STUDIES ON INHERITANCE OF R_1, E AND J BLOOD ANTIGENIC FACTORS IN ONGOLE AND ZEBU-JERSEY CROSSBRED CATTLE IN INDIA

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B. V. Sc. & A. H.

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THESIS

Submitted to Agra University, Agra,
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Head, Division of Animal Genetics,

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April 18, 1968.

Certified that the research work embodied in the thesis "Studies on inheritance of R_1, E and J blood antigenic factors in Ongole and Zebu-Jersey crossbred cattle in India" by Shri V. Prabhakar Rao is an original piece of work carried out by him in this Division under my guidance and supervision.

Prabhu S.S.

(S.S. PRABHU)

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THE AUTHOR WISHES TO PLACE ON RECORD

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C O N T E N T S

INTRODUCTION

Blood group studies in cattle had made great strides during the last 2-3 decades. Today 12 distinct systems have been recognised in cattle. They are A, B, C, F-V, J, L, M, N, SU, Z, R'S', and T' (Rendel, 1967), Each system carrying varying number of blood group antigenic factors each of which found to be inherited in a typical Mendelian manner, the presence acting as dominant to absence. In the present study, the incidence, inheritance and association with economic traits of 3 blood group factors had been investigated and results presented. The factors were R_1, E and J. In addition, incidence of haemoglobin variants was also studied. The breeds selected were Ongole and Jersey-Zebu crosses.

REVIEW OF LITERATURE

Landois (1875) observed clumping of red blood corpuscles when samples of blood from different species were mixed together. Thus he confirmed the existence of species specificities and further suggested the presence of similar differences within species. Bordet (1898) found that species specific antisera could be obtained when the blood from an animal of one species was injected into the body of an animal of another species. The antisera so formed were found to react with the red cells of donor and with those of other animals of the same species, causing haemolysis. He suggested the use of serological methods in recognising species specificities in erythrocytes of animals.

At the beginning of the present century Landsteiner (1900) made his fundamental discovery that the bloods of some of his coworkers were able to agglutinate the red cells of the others. In the same year, the Mendelian principles of heredity were rediscovered and not long after before a close association between immunology and genetics was established.

Von Dungern and Hirzfield (1910) studied the inheritance of Landsteiner's ABO blood system in man and demonstrated

that the blood groups were inherited in Mendelian fashion.

The studies on animal blood groups were initiated about the same time when the individual differences in the blood of goats were demonstrated by Erlich and Morgenroth (1900). They also developed Bordet's immunisation technique which had been since utilised in studies on animal blood groups.

Todd and White (1910) discovered that the red blood cells of any individual were characterised by a definite individuality of their own and could be distinguished from those of any other individual. They also speculated that the serological properties of blood cells were probably inherited. Their later work confirmed this concept.

Ottenburg and Friedman (1911) mixed the serum of various cows with the red blood cells of others. According to the agglutination reaction observed, the bloods of cattle were divided into three groups viz. Ao, $O\alpha$, Oo.

Wesceszky (1920), Kunz (1923), Schermer (1928) also reported similar iso-agglutinations in cattle.

Karshner (1928) studied fifty heads each of Holstein and Durham cattle and reported two groups : one with

unagglutinable cells and others whose cells were agglutinable with sera from first group.

Little (1929) with modification of the existing agglutination technique demonstrated the existence of three classes of individuals (Ao, Oα and Oo). He further elucidated the need of complement in iso-agglutination. He indicated that the titre of natural antibodies was rarely high and might often vary from time to time in the same individual and from animal to animal.

Naturally occurring antibodies did not prove very valuable in advancing the study of bovine blood groups. Hence immunisation and absorption techniques were used. By using this technique Ferguson (1941) identified 9 antigens which he named as A, B, C, D, E, F, G, H and I in order of their discovery. B, D, C and F were probably identicals. Each was seen to be inherited as if controlled by one gene.

Later, Ferguson in collaboration with Stormont and Irwin (1942) reported 40 antigens on the erythrocytes of cattle. Stormont and Cumley (1943) gave the pictorial representation of inheritance of 30 antigens of red cells of cattle and their use in disputed parentage.

Stormont, Irwin and Owen (1945) could distinguish

more than 50 antigenic factors in cattle blood by using antisera produced by immunizing cattle and rabbits with cattle red blood cells (iso- and hetero-immunisation).

Stormont (1950) further detected the additional gene controlled antigen on bovine erythrocytes and the notation used to characterise the additional antigenic factors were I', J', K', L', Z' and F. He also suggested the overlapping of serological relationships (cross reaction) among the antigens. In accordance with the evidence, serological sub-types of each of antigenic factors as T (subtypes T_1, T_2), U (U_1, U_2), X (X_1, X_2), O (O_1, O_2, O_3) and E' (E'_1, E'_2, E'_3) were proposed. In accordance with this system, C_2 and C should be called C_1 and C_2 and YC' and Y should be called Y_1 and Y_2 respectively.

Stormont (1950) recommended the use of rabbit serum as complement source instead of guinea-pig serum.

Schermer and Otte (1953), Forschner (1955), Tolle (1956), Mitscherlich et al. (1956) made the study on cattle blood groups and were able to detect 8 blood group factors designated as A, B, C, D, E, F, G and Z.

Mahanjko (1956) with the aid of iso-agglutination

test in adult Simmental and crossbred cattle established three blood groups Co, Ao and O. Erythrocytes of calves of Simmental breed were found to be non-agglutinable upto the first year of life.

Tolle and Urbaschek (1956b) using 366 families of different breeds found the factors A, B, C and E were due to single gene and the presence of each factor was dominant over its absence. The factors A and E were probably linked. The F_1 generation possessed only such blood groups which were present in one or both parents.

Rendel, Tolle and Neimann-Sorensen (1957) reported still another normally occurring antibody. They reported the results of a comparison of the properties of the iso-agglutinins with those of the iso-haemolysins. The comparison of natural antibodies with specific immune sera revealed that seven of these were identical to anti-J.

For a long time it was accepted that presence or absence of the antigenic factors were controlled by genes on 11 loci and corresponding to this 11 blood group systems were defined. The A, B, C, F-V, J, L, M, SU, Z, H' and Z' systems (Irwin, 1956 and Rendel, 1958).

Stormont (1958) pointed out that H' did not constitute

a separate system but belonged to the SU system and the factors S and H' were termed as S_1 and S_2 respectively. Lately it was reported that Z' factor was presumably part of A system (Stormont, 1961). Hence the 11 blood group systems, as pointed out earlier, had been reduced to 9 systems.

Stone et al. (1958) recommended the use of complement instead of isotonic saline solution, for preparation of erythrocytic suspension to ensure the addition of complement.

Braend (1961) and Larsen and Moustgaard (1961) recommended the use of plastic plates as replacements for the usual, Wasserman racks and 10 x 75 mm glass tubes.

Stormont (1962) reported the existence of 11 blood group loci in cattle, forming 11 blood group systems. These cattle systems were named A, B, C, F-V, J, L, M, N, S, Z and R'-S'. He also reported that approximately 100 specifically different bovine blood typing reagents were in use.

A-H system

Ferguson (1941), Ferguson et al. (1942) were first to report and isolate A and H antigenic factors.

The H factor was first described by Ferguson (1941)

and it was shown to be inherited as a dominant to its absence (Ferguson et al, 1942). Stormont et al. (1951) demonstrated that H was transmitted independently of the blood groups of the B and C systems. Hence initially the A-H system was described as a four allele system viz. a, a^A, a^H and a^{AH}. Ferguson (1955), Irwin (1956) described A-H system with four allelic genes controlling the manifestation of A and H factors.

As a result of typing American buffalo (Bison bison) Stormont and Suzuki (1956) recognised phenogroup (S) with reagent D and found this was a frequent bovine factor. It was observed that blood factor D belonged to the A system rather than to a separate system by itself. Stormont, Miller and Suzuki (1960), Larsen (1961) observed that A_1, A_2, D and H were members of A system.

