet Federative Socialist Republic, parentage has only been confirmed in 50 7 per cent of all bulls

Bukarov and Sorkovoi (1987) discussed the use of blood groups in detecting errors in parentage records Studies on

AI service in Ireland, using 75 internationally compared monospecific blood group antisera and transferrin, amylase and carbonic anhydrase showed that the percentage of mis identified progeny varied from 0-44 per cent between bulls (Beechinor and Kelly, 1987)

Parentage investigations on imported embryo transfer calves using blood antigens by Wu et al (1988) showed that the blood types of the calves were not inconsistent with those of their parents in USA

Akhmedov et al (1988) studied the immunogenetic characters of Uzbek cattle at a breeding station The recorded paternity was shown to be erroneous in 67 5 per cent of cases by blood typing

Ozbeyaz et al (1990) used blood protein polymorphisms at five different loci in parentage tests and detected false pedigrees in 18 4 per cent cases The probability of excluding a parent using informations at five polymorphic loci was estimated to be 65 7 per cent

Materials and Methods

MATERIALS AND METHODS

One hundred and sixty eight cross-bred cattle maintained at University Livestock Farm, Mannuthy whose blood groups are known, formed the experimental animals for producing blood typing reagents The blood typing of the above animals was carried out at Institute for Animal Breeding, University of Bern, Bern, Switzerland, using the reagents listed in Table 2

3 1 Production of Blood typing reagents by iso-immunisation

The blood group reagents were produced by iso immunisation and hetero-immunisation in rabbits Mostly iso immunisations were carried out so that absorption studies were easy, though high titred anti-serum can be obtained through hetero immunisation

Donor and recipient animals for iso immunisation were selected in such a way that they differed only in a few factors in their blood types The fundamental principle involved is that, antibodies are produced in the serum of recipient animal to those antigenic blood group factors that are absent in the recipient, but present in the donor

Table 2 List of blood typing reagents used in the Blood typing laboratory - Bern Switzerland

Systems	Reagents
A	A, H
B	B G_1, G_2 G_3, I, K, O_1, O_2, O_3, P, Q, T, Y_2, A D E_1, E_2, E_3, G_3, I , J , K , O , Q , Y
C	C_1, C_2 R W, X_1 X_2, L
F	F, V
J	J
L	L
M	M
S	S, U_1, H , U H
Z	Z
R	R
N T	N ,T

Usually dam-daughter pairs were selected to minimise the difference in the antigenic factors. When they are not available, animals with minor differences in the blood group factors were selected as donor recipient pairs.

Blood samples were collected aseptically by jugular vein puncture, using a 16 G hypodermic sterile needle. In the case of donor animals, whole blood (5-10 ml) was collected in a sterile tube with freshly prepared anticoagulant (isotonic solution of sodium citrate). Samples were collected for serum separation in a dry clean test tube, from the recipient animal. Samples with anti-coagulant were also collected from recipient animals and these samples were stored under refrigeration until further use.

All the animals were screened for the presence of any antibodies in their serum before the start of immunisations. This was done by standard haemolytic test as described by Ferguson (1941) with a panel of erythrocytes. A positive reaction (one half or more of the cells lysed) indicated presence of antibodies. Such positive animals were excluded from the immunisation.

The haemolytic tests were set up in microtitre plates (Laxbro) having 96 wells The tests were set up at room temperature but the incubation was done at 37°C Freshly collected serum from rabbits was used as the complement source The sera from rabbits were checked for the presence of antibodies against cattle red blood cells before using them as complement The haemolytic test consisted of incubation of fifty micro litre of test serum with 25 micro litre of two percent suspension of thrice washed red blood cells and 25 micro litre of complement Complement and saline controls were always set up with each test The shaking of the plates was done by Microshaker

The first reading was taken after 30 minutes of the incubation and was recorded as follows with a lead pencil

0 All cells intact, supernatant saline is clear and colourless

± About 50 per cent of the cells were lysed and much light passed through the fluid

+ All cells were lysed liquid transparent with sparkling red colour

Second reading was taken 90 minutes after the first reading and was marked in blue ink Third reading was taken 90 minutes after the second and was marked with a red pen The degrees of lysis were recorded from 0 (no visible haemolysis) to 4 (complete haemolysis) The second and third readings were recorded as follows

0 - All cells intact and settled at bottom supernatant clear

0+ - Almost all cells intact and settled at bottom Supernatant slightly reddish

Tr (Trace) Nearly ten per cent of cells were lysed super natant was reddish coloured

1 - Twenty per cent of cells were lysed supernatant was red

2 More than 50 per cent of cells were lysed, the unlysed cells settled at the bottom in the form of a small button or ring

3 - Nearly all cells were lysed, supernatant was bright red, when the plate was shaken liquid became cloudy

4 All cells were lysed, liquid was sparkling red and remained so even after shaking

The recipient animals were immunised by injecting the thrice washed erythrocytes of donor animals through intra muscular route The whole blood was centrifuged at 1030 g for ten minutes for packing the cells The dose of donor cells was increased in each subsequent injection Two injections were carried out in each week and usually six injections were necessary to produce sufficient antibody titre. The schedule of immunisation followed is presented in table 3

The serum samples collected during each immunisation were diluted in a serial two fold manner in normal saline solution These were tested against the donor cells by the haemolytic technique described earlier The highest dilution in which there was definite lysis was taken as the titre of the anti-serum

When titre of antibodies reached > 1/16, the recipient animal was bled for production of the immune serum Re-immunisations were performed in those animals which did not show requisite titre even after five to six injections The same was carried out after two to three months of first series of immunisations Usually a litre of whole blood was collected for the separation of immune antibodies The separated serum was kept at 20°C until used

Table 3 Immunisation Schedule

Day	Quantity of packed erythrocyte used (ml)	Quantity of NSS (ml)	Route of administration
1	2	2	1/m
4	4	2	,,
7	8	2	,
10	10	2	,,
13	12	2	
16	14	2	,

Once a high titred anti-serum was obtained it was suitably diluted for antibody absorption technique (usually 2 dilutions lower than the highest titre of the poly valent anti-serum)

The unabsorbed polyvalent anti-serum was tested against a series of cells (whose blood types are known) which were positive and negative for the expected factors in the immune serum From the results of the haemolytic test, the antibodies present in the immune serum were determined Cells showing weak reactions were utilized for absorption studies (Figs 1,2,3)

Absorptions were carried out in polystyrene plastic centrifuge tubes in aliquotes of four millilitres of diluted immune serum samples One millilitre of washed packed cells from each animal (20 per cent of the diluted serum sample) was added to each tube which was considered as different absorptions Each sample was mixed thoroughly by gentle inversion of the tubes closing the mouth of the tube Samples were incubated at room temperature for 30 minutes with an intermittent mixing after 15 minutes of incubation

ABSORPTIONS AND TEST FOR UNITY OF L' REAGENT

IMMUNOGENETICS LABORATORY

CENTRE FOR ADVANCED STUDIES IN ANIMAL GENETICS & BREEDIN

KERALA AGRICULTURAL UNIVERSITY

Mannuthy Trichu Dist Kerala

FIG. 1. RECORD OF HAEMOLYTIC TESTS

Date	Time Set up		Time	Temp	Read by
30 4 91	10 30 AM	1st Reading	11.00	33	MJV
		2nd Reading	12.30	33	MJV
		3rd Reading	2 00	33	MJV

Reagent / Animal No	1	2	3	L 4	5	6	7	8	9	10	1	12	13	14	15	16	L POSITIVE	Controls Complement	Controls Saline
	TEST FOR UNITY																		
1001	4	4	4	4	4	4	4	0	0	0	0	0	0	0			+	0	0
2002	4	4	4	4	4	4	4	0	0	0	0	0	0	0			+	0	0
3046	4	4	4	2	2	2	2	0	0	0	0	0	0	0			+	0	0
4051	4	0	4	0	0	0	0	0	0	0	0	0	0	0			+	0	0
5068	4	4	4	0	0	0	0	0	0	0	0	0	0	0			-	0	0
6078	4	4	4	4	4	4	4	0	0	0	0	0	0	0			+	0	0
7096	Tr	0	0	0	0	0	0	0	0	0	0	0	0	0			+	0	0
8126	4	0	3	0	0	0	0	0	0	0	0	0	0	0			-	0	0
9157	4	0	3	0	0	0	0	0	0	0	0	0	0	0			-	0	0
10207	4	0	0	0	0	0	0	0	0	0	0	0	0	0			-	0	0
11216	4	0	4	0	0	0	0	0	0	0	0	0	0	0			+	0	0
12248	0	0	0	0	0	0	0	0	0	0	0	0	0	0			-	0	0
13269	Tr	0	0	0	0	0	0	0	0	0	0	0	0	0			-	0	0
14293	4	0	Tr	Tr	Tr	Tr	Tr	0	0	0	0	0	0	0			+	0	0
15348	4	4	4	1	2	2	2	0	0	0	0	0	0	0			+	0	0
16416	4	4	4	1	2	2	2	0	0	0	0	0	0	0			+	0	0
17437	4	0	0	Tr	Tr	Tr	Tr	0	0	0	0	0	0	0			-	0	0
18453	4	0	0	0	0	0	0	0	0	0	0	0	0	0			-	0	0
19474	4	4	0	0	0	0	0	0	0	0	0	0	0	0			-	0	0
20600	4	4	4	4	4	4	4	0	0	0	0	0	0	0			+	0	0
21626	4	4	4	4	4	4	4	0	0	0	0	0	0	0			+	0	0
22704	4	4	4	4	4	4	4	0	0	0	0	0	0	0			+	0	0
23705	4	4	0	0	4	4	4	0	0	0	0	0	0	0			-	0	0
24731	4	4	0	0	4	4	4	0	0	0	0	0	0	0			-	0	0
25743	4	0	0	0	0	0	0	0	0	0	0	0	0	0			-	0	0
26749	3	0	0	0	0	0	0	0	0	0	0	0	0	0			-	0	0
27814	0	0	0	0	0	0	0	0	0	0	0	0	0	0			-	0	0
28826	4	4	4	4	4	4	4	0	0	0	0	0	0	0			+	0	0
29840	4	4	0	0	0	0	0	0	0	0	0	0	0	0			-	0	0
30 24001	4	4	4	4	4	4	4	0	0	0	0	0	0	0			+	0	0
31 24159	4	0	4	0	0	0	0	0	0	0	0	0	0	0			-	0	0
32					(068)	(126)	(24159)	(001)	(002)	(293)	(348)	(416)	(487)	(24001)					
33	(814) 1/16			(474)	+	+	+	+	+	+	+	+	+	+					
34		30%	30%		(474)	(474)	(474)	(474)	(474)	(474)	(474)	(474)	(474)	(474)					
35				+	+	+	+	+	+	+	+	+	+	+					
36	826	(157)	(474)	(157)	(157)	(157)	(157)	(157)	(157)	(157)	(157)	(157)	(157)	(157)					

KAUP 69 000 4 87

↑ Reagent

Sorbing cells used

ABSORPTIONS AND TEST FOR UNITY OF F REAGENT

IMMUNOGENETICS LABORATORY

CENTRE FOR ADVANCED STUDIES IN ANIMAL GENETICS & BREEDING

KERALA AGRICULTURAL UNIVERSITY

Mannuthy Trichur Dist Kerala

FIG.2. RECORD OF HAEMOLYTIC TESTS

Date	Time Set up		Time	Temp	Read by
21 6 91	10 45 AM	1st Reading	11 15	33	MJV
		2nd Reading	12 45	33	MJV
		3rd Reading	2 15	33	MJV

Reagent / Animal No	1	F_2	F_3	F_4	F_5	6	7	8	TEST FOR UNITY 9	10	11	12	13	14	FV Blood type	16	Controls Complement	Saline
1 001	4	3	4	3	3	2	3	4	0	0	0	0	0		F F		0	0
2 002	4	4	4	4	4	4	4	4	0	0	0	0	0		F F		0	0
3 025	4	0	0	0	0	0	0	0	0	0	0	0	0		V V		0	0
4 046	4	0	0	0	0	0	0	0	0	0	0	0	0		V V		0	0
5 068	4	2	1	2	2	2	2	1	0	0	0	0	0		F V		0	0
6 078	4	2	1	1	2	0	0	Tr	0	0	0	0	0		F V		0	0
7 207	4	4	4	4	4	3	3	4	0	0	0	0	0		F F		0	0
8 207	4	4	3	1	4	4	3	2	0	0	0	0	0		F F		0	0
9 211	4	0	0	0	0	0	0	0	0	0	0	0	0		V V		0	0
10 248	4	2	1	1	1	0	0	0	0	0	0	0	0		F V		0	0
11 293	4	4	4	4	4	4	4	4	0	0	0	0	0		F V		0	0
12 335	4	3	4	2	3	3	3	3	0	0	0	0	0		F F		0	0
13 316	4	2	1	1	1	0	0	0	0	0	0	0	0		F V		0	0
14 400	4	4	4	4	4	4	4	2	0	0	0	0	0		F F		0	0
15 26	4	2	3	3	3	2	2	2	0	0	0	0	0		F F		0	0
16 704	4	2	2	2	2	1	1	0	0	0	0	0	0		F V		0	0
17 717	4	3	3	3	3	0	0	2	0	0	0	0	0		F V		0	0
18 749	4	4	2	3	3	2	2	3	0	0	0	0	0		F F		0	0
19 814	4	0	0	0	0	0	0	0	0	0	0	0	0		F V		0	0
20 840	4	0	0	0	0	0	0	0	0	0	0	0	0		V V		0	0
21 4001	4	3	2	2	4	2	1	2	0	0	0	0	0		F F		0	0
22 004	4	4	3	3	3	3	3	3	0	0	0	0	0		F F		0	0
23 013	4	4	4	4	4	4	4	4	0	0	0	0	0		F F		0	0
24 016	4	3	3	4	2	2	2	3	0	0	0	0	0		F F		0	0
25																		
26																		
27																		
28																		
29																		
30																		
31																		
32																		
33																		
34						(211)	(211)	(840)	(001)	(002)	(131)	(600)	(626)					
35						+	+	+	+	+	+	+	+					
36																		
	293 (L79 RABBIT) 1 512 UA	(025) 20%	(211)	(840)	(046)	(025)	(046)	(211)	(025)	(025)	(025)	(025)	(025)					

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↑ ↑ ↑

ent

A orbing cells used

ABSORPTIONS AND TEST FOR UNITY OF NEW REAGENT M36

IMMUNOGENETICS LABORATORY

CENTRE FOR ADVANCED STUDIES IN ANIMAL GENETICS & BREEDING

KERALA AGRICULTURAL UNIVERSITY

Mannuthy Trichur Dt Kerala

FIG 3 RECORD OF HAEMOLYTIC TESTS

Date	Time Set up	s Reading	Time	Temp	Read by
4 6 91	11 00 AM	2nd Reading	11 30	33	MJV
		3 d Reading	1 00	33	MJV
			2 00	33	MJV

Reagen / Animal No		2	3		5	M36 TEST FOR UNITY 6		8	9	0	1	EXP FACTORS 12 G_3	13 A^1	14 E^1_3	15 O^1	16 F_1	Control Complement	Serum
001	4	4	2	4	4	3	4	4	0	0	0	-	+	-	-	+ +	0	0
002	4	Tr	0	0	0	0	0	0	0	0	0	+	+	+	+	+ +	0	0
023	1	Tr	0	0	0	0	0	0	0	0	0	+	-	-	+	+ -	0	0
042	1	Tr	0	0	0	0	0	0	0	0	0	-	+	+	+	+	0	0
056	1	Tr	0	0	0	0	0	0	0	0	0	-	-	+	+	+	0	0
068	1	Tr	4	4	4	4	4	3	0	0	0	+	+	-	-	+ -	0	0
072	1	Tr	0	0	0	0	0	0	0	0	0	+	+	+	+	+ -	0	0
078	1	Tr	0	0	0	0	0	0	0	0	0	-	-	*	-	+ +	0	0
096	0	0	0	0	0	0	0	0	0	0	0	-	-	+	-	+ +	0	0
118	1	0	0	0	0	0	0	0	0	0	0	+		+	+	+	0	0
131	4	1	0	0	0	0	0	0	0	0	0	+	+	+	+	+	0	0
207	4	2	0	0	Tr	0	0	0	0	0	0	-	+	+	+	+ +	0	0
207	3	Tr	0	0	0	0	0	0	0	0	0		+	-	+	+	0	0
268	3	0	0	0	0	0	0	0	0	0	0	+	+	-	+	+ -	0	0
335	3	1	0	0	Tr	0	0	0	0	0	0	+	-	+	+	-	0	0
414	3	2	0	0	0	0	0	0	0	0	0	+		+	+	+ -	0	0
453	3	3	0	0	0	0	0	0	0	0	0	+	-	-	+	+ -	0	0
474	3	1	0	0	0	0	0	0	0	0	0	-	-	+	+	+ -	0	0
800	3	1	0	0	0	0	0	0	0	0	0	-	+	-		+ -	0	0
826	4	3	0	0	0	0	0	0	0	0	0	+	+	+	+	+ +	0	0
704	4	1	0	0	0	0	0	0	0	0	0	+	-	+	+	+ +	0	0
717	4	0	0	0	0	0	0	0	0	0	0	-	±	+	-	+ +	0	0
731	4	3	3	4	0	0	0	0	0	0	0	+	+	-	+	+ -	0	0
743	4	2	0	0	0	0	0	0	0	0	0	+	+	+	+	+ -	0	0
749	3	0	0	0	0	0	0	0	0	0	0	+		-	+	+ -	0	0
812	3	Tr	0	0	0	0	0	0	0	0	0		-	+	-	+ +	0	0
814	3	2	0	0	0	0	0	0	0	0	0			-	+	+ -	0	0
24001	3	0	0	0	0	0	0	0	0	0	0	+	+	+	+	+ +	0	0
24159	3	Tr	0	0	0	0	0	0	0	0	0		-	-	-	+	0	0
30013	4	4	3	4	3	3	3	3	0	0	0	+		+	±	+	0	0
31							(042)	(731)	(001)	(068)	(013)							
32	UA						+	+	+	+	+							
33	1/16	20%	(002)	(207)	(600)	(626)	(626)	(626)	(626)	(626)	(626)							
34			+	+	+	+	+	+	+	+	+							
35	(840)	(24001)	(24001)	(24001)	(24001)	(24001)	(24001)	(24001)	(24001)	(24001)	(24001)							
36	207																	