Stormont et al. (1961) obtained anti-Z' from a Holstein-Friesian cow, immunised against a Zebu bull and also observed that Z' too was a member of A system. Hence the number of alleles of the A system were extended to ten viz. a^{A_1}, a^D, a^H, a^{A_1D}, a^{A_2D}, a^{A_1H}, a^{DH}, a^{A_1DH}, $a^{A_1D_2Z'}$ and a^{A_2DH} (Stormont, 1962).

The allele H occurred only in American Brahman cattle

(Bos indicus), where it occurred with a frequency of 0.42. The allele $a^{A_1D_2Z'}$, which occurred in Brahmans with a frequency of 0.09, was extremely rare or absent in most of the breeds of Bos taurus in United States (Stormont, 1962).

Bent Larsen (1961) reported that 7 of the 8 possible elleles of A system were present in Danish breeds of cattle. The only allele not found in Danish cattle (A^H) was found to be present in Indian Kumauni Hill breed.

Sinclair (1963) reported that variation in degree of reaction of H positive cells were due to quality of genetically determined H antigen on cells. A small statistically significant gene dosage effect was seen.

B and C systems

Stormont, Irwin and Owen (1945) recognised 40 distinct antigens and observed genetical association between B, G and K as previously noted by Ferguson (1941) for C and E antigens, who suggested that a single allele, or gene complex may govern the production of two or more immunologically distinct antigens.

Stormont, Owen and Irwin (1948) recorded association

of 21 antigens in various combinations. Each combination involved from 2 to 8 of these antigenic characters but 7 of these were found to occur singly.

Stormont, Owen and Irwin (1951) concluded from a number of sire family studies that 21 of 38 antigenic factors studied were governed by multiple alleles at one locus, the B locus. Another seven factors belonged to C system. The alleles at these two loci antigens which were characterised by a varying number of antigenic specificities. Eight phenogroups in B system and 22 in C system were recognised.

Stormont (1952b) reported 24 alleles in C system and 90 alleles in B system.

Neimann-Sorensen _et al_. (1956), Rendel (1956), Bouw (1958), Braend (1956, 1959), Gasparski _et al_. (1960), Muller (1960) reported new phenogroups.

While working on Danish cattle breeds Bent Larsen (1961) encountered 3 new alleles in B system viz. D_2, D_4 and D_{12}. The D_2 appeared as a non-linear subtype of Y_1 and Y_2, where Y_2 was complementary to the two others. The D_4 was probably a subtype of B, and the D_{12} apparently was closely related to the factors E'_1, E'_2 and E'_3.

As evident from the above reports the B system of cattle was one of the most complex blood group system known in any species. There were more than 300 reported phenogroups of B system and 35 phenogroups in C system (Stormont, 1962).

Larsen (1964) presented family data showing aberrant transmission of a phenogroup in the B system in Danish cattle. He maintained that the appearance of the group $O_2^{A'}1^{I'}$, unknown in Danish cattle by that time could be explained only as a result of irregularity in the transmission of the phenogroup $BO_3^{A'}1^{I'}O_4$.

Nasrat (1965) gave details of 2 cases of abnormal transmission of blood groups in the C system. To explain these cases, it was suggested that the C phenogroups were controlled by a complex locus consisting of a chain of closely linked genes.

F-V and Z systems

The F-V system of cattle was a two blood factor (F and V) two allele (f^F and f^V), three phenotypic system (F, F-V and V).

Ferguson (1941) identified F antigen on the red blood

cells and prepared isolysin for it.

Stormont (1948) differentiated heterozygotes from homozygotes for genes Z and F, with heteroimmune serum produced in rabbits, on the basis of rate of reaction. The cells from homozygotes were found to be haemolysed more rapidly. The gene frequency analysis and inheritance data were also found to be consistant with the above findings.

Rendel (1958) indicated that he had developed anti-sera,which distinguished between a strong V type (V_1) and weaker one (V_2), but later Miller (1960) isolated V_1 and V_2.

Neimann-Sorensen (1958) was unable to establish any serological relationship between M-N system of humans and F-V systems of cattle, although genetical similarity was evident.

Neimann-Sorensen (1958) was the first to prepare a F subtyping reagent designated F_2.

J system

Ferguson (1941) described J as a cellular character but it was found by inhibition tests that J positive cells contained soluble J substance.

Ferguson et al. (1942) reported that the J substance of cattle was a soluble blood group substance found in various body fluids and tissues. It was first described as a Mendelian dominant antigenic character of the erythrocytes (cells) detected only by naturally occurring antibodies in the serum of cattle lacking the J substance.

Stormont (1949) reported J factor to be a soluble serologically reactive substance, elaborated by tissues into serum. The erythrocytes acquired it secondarily. It could be useful in diagnosing monozygosity. New born calves (J^{cs}) lacked J substance on cells. He concluded it was not produced by the same tissues that produced the cells.

Stormont, Owen and Irwin (1951) observed that the J system was composed of two principal phenotypes, namely J positive and J negative, with evidence for a variety of inter-grades in the J positive category ranging from very weakly positive to very strongly positive.

Stone (1953), Stone and Irwin (1954) proposed that 3 alleles at J locus determined three types, J^{cs} would give J substance on the cells and in serum, J^{s} animals had J substance in the serum, but not detectable on the cells and J^{a} represented no J substance neither in serum nor on

cells. The alleles responsible for J-antigen were in descending order of dominance. J^{cs}, J^{s} and J^{a}. They further observed a decrease in titre of anti-J for a short period at calving.

Neimann-Sorensen, Rendel and Stone (1954) demonstrated that anti-J sera after removal of species specific antibodies still showed specific reactions with erythrocytes from sheep of type R and human of group A.

Stone and Irwin (1954), Patel (1958) observed a higher concentration of J substance in sera of J^{cs} cattle than J^{s} cattle and concluded that amount of J substance on cells was also determined by concentration of J in serum in part.

Stone (1956) reported the seasonal variation in the titre of normally occurring anti-J. He found highest titres during the late summer and early autumn and the lowest titres during the late autumn and early winter. Braend (1959) confirmed these findings for the animals in the Northern hemisphere. Osterhoff (1962) observed highest titres during the late summer and the lowest titres during the late winter, in the Southern hemisphere. He did not notice any difference between sexes and among breeds in this

seasonal variation, which was uninfluenced by feeding.

Patel and Stone (1957) found the J substance also on the J^s cells by absorption studies.

Rendel (1957) found presence of J both on the spermatozoa and seminal plasma of bulls positive for J, but were absent in J negative bulls. Matousek (1961) confirmed the presence of J in seminal plasma, serum, saliva and milk, but no J antigen could be demonstrated on spermatozoa.

Tolle (1960) described the production of anti-J by hetero-immunisation in rabbits. Studies by Jamieson (1960) on British breeds showed a large variation in the concentration of the soluble J substance both in the J^{cs} and J^s class, thus suggesting a relatively large number of alleles.

Coneally *et al.* (1962) observed strong evidence for multiple alleles controlling different amounts of J substance. Evidence was obtained for at least 8 multiple alleles. Rendel (1963) found that a relatively large number of alleles influenced the variations in the concentration of J in serum.

Oc factor

Stormont (1951) encountered a serum which had the

unique property of reacting with the red cells of group O (i.e. R negative) sheep but not those from group R sheep. With the aid of this serum, called natural bovine anti-sheep O, he showed that group O was inherited as a recessive to R. This was confirmed by Rendel et al. (1954) and Rendel (1957).

Sprague (1958) carried this work further and was successful in recognising a soluble blood group property of some cattle sera detected only by inhibition test. This property had been designated as 'Oc' which was related both serologically and genetically to J. The Oc property appeared to be inherited as a recessive character. As a consequence of these observations, the J system of cattle had been extended to include four more phenotypes J^{Oc}, J, Oc and —. A minimum of four alleles namely J^{JOc}, J^{J}, J^{Oc} and j had been accounted for the observed phenotypes.