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Reagent Absorbing cells used

The contents were centrifuged after incubation and the supernatant saved and stored at 4 C in a refrigerator until test was carried out

Haemolytic test of absorbed serum was carried out using all the cells involved in the absorption study The donor and recipient cells and a few cells selected at random (with known blood type) that possessed factor against which the absorption was directed, were used The test results were scrutinised for any weak reactions and for complete exhaustion of the specific antibodies demonstrated by a negative lytic test with the absorbing blood

In cases of incomplete absorptions, the absorptions were repeated by increasing the quantity of absorbing cells

The weakly reacting cells after the absorption were used in addition to the first cells and the entire process of absorption was repeated until a clear univalent reagent obtained (indicated by 0 and 4 reactions only in the haemolytic test of absorbed serum)

The absorbed serum was then subjected to test for unity For this, the absorbed serum (suspected for a monospecific reagent) was again absorbed with each of the cells showing reaction The resultant antiserum was then tested with all the cells involved in the absorption studies and the donor and recipient cells If the serum showed no reaction with any of the cells in the haemolytic test it was concluded that the absorbed serum contained antibodies against only one antigenic factor and that was considered as an unit reagent

If the absorptions were not successful, several combinations of cells and their concentrations and dilutions of serum were tried until the serum containing a unit reagent was obtained

Once a unit reagent was identified, large quantities of the same was produced by carrying out the absorptions in bulk quantities The reagent samples were stored in aliquotes of four milli litres in screw capped plastic serum storage vials (Laxbro) at -70°C in a deep freezer, to avoid frequent thawing and freezing

3 2 Production of blood typing reagents by Hetero-immunisation

Six rabbits belonging to New Zealand White and Soviet chinchilla cross-breds were tested for naturally occuring antibodies against bovine red cells by standard haemolytic test

Fresh blood from the donor cows was washed three times in normal saline and 20 per cent cell suspension in saline was made The same was injected through the external marginal vein of the rabbit at the rate of one milli litre per kilogram body weight

The injections were repeated weekly once until sufficient titre was obtained Titration of serum samples and absorption studies were carried out in the same manner as described earlier for iso-immunisation

3 3 Production of blood typing reagent from colostrum of immunised cow

Procedure described by Duneic et al (1979) was followed with modifications for preparing blood typing reagent from colostrum of an immunised cow Re

immunisations were carried out in one pregnant animal (No 814) three weeks before the expected date of calving Blood samples (about 10 ml) and colostrum samples (about 2 litres) were taken on the day of calving The colostrum was skimmed twice at 5000 rpm for 15 minutes for removing the fat The skimmed colostrum was then treated with calf rennet at the rate of five grams per litre of whey and incubated at 45°C for two hours in a water bath The coagulum was separated and filtered using Whatman filter paper No 1 The filtrate was then inactivated at 56°C for 30 minutes and stored in aliquotes of 10 ml at -70°C in deep freezer

The whey was titrated together with the anti serum obtained from the same recipient cow on the day of calving for comparison of antibody titre The whey was subjected to absorption techniques as described earlier for anti serum

3 4 Naturally Occuring antibodies

Serum from two animals (animal Nos 248 and 743) were identified to possess naturally occurring antibodies against the J substance by the Institute for Animal Breeding, University of Bern, Switzerland These sera samples were used as J reagent for blood typing The two sera samples

were titrated fortnightly by the haemolytic test to assess the variations in the anti-J substance in serum

Blood typing

Four hundred and eleven cross bred cattle maintained at University Livestock Farm, Mannuthy and Cattle Breeding Farm, Thumburmuzhi were blood typed The population included 113 Jersey crosses 162 Brown swiss crosses and 136 Holstein Friesian crosses

Animals were typed for erythrocytic antigens using 28 serologically different blood group reagents (14 internationally comparable and 14 new reagents listed in Table 4) by standard haemolytic test as described earlier (Fig 4) Of this seventeen reagents were already available in the laboratory and eleven were produced during the course of this study The relative frequencies of occurrence of the various blood group factors in the cross bred animals were estimated

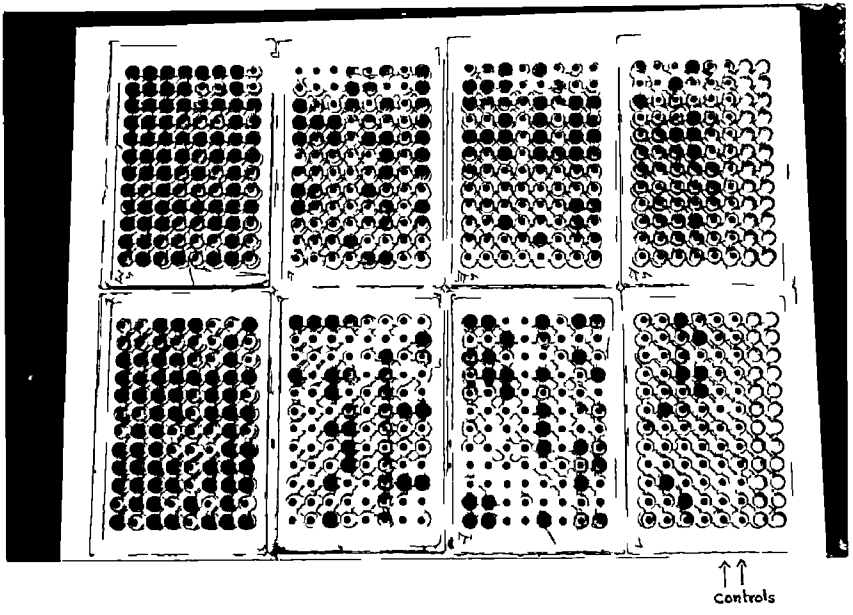


Fig 4. Blood typing of 24 cows with 28 reagents

Table 4 Reagents used in blood typing

Donor	Recipient	Dilution of Absorption	Blood cells used in Absorption	Source	provisional Designation	Remarks
814	626	1/16	(002) or (131)	Iso immune serum	M_1	--
826	814	1/16	(002) or (S_3) 207 each	,	M_2	--
248	--	-	Naturally Occuring	-	M_3	J
815	157	1/16	(329)+ (474)+ (704) + (743)	Iso-immune serum	M_9	-
815	157	1/16	(626) + (001)+ (749)	,	M_{10}	-
207	840	1/16	(24154)10/+ (626)10/ + (009)5/	,	M_{12}	--
743	-	-	Naturally occuring	-	M_{14}	J
24154	126	1/16	(207) 207	Iso-immune serum	M_{15}	--
704	453	1/16	(474) + (002)	,,	M_{16}	--

(Contd)

(Table 4 Contd)

Donor	Recipient	Dilution of Absorption	Blood cells used in Absorption	Source	provisional Designation	Remarks
126	24154	1/16	(002)		M_{17}	--
705	24001	1/16	(207) 20/	,	M_{18}	--
705	24001	1/16	(749) or (453) or (626)20/ each	,,	M_{19}	--
453	704	1/32	(002) 25/ + (T 622)20%+ (046) 20%	Iso immune serum	M_{20}	B
HF_2	139	1/32	(248) 20%	,,	M_{21}	Z
HF_2	139	1/32	(002) 20%		M_{22}	V
001	002	1/16	(207) 20%	,,	M_{23}	--
704	453	1/16	(046) 40/	,	M_{24}	E_3
704	453	1/16	(001) + (002)+ (042) 10%		M_{25}	S
826	814	1/16	(474) + (157) 30% each	,,	M_{26}	L
335	187	1/16	(126) 20%	,,	M_{27}	H

(Contd)

(Table 4 Contd)

Donor	Recipient	Dilution of Absorption	Blood cells used in Absorption	Source	provisional Designation	Remarks
731	749	1/16	(001) or (040) 30% each	,,	M_{28}	Y_2
815	157	1/16	(24159) + (626) 20% each	,	M_{29}	C_2
814	626	1/32	(437) 30%	,,	M_{31}	X_1
248	042	1/32	(348) + (743) 20% each	,	M_{32}	--
815	157	1/16	(704)+(743)+ (474)+(24001) 20% each	Iso-immune serum	M_{33}	--
293	L_{79}	1/512	(025) 20/	Hetero-immune serum	M_{34}	F
24001	705	1/64	(001)+(078)+ (T 207) 20% each	Iso immune serum	M_{35}	R
207	840	1/16	(24001)+(626) 20% each	,,	M_{3o}	--

Haemoglobin polymorphism

The red cells were washed three times in normal saline and ten per cent haemolysate was prepared Haemoglobin separation was accomplished by polyacrylamide gel electrophoresis in horizontal dimension as described by Gahne et al (1977) with modifications

Buffers and solutions

a Composition of electrode buffer

Tris	40 4 g
EDTA	4 0 g
Boric acid	3 0 g
Distilled water ad	2 L

The above ingredients were dissolved in distilled water and the pH was adjusted to 8 9 with 4 per cent boric acid solution

b Acrylamide stock solution (A)

Thirty two grams of Acrylamide (Sisco) and 0 8 g of N - N methylene bis-acrylamide (Sisco) were dissolved in 100 ml distilled water and filtered

c Gel buffer stock solution (B)

To 12 5 ml of 1 5M Tris solution (2 27 g of Tris hydroxy methyl amino-methane in 12 5 ml distilled water) were added 11 25 ml of distilled water, 0 075 ml of N N N N tetra methylene diamine (TEMED) and 0 04 ml of 2 mercapto ethanol The pH was adjusted to 8 3 with conc H_2SO_4 (approximately 0 3 ml) and the final volume was adjusted to 25 ml with distilled water

d Ammonium per sulphate (C)

Hundred grams of Ammonium per sulphate was dissolved in 50 ml distilled water

The above solutions were prepared and used on the same day

Working gel solution

The working gel solution was prepared just before use The composition of the solution for 10 per cent acrylamide solution

Acrylamide (A)	6 64 ml
Gel buffer (B)	5 32 ml
Distilled water	4 00 ml
TEMED	0 03 ml
Amm Per sulphate (C)	5 32 ml

e Fixing solution

Methanol	250 ml
Acetic acid	60 ml
Distilled water	1000 ml

f Staining solution

Coomassie brilliant blue R 250	1 25 g
Methanol	227 00 ml
Glacial Acetic acid	46 00 ml
Distilled water	227 00 ml

Dye was dissolved in solution of Methanol and distilled water Acetic acid was then added and stored in dark bottles

g Destaining solution

Ethanol	1500 ml
Acetic acid	500 ml
Distilled water	5000 ml

h Preserving solution

Ethanol	300 ml
Acetic acid	100 ml
Glycerol	100 ml
Distilled water to make upto	1000 ml

Casting of the gel

A continous buffer system of 10 per cent Acrylamide solution at pH 8 3 was used

The cell was made with two plates of the same size One of the plates used was an acrylic sheet with slots on it The plate had a frame on all sides with 1 5 mm thickness which formed the thickness of the gel The other was a thick glass plate the two plates were held together with vacuum grease on all sides to ensure tight sealing Paper clips were applied on all four sides and placed vertically while casting the gel

The working gel solution was prepared just before use with the addition of Ammonium per sulphate at the last. The solution was mixed carefully without introducing too much air. This solution was poured into the cell through the funnel at the top. Air bubbles if any, were removed by tapping. Polymerisation reaction was completed in 30 minutes.

Electrophoresis

When the polymerisation was completed, the gel was separated from the Acrylic sheet and washed with distilled water. The glass plate with the gel was kept on the electrophoretic chamber with the buffer. Proper connections were made with the chamber and the electrophoretic powerpack. The circuit was completed when the gel was connected to the buffer solution using wet wicks. The wicks were made using whatman filter paper sheets No.1. They overlapped the gel by 10 to 12 mm. The edges of the layers of filter paper sheets were made level and the opposite wicks were kept parallel to each other to ensure uniform voltage gradient along the gel.

Pre-electrophoresis was carried out before loading the sample to remove unwanted ions or charged particles on the

gel This was done after setting the voltage at 250 V (for nine samples) and adjusting the current to 15 mA either by adding or removing electrode buffer in the chamber or by altering the number of layers of filter paper used as wick

Twenty micro litres of each sample was charged into the slots made on the gel using a micro-syringe as quickly as possible to avoid diffusion of the initial samples

The samples were subjected to electrophoresis at 250 V at 15 mA Once a clear separation of the protein variants was observed, which usually occurred with in $1\frac{1}{2}$ 2 hours, the electrophoresis was stopped and the gel was transferred to the fixative

Gel fixation and staining

The protein variants were fixed on to the gel by immersing the gel in Fixing solution for one to two hours at room temperature This avoided loss of soluble proteins and minimised the diffusion The gel was kept in the staining solution for two hours

Destaining

The gel was then transferred to the destaining solution and kept for 3-4 hours The solution was frequently changed until the bandless portions of the gel became colourless

Preservation

The gels were preserved in the preserving solution for sufficiently long duration

Genetic studies

Blood groups

Frequencies of alleles in the co dominant system (FV) were calculated by direct counting method

Simple dominant systems containing 2 alleles (L and Z) were analysed by obtaining the square root of the frequency of the homozygous recessive genotype (Li, 1955) The following formula was used $q \sqrt{\frac{R}{G}}$ where, q is the frequency of R recessive group (the class with no detectable antigenic factor) in G population

The population was tested for genetic equilibrium with respect to FV locus by methods described by Falconer (1981)

The mode of inheritance of cellular antigens were studied from the data on 88 Sire-dam offspring sets (Ferguson, 1941) The matings were divided into three types viz a) those in which both parents possessed the particular antigen, b) those in which only one parent had the antigen and c) those in which the cells of neither parent contained the antigen The offsprings of each mating were classified into two groups those with antigens and those without antigens and was compared with Mendelian ratios of inheritance

Haemoglobin

The genotype and gene frequencies were estimated by direct counting method

The heterozygosity at haemoglobin locus was estimated as per the method described by Nei and Roy Choudhury (1974) The hetero-zygosity of k^{th} locus (h_k) was defined as

h_k 1 - jk

where jk x_1^2 is the frequency of homozygotes at k^{th} locus

x_1 $\frac{n_1}{n}$ denotes the gene frequency of the 1^{th} allele at the k^{th} locus

The population was tested for genetic equilibrium at haemoglobin locus by employing x^2 test of significance

The mode of inheritance of haemoglobin variants were studied from the data on 88 sire-dam-offspring sets

Parentage studies

Eighty eight sire dam offspring sets belonging to seven sire families from the two farms cited earlier, were used in the study All the calves tested were above 3 months of age The accuracy of pedigree records wase checked using blood factors and haemoglobin types of these animals

RESULTS

4 1 Production of blood typing reagents by iso immunisations

All the animals were screened for the presence of any antibodies against the bovine red blood cells before immunisation and all were found to be negative The donor and recipient animals were selected for each immunisation and the expected antibodies in each immunisation and the titre obtained at the time of harvesting are presented in table 5 (i), 5 (ii) and 5 (iii) In all, thirty one iso-immuniations were carried out and twenty two polyvalent sera were harvested Of this in eleven cases the polyvalent anti-sera were harvested after the primary immunisation and the titre varied from 1/16 (in Animal Nos. 025 211 and 235) to 1/512 (n Animal No V 024) In all other cases the recipient animals were re-immunised Only eleven animals responded to this and showed sufficient antibody titre varying from 1/64 (Animal No 072) to 1/512 (Animal No 81+ and V 016)

Apart from the twenty two polyvalent iso-immune sera produced during the period of study, six high titred polyvalent immune sera already harvested and stored in the blood group laboratory, were also subjected to absorptions for producing the monovalent blood typing reagents (Table 6)