Kiharay et al. (1964) detected four antibodies, anti-J, anti-Z', anti-Ch_1, and anti-Ch_2, in normal sera in the Japanese breed of cattle. In relation to the J and Z' substances cattle sera fell into 3 classes, those having antigens (1) both in sera and cells (J^{cs}, Z'^{cs}), (2) in sera, but not on the cells (J^{s}, Z'^{s}) or (3) neither in sera nor on cells (J^{a}, Z'^{a}). The percentage frequency of these classes

among 263 Japanese cattle was as follows. 11 for J^{cs}, 32.7 for J^{s} and 56.3 for J^{a}, 42.2 for Z'^{cs}, 35.0 for Z'^{s} and 22.8 for Z'^{a}. These frequencies differed from those found in European breeds.

S-U system

Ferguson, Stormont and Irwin (1942) reported S and U factors. Stormont (1950) found sub-group for the U factor. Ferguson (1955), Neimann-Sorensen (1958), Rendel (1958) assigned S, U_1 and U_2 groups to a separate genetic system viz. S-U system. Neimann-Sorensen and Spryzak (1959) assigned H' to S-U system. Stormont, Miller and Suzuki (1960, 1961b) confirmed and offered genetic proof for existence of the five phenogroups. Stone and Miller (1963) found naturally occurring antibodies which detected specifically U' factor possessed by U_2 and not by U_1 cells.

Grosclaude (1963) isolated a new antibody called anti-S" which sub-divided the late phenogroup S_1H' into two new phenogroups S_1S'' H' and S_1H'; moreover anti-S" detected a phenogroup S" H' encountered in Normande and Charolaise breeds where it was rather rare. Grosclaude

and Millot (1963) found three new phenogroups U_1U'' H', U" H' U_2^BH'.

Other systems

Stormont (1952) discovered L and M blood factors. The M was found to occur on the cells in two forms M_1 and M_2.

Rendel (1958a) found that M belonged to a separate system by itself. According to Stormont (1962) it was two blood factor, three allele, three phenotype system.

Miller (1962) reported two new systems, viz. one blood factor N-system and R'-S' system a two factor system. The R'-S' system was similar to the F-V system.

Grosclaude (1965) reported to have produced a new antibody in a Normande bull and a Charolais cow by isoimmunisation. The new factor was denoted as T', which appeared to be under the control of a locus different from the 10 blood group loci already known in cattle. The data were insufficient to determine linkage relationships. The frequency of the antigen T' was 0.10 for Normande, 0.20 for Charolais and 0.02 for Friesians.

Maijala and Lindstrom (1965) detected a new blood group factor, named SF_3 as a result of iso-immunisation. The SF_3 appeared to be controlled by a single autosomal dominant gene. This factor was genetically independent from A, B, C, F-V, J, L, SU and Z, but as suitable material was not available in Finland, its relationship with loci R'-S', M and N was not studied.

Ianelli (1965) reported a new antigen called N_f which was controlled by a single dominant gene. Bouw et al. (1965) studied the inheritance of a new blood group factor H_5, in Dutch Friesian breed. The factor appeared not to be sex linked, its presence was dominant to its absence, and it was not controlled by any one of the known blood group loci. The frequency in Dutch Friesian breed was estimated to be 0.06. Based on this frequency it was concluded that this factor was not likely to be identical to N also.

Rendel (1967) reported that with regard to red cell antigens, about 80 antisera or reagents which reacted with the same number of antigenic factors on the red cells had been described and these factors were controlled by 12 or 13 loci of varying specificity.

Parentage studies

Ferguson (1941), Ferguson et al. (1942) indicated the possible value of blood groups in establishing parentage. Stormont and Cumley (1943) with a pictorial representation of method described its use in establishing the paternity in disputed cases.

Ferguson (1947) indicated that possibility of confusing identity had been greatly increased due to popularity of A.I. and gave illustrated account of disputed parentage cases with possible solutions. Willett et al. (1951, 1953) indicated the use of blood groups in identifying calf born to a foster dam following transplantation of a fertilised egg.

Humble (1952) analysed 114 cases of paternity where the calvings had occurred 3 or more days too early in relation to the second matings (standard gestation period 280 days) and found that 78% of the calves had resulted from second mating and 22% from the first.

Irwin (1956) found the blood group tests reliable as finger prints in man and presented some examples of paternity problems during A.I. work.

Neimann-Sorensen et al. (1956) solved most cases of disputed paternity, but were unable to discriminate between suspected bulls in 14% of cases.

Rendel (1956), Larsen (1957), Rendel (1958b) studied the accuracy of breeding records in practice and 4.2% were found to be in error. The 78.4% cases were solved among 260 disputed parentage cases studied.

Rapacz and Dubiski (1958) were able to exclude by using a polyvalent serum about 89% of animals not offspring of couple whose red cells were used for antibody. Efficiency could be increased by using an additional polyvalent serum.

Rendel and Gahne (1961) found that the combined use of cellular antigen and transferrin types considerably increased the efficiency in solving paternity cases.

Hosoda, Abe and Kosaku (1963) used polyvalent antisera to bovine red cells prepared in rabbits for parentage determination and concluded that they were useful in absence of a comprehensive range of specific antisera.

Tolle and Beuche (1958), Gasparski and Gasparska (1960), Schmid (1962), Beuche (1963) found similar results in comparable studies.

Kova'cs (1965) typed 712 progeny supposedly sired by 10 bulls to study the validity of their parentage. The supposed parentage was not confirmed for 22% of the progeny. He advocated the blood testing before progeny testing.

Vagonis et al. (1966) in a parentage study of 903 bulls in 1964 and 577 bulls in 1965 were able to confirm the paternity in 72% of cases in 1964 and were not able to confirm 23.1% and 16% of cases respectively.

Maijala and Lindstrom (1966) were of the opinion that there existed a constant need for pedigree control in A.I. and the blood typing technique seemed to be a satisfactory and continously improving method for meeting this demand.

Breed studies

Ferguson (1941) indicated blood group frequency differences in some breeds. Owen et al. (1944, 1947) immunogenetically analysed Holstein-Friesian and Guernsey with 30 reagents and reported marked difference between these two breeds regarding frequencies of occurrence of some antigens. Ferguson et al. (1950) confirmed the observations of Owen et al. (1947) by reporting more results on Holstein,

Guernsey, Jersey, Brown Swiss and Ayrshire breeds.

Stormont (1952a) found differences in the frequencies of alleles in F-V and Z system in the same two breeds and further demonstrated that two breeds were in genetic equilibrium with regard to these loci.

Neimann-Sorensen (1956) analysed RDM, SDM and Jersey and found obvious and in general highly significant breed differences. 47 different B alleles were found in three breeds and their relative frequency estimates revealed that most of them were characteristic for one breed only. The gene frequencies of the F-V and the Z system for all breeds were in genetic equilibrium.

Tolle and Urbaschck (1956a) tested about 2000 animals of different breeds and found significant breed differences regarding frequency of different factors.

Rendel (1958d) analysed Swedish breeds and found samples in genetic equilibrium at F-V and Z loci. The frequency of the various blood group genes varied considerably between breeds. The B system was particularly effective in distinguishing between breeds. Large differences were reported to exist between the four lines of one breed studied.

Osterhoff (1959) reported a low frequency of F in Africander breed and reported 3 new antibodies. Neimann-Sorensen and Spryzak (1959) found that existence of 9 new B blood group alleles distinguished Polish Red breed from all other investigated by that time.

Fiorentini and Rognoni (1960) reported percentage phenotypic frequencies of 10, 73 for R_1 and E factors respectively. The gene frequency of J was 0.197. For these factors and genes the frequencies differed from those reported for other breeds of cattle in Italy.

Muller (1960) in Red Spotted Simmental breed, Schindler (1961), Matousek et al. (1960) in Czechoslovak Red Spotted; Gasparski et al. (1960) in Polish Red, Chet Ram and Khanna (1961) in Hariana and Kumauni hill, Buschmann (1962) in German cattle, Grosclaude and Millot (1963), Salerno (1962), Salerno and Gatti (1963), Ianelli (1963), Schmid and Erhard (1963) in Malcho cattle and other various cattle, have reported similar breed differences in their respective studies.