Table 5(iii) Re-iso immunisations non-responders

Donor Animal No	Recipient Animal No	Expected Antibodies	Titre of Antiserum after Booster Dose
480	490	H, B, I , X_1, M	1/16
416	078	A, H, P, G , X_2, S	1/32
480	178	A, B G_3, V, M	Nil
480	844	I , Q', M	,,
335	040	G_1, E_3, O , C_2, H	
001	080	Q, N, L U , Z	
V016	V013	A, I, J , L	
T-207	A-269	Q, Y_2 D , Y W, R T	,,
078	416	G_2, Y_2, T , J	,
703	056	X_1, F	,,
T-056	703	T, S	,,

Table 6 Polyvalent iso-immune sera harvested earlier and used for present study

Donor Animal No	Recipient Animal No	Expected Anti-bodies	Maximum titre
815	157	Q, D', Q , C_2 X_1, X_2, L, S,N', T	1/256
814	626	A, O_1, C_2, R, X_1, X_2,	1/128
24001	705	A H, Y_2, A , K , R, X_1, L , L, S, T R	1/128
248	042	J , C_2, R, X_1 L	1/64
705	24001	W, O , V	1/32
207	840	G_3, A , E_3, O , F, L	1/128

Eight blood typing reagents were produced from isoimmune antisera and were tentatively designated as M_{27}, M_{28}, M_{29}, M_{31}, M_{32}, M_{33}, M_{35} and M_{36} Of these five are comparable to internationally accepted reagents viz H' (M_{27}), Y_2 (M_{28}), $C_2(M_{29})$, X_1 (M_{31}) and R(M_{35}) The donor and recipient animals the titre of immune serum and absorbing cells for each reagent are presented in table 7

4 2 Production of blood typing reagents by hetero-immunization

Eight hetero-immunizations were carried out in rabbits and the details of hetero-immunisation are presented in Table 8 Of these, seven rabbits showed sufficient antibody titre varying from 1/256 (No 2864) to 1/4096 (No L_{79}) after the primary immunisation One animal (D_{55}) did not show any response Out of the seven polyvalent hetero-immune sera harvested, only two reagents (M_{30} and M_{34}) could be produced (Table 7) The reagent M_{34} was comparable with the F reagent

Table 7. Blood typing reagents produced

Donor	Recipient	Dilution of Absorption	Blood cells used in Absorption	Source	provisional Designation	Remarks
826	814	1/512	(474) + (157) 30% each	Iso-Immunisation Colostrum	M_{26}	L
335	187	1/16	(126) 20%	Iso-immune serum	M_{27}	H''
731	749	1/16	(001) or (040) 30% each	,,	M_{28}	Y_2
815	157	1/16	(24159) + (626) 20% each	''	M_{29}	C_2
216	133	1/128	(096) 20%	Hetero-immune serum	M_{30}	--
814	626	1/32	(437) 30%	Iso-immune serum	M_{31}	X_1
248	042	1/32	(348) + (743) 20% each	,,	M_{32}	--
815	157	1/16	(704) + (743) + (474) + (24001) 20% each	,,	M_{33}	--
293	L_{79}	1/512	(025)or (211)or (840) or (046) 20% each	Hetero-Immune serum	M_{34}	F
24001	705	1/64	(001)+ (078)+ (T-207)20% each	Iso-immune serum	M_{35}	R
207	840	1/16	(24001) + (626) 20% each	''	M_{36}	--

Table 8. Hetero - immunisations carried out in Rabbits

Donor (Bovine)	Recipient (Rabbits)	Titre of anti-serum (Ist immunisation)	Titre of anti-serum (IInd immunisation)
HF_2	L_{79}	1/1024	--
,,	D_{55}	1/512	--
293	L_{79}	1/4096	--
,,	D_{55}	--	--
216	133	1/2048	1/4096
,,	D_7	1/2048	--
056	2864	1/256	--
,,	D_{60}	1/2048	--

4.3. Production of blood typing reagents from colostrum

One pregnant cow (Animal No. 814) was immunised with erythrocytes of the donor animal (Animal No. 826) three weeks before the expected calving date. The titre of this colostrum was 1/4096. The colostrum was then subjected to absorption in a dilution of 1:512, with cells of 474 and 157 animal numbers, at a concentration of 30 per cent each of the diluted colostrum. The resultant reagent was designated as M_{26} and was comparable with the L reagent (Table 7).

All the reagents produced were properly labelled and stored in aliquotes of four millilitre in screw capped plastic storage vials at -70°C in an Ultra Low Temperature deep freezer.

4.4. Naturally occurring antibodies

The sera from animal Nos. 248 and 743 were used as J reagent for blood typing. It can be seen from the Fig. 5 that the serum of 743 reacted with only a few cells positive for anti-J from 248. Variations were observed in both cases when the anti-J titre was assessed fortnightly (Table 9). No association could be noticed with the seasons of the year (Fig. 6).

Fig.5. REACTION PATTERN OF ANTI-J FROM ANIMAL No.s 248 & 743

IMMUNOGENETICS LABORATORY

CENTRE FOR ADVANCED STUDIES IN ANIMAL GENETICS & BREEDING

KERALA AGRICULTURAL UNIVERSITY

Mannuthy, Trichur Dist. Kerala

RECORD OF HAEMOLYTIC TESTS

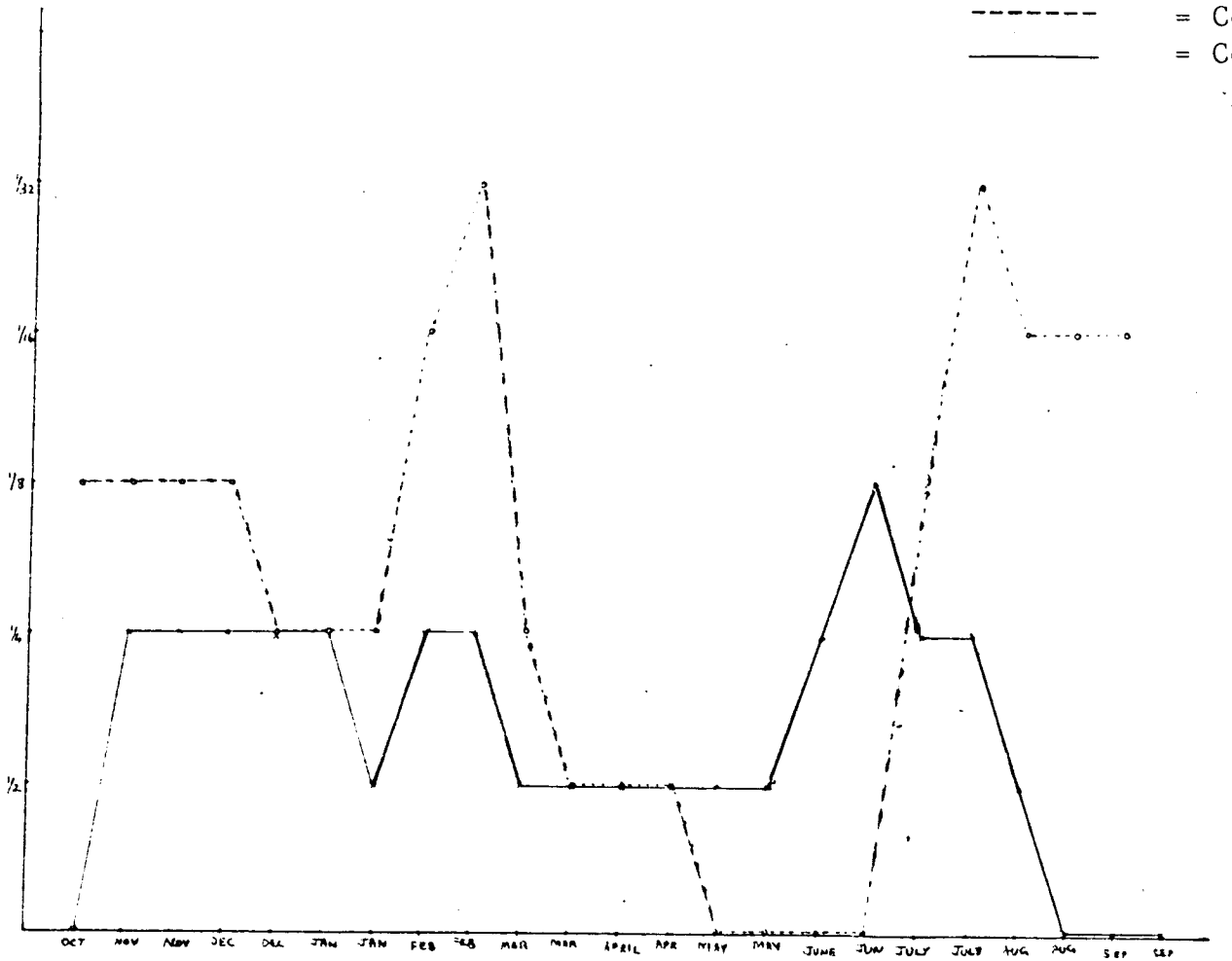
Date	Time Set up		Time	Temp.	Read by
		1st Reading	11.00	37	MJV
		2nd Reading	12.30	37	MJV
8.1.92	10.30 A.M	3rd Reading	2.00	37	MJV

Reagent	J REAGENT																Controls	
Animal No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Complement	Saline
	248		743															
1 002	3		0														0	0
2 022	0		0														0	0
3 023	4		0														0	0
4 046	0		0														0	0
T 5 046	4		2														0	0
6 051	4		0														0	0
7 093	0		0														0	0
B 8 104	0		0														0	0
B 9 119	3		2														0	0
T 10 134	0		0														0	0
B 11 140	4		0														0	0
12 160	4		0														0	0
T 13 162	4		0														0	0
M 14 179	0		0														0	0
15 182	0		0														0	0
M 16 207	3		0														0	0
17 211	4		4														0	0
18 216	4		0														0	0
19 234	3		0														0	0
20 235	2		0														0	0
M 21 235	4		2														0	0
22 239	4		4														0	0
23 262	4		4														0	0
24 348	4		0														0	0
25 349	4		0														0	0
26 437	3		2														0	0
27 453	4		3														0	0
28 469	4		3														0	0
29 600	4		0														0	0
30 626	4		4														0	0
31 640	1		0														0	0
32 667	0		0														0	0
33 717	0		0														0	0
34 749	4		0														0	0
35 826	4		0														0	0
36 24001	0		0														0	0

KAUP 89/1000/4/87

Fig. 6

X AXIS 2 cm = 1 month
Y AXIS 3 cm = 2 fold dilution of serum
------- = Cow No.248
——— = Cow No.743



VARIATION IN ANTI-J TITRE OBSERVED IN CROSS-BRED COWS OVER A PERIOD OF 12 MONTHS AT ULF-MANNUTHY

Table 9. Naturally occurring antibodies and its variation

Date of collection of serum	Animal No. 248	Animal No. 743
7- 9-'90	1/4	--
19- 9-'90	1/2	--
22-10-'90	1/8	--
5-11-'90	1/8	1/4
22-11-'90	1/8	1/4
7-12-'90	1/8	1/4
15-12-'90	1/4	1/4
4- 1-'91	1/4	1/4
18- 1-'91	1/4	1/2
31- 1-'91	1/16	1/4
15- 2-'91	1/32	1/4
7- 3-'91	1/4	1/2
19- 3-'91	1/2	1/2
2- 4-'91	1/2	1/2
12- 4-'91	1/2	1/2
3- 5-'91	--	1/2
15- 5-'91	--	1/2
3- 6-'91	--	1/4
17- 6-'91	--	1/8
29- 6-'91	1/8	1/4
15- 7-'91	1/32	1/4
3- 9-'91	1/16	--
17- 9-'91	1/16	--
28- 9-'91	1/16	--

Blood typing

Four hundred and eleven cross-bred cattle belonging to three different genetic groups (Jersey cross-bred, Brown-Swiss cross-bred and Holstein-Friesian cross-bred) were blood typed using twenty eight serologically different blood group reagents. The relative frequency of occurrence of various blood factors are presented in table 10.

In Jersey cross-breds, the blood factor F had the highest frequency (0.90) and the factor M_{15} had the lowest frequency (0.10). In Brown-Swiss cross-breds the highest frequency was obtained for C_2 factor (0.78) and lowest frequency for M_{15} (0.05). In Hostein-Friesian cross-breds F was the most frequent factor (0.94) and factor M_{15}, the least frequent (0.10). In the pooled population the highest frequency of 0.79 and the lowest frequency of 0.08 was observed for E'_3 and M_{15} respectively. This showed that the new factor M_{15} was very rare in the cross-breds presently studied.

Table 10(a) Relative frequencies of erythroeytic antigens among cross-breds

Population	Total No. of Animals	B			C			FV		J	L	S		Z
		B	Y_2	E'_3	C_2	R	X_1	F	V	J^{cs}	L	S	H''	Z
Jersey cross-bred	113	0.59 (67)	0.55 (62)	0.87 (98)	0.79 (89)	0.23 (26)	0.65 (73)	0.90 (102)	0.67 (76)	0.43 (49)	0.49 (55)	0.35 (39)	0.11 (12)	0.68 (77)
Brown-swiss cross-bred	162	0.66 (107)	0.51 (83)	0.75 (122)	0.78 (127)	0.23 (37)	0.55 (89)	0.28 (45)	0.63 (102)	0.44 (71)	0.51 (82)	0.41 (66)	0.14 (23)	0.65 (106)
Holstein Friesian cross-bred	136	0.72 (98)	0.59 (80)	0.76 (104)	0.74 (101)	0.22 (30)	0.60 (81)	0.94 (128)	0.60 (82)	0.37 (50)	0.58 (79)	0.38 (52)	0.16 (22)	0.68 (93)
Pooled cross-breds	411	0.66 (272)	0.55 (225)	0.79 (324)	0.77 (317)	0.23 (93)	0.59 (243)	0.67 (275)	0.63 (260)	0.41 (170)	0.53 (216)	0.62 (157)	0.14 (57)	0.67 (276)

Figures in parenthesis indicate the number of observations.

Table 10 (b) Distribution of erythrocyte anti. ... among cross breds

Population	Total No. of Animals	M_1	M_2	M_9	M_{10}	M_{12}	M_{15}	M_{16}	M_{17}	M_{18}	M_{19}	M_{23}	M_{32}	M_{33}	M_{36}
Jersey cross-bred	113	0.20 (23)	0.53 (60)	0.50 (56)	0.27 (31)	0.26 (29)	0.10 (11)	0.45 (51)	0.19 (21)	0.55 (62)	0.39 (44)	0.26 (29)	0.19 (22)	0.27 (30)	0.22 (25)
Brown-swiss cross-bred	162	0.10 (16)	0.52 (84)	0.39 (63)	0.23 (37)	0.24 (39)	0.05 (8)	0.46 (75)	0.14 (23)	0.52 (84)	0.42 (68)	0.27 (43)	0.08 (13)	0.20 (32)	0.19 (31)
Holstein Friesian cross-bred	136	0.13 (18)	0.54 (74)	0.32 (44)	0.27 (37)	0.28 (38)	0.10 (13)	0.47 (64)	0.16 (22)	0.57 (78)	0.41 (56)	0.23 (31)	0.10 (14)	0.21 (29)	0.23 (31)
Pooled cross-breds	411	0.14 (57)	0.53 (218)	0.40 (163)	0.26 (105)	0.26 (106)	0.08 (32)	0.46 (190)	0.16 (66)	0.55 (224)	0.41 (168)	0.25 (103)	0.12 (49)	0.22 (91)	0.21 (87)

Figures in parenthesis indicate the number of observations

Haemoglobin polymorphism

Four hundred and eleven crossbred cattle were typed for haemoglobin variants. The animals were found to possess two types of haemoglobin variants - fast moving B and slow moving A type. Three haemoglobin phenotypes viz.Hb AA, Hb AB and Hb BB were observed during the present study (Fig. 7).

Genetic Studies

Blood groups

a) Estimation of gene frequencies

The gene frequencies for each blood group factor were calculated and are presented in table 11.

Alleles at B locus

Among the alleles recognised under B blood group system, E'_3 had the highest frequency in all the cross-bred populations. The frequency of E'_3 was 0.87 (Jersey cross-breds), 0.75 (Brown-Swiss cross-breds) and 0.76 (Holstein-Friesian cross-breds). The lowest frequency was obtained for e'_3. The frequencies were 0.13, 0.25 and 0.24 in Jersey cross-breds, Brown-Swiss cross-breds and Holstein-Friesian cross-breds respectively.