Osterhoff (1965) studied the frequency of occurrence of natural antibodies in 17 breeds of cattle, and showed marked differences between indigenous and imported breeds.

Singh (1965) studied the frequency of occurrence for the Hariana and Jersey-Sindhi crossbreds with respect to 15 antigens. Highly significant differences were noticed with respect to six (C, R, W, J, M, Iz_{33}) antigens and significant difference to Z' factor.

Grosclaude (1965) estimated allelic frequencies of antigenic factors belonging to S system in 5 French cattle breeds. Main breed differences were reported in respect of alleles SU, U"H' which was not found in the Flemish and Normande breeds, but was found to be present in Charolais, Blonde d' Aquitaine and Salers cattle.

Miller (1966) reported 27 phenogroups of B alleles in American Long Horns, only 5% of which had been previously reported in American breeds of cattle. The m^M and F_2 alleles which were found in some breeds of American cattle were not found in Long Horns.

Millot (1966) indicated a relationship between the Charolais and the French Friesian, Mountbeliard, Eastern Spotted and Limousin breeds, but little relationship with Normande breed.

Rendel (1967) discussed the possibility of making use of blood group loci as markers to study the similarities

and differences between and within breeds.

Inter-species studies

Stormont et al. (1961) presented evidence which indicated that at least nine genetic systems of blood groups now recognised in cattle had their homologues in American bison. In addition, the bison had also got the soluble blood group substances serologically indistinguishable from the J and Oc properties of cattle. They indicated the existence of a close toxonomic effinity between two species.

Dutta and Stone (1963) tested the Indian water buffalo with 4 cattle blood reagents, only J reacted in some cases but F, V and Z failed to react. This showed that serological relation between buffalo and cow was much more wider.

Chet Ram, Khanna and Prabhu (1964) typed the buffalo blood with 35 cattle blood group reagents. Out of 35 reagents used in the test, only 16 reacted positively with buffalo blood. All the 16 antigenic factors present on buffalo blood cells, except three, occurred much less frequently in buffalo than cattle. Three antigenic factors namely, Iz_3, Iz_8 and J were found to occur more frequently in buffaloes than in

cattle and in two cases, the differences were highly significant. It was noted with interest that the J antigen was found to be present in both the species in an almost equal frequency. Anti-J had been isolated from normal sera of cattle as well as buffaloes.

Twin studies

Owen (1945) recorded identical blood types in twins of unlike sex resulting from a case of superfecundation. He further found that most of fraternal twins had identical blood types and that these twins had an admixture of two kinds of erythrocytes which, he explained by proposing exchange of premordial blood cells due to foetal anastomosis. Owen et al. (1946) introduced the term erythrocytic mosaicism for this blood admixture.

Stormont (1949) demonstrated that twins genetically different for J remained phenotypically different even if they had mosaicism and identical types.

Stone and Palm (1952) described a case in which certain B factors appeared in the calves in spite of the fact that they were absent in possible parents. On re-examination, it was found that the dam was a twin and the

proportion of the blood from other was so greatly in excess that her own type practically defied detection.

Stone et al. (1952) demonstrated that the blood grouping furnished a simple method of predicting whether a heifer would be sterile, or fertile. In twins of unlike sex with different blood types heifers were normal while those having mosaicism were freemartins.

Stormont (1954) observed erythrocyte mosaicism in a heifer recorded as a single birth. It was concluded that co-twins must have died in uterus.

Neimann-Sorensen et al. (1956) determined blood type of 126 twin pairs of opposite sex and only 10% were found to contain separate blood type. This result agreed with percentage of freemartins found on basis of anatomical findings.

Rendel (1958c) found that out of 53 pairs of dizygous twins, mostly the pairs had almost same properties of each erythrocyte type. Four cases had only 10% erythrocytes of their own genotype. Out of 441 pairs of unlike sex only 93% were verified as dizygous. Out of 460 like sexed twins, about 83% were classified as dizygous. Further, it was

concluded that samples of monozygous twins diagnosed both morphologically and with aid of blood grouping contained only 1% dizygous pairs.

Brannang and Rendel (1958) classified 41 pairs of twins and triplets morphologically as well as blood groups and then studied their differential growth. The two methods showed close agreement.

Stone et al. (1960) found that erythrocyte mosaicism in each twin did not demonstrably effect either the cellular antigens formed, or genes for the cellular antigens transmitted by each twin to its progeny.

Miller and Morris (1961) reported a case of fertile heifer, co-twin with bull having blood type mosaicism, but each had different blood type suggesting that during the beginning of pregnancy one or two additional foetuses existed.

Rendel et al. (1962) reported a set of five egg quintuplets. Each animal in set carried 4 different erythrocyte types originated from at least four eggs. But studies on albumin and transferrin types showed that five eggs were involved. All females were freemartins, but there was a close parallelism between the degree of intersexuality and proportion of various erythrocyte types.

Rendel (1962) advised the use of serum protein groups along with blood group study in detecting fraternal twins. Rendel (1963) also made detailed study on J antibody by studying monozygotes, dizygotes, half-sibs and unrelated animals. The concentration of J substance was determined in all pairs in which both members were J positive which has J substance in serum and on cells (J^{cs}).

Kumazak et al. (1963) typed the blood of 8 unisexual twin pairs using 21 sera. The only within pair difference was in respect of J antigen in unisexual pairs. Three of the unisexual pairs gave incomplete haemolysis with one or both of the R and U_2 reagents. It was proved that these unisexual pair were not monozygotic.

Stone et al. (1964) made a study of the proportions of erythrocyte types in cattle twins showing erythrocyte mosaicism. When one chimera reached 8 years of age 96% of the red cells were found to be of a new 'recombinant' type, presumably resulting from some kind of somatic cell mating of the 2 haemopoitic stem cells.

Association with economic characters

Dunlop (1951) studied the correlation between the

presence of 11 antigenic factors and type score for ribs, udder and teats in Guernsey herd. In only one out of 33 analysis did the difference between the means of the scores of those animals possessing a certain antigenic factor and the mean of those not possessing it reached the 0.05 level of significance.

Meclure (1952) observed no correlation between the presence of A substance of cattle cells and variations of the butter fat test in Friesian cattle.

Nair et al. (1955) found statistically significant evidence for an association between certain type defects and the blood type antigens M, J, C_2.

Morton, Stone and Irwin (1956) found no apparent effect of presence or absence of any blood factor on fertility as measured by the number of calves tested.

Nair (1957) reported a significant correlation between fat percentage and the presence of certain antigens in the B system in 922 high-yielding Holstein-Friesian cows.

Laben and Stormont (1958) found a slight excess of heterozygotes at B, F-V and Z loci in an inbred Jersey herd. In two instances of limited numbers, cows heterozygotes at

B locus out performed their homozygous paternal half sisters of similar inbreeding. No other important differences were noted between heterozygotes and homozygotes regarding birth weight, F.C.M. yield, reproductive efficiency and disposal etc.

Mitscherlich, Tolle and Walter (1959) investigated 242 German Friesian heifers at bull testing stations. Analysis within sires and herds showed that animals with blood factor M yielded less milk than M negative contemporaries. When also the influence of factor M was eliminated the factor O_3 (B system) and R (C system) were found to be associated with decreased milk yield, the factor Y_2 (B system) and L (L system) with lowered fat percentage and Q (B system) with high fat percentage. These findings were retested on a new independent material. Confirming results were observed, but in parts of the data, the previously found influence of factor M and Y_2 were confirmed (Tolle, 1959).

Rendel (1959, 1961) compared the fat percentage of milk, total fat yield and the heart girth measurements of paternal half sibs (of the SRB breed) having and lacking certain blood group alleles of eight different blood group systems. Statistically significant associations were found

between some blood group genes and fat percentage especially the B allele $B^{BO_1YD'}$. Animals heterozygous at the B locus also had a significantly higher fat percentage than homozygous ones. J and M genes showed an increasing effect on fat percentage of milk while L gene had a strongly depressive effect. No cumulative effect of Z gene was found. Larsen et al. (1959) and Conneally (1962) confirmed the result of Rendel though in a limited study.

Tolle (1959) observed that M factor seemed to have an arresting effect on the length of lactation period. Antigens A_1, Y_2 and I' showed a positive effect on milk yield. The effect of M, U_2 and Z were highly significant. The factor A_1 and Y_2 also showed a depressing effect on butter-fat percentage.

Neimann-Sorensen and Robertson (1961) investigated possible association between blood groups and 9 production characters. In Red Danish breed the cows having the B alleles $B^{BO_1Y_1D'}$ had an average fat percentage above that of their contemporaries by 0.064 ± 0.015. In Jerseys $B^{GD'}$ allele was accompanied by increase in fat percentage of 0.136 ± 0.049 while $B^{O_1TE'3K'}$ suppressed fat percentage by 0.200 ± 0.066. He further found that in all cases in which the

effect of a B allele on milk yield or fat content approached significance, the effect for these two characters was in opposite directions.

Smith and Pfaw (1962) found fat percentage to be associated only with the S_1 factor of the SU system. The J allele significantly decreased withers height at birth by about 1.5 cms.

Car (1962) in a preliminary analysis observed Z to be significantly linked with higher production ($P < 0.05$) in Dutch-Friesian and Simmental cattle. Munkaesi (1962) studied the relationship of blood group factors with milk yield in various dairy breeds, but no linkage between blood group genes and genes for production characters was detected.

Salerno (1963) investigated the relationship of blood group with age at first calving, calving interval and service period. He found heifers with A alleles calved 57 days later than those lacking it and animals with F allele calved 72 days later than those with V allele, both differences were highly significant. Animals with S_1 alleles had calving interval significantly shorter by 28 days than those with S_2 alleles. Animals having J alleles had service periods highly significantly longer by 12 days than those

without it.

Salerno (1964) studied the relationship of certain blood group antigenic factors with the weight at various ages. At birth, females carrying V and S_1 genes were 6.73 kg and 4.38 kg heavier than those with genes F and those allelic to S_1 respectively. At 6 months of age, females carrying V and L genes were heavier by 89.8, 45.6 kg respectively than those carrying the alleles F and L. At 18 and 24 months, males with the M gene were lighter by 154.9 kg and 212.9 kg respectively than those carrying the allelic genes. All the above differences were statistically significant.

Hogrevee (1965) reported that the factor E_2 had an unfavourable effect and V_2 a favourable one ($P < 0.01$) on lifetime production in Black Pied Lowland cattle. He confirmed the results obtained by Mitscherlich et al. (1959).

Singh (1965) studied the relationship of 15 antigenic factors with 7 economic characters in Hariana and Jersey-Sindhi crosses. He reported a highly significant association of age at 1st calving with M and Iz_{35} factors. Wither height at birth was associated with C factor in Hariana, and

J and Iz_{36} in crossbreds. Milk yield during first lactation was highly significantly associated with W and X_1 in Hariana. Birth weight was significantly associated with antigens A and Iz_{34}, in crossbreds. The blood group factor A showed a significant association with calving interval in crossbred.

Gupta (1965) reported significant association of Z' and N factors with gestation period, factor R and V with number of services per pregnancy and factors Iz_{33} and Iz_{34} with dry period.

Salerno (1965) reported that at 18 and 24 months of age males which carried the M gene had a significantly higher body weight than those with the 'm' allele.

Coneally and Stone (1965) observed a significant effect of B phenogroup $B^{O_1Y_2D'}$ on the fat percentage. The fat percentage of the cows with the $B^{BO_1Y_2D'}$ allele in Holstein-Friesian (the great majority being heterozygous) was 0.33 higher than in those lacking it. Hogrevee and Koch (1966) observed that factors D^6 and M occurred significantly more frequently in cow groups with high fat content and the factor L more frequently in cows with low fat content in Black Pied Lowland cattle.

Osterlee (1965) presented a review on some aspects of studies on relationship between blood groups and economic characteristics in farm animals.

Blood group research in India

In India, work on bovine blood groups was initiated for the first time at the Indian Veterinary Research Institute in the year 1942 and the first report was published by Singh (1945). He presented the results of studies in blood groups in 11 Indian and 2 foreign breeds. He found that blood group antigens behaved as independent units and indicated possible common origin and relationship with these breeds.

Systemic studies on the subject, however, were taken in hand only in the year 1955 with the opening of an Immunogenetics Laboratory in the Division of Animal Genetics of Indian Veterinary Research Institute, Izatnagar.

Chet Ram and Khanna (1961) tested blood of Hariana cattle with 26 different blood group reagents prepared at the laboratory itself. They found that Hariana cattle carried in their blood almost all the antigenic factors for which the tests had been made, including the rare factor Z'.

A comparison of frequency of occurrence of these factors were made with Kumauni hill breed. It was found that there existed significant difference in the occurrence of some antigenic factor in the two breeds.

Chet Ram, Khanna and Prabhu (1964) and Chet Ram et al. (1964) tested the blood of cattle, buffaloes and camel with blood typing reagents produced against cattle. They tested 35 reagents against buffaloes and 38 reagents against camel red blood cells. It was found that 16 reagents in buffaloes and 2 in camel were similar or identical with those found in cattle. They also reported the presence of several antigens in cattle blood cells which could not be fitted in with those reported by European and American workers. These were designated as Iz_1, Iz_2 and so on.

Khanna, Chet Ram and Prabhu (1964) also conducted studies on the relationship between blood group factors and first lactation yields and total fat production in Hariana and found that antigens Iz_{15} and BF_2 were significantly related with first lactation yield. BF_2 and Iz_6 were found to be significantly associated with total fat production. Similarly they found a significant relationship between blood group factors Y and Iz_9 and age at first calving.

To study the breed differences in the frequency of occurrence of blood group antigens, 11 different cattle breeds have been blood typed by Khanna, Chet Ram and Prabhu (unpublished). The results showed that many of the blood group antigens differ significantly in their occurrence in different breeds.

Singh (1965) had studied the occurrence of 15 blood group antigens in Hariana and Jersey-Sindhi crossbreds and their relationship with 7 economic characters. He observed significant differences between Hariana and crossbreds in respect to 6 antigens.

Gupta (1965) also studied occurrence and association of 15 antigenic factors with 6 economic characters in Hariana.

Naik et al. (1965) produced 2 new anti-sera by iso-immunisation and one by hetero-immunisation. The iso-immunisation sera were called anti-Ki and anti-AKi and the hetero-immune sera anti-AK. Comparison with standard reagents showed that anti-Ki belonged to the B system and the other two most probably belonged to the SU system. The gene frequencies for these factors were calculated in 5 Indian breeds (Malvi, Khillari, Kankrej, Dangi and Gir).

Haemoglobin polymorphism in cattle

Pauling et al. (1949) investigating Hb of human by means of paper electrophoresis found a fraction (HbS) which was different from the normal Hb. This finding directed the interest to the electrophoretic study of Hb and shortly afterwards a great number of papers dealt with animal haemoglobins as well.

Individual differences in the haemoglobin type of cattle were first detected by Cabannes and Serain (1955) by means of paper-electrophoresis. In some animals only a fast-Hb fraction (b) while in others only a slow one (a) was found, but there were individuals in which both components (a and b) were present.

Bangham (1957) proposed the designation HbA (slow), HbB (fast) and HbAB in which both fractions were present. He supposed that there were two co-dominant autosomal alleles (Hb^A and Hb^B) controlling the three haemoglobin types

Genotypes	Phenotypes
Hb^A/Hb^A	HbA
Hb^B/Hb^B	HbB
Hb^A/Hb^B	HbAB

To support this hypothesis, he determined the Hb types of the offspring from 150 matings and obtained the following results.

Mating types	Hb type resulting in the offspring		
A x A	A	-	-
A x AB	A	AB	-
AB x AB	A	AB	B
A x B	-	AB	-
AB x B	-	AB	B
B x B	-	-	B

These findings were soon confirmed by others and further data were published. Grimes et al. (1957) reported that the heterozygous type HbAB showed equal concentrations of the A and B components, thus they supposed the equal dosage effect of the alleles.