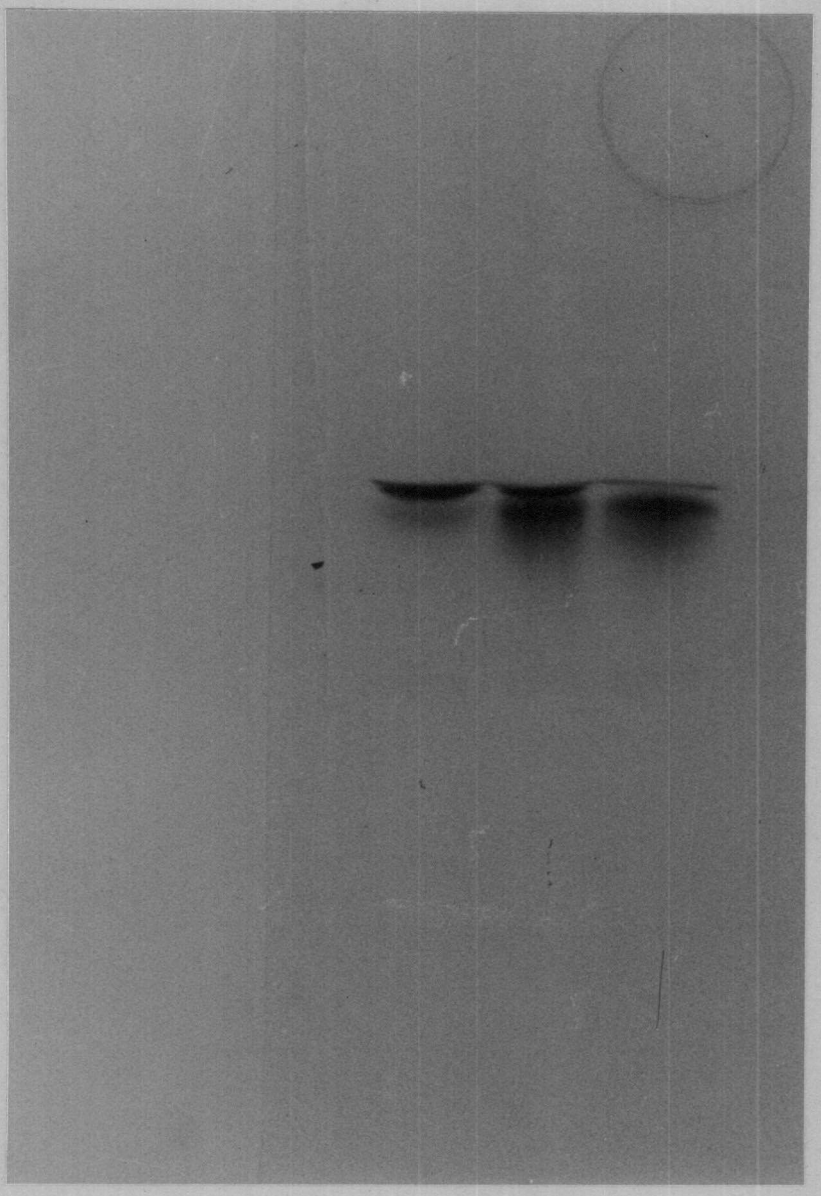


Fig.7. Haemoglobin phenotypes in cross-bred cattle

Table 11. Estimated gene frequencies of various blood group factors

Blood group system	Blood group factors	alleles	Jersey cross (113)	Borwn-swiss cross (162)	Holstein-Friesian cross (136)	Pooled cross breds (411)
B	B	B	0.59	0.66	0.72	0.66
		b	0.41	0.34	0.28	0.34
	Y_2	Y_2	0.55	0.51	0.59	0.55
		y_2	0.45	0.49	0.41	0.45
	E'_3	E'_3	0.87	0.75	0.76	0.79
		e'_3	0.13	0.25	0.24	0.21
C	C_2	C_2	0.79	0.78	0.74	0.77
		c_2	0.21	0.22	0.26	0.23
	R	R	0.23	0.23	0.22	0.23
		r	0.77	0.77	0.78	0.77
	X_1	X_1	0.65	0.55	0.60	0.59
		x_1	0.35	0.45	0.40	0.41
FV	F	F	0.615	0.633	0.669	0.640
	V	V	0.385	0.367	0.331	0.360
J	J	J^{cs}	0.43	0.44	0.37	0.41
		J^s J^a	0.57	0.56	0.63	0.59

(Contd....)

(Table 11 Contd.....)

Blood group system	Blood group factors	alleles	Jersey cross (113)	Borwn-swiss cross (162)	Holstein-Friesian cross (136)	Pooled cross breds (411)
L	L	L	0.28	0.30	0.35	0.31
		l	0.72	0.70	0.65	0.69
S	S	S	0.35	0.41	0.38	0.38
		s	0.65	0.59	0.62	0.62
	H''	H''	0.11	0.14	0.16	0.14
		h''	0.89	0.86	0.84	0.86
Z	Z	Z	0.44	0.41	0.44	0.43
		z	0.56	0.59	0.56	0.57
	M_1	M_1	0.11	0.05	0.07	0.08
		m_1	0.89	0.95	0.93	0.92
	M_2	M_2	0.32	0.31	0.33	0.32
		m_2	0.68	0.69	0.67	0.68
	M_9	M_9	0.29	0.22	0.18	0.22
		m_9	0.71	0.78	0.82	0.78
	M_{10}	M_{10}	0.15	0.12	0.15	0.14
		m_{10}	0.85	0.88	0.85	0.86

(Contd...)

(Table 11 Contd.....)

Blood group system	Blood group factors	alleles	Jersey cross (113)	Borwn-swiss cross (162)	Holstein-Friesian cross (136)	Pooled cross breds (411)
	M_{12}	M_{12}	0.14	0.13	0.15	0.14
		m_{12}	0.86	0.87	0.85	0.86
	M_{15}	M_{15}	0.05	0.03	0.05	0.04
		m_{15}	0.95	0.97	0.95	0.96
	M_{16}	M_{16}	0.26	0.27	0.27	0.27
		m_{16}	0.74	0.73	0.73	0.73
	M_{17}	M_{17}	0.10	0.07	0.08	0.08
		m_{17}	0.90	0.93	0.92	0.92
	M_{18}	M_{18}	0.33	0.31	0.35	0.33
		m_{18}	0.67	0.69	0.65	0.67
	M_{19}	M_{19}	0.22	0.24	0.23	0.23
		m_{19}	0.78	0.76	0.77	0.77
	M_{23}	M_{23}	0.14	0.14	0.12	0.13
		m_{23}	0.86	0.86	0.88	0.87
	M_{32}	M_{32}	0.11	0.04	0.05	0.06
		m_{32}	0.89	0.96	0.95	0.94
	M_{33}	M_{33}	0.15	0.11	0.11	0.12
		m_{33}	0.85	0.89	0.89	0.88
	M_{36}	M_{36}	0.12	0.10	0.12	0.11
		m_{36}	0.88	0.90	0.88	0.89

Alleles at C locus

In the three cross-breds presently studied, the alleles C_2 had the highest frequency in Jersey cross-breds (0.79) and Brown-Swiss cross-breds (0.78). In Brown-Swiss cross-breds the r allele was found to be the predominant one.

Alleles at FV locus

The F allele was predominant in all the cross-breds and the frequency varied from 0.62 (Jersey cross-breds) to 0.67 (Holstein-Friesian cross-breds).

Alleles at J locus

All the cross-breds had higher frequency of J^s or J^a and the frequencies were 0.57, 0.56 and 0.63 in Jersey cross-breds, Brown-Swiss cross-breds and Holstein-Friesian cross-breds respectively.

Alleles at L locus

The l allele which do not produce the L antigen was predominant in all the cross-breds. The frequency of l were 0.72 (Jersey cross-breds), 0.70 (Brown-Swiss cross-breds) and 0.65 (Holstein-Friesian cross-breds).

Alleles at S locus

Among the alleles at S locus, h" had the highest frequency of 0.89, 0.86 and 0.84 in Jersey cross-breds, Brown-Swiss cross-breds and Holstein-Friesian cross-breds respectively.

Alleles at Z locus

The z allele was predominant in all the cross-breds and its frequency was 0.56 (Jersey cross-breds and Hostein-Friesian cross-breds) and 0.59 (Brown-Swiss cross-breds).

Among the alleles which could not be grouped under any blood group system, m_{15} had the highest frequency in all the cross-breds. The frequencies were 0.95 (Jersey cross-breds and Holstein-Friesian cross-breds) and 0.97 (Brown-Swiss cross-breds).

b) Test for genetic equilibrium

The populations were tested for genetic equilibrium at the FV locus using X^2 test (Table 12).

Table 12. Observed and expected genotype and gene frequencies of the FV blood group system in different genetic groups of cattle.

Population		Genotypes			Gene Frequencies		X^2 Values 2 df
		FF	FV	VV	q^F	q^V	
Jersey cross	Obs.	37.0	65.0	11.0	0.615	0.385	5.212
	Exp.	42.74	53.51	16.75			
Brown-swiss cross	Obs.	60.0	85.0	17.0	0.633	0.367	2.694
	Exp.	64.91	75.27	21.82			
Holstein-Friesian cross	Obs.	54.0	74.0	8.0	0.669	0.331	7.118*
	Exp.	60.87	60.23	14.9			
Pooled cross-breds	Obs.	151.00	224.00	36.00	0.640	0.360	13.706**
	Exp.	168.35	189.39	53.26			

* $P < 0.05$

** $P < 0.01$

A good agreement was observed between the observed and expected numbers in each phenotypes in all the populations except in Holstein-Friesian cross-breds ($P < 0.05$).

c) Mode of inheritance of blood group factors

The pattern of inheritance was studied in 88 sire-dam-offspring sets (Table 13). Many of these factors expressed a ratio of 3:1 in the first set of mating types where both parents possessed the antigen. In all these cases the number of offsprings lacking the antigen were more.

In the second set, where one of the parents possessed the antigen, the ratio of 1:1 could be obtained only in case of M_2, M_{16} and M_{19}. In the B system, Z system and M_{18} the number of offsprings possessing the antigen were more while in all the remaining cases, offsprings lacking the antigen were drastically high in number.

Among the third type of mating, where both the parents lacked the antigen, the results were against the expectations. Many offsprings showed presence of antigen.

Table 13 Inheritance of cellular antigens.

Antigen (Ag)	Type of matings	No. of matings	Number of offsprings	
			Having Ag	Lacking Ag
B	+ x +	39	29	10
	+ x -	40	28	12
	- x -	9	3*	6
Y_2	+ x +	23	16	7
	+ x -	38	27	11
	- x -	27	7*	20
E'_3	+ x +	49	41	8
	+ x -	35	28	7
	- x -	4	0	4
C_2	+ x +	69	55	14
	+ x -	18	8	10
	- x -	1	0	1
R	+ x +	1	1	0
	+ x -	32	8	24
	- x -	55	3*	52
X_1	+ x +	43	28	15
	+ x -	36	15	21
	- x -	9	2*	7

(Contd.....)

(Table 13 Contd.....)

Antigen (Ag)	Type of matings	No. of matings	Number of offsprings	
			Having Ag	Lacking Ag
J	$J^{cs} \times J^{cs}$	15	8	7
	$J^{cs} \times 0$	38	16	22
	0×0	35	4*	31
L	+ x +	30	15	15
	+ x -	39	17	22
	- x -	19	7*	12
S	+ x +	20	12	8
	+ x -	48	20	28
	- x -	20	3*	17
H''	+ x +	4	2	2
	+ x -	23	2	21
	- x -	61	5*	56
Z	+ x +	27	19	8
	+ x -	53	39	14
	- x -	8	5*	3

(Contd.....)

(Table 13 Contd.....)

Antigen (Ag)	Type of matings	No. of matings	Number of offsprings	
			Having Ag	Lacking Ag
M_1	+ x +	4	3	1
	+ x -	22	3	19
	- x -	62	3*	59
M_2	+ x +	19	13	6
	+ x -	47	23	24
	- x -	22	3*	19
M_9	+ x +	10	3	7
	+ x -	47	12	35
	- x -	31	2*	29
M_{10}	+ x +	4	2	2
	+ x -	31	10	21
	- x -	53	6*	47
M_{12}	+ x +	1	0	1
	+ x -	23	8	15
	- x -	64	10*	54
M_{15}	+ x +	1	0	1
	+ x -	26	2	24
	- x -	61	1*	60
M_{16}	+ x +	26	20	6
	+ x -	42	20	22
	- x -	20	5*	15

(Table 13 Contd.....)

Antigen (Ag)	Type of matings	No. of matings	Number of offsprings	
			Having Ag	Lacking Ag
M_{17}	+ x +	9	2	7
	+ x -	38	4	34
	- x -	41	3*	38
M_{18}	+ x +	20	14	6
	+ x -	50	36	14
	- x -	18	9*	9
M_{19}	+ x +	19	15	4
	+ x -	39	19	20
	- x -	30	5*	25
M_{23}	+ x +	9	3	6
	+ x -	45	5	40
	- x -	34	1*	33
M_{32}	+ x +	3	1	2
	+ x -	15	5	10
	- x -	70	3*	67
M_{33}	+ x +	0	0	0
	+ x -	18	8	10
	- x -	70	4*	66
M_{36}	+ x +	0	0	0
	+ x -	20	8	12
	- x -	68	8*	60

The mode of inheritance in the co-dominant system FV was studied (Table 14) and compared with the expected values by X^2 test of significance. The values for FV x FV mating type was significantly different ($P < 0.05$).

Haemoglobin

a) Phenotype and gene frequencies

Table 15 shows the population-wise difference in the haemoglobin phenotypes. The frequency of HbAA phenotype was highest in Holstein-Friesian cross-breds (0.6985) and lowest in Jersey cross-breds (0.4071). Very few of the Holstein-Friesian cross-breds possessed Hb BB phenotype. Heterozygotes occurred with a frequency of 0.5123 in Brown swiss crosses which was higher than that of the other two genetic groups. In pooled population, the frequency of HbAA, HbAB and HbBB were 0.5036, 0.4037 and 0.0657, respectively.

The allele Hb^A was predominant in all the populations. The gene frequency of Hb^A was highest in Holstein-Friesian cross-breds (0.83) followed by Jersey cross-breds (0.67) and Brown-Swiss cross-breds (0.66).

Table 14. Inheritance of factors in FV system.

Mating type	No. of matings	Genotypes FF obs.	FF exp.	FV obs.	FV exp.	VV obs.	VV exp.	X^2 value 2 df
FF x FF	6	6.0	6.00	--	--	--	--	0.0000
FF x FV	30	12.0	15.00	18.0	15.0	--	--	1.2000
FF x VV	2	--	--	2.0	2.0	--	--	0.0000
FV x FV	37	13.0	9.25	22.0	18.5	2.0	9.25	7.8649*
FV x VV	8	--	--	6.0	4.0	2.0	4.00	2.0000
VV x VV	0	0.0	0.00	0.0	0.0	0.0	0.00	0.0000

* $P < 0.05$

Table 15 Phenotype and gene frequencies of haemoglobin types in different genetic group of cattle

Population	Sample Size	Phenotype frequencies			Gene frequencies	
		Hb AA	Hb AB	Hb BB	Hb^A	Hb^B
Jersey cross	113	0.4071 (46)	0.5133 (58)	0.07096 (9)	0.67	0.33
Brown-swiss cross	162	0.4074 (66)	0.5123 (83)	0.0802 (13)	0.66	0.34
Holstein Friesian cross	136	0.6985 (95)	0.2647 (36)	0.0368 (5)	0.83	0.17
Pooled cross breds	411	0.5036 (207)	0.4307 (177)	0.0657 (27)	0.72	0.28

b) Heterozygosity at the haemoglobin locus

The genetic variability of the population at the haemoglobin locus was measured by heterozygosity (Table 16). The Jersey and Brown-Swiss cross-breds showed more heterozygosity at Hb locus (0.44) than the Holstein-Friesian crosses (0.28).

c) Test for genetic equilibrium at Hb locus

All the genetic groups studied were in genetic-equilibrium with respect to the Hb locus. The observed number of heterozygotes were more than the expected values in Jersey and Brown-Swiss cross-breds. But in Holstein-Friesian cross-breds the number of homozygotes was more than the expected value. But the difference was not statistically significant (Table 17).

d) Mode of inheritance of haemoglobin variants

The mode of inheritance of haemoglobin alleles was studied in 80 matings consisting of six mating classes (Table 18). The observed and expected number of offsprings in different phenotypes did not differ significantly. The

Table 16 Heterozygosity at Haemoglobin loci in different cross-bred cattle

Population	Heterozytosity
Jersey cross	0.4422
Brown-swiss cross	0.4488
Holstein-Friesian cross	0.2822
Pooled cross-bred	0.4032

Table 17. Observed and expected phenotype and gene frequencies of haemoglobin in cross-bred cattle

Population	Total No. of animals tested	Haemoglobin phenotypes						X^2 value 2 df	Gene frequencies	
		AA		AB		BB			Hb^A	Hb^B
		obs.	exp.	obs.	exp.	obs.	exp.			
Jersey cross	113	46.0	50.73	58.0	49.97	9.00	12.3	2.6167	0.67	0.33
Brown swiss cross	162	66.0	70.56	83.0	72.71	13.00	18.73	3.481	0.66	0.34
Holstein-Friesian cross	136	95.0	93.69	36.0	38.38	5.0	3.93	0.4572	0.83	0.17
Pooled cross-breds	411	207.0	213.06	177.0	165.72	27.0	32.22	1.7859	0.72	0.28

Table 18. Inheritance of Haemoglobin types

Mating type	No. of matings	Phenotype of progeny						X^2 value 2 df
		AA		AB		BB		
		obs.	exp.	obs.	exp.	obs.	exp.	
AA x AA	32	32	6.00	0.0	0.0	0	0.00	0.0000^{NS}
AA x AB	39	27	19.50	12.0	19.5	0	0.00	5.7692^{NS}
AA x BB	6	0	0.00	6.0	6.0	0	0.00	0.0000^{NS}
AB x AB	3	2	0.75	1.0	1.5	0	0.75	2.9999^{NS}
AB x BB	0	0	0.00	0.0	0.0	0	0.00	0.0000^{NS}
BB x BB	0	0.0	0.00	0.0	0.0	0	0.00	0.0000^{NS}
Total	80	61.0	52.25	19.0	27.0	0	0.75	4.5857^{NS}

NS Non-significant

observed Hb AA phenotype (61) was more than the expected (52.25). But the differences were not significant statistically. When the mating between AA x AB occurred more of AA individuals were born instead of an equal distribution of AA and AB individual. Similarly instead of 1:2:1 ratio among the offsprings of AB x AB crosses, a ratio of 2:1 was noticed among AA, AB offsprings. No offsprings were born with BB phenotypes from the four different mating types though the expected number was 0.75. The observed and expected values of the phenotypes of progeny from the possible mating types did not differ significantly when tested.

Parentage studies

The blood types of 88 sire-dam-offspring sets are given in table 19. Among the 88 progenies studied under seven sire families, 50 were found to have correct parentage while in 38 cases the recorded parentage was found to be incorrect (Table 20).

The average error in the recorded parentage was estimated to be 43.18 per cent where the error ranged from 0 (Bull No. 352) to 100 (Bull No. 174) per cent in individual

Table 19. Blood types of sire-dam-offspring sets in seven sire families.

(i) Half-sibs of Sire No. 204

	B			C			F/V	J	I	S		Z	M_1	M_2	M_9	M_{10}	M_{12}	M_{15}	M_{16}	M_{17}	M_{18}	M_{19}	M_{23}	M_{32}	M_{33}	M_{36}	Hb
	B	Y_2	E'_3	C_2	R	X_1				S	H''																
Sire 204	+	–	+	+	–	+	FV	+	+	+	–	+	–	–	+	–	–	–	+	+	+	+	+	–	–	–	AA
Dam IM-41	+	+	+	+	+	+	FV	(+)	+	–	–	+	–	–	+	+	–	+	–	+	+	+	–	–	+	+	AA
Calf 288	+	+	+	+	+	+	FV	–	+	–	–	(+)	–	–	–	+	+	(+)	+	–	+	+	–	–	+	–	BB
Dam 840	+	–	–	+	–	+	VV	+	–	–	–	(+)	+	+	+	–	–	+	+	–	–	+	–	+	–	–	AB
Calf 388	(+)	–	+	–	+	+	FV	+	+	–	+	+	–	+	+	+	+	(+)	+	–	+	–	(+)	–	–	–	AB
Dam 137	–	–	+	+	–	+	FV	–	–	+	–	+	–	(+)	–	–	–	–	+	–	+	–	–	–	+	–	AA
Calf 396	+	+	+	+	–	–	FF	–	–	+	+	–	–	+	+	+	+	–	(+)	(+)	–	–	–	–	–	+	AB
Dam 837	–	–	–	+	–	+	FV	–	+	(+)	(+)	–	–	+	–	–	+	–	–	–	+	+	–	–	–	+	AB
Calf 406	+	+	+	–	–	–	FF	–	–	–	–	–	–	–	–	–	+	–	+	–	–	–	–	–	–	+	AB
Dam 395	+	+	+	–	–	–	FV	–	+	–	–	(+)	–	–	–	–	+	–	(+)	–	+	–	–	–	–	+	AA
Calf 414	–	+	+	–	–	–	FF	–	+	–	–	–	–	–	–	–	+	–	(+)	–	+	–	–	–	–	+	AA
Dam 244	+	–	+	+	–	+	FF	–	+	–	–	+	–	–	+	–	–	–	–	–	+	–	–	–	–	–	AB
Calf 418	+	–	–	+	–	–	FV	–	–	+	–	+	–	–	+	–	–	–	–	–	+	+	–	–	–	–	AB

Contd...

(i) Contd....

	B			C			F/V	J	L	S		Z	M_1	M_2	M_9	M_{10}	M_{12}	M_{15}	M_{16}	M_{17}	M_{18}	M_{19}	M_{23}	M_{32}	M_{33}	M_{36}	Hb
	B	Y_2	E'_3	C_2	R	X_1				S	B"																
Dam 188	-	-	+	+	-	(+)	FF	-	+	-	-	(+)	-	-	+	-	-	-	-	-	+	+	-	-	-	-	AA
Calf 427	+	+	+	+	-	+	FV	-	-	-	-	-	-	(+)	-	-	-	-	-	-	+	(+)	-	-	-	-	AA
Dam 704	-	-	+	+	-	-	FV	-	+	(+)	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	AB
Calf 429	+	-	-	+	-	-	FV	-	+	+	-	+	-	-	-	-	-	-	(+)	-	+	(+)	-	-	-	-	AA
Dam 136	-	-	+	-	+	-	FF	-	+	-	-	+	-	(+)	+	+	-	-	-	-	+	-	-	-	+	-	AB
Calf 432	-	-	+	-	+	-	FF	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	+	-	+	+	AA
Dam 474	-	-	+	+	-	-	FV	+	-	+	-	-	-	+	-	-	-	-	-	-	-	-	(+)	-	-	-	AA
Calf 434	+	-	+	+	-	-	FV	+	+	+	-	+	-	-	-	-	-	-	(+)	-	+	(+)	+	-	-	-	AA
Dam 296	+	-	+	+	-	+	FV	+	+	+	-	+	-	+	+	+	(+)	-	+	-	+	+	+	-	+	-	AB
Calf 435	-	-	+	-	-	-	FV	-	+	-	-	+	-	-	-	-	-	-	-	-	+	(+)	-	-	-	-	AA
Dam 031	+	-	(+)	-	(+)	(+)	VV	+	-	(+)	-	(+)	-	+	-	-	(+)	-	+	-	+	+	-	-	-	+	AA
Calf 437	+	-	+	+	-	+	VV	+	-	+	-	+	-	-	-	-	-	-	(+)	-	+	+	-	-	-	-	AA

Contd....

(ii) Contd...

	B			C			F/V	J	I	S		Z	M_1	M_2	M_9	M_{10}	M_{12}	M_{15}	M_{16}	M_{17}	M_{18}	M_{19}	M_{23}	M_{32}	M_{33}	M_{36}	Hb
	B	Y_2	E'_3	C_2	R	X_1				S	H"																
Dam T-131	(+)	+	(+)	+	–	+	FV	+	+	–	–	–	–	–	–	–	–	–	–	(+)	+	+	–	–	–	–	AA
Calf 444	–	–	–	+	–	+	FF	+	–	+	–	+	–	–	–	–	–	–	(+)	–	+	–	–	–	–	–	AB
Dam 162	+	+	(+)	+	–	–	FF	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	AA
Calf 450	+	+	(+)	–	–	–	FF	+	–	–	–	(+)	–	–	(+)	–	–	–	–	–	–	–	–	–	–	–	AB
Dam 160	+	+	+	+	–	+	FV	+	+	(+)	–	–	+	+	–	–	–	–	–	–	–	–	–	+	–	–	AA
Calf 472	+	–	+	+	–	–	FV	–	+	–	–	–	–	+	–	–	–	–	–	–	+	–	–	–	–	–	AA
Dam 685	–	+	–	–	–	+	FF	–	–	–	–	(+)	–	+	–	–	–	–	–	(+)	–	–	–	–	–	–	AA
Calf 475	+	+	+	–	–	+	FF	–	–	–	–	(+)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	AA
Dam T-211	+	+	+	+	+	+	FF	–	+	–	–	(+)	–	+	+	+	–	–	–	–	–	(+)	–	–	+	–	AB
Calf 477	+	+	+	+	–	+	FV	–	–	–	–	+	+	+	–	(+)	–	–	–	–	+	+	(+)	+	–	–	BB
Dam M-235	+	–	–	+	–	+	FV	+	–	+	–	–	–	–	+	–	–	–	+	–	–	–	+	–	–	–	AB
Calf 483	+	–	–	+	–	+	VV	+	+	+	–	–	–	–	–	–	–	–	+	–	+	+	–	–	–	–	AA

Contd...

(i) Contd...

	B						F/V	J	I	S		Z	M_1	M_2	M_6	M_{10}	M_{12}	M_{15}	M_{16}	M_{17}	M_{18}	M_{19}	M_{23}	M_{32}	M_{33}	M_{36}	Hb
	B	Y_2	E'_3	C_2	R	X_1				S	H"																
Dam 693	+	-	+	-	-	(+)	FV	-	-	-	-	+	+	(+)	-	+	-	-	-	-	-	-	-	+	+	-	BB
Calf 484	+	-	+	+	-	+	FV	+	-	-	-	+	+	(+)	-	+	-	-	-	-	-	+	-	-	+	-	AB
Dam 707	+	-	-	+	-	-	VV	+	-	-	-	-	-	-	-	-	-	-	+	-	+	+	-	-	-	-	AB
Calf 490	-	-	+	+	-	-	FV	+	-	-	+	-	-	-	-	-	-	-	+	-	+	(+)	-	-	-	-	AA
Dam 328	+	-	+	+	+	+	FF	-	+	-	(+)	-	-	+	-	-	+	+	+	-	-	-	-	-	-	+	BB
Calf 499	+	-	-	+	-	-	FV	+	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-	+	AB
Dam 177	(+)	+	+	+	-	+	FV	-	+	-	-	-	-	+	-	-	-	-	-	-	+	+	-	-	-	-	AB
Calf 503	+	-	+	+	-	-	FV	+	+	+	-	-	-	-	-	-	-	-	(+)	-	+	+	-	-	-	-	AB
Dam 206	(+)	+	+	-	-	-	FF	-	-	(+)	-	+	-	-	-	-	-	+	(+)	(+)	-	-	+	-	-	+	AB
Calf 511	-	-	+	+	-	+	FV	+	+	+	-	(+)	-	-	-	-	-	-	+	-	+	+	+	-	-	-	AB

(111). Half-sibs of Sire No. 352

	B			C			F/V	J	I	S		Z	M_1	M_2	M_9	M_{10}	M_{12}	M_{15}	M_{16}	M_{17}	M_{18}	M_{19}	M_{23}	M_{32}	M_{33}	M_{36}	Hb
	B	Y_2	E'_3	C_2	R	X_1				S	H"																
Sire 352	+	-	+	+	-	+	FV	+	+	+	+	+	-	-	(+)	-	-	+	+	(+)	+	+	+	-	-	+	AA
Dam 183	+	+	+	+	-	+	FV	-	-	-	-	+	(+)	+	-	-	-	-	+	+	+	+	-	-	-	-	AA
Calf 464	+	+	+	+	-	+	FV	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	AA
Dam 211	-	-	+	-	-	(+)	FV	+	+	(+)	-	+	-	+	-	-	-	-	-	-	+	+	-	-	-	-	BB
Calf 525	-	-	(+)	+	-	-	FF	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	AB

(iii). Half-sibs of Sire No. 449

	B			C			F/V	J	I	S		Z	M_1	M_2	M_9	M_{10}	M_{12}	M_{15}	M_{16}	M_{17}	M_{18}	M_{19}	M_{23}	M_{32}	M_{33}	M_{36}	Hb
	B	Y_2	F'_3	C_2	R	X_1				S	H"																
Sire 449	-	-	-	+	-	-	FF	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	AA
Dam 146	+	+	-	+	-	-	FF	-	-	-	-	(+)	-	+	(+)	+	+	-	(+)	-	-	-	-	-	+	+	AA
Calf TM-47	+	-	-	+	-	-	FF	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	AA
Dam T-078	-	+	-	+	+	-	FV	+	+	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	AA
Calf TM-50	-	+	-	-	-	-	FV	+	-	-	-	+	-	-	-	-	-	-	-	-	+	(+)	-	-	-	-	AA
Dam 132	+	-	-	+	-	+	FV	-	-	-	-	(+)	+	+	-	-	+	-	(+)	-	-	-	-	+	-	+	AB
Calf TM-55	+	-	-	-	-	-	FV	-	-	-	-	+	-	-	-	-	-	-	+	-	+	-	-	-	-	-	AA
Dam 140-B	-	-	+	+	+	+	FF	+	+	-	-	-	-	(+)	+	+	-	-	-	-	-	-	-	-	+	-	AA
Calf TM-57	-	-	+	+	-	+	FV	+	-	-	-	+	-	(+)	-	-	-	-	-	-	+	-	-	-	-	-	AA
Dam 268	+	+	+	+	-	+	FV	(+)	-	-	-	+	-	+	-	(+)	+	-	+	+	+	+	-	-	-	+	AB
Calf TM-63	+	-	-	-	-	-	FF	-	-	-	-	(+)	-	+	-	-	(+)	-	+	-	-	-	-	-	-	+	AA
Dam 132-A	+	-	-	+	-	+	FV	-	-	-	-	(+)	+	+	-	-	+	-	(+)	-	-	-	-	+	-	+	AB
Calf TM-64	+	-	-	-	-	-	FF	-	-	+	-	(+)	-	+	-	-	+	-	+	-	-	-	-	-	-	+	AA

Contd....

(iii) Contd....

	B			C			F/V	I		S		Z	M_1	M_2	M_9	M_{10}	M_{12}	M_{15}	M_{16}	M_{17}	M_{18}	M_{19}	M_{23}	M_{32}	M_{33}	M_{36}	Hb
	B	Y_2	F'_3	C_2	R	S_1				S	B''																
Dam 324	+	+	+	+	–	+	FV	+	+	+	–	–	+	+	+	+	+					+	+	–	+	+	–	AB
Calf TM-65	+	–	+	+	–	+	FV	–	+	–	–	–	–	+	+	+	–	–	+	–	+	+	–	–	+	+	–	AB
Dam TM-43	–	+	+	–	+	(+)	FV	–	+	–	–	–	–									(+)	–					AA
Calf TM-67	–	+	+	–	+	–	FF	–	–	–	–	–	–	+	+	+	–							–	–	+	–	AA
Dam 484	+	+	+	–	–	–	FV	+	+	+	–	–	–	–	–	–	+	–	+		+	+	–	–	–	–	–	AA
Calf TM-202	+	+	+	–	–	–	FV	–	–	+	–	+	–	–	–	–	–	–	+	–	+	+	–	–	–	–	+	AA
Dam 809	+	+	+	+	–	+	FV	–	+	–	–	+	–	+	+	–	+	–	+	+	+	+	–	–	–	–	+	BB
Calf 268	+	+	+	+	–	+	FV	+	–	–	–	+	–	+														AB
Dam 492	+	+	+	+	–	+	FF	–	+	+	–	+	–	+	+	–	–	–	–	+	–	+	–	+	–	–	–	AB
Calf 271	+	+	+	–	–	–	FV	+	–	–	–	(+)	–	+	+	–	–	–	–	–	–	+	+	–	–	–	–	AA
Dam 428	+	+	+	–	–	+	FF	–	+	–	–	+	–	–	+	–	–	–	–	–	–			–	–	–	–	AB
Calf 272	–	+	+	–	–	–	FF	–	+	–	–	–	–	–	+	–	–	–	+	–	–							AA
Dam 485	+	–	+	–	–	–	FV	–	–	–	–	–	–	–								(+)	+	–	–	–	–	AA
Calf 273	+	+	+	–	–	–	FF	–	+	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	AA

Contd...

(iii) Contd...

	B			C			F/V	J	I	S		Z	M_1	M_2	M_9	M_{10}	M_{12}	M_{15}	M_{16}	M_{17}	M_{18}	M_{19}	M_{23}	M_{32}	M_{33}	M_{36}	Hb
	B	Y_2	E'_3	C_2	R	X_1				S	H"																
Dam 219	-	+	+	-	-	(+)	FF	+	-	-	-	(+)	-	+	-	-	-	-	-	-	-	-	-	-	-	-	AA
Calf 278	-	-	+	+	-	-	FF	+	+	-	-	+	-	+	+	-	-	-	+	-	-	-	-	-	-	-	AB
Dam 093	-	-	+	+	-	+	VV	-	-	+	-	(+)	-	-	-	-	-	-	+	-	-	-	-	-	+	+	AB
Calf 283	+	-	-	-	+	+	FV	-	+	+	-	-	-	+	-	+	+	-	-	+	-	+	-	-	-	+	AB
Dam 745	+	+	-	+	+	-	FF	+	-	-	-	(+)	-	-	+	+	-	-	-	-	+	-	-	-	+	-	AA
Calf 296	+	+	-	+	+	-	FF	-	-	(+)	-	+	-	-	+	+	-	-	-	(+)	-	+	+	-	-	-	AA
Dam 738	+	-	-	+	-	(+)	FV	-	-	(+)	-	-	-	-	-	-	-	-	-	-	+	-	+	+	-	-	AA
Calf 301	+	-	-	+	-	-	FV	-	-	+	-	+	-	-	-	-	-	-	+	+	-	(+)	-	+	+	-	AB
Dam 192	+	-	+	+	+	+	VV	-	+	-	-	(+)	+	-	+	+	-	-	+	+	-	-	-	-	+	-	AA
Calf 316	-	(+)	+	+	-	+	FV	-	-	+	+	+	+	+	-	-	-	+	+	-	-	-	-	-	-	-	AA
Dam 765	-	-	+	+	-	-	FV	+	-	+	(+)	+	+	-	+	-	-	-	+	-	+	-	-	-	+	+	AA
Calf 323	-	-	+	+	+	-	FV	+	+	+	-	+	-	-	-	+	+	-	+	-	+	-	-	-	-	+	AB
Dam 844	+	+	+	+	-	+	FV	-	-	-	-	-	-	+	+	-	+	-	(+)	(+)	-	-	-	-	-	+	Ab
Calf 379	-	+	+	-	-	-	FV	-	-	+	-	+	-	+	-	-	+	-	+	-	-	-	-	-	-	-	

Contd.....