In young calves a diffuse Hb fraction was found by Salisbury and Shreffler (1957, 1959). This fraction remained behind fraction B in its electrophoretic mobility. It was designated HbF (foetal). This fraction gradually disappeared by 80th day of extra-uterine life.

Grimes et al. (1958) found that the ratio of HbF at

birth was ranging from 41-100% of the total, and that the synthesis of HbF started in most cases before the parturition.

A third haemoglobin HbC was reported by Vella (1958) in Bos indicus. The existence of HbC was later confirmed by Crockett et al. (1963), Naik and Sanghvi (1965) and Osterhoff and VanHardeen (1965) in cattle of Indian and African origin.

Bangham and Blumberg (1958) showed that bovine haemoglobin B was present in Southern European and African breeds of cattle. Similar haemoglobins were previously reported in cattle of United States (Grimes et al, 1957; Salisbury and Shreffler, 1957).

The occurrence of HbB in Jersey and in some other European and African breeds appeared to support the theory that Jersey cattle originated from Bos indicus (Bangham and Blumberg, 1958) and that their ancestors migrated from the Indus Valley to Europe through Africa (Boston, 1954). It had been suggested that HbB was associated with tolerance of tropical climate (Evans, 1963).

Bungham and Blumberg (1958) tested the possible correlation between the absence of bovine HbB and relative high tolerance to trypanosomiasis as an anology with role

of haemoglobin S in Malaria in humans, but found no evidence of any excess of heterozygotes. They, however, pointed out a parallel between increased resistance to East coast fever of calves, compared to adults to the comparative malaria resistance of human infants. Lehman and Rollison (1959) failed to show such correlation in Ankole breed.

Shreffler and Salisbury (1959) reported the presence of bovine HbB in Brown Swiss, Sindhi, Guerensey and Jersey cattle. The genetic control had been assigned to the autosomal alleles fully expressed in heterozygote.

Lehman (1959) examined the gene frequencies in Indian Gir cattle from randomly selected breeds and found the frequencies of bovine haemoglobin A and B to be equal. There was also significant excess of heterozygous phenotypes which suggested that in Gir cattle natural selection favoured the heterozygotes.

Shreffler and Salisbury (1959) made an attempt to correlate the haemoglobin polymorphism to health, vigour, livability, conformation or production but could not find differences in polymorphic variants within a breed.

Moustgaard et al. (1960) and Hojgard et al. (1960) reported B phenotypes in Brown Swiss, Hariana, and Kumauni Hill

breeds from India and in Africander cattle from South Africa and that in Jersey cattle bovine B is the most frequently observed.

Braend et al. (1962) reported the occurrence of only HbA in Icelandic cattle of Norway.

By paper electrophoretic investigations of the Indian Brahman breed a new fraction (C) was detected by Crockett et al. (1963). This haemoglobin had been found in individuals derived from crossings of Brahman with Herefords. Concerning its electrophoretic position it was found in between the HbA and HbF fractions. As it was only found in the form of HbAC, it was suggested that the HbC and HbCB were lethal types. In types AC the two components (A and C) showed a similar concentration on which basis it was supposed that the HbC gene was allelic with the Hb^A and Hb^B genes, but its frequency was very low.

Carr (1964) reported yet another new haemoglobin variant, HbC Rhodesia in the breeds of Angoni, Barotse and Tonga of African origin. This new haemoglobin migrates at a rate intermediate between bovine A and bovine B and more slowly than foetal haemoglobin.

Markus *et al.* (1965) investigated the haemoglobin polymorphism in 480 Hungarian *Spotted* cattle. Out of these 72.7% were of HbA type 25.41% of HbB type and 1.88% were of HbAB type. Frequencies of A and B alleles were 0.8542 ± 0.0011 and 0.1458 ± 0.0011 respectively.

Naik *et al.* (1965) reported the discovery of a new variant in the *Zebu* cattle, which was named as HbX. This was similar to HbC reported by Crockett *et al.* (1963). Naik and Sanghvi (1965) reported yet another haemoglobin type in a *Khillari* bull. Accordingly, it was called Hb *Khillari*. This fraction had a slower mobility than HbA. The new heterozygote phenotype showed an almost equal amount of HbA and Hb *Khillari* indicating a similar dosage effect of alleles. They had studied 1060 haemoglobin samples from five Indian cattle breeds which revealed 321 HbA, 498 HbAB, 236 HbBB, 4 Hb-AX and 1 Hb A + *Khillari*.

Efremov and Braend (1965) found a fourth haemoglobin in adult cattle (HbD) of African *Moturu* breed. The HbD had a slightly slower rate of migration by electrophoresis than HbA. The D haemoglobin was further more characterised in having a second band migrating slightly slower than the D band. This second band was called as D_2. The Hb^D was found

alone in two animals of the African Moturu breed. In combination with Hb^A it occurred in 6 animals. They suggested the occurrence of fourth gene Hb^D.

Braend et al. (1965) studied the inheritance of bovine haemoglobin D (HbD) in a total of 107 animals from three herds of Moturu breed in Nigeria. They observed phenotypic distribution agreed with the genetic theory of a codominant allele being responsible for the variant. The frequency of HbD for three herds were 0.26, 0.21 and 0.13 respectively. In samples for 63 N' Dama cattle HbD was not found.

Srivastava (1965) reported haemoglobin types in Holstein, Jersey, Brown Swiss and European and Sindhi crosses. Holstein blood contained only Hb^A. The frequency of the gene for bovine Hb^B was greater in cattle of Sindhi leniage than European crosses.

Osterhoff and Van Herdeen (1965) differentiated the HbA into several types by using a starch gel electrophoresis running time of 2½ hours instead of normal 1 hour. A_1/A_1 was identical with the A/A originally described, and A_2/A_2, A_3/A_3 moved faster and more slowly respectively than A_1/A_1. They studied the following 9 phenotypes of haemoglobin

namely, A_1/A_1, A_2/A_2, A_3/A_3, A_1/A_2, A_1/A_3, A_{1+3}/B, B/B, A_1/B and A/B.

Carr (1965) reported the occurrence of Hb^D in Angoni type of East African Short Horn Zebu cattle. One example each of the phenotypes AD and BD were found in 155 cattle. Weber (1965) reported the rare occurrence of HbB in the 4 Swiss cattle breeds examined. The frequency of AA in Swiss Brown, Simmentals, Fribourg and Herens was 73.2%, 75.8%, 81.5% and 94.4% respectively. Bernoco and Sarore (1965) studied the haemoglobin types in 261 Red Pied Aostas in 5 herds. The percentage of cattle of Hb types A, AB and B was 74, 23 and 3 respectively, and the frequency of the Hb^A and Hb^B genes was 0.854 and 0.146 respectively.

Larsen (1965) tested the linkage of the genes for Hb types and the loci controlling the A, B, C, F-V, J, L, M, SU, Z and R'S' blood group system and transferrin types using Morton's sequential probability ratio. It was found that seggregation was closely connected with that of the genes of the A blood group system. Assuming linked loci the recombination frequency was estimated to be 3 ± 1.7%. Close linkage was excluded in the other genetic systems tested. In a similar study, Hessenholt et al. (1965) calculated the recombination values for genes controlling haemoglobin

and B, C, L and M blood group systems and haemoglobin F-V and Z systems. The recombination values were 20% and 10% respectively. The recombination value for transferrin and haemoglobin was estimated to be 5%.

Larsen and Moustgaard (1965) investigated the blood, transferrin and haemoglobin types of cattle from middle East. 147 blood samples from Cypriot, 118 from Egyptian and 199 from Syrian cattle were used.

Evans and Crowley (1966) studied blood samples from 25 Kerry cattle all of which contained the commonly occurring A type. They suggested that Kerry breed had no link with an ancestral line of channel Island cattle (with B haemoglobin) supposedly of oriental origin, but that it had an origin similar to that of other Northern European breeds.