(iii) Contd...

	B			C			F/V	J	L	S		Z	M_1	M_2	M_9	M_{10}	M_{12}	M_{15}	M_{16}	M_{17}	M_{18}	M_{19}	M_{23}	M_{32}	M_{33}	M_{36}	Hb
	B	Y_2	E'_3	C_2	R	X_1				S	H"																
Dam 739	(+)	–	+	+	–	+	FF	–	–	–	–	(+)	–	+	+	–	–	–	–	–	–	–	–	–	–	–	AB
Calf 530	+	+	+	–	–	–	FV	–	+	–	–	+	–	+	–	–	–	–	(+)	–	+	+	–	–	–	–	BB
Dam 211	–	–	+	+	–	(+)	FV	+	+	(+)	–	+	–	(+)	–	–	–	–	–	–	+	+	–	–	–	–	BB
Calf 549	–	–	–	+	–	+	FV	–	–	+	–	+	–	–	–	–	–	–	+	–	+	+	–	–	–	–	AA
Dam 209	(+)	(+)	(+)	+	–	+	FV	–	+	+	–	(+)	–	+	+	+	+	–	+	–	–	(+)	–	–	–	+	AB
Calf 550	–	–	–	+	–	(+)	FV	+	–	(+)	(+)	(+)	–	–	–	–	–	–	+	–	+	+	–	–	–	–	AB
Dam 214	+	+	+	–	–	–	FF	–	–	–	–	+	–	(+)	–	–	–	–	–	–	+	–	–	–	–	–	AB
Calf 559	+	+	+	+	–	+	FV	–	–	–	–	–	+	–	–	(+)	+	–	+	–	–	–	–	+	–	+	AA
Dam 273	+	+	+	–	–	–	Ff	–	+	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	AA
Calf 563	–	+	(+)	+	–	–	FF	–	+	–	–	(+)	–	–	–	–	–	–	–	–	+	–	–	–	–	–	AA
Dam 244	+	–	+	+	–	+	FF	–	+	–	–	+	–	–	+	–	–	–	–	–	+	–	–	–	–	–	AB
Calf 564	+	–	+	+	–	–	FF	–	+	(+)	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	AB
Dam 207	–	–	+	+	+	–	FV	+	+	+	–	+	–	+	+	–	–	–	+	–	–	–	+	–	–	–	AB
Calf 840	+	–	–	+	–	+	VV	+	–	–	–	(+)	+	+	+	–	–	–	–	–	–	+	–	+	–	–	BB

(iv). Half-sibs of Sire No. 208

	B			C			F/V	J	I	S		Z	M_1	M_2	M_9	M_{10}	M_{12}	M_{15}	M_{16}	M_{17}	M_{18}	M_{19}	M_{23}	M_{32}	M_{33}	M_{36}	Hb
	B	Y_2	E'_3	C_2	s	x_1				S	H''																
Sire 208	+	+	+	+	+	+	FV	-	+	-	+	-	-	+	-	+	-	+	-	+	-	-	+	-	-	-	AA
Dam 207	+	+	+	+	-	-	FV	-	+	-	-	-	(+)	+	-	-	-	-	-	-	+	-	-	-	-	-	AA
Calf TM-66	+	-	+	+	-	+	FV	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	AA
Dam 443	(+)	-	+	+	-	-	FV	-	-	+	-	+	-	-	-	-	-	-	-	-	-	+	+	-	-	-	AB
Calf TM-71	+	+	+	+	-	+	FV	-	-	+	-	+	-	-	-	-	-	-	-	-	+	+	-	-	-	-	AA
Dam M-246	+	+	+	+	-	+	FV	+	+	(+)	-	+	+	+	+	+	-	-	-	-	+	-	-	+	+	-	AB
Calf TM-201	+	+	+	+	-	+	FF	+	-	-	-	-	-	+	-	-	-	-	-	+	+	-	-	-	-	-	AA
Dam 120	+	+	-	+	-	+	FF	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	AA
Calf TM-203	+	+	-	+	-	+	FF	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	AA
Dam 385	-	-	-	+	-	-	FV	-	+	+	-	+	-	-	-	-	-	-	+	-	+	+	-	-	-	-	AB
Calf TM-207	-	+	+	-	-	-	FF	-	+	+	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	AB

Contd....

(iv) Contd....

	B			C			F/V	J	I	S		Z	M_1	M_2	M_9	M_{10}	M_{12}	M_{15}	M_{16}	M_{17}	M_{18}	M_{19}	M_{23}	M_{32}	M_{33}	M_{36}	Hb
	B	Y_2	E'_3	C_2	R	X_1				S	H"																
Dam 206	-	+	+	+	-	-	FV	+	-	(+)	-	+	-	-	(+)	-	+	-	+	-	+	+	+	-	-	-	AB
Calf TM-210	-	+	+	+	-	+	FF	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	AA
Dam TM-58	(+)	-	-	-	-	(+)	FV	-	+	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	AA
Calf TM-212	-	+	+	-	-	-	FF	-	-	-	-	(+)	-	-	-	-	-	-	-	-	+	-	-	-	-	-	AA
Dam 204	+	+	+	+	-	+	FV	-	+	+	(+)	(+)	+	+	-	(+)	-	+	+	+	+	-	+	+	-	-	AA
Calf TM-213	+	+	+	+	+	+	FF	-	+	+	-	(+)	-	(+)	-	+	-	-	+	(+)	+	-	-	-	+	-	AA
Dam 208	+	+	+	+	-	+	FV	-	+	-	-	+	-	+	-	-	-	-	-	+	+	-	-	-	-	-	AA
Calf TM-214	+	-	+	+	-	+	FF	-	+	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	AA
Dam 222	+	+	+	+	-	+	VV	+	+	(+)	-	(+)	-	-	-	-	(+)	-	+	-	+	+	-	-	-	-	AB
Calf TM-215	-	+	+	-	-	-	FV	+	-	+	-	-	-	-	-	-	-	-	+	-	+	+	-	-	-	-	AA
Dam 207	+	+	+	+	-	-	FV	-	+	-	-	-	(+)	+	-	-	-	-	-	-	+	-	-	-	-	-	AA
Calf TM-216	+	+	+	+	-	-	FF	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	AA

Contd....

(iv) Contd....

	B			C			F/V	J	I	S		Z	M_1	M_2	M_9	M_{10}	M_{12}	M_{15}	M_{16}	M_{17}	M_{18}	M_{19}	M_{23}	M_{32}	M_{33}	M_{36}	Hb
	B	Y_2	E'_3	C_2	R	X_1				S	H"																
Dam 133	+	+	+	+	–	+	FV	(+)	+	–	(+)	+	+	+	+	–	–	–	–	+	+	–	–	–	–	–	AB
Calf 299	+	+	+	+	–	+	FV	(+)	+	–	–	+	–	+	+	+	–	–	–	+	+	–	–	–	–	–	AB
Dam 433	+	+	+	+	–	(+)	FV	–	+	–	–	(+)	–	+	–	–	–	–	–	(+)	+	+	–	–	–	–	AB
Calf 300	+	+	+	+	–	+	FV	–	+	–	–	–	–	–	–	–	+	–	+	–	+	+	–	–	–	+	AA
Dam 138	+	+	–	–	+	–	FF	+	–	–	+	+	–	–	+	+	–	–	+	–	–	–	–	–	+	–	AA
Calf 336	+	+	+	–	+	+	FF	–	–	+	+	+	–	(+)	+	+	+	–	+	–	–	–	–	–	+	+	AA
Dam 155	–	–	+	+	–	+	FV	–	+	–	–	+	–	+	+	–	–	–	–	–	–	+	–	–	–	–	AB
Calf 372	–	+	+	+	–	+	FV	+	–	–	–	+	–	+	(+)	–	–	–	–	–	+	+	–	–	–	–	AA
Dam T-414	+	+	+	+	–	–	FV	–	–	+	–	–	–	–	+	+	+	–	+	–	–	–	–	–	+	+	AB
Calf 397	–	–	–	+	–	–	FV	–	–	+	–	+	–	–	–	–	–	–	+	–	+	+	–	–	–	–	AA

(v). Half-sibs of Sire No. 215

	B			C			F/V	J	I	S		Z	M_1	M_2	M_9	M_{10}	M_{12}	M_{15}	M_{16}	M_{17}	M_{18}	M_{19}	M_{23}	M_{32}	M_{33}	M_{36}	Hb
	B	Y_2	E'_3	C_2	R	X_1				S	B"																
Sire 215	+	+	+	+	–	+	FV	+	–	–	–	+	–	+	–	–	–	–	–	–	+	+	+	–	–	–	AA
Dam 252	–	–	+	–	–	(+)	FV	+	+	(+)	–	(+)	–	+	–	–	+	–	(+)	–	+	+	–	–	–	+	AB
Calf 338	+	+	+	+	–	+	FF	–	+	–	–	+	–	–	–	–	+	–	+	–	–	–	–	–	–	+	AA
Dam 232	–	+	+	+	–	–	FF	–	(+)	+	–	(+)	–	+	–	–	–	–	–	–	–	–	–	–	–	–	AA
Calf 339	–	–	–	+	–	+	FF	–	+	+	–	(+)	–	–	–	–	–	–	+	–	+	–	–	–	–	–	AA
Dam T-206	+	+	+	+	+	+	FF	+	+	(+)	–	(+)	–	+	(+)	(+)	–	–	–	–	–	(+)	–	–	–	–	AA
Calf 394	+	+	+	+	–	+	FV	+	–	+	–	+	–	+	–	–	–	–	–	–	+	+	–	–	–	–	AA
Dam 151	+	+	+	–	–	–	VV	–	–	+	–	(+)	–	–	–	–	–	–	+	–	–	(+)	+	–	–	–	AA
Calf 400	+	+	+	–	–	–	VV	–	–	+	–	+	–	(+)	–	–	–	–	+	–	+	+	+	–	–	–	AA
Dam B-132	–	–	+	+	–	+	FV	+	+	–	–	+	–	+	+	–	+	–	+	–	+	–	–	–	–	+	AA
Calf 421	+	+	+	+	–	+	FV	–	+	–	–	+	–	+	–	–	–	–	–	+	+	–	+	–	–	–	AA

Contd.....

(v) Contd....

	B			C			F/V	J	L	S		Z	M_1	M_2	M_9	M_{10}	M_{12}	M_{15}	M_{16}	M_{17}	M_{18}	M_{19}	M_{23}	M_{32}	M_{33}	M_{36}	Hb
	B	Y_2	E'_3	C_2	R	X_1				S	H"																
Dam 079	+	+	+	+	–	+	VV	–	+	–	–	–	–	+	+	–	–	–	–	+	+	+	–	–	–	–	AA
Calf 460	+	–	–	+	–	+	FV	–	+	–	–	–	–	–	–	–	–	–	–	–	+	+	+	–	–	–	AA
Dam 013	+	+	+	+	–	+	FV	+	–	–	–	–	+	+	–	–	–	–	(+)	–	–	–	+	+	–	+	AB
Calf 467	+	–	+	+	–	+	FV	–	–	–	–	–	–	+	–	–	+	–	–	–	–	–	–	–	–	–	AB
Dam 174	–	–	+	–	–	+	FV	+	+	(+)	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	BB
Calf 480	+	+	+	–	–	–	FV	+	+	–	–	+	–	–	–	–	–	–	–	–	+	+	–	–	–	–	AB
Dam B-122	+	–	+	+	(+)	+	VV	–	–	–	–	(+)	–	–	(+)	+	–	–	–	–	–	+	–	–	(+)	–	AA
Calf 486	–	–	+	–	–	+	FV	–	–	–	–	+	–	–	–	–	–	–	–	–	+	(+)	–	–	–	–	AA
Dam 192	(+)	–	+	+	+	+	VV	–	+	–	–	(+)	+	–	+	+	–	–	+	+	–	(+)	–	+	+	–	AB
Calf 510	(+)	+	–	+	–	+	FV	–	–	–	–	–	+	–	–	–	–	–	–	–	+	+	–	+	–	–	AA

(vi) Half-sibs of Sire No. 174

	B			C			F/V	J	L	S		Z	M_1	M_2	M_9	M_{10}	M_{12}	M_{15}	M_{16}	M_{17}	M_{18}	M_{19}	M_{23}	M_{32}	M_{33}	M_{36}	Hb
	B	Y_2	E'_3	C_2	R	X_1				S	H"																
Sire 174	+	+	+	+	-	+	FV	-	-	-	-	+	+	-	-	-	-	+	-	-	+	-	-	+	-	-	AB
Dam 822	+	-	+	-	(+)	+	FF	-	-	(+)	-	(+)	-	-	+	+	-	-	(+)	-	-	-	-	-	(+)	-	AB
Calf 284	+	+	+	+	-	+	FF	-	-	+	-	+	-	+	-	-	-	-	+	-	-	-	-	-	-	-	AA
Dam B-141	+	+	+	+	-	+	FV	+	-	-	-	+	+	+	+	-	-	-	-	-	+	+	-	+	-	-	AB
Calf 316	-	+	+	+	-	+	FV	-	-	-	-	+	+	+	-	-	-	-	+	-	-	-	-	+	-	-	AA
Dam 731	-	+	-	+	+	+	FV	+	-	+	-	+	-	+	+	+	+	-	+	-	+	+	-	-	-	+	AA
Calf 359	-	-	+	+	+	-	VV	+	+	+	+	-	-	-	+	+	-	-	+	-	+	+	-	-	+	-	BB
Dam 314	-	-	+	+	-	+	FV	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	AB
Calf 478	+	+	+	+	-	-	FV	-	+	+	-	+	-	+	-	-	-	-	+	-	+	+	+	-	-	-	AB
Dam 134	(+)	+	-	+	-	+	FV	+	+	-	(+)	-	-	-	(+)	-	(+)	-	-	-	(+)	-	-	-	-	-	AA
Calf 479	+	(+)	-	+	-	+	FV	+	+	+	-	+	-	-	-	-	-	-	+	+	+	-	-	-	-	-	AA

(vii). Half-sibs of Sire No. 225

	B			C			F/V	J	I	S		Z	M_1	M_2	M_9	M_{10}	M_{12}	M_{15}	M_{16}	M_{17}	M_{18}	M_{19}	M_{23}	M_{32}	M_{33}	M_{36}	Hb
	B	Y_2	E'_3	C_2	R	X_1				S	H"																
Sire 225	-	+	+	+	-	+	FF	-	+	-	+	+	(+)	+	-	-	(+)	-	-	-	-	-	-	-	-	-	AA
Dam 345	+	+	+	+	-	+	FV	-	+	-	-	-	+	+	-	-	-	-	-	-	+	+	-	+	-	-	AB
Calf TM-69	+	+	+	+	-	+	FV	-	+	-	-	+	+	+	-	-	-	-	-	-	+	-	-	+	-	-	AA
Dam T-080	+	-	+	+	-	+	FF	-	-	(+)	(+)	+	-	-	-	-	+	-	+	-	+	(+)	(+)	-	-	+	AA
Calf 318	+	-	+	+	-	-	FV	-	+	+	(+)	(+)	-	(+)	-	-	-	-	+	-	+	+	+	-	-	-	AA
Dam 285	+	+	+	+	-	-	FV	-	(+)	-	-	+	+	+	-	-	-	-	-	-	-	(+)	-	+	-	-	AB
Calf 327	+	+	-	+	-	+	FV	-	+	-	+	+	+	+	-	-	-	-	-	-	-	-	+	-	-	-	AA
Dam TM-140	-	+	+	+	-	+	FV	-	+	-	-	-	-	+	+	+	-	-	-	-	+	-	-	-	+	-	AA
Calf 350	+	+	+	+	-	-	FF	-	-	-	-	+	-	+	+	+	-	-	-	(+)	+	-	-	-	(+)	-	AA
Dam 707	+	-	-	+	-	-	VV	+	-	+	-	-	-	-	+	-	-	-	+	-	+	+	-	-	-	-	AB
Calf 360	+	+	+	+	-	+	FF	-	+	-	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	AA

Table 20. Results from complete paternity cases

Bull No.	No. of recorded parentage	No. of correct parentage	percent error in records	No.of wrong parentage	Bulls excluded as sires						All the bulls excluded as sire
					6bulls excluded	5bulls excluded	4bulls excluded	3bulls excluded	2bulls excluded	1bulls excluded	
449	27	13	51.85	14	4	1	-	3	-	-	6
204	23	14	39.13	9	4	-	-	-	-	-	5
2.8	16	12	25.00	4	3	-	1	-	-	-	-
215	10	7	30.00	3	2	1	-	-	-	-	-
174	5	0	100.00	5	1	2	1	-	-	-	1
225	5	2	60.00	3	1	1	-	-	-	-	1
352	2	2	0.00	-	-	-	-	-	-	-	-
Total	88	50	43.18	38	15	5	2	3	-	-	13

sire families. Maximum number of complete paternity cases could be obtained under sire 449 (with 27 offsprings).

All the offsprings with wrong parentage recorded were checked with all other possible bulls tested. Hence it could be noted that in 15 cases only one of the bull could be the possible sire, in five cases any two bulls could be the sire of the offsprings, in two cases any three bulls and in three cases any four bulls could be the male parent. All the bulls under test could be excluded as the sire in 13 cases out of the 38 cases of wrong parentage detected.

The attributes of disputes in each of the 38 cases has been listed in Table 21. The main character being blood group factors and very few (six cases) due to haemoglobin variants.

The dispute occurred mainly in the inheritance of new blood group factors which were identified locally (Table 22). The factor M_{12} could detect 26.3 per cent of the error, next to which was factor M_{36} (21.1 per cent).

The factors Y_2 L, and M_{18} could also detect 18.4 per cent of the error in parentage. Factors E'_3 and C_2 could detect no error in the records from their inheritance pattern.

Table 21. Disputed parentage

Sire No.	Total No. of pairs of dam offspring phenotype studied	No. of Dam-offspring pairs found to be in Dispute		Phenotype variations seen in	
		Dam	Offspring	blood factors	Hb
1	2	3	4	5	6
449	27	1. 132	TM-55	M_{18}	
		2. 492	271	FV,J,M_9,M_{19}	
		3. 485	273	Y_2,L,Z	
		4. 219	278	L,M_9,M_{18}	
		5. 093	283	B,Y_2,R,L,M_2,M_{10} M_{12},M_{18},M_{33},M_{36}	
		6. 192	316	Y_2,H'',M_2,M_{15}	
		7. 765	323	R,L,M_{10},M_{12},M_{33}, M_{36}	
		8. 844	379	Z	
		9. 739	530	Y_2,FV,L,M_{18},M_{19}	
		10.211	549	--	AAxBB->AA
		11.209	550	J,H'',M_{18}	
		12.214	559	X_1,FV,M_1,M_{10}, M_{12},M_{32},M_{36}	
		13.273	563	M_{18}	
		14.207	840	B,X_1,FV,M_1, M_{19},M_{32}	AAxAB->BB

(Contd....)

(Table 21 Contd....)

1	2	3	4	5	6
174	5	1. 822	284	M_2	
		2. 141-B	316	M_{16}	
		3. 731	359	L,H'',M_{33}	ABxAA->BB
		4. 314	478	L,S,M_{16},M_{19},M_{23}	
		5. 134	479	S,M_{16},M_{17}	
204	23	1.TM-41	288	M_{12},M_{36}	
		2. 840	388	$R,H'',M_{10},M_{12},M_{33},M_{36}$	
		3. 137	396	$Y_2,H'',M_{10},M_{12},M_{33}$	
		4. 837	406	Y_2	
		5. 188	188	427	
		6. 136	432	M_{12},M_{36}	
		7. T-131	444	-	AAxAA->AB
		8. 162	450	-	AAxAA->AB
		9. T-211	477	M_1,M_{32}	AAxAB->BB

(Contd....)

(Table 21 Contd....)

1	2	3	4	5	6
208	10	1. 433	300	M_{12},M_{16},M_{36}	
		2. 138	336	S,M_{12},M_{36}	
		3. 155	372	J	
		4.T-414	397	Z,M_{18},M_{19}	
215	10	1. 232	339	M_{16}	
		2. B-132	421	M_{17}	
		3. 013	467	M_{12}	
225	5	1. T-080	318	FV	
		2. T-140	350	B,M_{17}	
		3. 707	360	FV	

Table 22. Efficiency of different blood group and Hb polymorphic system in parentage determination

Blood group systems	Blood factors	No. of sire-dam-offspring sets in dispute	Percentage of error determined
1	2	3	4
B	B	3	7.9
	Y_2	7	18.4
	E'_3	-	0.0
C	C_2	-	0.0
	R	3	7.9
	X_1	2	5.3
FV	FV	6	15.8
J	J	3	7.9
L	L	7	18.4
S	S	3	7.9
	H''	5	13.2
Z	Z	3	7.9

(Contd...)

(Table 22 Contd...)

1	2	3	4
M_1	M_1	3	7.9
M_2	M_2	4	10.5
M_9	M_9	2	5.3
M_{10}	M_{10}	5	13.2
M_{12}	M_{12}	10	26.3
M_{15}	M_{15}	1	2.6
M_{16}	M_{16}	5	13.2
M_{17}	M_{17}	3	7.9
M_{18}	M_{18}	7	18.4
M_{19}	M_{19}	5	13.2
M_{23}	M_{23}	1	2.6
M_{32}	M_{32}	3	7.9
M_{33}	M_{33}	5	13.2
M_{36}	M_{36}	8	21.1
Hb	--	6	15.8

Discussion

DISCUSSION

5 1 Production of blood group reagents by iso-immunisation

Earlier it was postulated that the antigens of the erythrocytes represent the immediate products of the genes that control them The one gene-one antigen relationship theory was based on two facts 1) are genes at more than one locus involved in the control of any particular antigenic specificity? and 2) is more than one antigenic specificity imposed by any particular gene? It is now established that there are eleven blood group systems in cattle Each blood group system consists of those products controlled by the alleles of one locus One gene determines several distinguishable serological characcteristics, but each unit specificity is usually associated with only a single locus The variety of separate specificities in any one system only represents the overlapping serological properties of a series of closely related antigens controlled by the alleles of a single gene

Great variations were noted in the recipient s response to immunisation with donor erythrocytes in cattle {Tables 5(i), 5(ii) and 5(iii)} Variations extended from nil response (even after booster dose) to a maximum antibodv titre of 1 in 512 During the course of iso immunisation,

only 33 per cent of the recipient animals showed production of the haemolysins In re-iso-immunisations, the response was better In general, antisera with higher titre were obtained in re-iso-immunisations (Table 5 - ii) The anamnestic for the blood group factors indicated that the cattle have good immunological memory for stimulus produced by the blood group factors The fitness of individual depends upon the average heterozygosity at different loci in the individual The degree of heterozygosity of the recipient animal represents the extent of combined genotype, and it is a symbol for resistance Good resistance to diseases indicates a well functioning immune apparatus, that reacts to all kinds of antigens including foreign erythrocytes Good correlation also exists on the factor differences between donor and recipient animals with the immune-response The amount of antibody production may decrease with increasing antigenic variety (Stur, 1979)

Many immunisations ended with nil response even after second or third booster doses (Table 5 - iii) This can be due to weak antigenicity of donor erythrocytes or increased antigenic variations between donor and recipient cells Similar nil response has been reported in cattle earlier (Stur, 1979)

5 2 Production of blood group reagents by hetero-immunisation

Responses to bovine erythrocytic antigens in rabbits were similar to that noted by many other Indian workers (Naik et al , 1963 and Khanna, 1968) The high titred polyvalent serum obtained by hetero-immunisation (Table 8) contained mostly of bovine species specific antibodies Since cattle was a Forssman negative species, no forssman antibodies were expected in rabbit s serum Most of the cattle F and Z reagents were produced by hetero-immunisation But it was very difficult to produce mono specific reagent by absorption technique from hetero-immune sera

More recently method, hybridoma technique has been developed (Tucker et al , 1986) for producing monoclonal antibodies to bovine erythrocyte specificities Since the production of iso-immune reagent is time consuming and require regular access to the herd for immunisation and as a source of red cells for absorptions, hybridoma technique is found to be more easy and quick But only a few mono-specific reagents could be produced by this and the difficulty in isolation of clones for other antigen may be

due to the known poor immunogenicity of at least a few of them Further occurrence of clones producing antibodies against epitopes shared by two or more antigenic factors, may cause failure to detect other specificities This method can be considered only as a complement to conventional production of polyclonal antibodies by iso immunisations

3 Preparation of blood group reagents from colostrum

It is well known that the antibodies occur not only in blood but also in other body fluids The number of antibodies in colostrum is particularly large and this is the reason why in the present study an attempt has been made to obtain cattle blood grouping reagents from the colostrum of an immunised cow Both colostrum and blood serum of an immunised cow were studied The titre of antibodies in the colostrum taken on the day of calving was higher than that of serum antibodies No anti-erythrocytic antibodies were detected in the milk examined on the 10th day after calving The L reagent produced from the colostrum showed identical reaction to that of the L reagent produced from the serum of recipients On the basis of the results obtained it may be supposed that colostrum taken from immunised cows on the day of calving may be a reliable source of blood typing reagent

An added advantage is that large quantities of reagents can be produced from a single immunisation Dunlec et al (1979) also reported similar findings

5 4 Naturally occurring antibodies

The anti J from different sources (Animal Nos 248 and 743) was found to possess sub type relationship ie Anti J from Animal No 743 reacting only with a few cells that react with Anti J from Animal No 248 Hence it can be assumed that Anti J from Animal No 743 was a sub type of Anti J from Animal No 248 (Fig 5) Variations in the anti J titre were noticed in two J negative cows (Table 9) and this was in agreement with the observations of Stone (1956), Prakash (1965) and Nagarajchar et al (1988) Though a wide range of variations from 1/2 to 1/32 dilution of normal sera of these animals could be detected, no associations were found with season or reproductive status of the animals (Fig 6) Instead,the titre found to increase, when the animals were fed with green Guinea grass and to decrease when fed with silage The increase in titre may be due to the presence of some lectins in the leaves of Guinea grass Studies in more number of animals in this field is

essential, since presence of anti-J in the serum of cows is related to the breeding efficiency of the animal

Blood typing

The relative frequencies of occurrence of various blood group factors in the cross breds are given in table 10 In general the factors that are comparable to the international reagents had higher frequencies in all the populations when compared to the new factors Some of the new factors (eg M_{12} M_{15} and M_{36}) occurred very rarely among the cross breds but none of the genetic groups showed complete lack of any of these new factors It will be too early to discuss anything on this observation until detailed studies are carried out on large number of animals Studies are also to be carried out on the inclusion of these new factors to various blood group systems

Haemoglobin variants

Only two haemoglobin variants - Hb^A and Hb^B could be noticed in the present study All the three expected

phenotypes viz , Hb AA Hb AB and Hb BB were observed in the population This was in accordance with the findings of Naik et al (1963) Singh and Bhat (1979) Nandakumaran et al (1979), Singh and Bhat (1980a,b), Shankar and Bhatia (1982) Singh et al (1983) and Khanna and Tandon (1987)

Other variants like Hb^C Hb^D and $Hb^{Khillari}$ were not observed in the cross bred population studied These variants were reported in Malvi Khillari Dangi Kankrej Rath Kumaoni and Sahiwal breeds by Naik et al (1969) Singh and Khanna (1973) and Shankar and Bhatia (1982) The absence of these rare variants in the population studied were expected since the genetic groups presently studied did not get any gene from the above mentioned breeds

Genetic studies

Blood groups

a) Estimation of gene frequencies

The estimated gene frequencies of various blood group factors are presented in table 11

Factor B This was a common factor in all the three different genetic groups In the pooled cross bred population, the dominant allele B exhibited a frequency of 0 66 The frequency of the same was in an increasing trend viz 0 59 (Jersey cross bred), 0 66 (Brown-swiss cross bred) and 0 72 (Holstein Friesian cross bred), among the three genetic groups The frequency of this allele was not reported earlier in any Indian cattle breeds

Factor Y2 The observed frequencies of Y_2 factor were 0 55, 0 51 and 0 59 in Jersey Brown Swiss and Holstein Friesian cross breds respectively Prabhu and Mishra (1972) reported that 48 4 per cent, 17 per cent and 48 7 per cent of the Hariana, Ongole and Tharparkar breeds, respectively reacted positively to Y_2 factor In the present study, the pooled cross bred population had a Y_2 gene frequency of 0 55 The increased frequency of this allele may be due to the fact that the present studies were carried out in cross bred cattle and this allele may be more frequent in the exotic breeds

Factor E 3 This factor was present in 324 out 411 cross bred animals typed and gene frequency of this factor was highest among other factors tested Jersey cross bred exhibited highest gene frequency for the dominant E_3

factor (0 87) while it was almost equal for the other two breeds viz Brown-Swiss cross (0 75) and Holstein-Friesian cross (0 76) Reports were not available on the occurrence of this factor in any Indian breeds

Factor C2 This was the most common factor in C system Frequencies of C_2 allele were 0 79 (Jersey cross bred) 0 78 (Brown Swiss cross bred) and 0 74 (Holstein-Friesian cross-bred) The factor was reported to be present in three Indian cattle breeds, viz Hariana, Ongole and Tharparkar with a relative frequency of occurrence 0 438, 0 134 and 0 442 respectively (Prabhu and Mishra, 1972)

Factor R There are earlier reports of occurrence of this factor in Gir, Hariana, Kangayam Kankrej, Sahiwal Sindhi Rathi and Tharparkar breeds with the percentage or positively reacting animals as 6 6 2 3, 19 2, 16 0 2 5 1 7, 13 3 amd 19 0 respectively (Prabhu and Mishra, 1972) The cross-breds in the present study also possessed this antigen with a low frequency The estimated gene frequency of R allele was 0 23 in the pooled cross-breds, with nearly equal gene frequency in the three genetic groups studied

Factor X1 Nine Indian cattle breeds were reported to have X_1 factor on their erythrocyte membrane out of the eleven breeds studied (Prabhu and Mishra, 1972) The incidence of this factor has not been reported in Ongole and Rathi cattle breeds (Prabhu and Mishra, 1972) The reports showed that the relative frequency of occurrence of this factor was highest (100 per cent) in Hallikar breed, the percentage of positively reacting animals in Gir, Hariana, Hill, Kangayam, Kankrej, Sahiwal Sindhi and Tharparkar varying from 70 to 94 per cent In the present study, the gene frequency for this dominant allele was estimated as 0 65, 0 55 and 0 60 in Jersey Brown-Swiss and Holstein-Friesian cross bred animals respectively

FV system This system has been studied extensively in eleven cattle breeds of Indian and two cross-bred cattle (Jersey x Sindhi and Sahiwal x Holstein-Friesian) by earlier workers The incidence was 100 per cent for the F factor in Gir breed and the factor V was present in all the eleven breeds studied till then (Prabhu and Mishra, 1972) The frequency of F allele was 0 928 in Gir cattle and was 0 633 in Rathi But the frequency of F allele was 0 833 and 0 685 in the cross-breds viz Jersey x S ndhi and Sahiwal x Holstein-Friesian cross-breds (Bhag. et al 1972) The

gene frequencies q^F and q^V for the pooled cross-breds under the present study were 0 64 and 0 36, respectively The frequency of F allele was much lower in all the genetic groups presently studied when compared to those for the cross breds reported earlier This may be due to the increased zebu lineage

Factor J Gene frequency for the allele J^{cs} has been estimated in the three cross bred populations, the values being 0 43 (Jersey cross-bred), 0 44 (Brown Swiss cross-bred) and 0 37 (Holstein Friesian cross bred) Prabhu and Mishra (1972) had reported that all the Indian cattle breeds studied, possessed the factor with wide range of relative frequency The incidence of this factor was lowest for Kankrej (5 7 per cent) and highest for Tharparkar (74 per cent) The gene frequencies for the J allele ranged from 0 029 (Kankrej) to 0 491 (Tharparkar) The gene frequencies were 0 182, 0 127 and 0 216 for the cross breds viz Jersey x Sindhi, Sahiwal x Holstein and Jersey x Ongole respectively (Prabhu and Mishra, 1972) Compared to the earlier reports, the gene frequencies obtained during the present study were high and the value might have been more, if the J^s animals were also typed and included in the J positive group

Factor L The dominant factor L was present in all the three genetic groups, with a gene frequencies of 0 28, 0 30 and 0 35 in Jersey, Brown Swiss and Holstein Friesian cross-breds, respectively The gene frequencies of this allele in different Indian breeds have been reported earlier (Prabhu and Mishra, 1972) The frequency was highest in Tharparkar (0 878) and lowest in Kangayam (0 494) This has not been reported in Ongole breed

Factor S The incidence of this factor was reported only in three per cent of Sahiwal but in 5 9 per cent of Hill cattle The gene frequencies calculated in the present study were 0 35, 0 41 and 0 38 in the Jersey cross bred, Brown swiss cross-bred and Holstein-Friesian cross bred populations, respectively There is a probability that factor S may be playing an important role in the adaptability of animals in hilly areas It is too early to make any conclusion until the occurrence of this allele is reported in many other Indian cattle breeds