Balakrishnan and Nair (1966) typed the haemoglobins of 53 Red Sindhi, 170 Sahiwal and 232 Tharparkar animals. The frequency of Hb^A gene in males of the three breeds was 0.700, 0.707 and 0.897 respectively and in females 0.625, 0.671 and 0.894 respectively. The breed differences were significant but the sex differences were not. The three populations were in genetic equilibrium. The Hb type was not significantly associated with age at first calving and first lactation milk yield.

Sen *et al.* (1966) studied blood samples of 317 adult *Hariana*, *Desi*, *Sahiwal*, *Gir*, *Tharparkar* and *Red Sindhi* cattle and 50 adult *Murrah* buffaloes, for haemoglobin variation. All the three types of Hb (A, AB and B) were present in the cattle examined. Type C was not detected. Although the law of genetic equilibrium was followed by *Hariana*, *Desi*, *Gir* and *Red Sindhi* cattle. There was an excess of AB individuals in all the breeds. This deviation was statistically significant for the total population and for the *Sahiwal* and *Tharparkar* breeds taken individually. The average frequency of the HbB gene was 0.353.

MATERIALS AND METHODS

Cattle red cells, unlike those of man, pigs and chickens are not readily agglutinated, even when saturated with multiple doses of blood typing antibodies. Consequently, lytic procedures are used in cattle blood typing tests (Robert Muir, 1909; Furguson, 1941; Stormont, 1950; Stone et al., 1958 and Braend, 1961).

Terminology

Some of the definitions and terms used in this study are described below.

Antigen

Antigens (abbreviation for anti-somatogens) are substances usually containing proteins, lipoid-substances and polysaccarides or combinations of these e.g. bacteria, viruses, toxins and erythrocytes. The latter are the antigens used in blood group studies.

Antibody

Antibodies are specific substances, probably produced in the reticulo-endothelial system as preventive agents against foreign substances introduced into the body. The chemical composition of antibodies is unknown, but they

are most probably euglobular and pseudoglobular proteins which are very specific i.e. they only react with the antigen by which they were produced. This characteristic is used to detect antigenic differences between red blood cells.

Antiserum

Antiserum is a serum containing antibodies. In blood group research, antisera are obtained by hyper-immunization with erythrocytes i.e. repeated transfusion of small quantities of blood. Antiserum may contain more than one populations of antibodies.

Test serum or reagent

This is an antiserum which has been rendered specific to a single antigenic factor. Each test serum is able to detect a corresponding antigenic factor on the erythrocytes.

Blood group factor

Antigenic factors or blood factors are the specific entities on the erythrocytes which react with the different reagents (antisera). Thus, erythrocytes reacting with the A reagent (anti-A) are said to carry the antigenic factor or blood factor A.

Blood group or phenogroup

Blood group denotes a gene determined unit, containing one, or more antigenic factors.

Blood group system

Blood factors, or blood groups which are determined by genes at the same locus and most probably on the same chromosome are said to belong to the same blood group system.

Blood type

Blood type of an animal is simply its complete antigenic formula, as determined with all the available reagents in the blood group determination.

A. Production of iso-immune sera

The immunisation absorption techniques for the preparation of blood group reagents in the present study are the same as described by Ferguson (1941) and Chet Ram and Khanna (1961).

(1) Selection of donors

Except anti-J the remaining two reagents viz. R_1 and E, have been prepared by iso-immunisation. The animals used for iso-immunisation belonged to the Animal Genetics Division,

Indian Veterinary Research Institute, Izatnagar.

Four donor-recipient pairs were made among the allotted cattle by referring to their blood group formulae. These donors and recipients were selected in such a way that the blood type of two animals differed only in few antigens including those against which it was desired to produce antiserum. This method allowed prediction of the antibodies produced if the immunisations were effective. In addition, absorption and analysis of the resulting antisera were also facilitated. Theoretically, antibodies would be expected against the antigenic components present in blood of donors but absent from the recipients, including those not detected previously. For example the animal No. 17 was immunised with blood of animal No. 4. The antibodies expected were X_2, Y_2 and E.

Donor (4)	-/-	Y_2/-	X_2/-	E/E	F/F	S_2/S_2	H'/H'	
Recipient (17)	A/-	Q_1/-	-/-	-/-	-/-	R_1/-	R_2/R_2	F/-
	-/-	-/-	S"/-	S_2/S_2	H'/H'			

Antibodies expected Y_2 X_2 E

(2) Iso-immunisation procedure

The term immunisation in the blood group study is

used for injection of blood from donor to the recipient for the production of immune sera. Blood for immunisation was collected, in sterile conical flasks from the external jugular vein of the donors. The animals were bled just prior to the immunisation with all possible aseptic precautions. The blood was collected directly into the flasks containing the adequate amount of anti-coagulant i.e. one part of anti-coagulant for every nine parts of blood. Throughout the period of this study, the anticoagulant of the following composition was used.

Sodium citrate	...	20 gms
Sodium chloride	...	5 gms
Distilled water to make		1000 cc

The donor's blood was then slowly (usually in 3-4 minutes) injected into the jugular vein of the recipient with sterile 50 cc side nozzled syringe. One animal may act as donor for more than one animal.

The procedure for iso-immunisation has been followed as weekly injections of 25 cc of citrated donor's blood was given to the recipient till the appearance of antibodies with a sufficient titre (1:32). Antibodies with sufficient titre usually appeared after 6 injections. Just prior to the injection of the blood into the recipient a small amount

of blood was taken in sterilised centrifuge tubes each time in order to find the antibody titre, developed in the serum of the recipient. After one hour the clot was broken and the serum was separated by centrifuging at 2000 r.p.m. for 15 minutes. The serum, thus separated was inactivated at 56°C for half an hour to destroy any non-specific factor. It was stored at -20°C. The serum from these samples were tested for lytic antibodies in a titre test against red blood cells of the donor. For this purpose the donors blood was collected in sterile centrifuge tubes having adequate amount of anti-coagulant. The blood was then centrifuged for 15 minutes at 2000 r.p.m. and the serum was thrown out. Then equal quantity of normal saline was added to the packed red blood cells and this was centrifuged again for 10 minutes at 2000 r.p.m. This procedure was repeated three times to obtain thoroughly washed red blood cells. Then a 2.5% suspension of these washed R.B.C. in saline was prepared. Throughout the period of this study normal saline of 0.9% strength was used. The technique for the titre testing is described below.

(3) Titre testing

Every antiserum was always titrated against donor cells or cells from a number of individuals with corresponding blood group factor. The highest dilution in which the serum

still produced the complete lysis of all cells was recorded as titre of serum. The tests were set up in wic-perplex haemagglutination plastic plates having eighty cavities. The serum was tested in two-fold increasing dilutions in normal saline. The pippetes used were standardised to give 20 drops per cc. Two drops (i.e. 0.1 cc) of normal saline was taken in each of second to seventh cavities of perplex plate. Two drops of the serum, whose titre is to be tested, were taken in each of first two cavities. After mixing the serum and saline thoroughly in second cavity 2 drops of the mixture were transferred from the second to the third cavity and again two drops from the third to fourth and so on upto seventh cavity, finally 2 drops of the mixture were discarded from the 7th cavity. Thus the serum in the 7th cavity was diluted 128 times (i.e. 1:128). This was followed by addition of one drop of 2.5% suspension in normal saline of thrice washed erythrocytes and a shaking. After 10 minutes, fresh rabbit serum as complement source was added and then again shaken. After 10 minutes a final shaking was given and after 2½ hours first reading was taken followed by shaking for 5 minutes and then 2nd reading was taken after 5 hours. Along with this test, two sets of controls were always kept. The saline control with 3 drops of saline and complement control had 3 drops of complement and one drop of cell suspension in each of this.

(4) Collection of antiserum

When sufficient titre of antibodies was observed in antiserum i.e. 1:16, the immunisations were stopped. In one pair [17 (4)] the required titre of antibodies developed after 3rd injection only, but in the other pair [8 (2)] it appeared after 5 injections. A week after the last injection, the animals showing sufficient titres, were bled one to two litres of blood from the jugular vein. The blood was centrifuged at 2000 r.p.m. for 15 minutes to get serum. This was kept in water bath for half an hour at 56°C to destroy complement and other non-specific antibodies. The serum was then stored at -20°C in deep-freeze for further analysis.