Factor H This factor occurred very rarely among the animals presently studied, the gene frequencies being 0 11, 0 14 and 0 16 in Jersey cross bred, Brown Swiss cross bred and Holstein-Friesian cross breds respectively The

incidence of this factor has not yet been reported in any Indian cattle breeds

Factor Z This simple dominant factor occurred in all the three cross-bred populations studied with a gene frequency of 0 44, 0 41 and 0 44 in Jersey cross-bred, Brown-Swiss cross-bred and Holstein-Friesian cross breds respectively The frequencies for this factor has not been reported earlier in any of the Indian cattle breeds

New Factors The gene frequencies for the new blood group factors have been estimated for the Jersey cross-bred, Brown-Swiss cross bred and Holstein Friesian cross-breds included in the present study and are listed in table 11 Since the factors are to be compared with internationally accepted reagents and no previous reports were available in these blood group factors comparison of gene frequencies of these new factors were impossible

The frequencies of various blood group factors obtained during the present study may give an indication of the genetic composition of the cross-bred population in this state with respect to the blood group loci

b) Test for genetic equilibrium

Holstein cross breds deviated much from the expected value and the Jersey cross-breds nearing the table value (Table 12) This was due to increased use of a few number of bulls which are homozygous (FF) at this locus However, the conclusion stands tentative until all the other alleles reported in this system elsewhere could be studied in the population

The frequency of allele (q^V) was much higher in the population presently studied, when compared to the earlier reports of Prabhu and Mishra (1972) in ten Indian and three cross-bred cattle, Bhagi et al (1972) in Jersey x Sindhi and Sahiwal x Holstein-Friesian crossbreds and Hines et al (1977) in Holstein cattle

c) Mode of inheritance of blood group factors

After a blood group factor has been established serologically as a unit, it is important to satisfy the genetic criteria for that single factor This is achieved by studying the results of segregation within the mating type However additional evidence can be obtained by using a gene

frequency analysis and by comparing the observed and expected number of mating types and the phenotypes of the offsprings The analysis pre supposes that the data are from a randomely mated population For the cattle blood antigenic factors which appear serologically as a unit, the distribution of each factor in the progeny from various kinds of mating was studied (Table 13) The expected distribution was according to the dominant inheritance The observed and expected phenotypic frequencies could not be compared as it was not possible to know the genotypes of the parents There were however 38 exceptions where offsprings exhibited a particular factor even when both the parents were negative The majority of such matings could be explained as cases of wrong entries in Pedigree records The chances of irregular transmission of the blood antigenic factors, however, could not be ruled out There are several reports where the irregular transmission of blood factors have been reported (Moustgaard and Neimann Sorensen, 1962, Stormont, 1963, Bouw et al , 1964) Irregular transmission was explained on the basis of crossing over within a blood group locus In the present study it was difficult to delineate the conditions of irregular transmission from disputed parentage cases with the available limited data

The results of comparison between the observed and expected frequency of mating and the phenotypic distribution in progenies in each mating based on analysis of autosomal unifactorial inheritance indicated a good agreement between the observed and expected ratios There were few deviations but such deviations were not unexpected because of small sample size It could be concluded in the results therefore that the cattle red cell antigenic factors were inherited as dominant over their absence

Haemoglobin polymorphism

a) Genotype and gene frequencies at haemoglobin locus

Three haemoglobin phenotypes HbAA, HbAB and HbBB controlled by two alleles, HB^A and Hb^B were observed No other variants could be observed in any of the genetic groups studied The frequency of Hb AA individuals was more in the pooled cross breds (0 5036) and the Hb BB type occurred very rarely (0 0657) The number of heterozygotes (Hb AB) were highest in Jersey cross (0 5133) followed by Brown-Swiss cross (0 5123) and Holstein Friesian crosses (0 2647)0 These may be the reasons for increased adaptability of Jersey cross breds and Brown Swiss

cross-breds under Kerala conditions than the Holstein cross-breds The superiority of Hb AB animals over to that of Hb AA and Hb BB animals in the adaptability was reported earlier by Singh et al (1983)

Earlier reports suggested that the Hb^A allele occurred in all cattle breeds and the less common Hb^B allele occurred mostly in Asian and African zebus and in Channel Island breeds, especially Jersey The present study supports the earlier findings The frequency of Hb^A allele was highest in Holstein cross breds (0 83) followed by Jersey cross-breds (0 67) and Brown-Swiss cross breds (0 66) The pooled cross breds had a Hb^A frequency of 0 72 The frequency of Hb^B allele was 0 17, 0 33 and 0 34 in the Jersey cross-bred, Brown swiss cross bred and Holstein Friesian cross-bred respectively

The gene frequency for Hb^A in Holstein Friesian crosses in the present study was 0 83 while the values observed by Singh and Bhat (1972) in 1/2 Friesian cross bred and 3/4 Friesian cross-breds were 0 787 and 0 886 respectively The high frequency of Hb^A allele in Holstein Friesian crosses is expected as the pure Holstein breed has a gene frequency of almost 1 0 This finding was in accordance with the earlier

reports of Singh and Bhat (1972) in cross bred cattle of India

The higher frequency of Hb^B allele may be due to the presence of Sindhi genes in the cross breds presently studied For upgrading the local cattle of Kerala, Red Sindhi was used in early 1940 s and 1950 s and this could have resulted in an increased flow of Sindhi gene to the cattle population of Kerala Srivastava (1965) reported that Hb^B allele had higher frequency in the cattle of Sindhi lineage

b) Heterozygosity at haemoglobin locus

Polymorphism in a population reflects genetic variability The variation in population provides scope for selection Blood groups and protein variants are useful tools for estimating variability between population Heterozygosity at haemoglobin locus was calculated in three cross bred populations Maximum heterozygosity was observed as 0 448 (Brown Swiss cross breds) followed by 0 442 (Jersey cross breds) and 0 282 (Holstein Friesian cross breds) In the pooled cross breds, the heterozygosity was estimated to be 0 403 From the results, it may be inferred that the heterozygosity in the different cross-breds varied

according to the exotic breeds used though the indigenous breeds remained common Higher heterozygosity at haemoglobin locus in Jersey cross breds and Brown-Swiss cross breds and lower heterozygosity in Holstein Friesian cross-breds were reported earlier by Nandakumaran et al (1982) Measurement of heterozygosity at one locus, may not reflect the true genetic variability of a breed or population But the present study is only a beginning and it is suggested that more extensive studies are to be carried out by including more number of polymorphic loci

c) Test for genetic equilibrium at haemoglobin locus

Good agreements between the observed and expected genotype frequencies at the haemoglobin locus were obtained This showed that the populations were in genetic equilibrium with respect to haemoglobin locus Selection of animals was based on their performance and other economic characters Any biochemical marker were not included in the selection criteria Hence the result obtained was in accordance with the expectations Earlier workers also could observe the same trend in the population they studied (Singh and Khanna 1971, Singh et al , 1972 and Nandakumaran et al , 1979)

d) Mode of inheritance of haemoglobin variants

A good agreement was obtained between the observed and expected number of offsprings with the different haemoglobin phenotypes This proved that the haemoglobin alleles Hb^A and Hb^B are co dominant and autosomal and showed equal penetrance of both the alleles in the population The results were consistent with the observations of Singh and Khanna (1971) who studied 120 offsprings from all the different haemoglobin mating types in Hariana cross-bred cattle

Parentage studies

Animal breeders have already stressed the need for identifying the actual parentage of progenies from elite cows and superior bulls in progeny testing programmes Early in 20th century animal breeders started using blood type of animals to exclude wrong parentage The reliability on this method in solving the problems of questionable parentage have been stressed by Stormont (1967) The accuracy can be increased when more polymorphic systems are included in the study (Singh and Nair, 1980)

When mis identification occurred in the recording of sires of progenies (more chance in artificial breeding programmes) substantial urder estimation of the heritabilty was noticed (Van Vleck, 1970 a and 1970 b) This reduction in the estimate of heritability from intra-sire correlation method appeared to be proportional to the square of the fraction of the cows whose sire was correctly identified

On perusal of the breeding records maintained at University Livestock Farm Mannuthy ard Cattle Breeding Farm, Thumburmuzhi 88 sets of sire dam offsprings could be blood typed in seven sire families The results from 88 complete paternity cases showed accuracy only in 50 cases while the remaining 38 cases were found to be erroneous

In thirteen cases, all the sires available for blood typing could be excluded This may be due to the erroneous recording of the female parents In 15 cases (wrongly recorded parentage) six bulls out of the seven available for testing, could be excluded as the male parent (Table 20) But the recording was found to be erroneous Confusion with the male parentage can be avoided by reducing the repeat breeding (Rendel, 1956 b) In a farm with two or three bulls only available for breeding insemination of a cow

with the semen from same bull in consecutive heat periods is difficult Hence, proper timing of insemination, proper recording of the bull number whose semen is used for insemination and early pregnancy etc are very important to reduce the error in pedigree records

The error estimated in the breeding records of University Livestock Farms, was 43 18 per cent (Table 20) This value was more than that expected But there were reports of even much higher percentage of error in pedigree records by many workers The error ranged from 23 5 to 49 5 per cent in Russian farms (Vsyakikh et al , 1973), 62 2 to 67 78 per cent in Spanish cattle breeds (Altarriba et al , 1983) and 67 5 per cent in Uzbek cattle (Akhmedov et al , 1988) However in most of the cases, the recorded error in parentage was between 10 and 30 per cent (Kovacs Gy, 1965, Schleger and Soos, 1967, Slepcanko, 1970, Singh and Nair, 1980 and Lazareva and Sukhova, 1985)

This observation throws light into the urgent necessity for adopting blood typing of all animals in a breeding farm as a routine procedure

Most of the blood group factors (except E_3 and C_2) and haemoglobin polymorphic system were involved in detecting the correct parentage with variations in the number of recorded parentage with each factor Of the thirty eight wrongly detected parentage cases, twelve exclusions were based one single factor inheritance, but the incidence of which was expressed by a strong antigen-antibody reaction In the remaining cases conclusions were made based on more than one factor

The relative efficiency of each factor in detecting the error was estimated (Table 22) This was found to be highest for the factor M_{12} which is a new factor produced in this laboratory The efficiercy of this factor was 26 3 per cent as compared to 21 1 per cent for M_{36} which is also a new factor identified in this laboratory The efficiency was much lower for factors that occurred only very rarely (M_{15} and M_{23})

When haemoglobin variants alone were considered, the efficiency was 15 8 per cent This is in confirmation with the earlier reports of Singh and Bhat (1981) who reported 7 to 18 per cent efficiency in different Indian cattle breeds In three cases, wrong parentage could be detected on the

basis of dispute in the inheritance of haemoglobin variants alone In three cases disparity could be noticed both in blood factors and haemoglobin variants Hence there was increase in error estimated by 8 per cent with the use of haemoglobin polymorphic system in addition to the factors in the blocd group systems This clearly showed that supplementing polymorphic systems to the blood group systems increased the efficiency of detecting the error in parentage (Rendel and Gahne, 1961) The additional use of the transferrin system resulted in a considerable increase in efficiency ard the combined use of tests for cellular antigens and transferrin could solve 84 per cent of the complete paternity cases with two possible sires The efficiency of any genetic system in solving parentage problem is dependent on the number of alleles in the system, their frequencies and whether the genotypes could be directly inferred from the phenotype The efficiency of checking the breeding records can be increased if more complex systems like transferrin ard amylase are included With two polymorphic systems, the error in recorded parentage was found to be 43 per cent The error may increase if more number of reagents are used for typing and more number of polymorphic loci are included

Identification of new born calves at the time of birth or as soon as possible on the day of birth itself is more important When more than one calving occurs in a day Proper identification of the calf with permanent ear marks or by other methods and accurate recording of the dam number are highly essential A systematic method should be followed in identifying the animals Care should be taken to avoid duplication of numbers It is also suggested that all the bulls used for breeding should be blood typed

Summary

SUMMARY

1 Thirty one iso immunisations and eight hetero-immurisations in rabbits were carried out for prcductior of blood typing reagents Iso-immune sera were comparatively low titred than that of the hetero-immune sera

2 Eleven bovine blocd typing reagents tentatively designated as M_{26} (L), M_{27} (H), M_{28} (Y_2), M_{29} (C_2), M_{30}, M_{31} (X_1), M_{32}, M_{33}, M_{34} (F), M_{35} (R) and M_{36} were produced Seven were comparable to internationally accepted reagents The remaining were new reagents and are to be compared with international reagents

3 Colostrum from immunised cows seemed to be the most promising source of antibody for bovire erythrccytes

4 Season had no effect on the naturally occurring antibody against J antigen

5 Four hundred and eleven cross bred cattle were blocd typed using a panel of 28 reagents

6 Gene frequencies for the above blood factors were estimated for the three different cross breds (Jersey, Brown swiss and Holstein Friesian)

7 The blood factors except in the FV system were found to be inherited in a Mendelian manner with simple dominance In the FV system, the alleles were co-dominant

8 Cross bred populations were tested for genetic equilibrium at FV blood group locus and haemoglobin locus The same was found to be in Hardy Weinberg equilibrium in both loci except for FV locus in Holstein Friesian crosses

9 Two haemoglobin variants Hb^A and Hb^B were detected in all the three crossbred populations Gene and genotype frequencies for haemoglobin variants were estimated for thre different cross breds Holstein Friesian crosses had greatest number of Hb AA individuals and hence the highest gene frequency for Hb^A allele

10 Heterozygosity at the haemoglobin locus was high for Jersey and Brown swiss cross breds

11 Studies on the inheritance of haemoglobin alleles revealed that Hb^A and Hb^B were co-dominant and autosomal

12 Exclusion of parentage was possible in 38 cases (43 18 per cent) studied with blood group factors and haemoglobin system Error could be detected with almost all the variants used, except E'_3 and C_2 blood factors

13 The efficiency of new blood group factors M_{12} and M_{36} was higher than the other factors in detecting false parentage Efficiency of Hb locus was 15 8 per cent in this regard

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PARENTAGE CONTROL IN CATTLE USING BLOOD TYPES

By

V. MARY JOHN

ABSTRACT OF A THESIS

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Master of Veterinary Science

Faculty of Veterinary and Animal Sciences
Kerala Agricultural University

Department of Animal Breeding and Genetics
COLLEGE OF VETERINARY AND ANIMAL SCIENCES
Mannuthy Thrissur

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ABSTRACT

Immunogenetic studies were carried out in four hundred and eleven cross-bred cattle maintained at the University Livestock Farm, Mannuthy and Cattle Breeding Farm, Thumburmuzhi The animals belonged to three genetic groups, viz Jersey cross breds, Brown-Swiss cross breds and Holstein Friesian cross breds The animals were typed for blood group factors and haemoglobin Blood group reagents were produced from iso immune sera, hetero immune rabbit sera and colostrum of an immunised cow Eleven reagents were produced by the above cited methods and seven of them were comparable to international reagents Serum from two animals (Animal Nos 248 and 743) were used as sources of anti J whose titres were being assessed periodically by haemolytic technique The titre varied from 0 to 1 32, but no association with seasons of the year could be noticed

Typing of cross bred animals was done with 28 blood group reagents (14 internationally comparable and 14 new reagents) The internationally comparable reagents were B, Y_2, E_3, C_2, R, X_1, F, V, J, L, S, H and Z Anti J from two different sources (Animal Nos 248 and 743) were used and one (Animal No 743) seemed to be the sub type of other (Animal No 248) Standard haemolytic test was carried out

for typing animals for their blood group factors The factors occurred in the three genetic groups with varying gene frequencies

A good agreement was observed between the observed and expected numbers in each genotypes with respect to FV locus in all the population except in Holstein Friesian cross breds ($P < 0.05$)

The mode inheritance of blood group factors showed that the cattle red blood cell antigenic factors were inherited as dominant over their absence

The cross-bred population was also typed for haemoglobin Electrophoresis was carried out in poly acrylamide gel Only two haemoglobin variants viz HbA and HbB and three phenotypes viz Hb AA, Hb AB and Hb BB were observed The gene frequencies of Hb^A allele was 0 67, 0 66 and 0 83 in Jersey cross breds, Brown Swiss cross breds and Holstein Friesian cross-breds, respectively The genotype frequencies at haemoglobin locus for the pooled cross breds were 0 5036 (Hb AA), 0 4307 (Hb AB) and 0 0657 (Hb BB), respectively Genetic variability of breeds was studied in terms of heterozygosity at Hb locus and

Friesian cross breds were found to have least heterozygosity, ie 0 2822

A good agreement was noticed between the observed and expected genotypes at haemoglobin locus and the populations were found to be in genetic equilibrium The two alleles Hb^A and Hb^B showed co dominance and equal penetrance when the inheritance pattern was studied

An attempt was made to find out whether there existed any error in the recording Exclusion of parentage was possible in 38 cases of recorded parentage and the error in breeding records was estimated to be 43 18 per cent

The efficiency of each factors in solving the disputed cases was found to range from 0 to 26 3 per cent with higher efficiency being recorded for new blood group factors Haemoglobin polymorphic system alone could detect three of the 38 disputed cases

This showed that supplementing protein polymorphic loci with the blood group loci will increase the efficiency of parentage control