B. Analysis of antisera

(1) Haemolytic test

The procedure for haemolytic test described by Stukovsky was followed throughout the period of this study. The technique followed is described here under.

The tests were set up in wic-perplex haemagglutination plastic plates having 80 cavities. With pipettes standardised to give 20 drops per cc two drops or 0.1 cc of antiserum

(absorbed fraction) and one drop of thrice washed, 2.5% cell suspension in isotonic normal saline were added in each cavity of the plate. After shaking, the mixture was allowed to stand for 10 minutes at room temperature and then one drop of complement was added to each cavity. The plates were thoroughly shaken again, and after 2½ hours of incubation the first reading was taken which was followed by a thorough shaking of plates. Then the final reading was taken after an interval of 2½ hours.

Three types of reactions have been differentiated and recorded.

(a) -Ve : All cells intact and settled, no colouring of supernatent (no lysis).

(b) ± or transient : Nearly half of the cells intact and settled and supernatent reddish (partial lysis).

(c) +Ve : Supernatent red coloured and transparent (complete lysis) and more than half cells lysed.

Two sets of controls were always included.

(i) Complement control - consists of 3 drops of complement and one drop of cells. This served as a test for haemolytic activity of complement.

(ii) Saline control - consists of 3 drops of normal saline and a drop of cell suspension. This assures the stability of cells in saline medium.

(2) Analysis of antisera

The antisera obtained after immunisation was generally polyvalent and had to be assessed for the type of antibodies present in it and their respective strength. For this purpose at least 16 different blood samples were collected whose blood types were known and cells were washed three times in normal saline. The packed cells were divided into two equal portions. To the first portion nearly half of the quantity of serum to be absorbed was added. The mixture was allowed to stand for 30 minutes at room temperature and then the serum was transferred to second portion of packed cells and allowed to stand for 30 minutes at 5^{o}C. After this the mixture of cells and antiserum was centrifuged for 15 minutes at 3000 r.p.m. and the serum was taken out. Cells thus left were thrown. These absorbed sera were tested against fresh three times washed cell suspension of same 16 animals whose cells were used for absorption and the haemolytic reaction was noted. The results were analysed to see the types of antibodies present in the antiserum. An example is given below. Antiserum No. 8 was collected by

Table No 1

Fractionation of antiserum 8, Antibodies expected R_1, F

S.No.	No of animals with relevent antigens on red cells	Expected antibodies left in absorbed fractions																	control	
		1 R_1	2 −	4 R_1	5 −	7 −	8 R_1F	14 R_1	15 R_1	17 −	18 −	25 R_1	26 −	28 R_1F	40 R_1	45 −	47 −	UNAB	Saline	compli-ment
1	1 F	−	−	−	−	−	+	−	−	−	−	−	−	+	−	−	−	+	−	−
2	2 R_1F	+	−	+	−	−	+	+	+	−	−	+	−	+	+	−	−	+	−	−
3	4 F	−	−	−	−	−	+	−	−	−	−	−	−	+	−	−	−	+	−	−
4	5 R_1F	+	−	+	−	−	+	+	+	−	−	+	−	+	+	−	−	+	−	−
5	7 R_1F	+	−	+	−	−	+	+	+	−	−	+	−	+	+	−	−	+	−	−
6	8 −	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
7	14 F	−	−	−	−	−	+	−	−	−	−	−	−	+	−	−	−	+	−	−
8	15 F	−	−	−	−	−	+	−	−	−	−	−	−	+	−	−	−	+	−	−
9	17 R_1F	+	−	+	−	−	+	+	+	−	−	+	−	+	+	−	−	+	−	−
10	18 R_1F	+	−	+	−	−	+	+	+	−	−	+	−	+	+	−	−	+	−	−
11	25 F	−	−	−	−	−	+	−	−	−	−	−	−	+	−	−	−	+	−	−
12	26 R_1F	+	−	+	−	−	+	+	+	−	−	+	−	+	+	−	−	+	−	−
13	28 −	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
14	40 F	−	−	−	−	−	+	−	−	−	−	−	−	+	−	−	−	+	−	−
15	45 R_1F	+	−	+	−	−	+	+	+	−	−	+	−	+	+	−	−	+	−	−
16	47 R_1F	+	−	+	−	−	+	+	+	−	−	+	−	+	+	−	−	+	−	−

immunisation from blood of 2. The antibodies expected were R_1 and F. The results of analysis are given in Table 1.

It can be seen from Table 1 that there are 3 types of reactions.

(1) Those cells which are having antigenic factors F only. During absorption these cells have absorbed F antibodies and left R_1 antibodies (1, 4, 14, 15, 25, 40).

(2) Those cells which are positive for both R_1 and F. These cells have absorbed both the fractions of antibodies (2, 5, 7, 17, 18, 26, 45, 47).

(3) Those cells which have no corresponding antigen on their red cells and after absorption left both the fractions intact (8, 28).

By carefully analysing the above reaction it was concluded that there were two fractions of antibodies in the antiserum [8 (2)]. They were anti-R_1 and anti-F. Then anti-R_1 was separated by selective absorption and its unitary behaviour was tested by an unitary test which was followed by a titre test.

(3) Absorption of anti-serum

Blood cells which were used for absorbing antiserum for the preparation of the reagent were selected in such a way that it contained all the polyvalent antigen, except the one against which reagent was to be prepared. The blood from such animals was collected in suitable quantity with anticoagulant solution and the cells were washed thrice in normal saline solution. The volume of cells was taken generally twice the antiserum which was to be absorbed. The packed cells were divided into 4 portions. To the 1st portion, antiserum was added and the two things were thoroughly shaked and were incubated at 25°C (room temperature) for 30 minutes. The whole mixture was centrifuged and supernatent was added to the next portion of cells. In this way 4 absorptions were given to the antiserum. Before final portion was centrifuged the mixture was kept for 30 minutes at 5°C in the refrigerator to absorb cold haemolysins if there were any. The new reagent thus prepared was kept at -20°C for further testing.

(4) Desorption

Desorption technique was also employed by the addition of normal saline (in equal amount to antiserum) to the recedual

cells after absorption. The mixture was kept every time at room temperature and at 5°C for 30 minutes alternately. The titre of both absorbed and desorbed factors were noted.

(5) Unitary test for reagent

A newly prepared reagent was further subjected to serological test for unity. This was done in the same way as was done for the analysis of antiserum by using at least 16 different red blood cell samples from unrelated animals as far as possible. When the test showed that absorptions with cells from any positive individual had removed all antibodies for cells from any one of the other positive individuals, and absorbed fraction from negative individuals did not remove any antibodies for the positive individuals, this fraction of antisera was taken as a reagent and criterion for that fraction was that could not be further subdivided into more fractions. An example for the unitary test is given in Table 2.

It is evident from Table 2 that only one type of reaction was given by all the absorbed fractions of sera, because all those animals positive for R_1 antigen have absorbed all the antibodies for other R_1 positive animals. The animals negative for R_1 antigen have left off antibodies

Table No:2
Unitary test of Anti-R_1 Reagent

S No.	cell suspensions of 16 animals	Absorbed fractions of reagent with red blood cells from different animals																control	
		1	2	4	5	7	8	14	15	17	18	25	26	28	40	45	47	UNAB	Saline	compliment
1	1	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
2	2	+	−	+	−	−	+	+	+	−	−	+	−	+	+	−	−	+	−	−
3	4	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
4	5	+	−	+	−	−	+	+	+	−	−	+	−	+	+	−	−	+	−	−
5	7	+	−	+	−	−	+	+	+	−	−	+	−	+	+	−	−	+	−	−
6	8	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
7	14	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
8	15	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
9	17	+	−	+	−	−	+	+	+	−	−	+	−	+	+	−	−	+	−	−
10	18	+	−	+	−	−	+	+	+	−	−	+	−	+	+	−	−	+	−	−
11	25	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
12	26	+	−	+	−	−	+	+	+	−	−	+	−	+	+	−	−	+	−	−
13	28	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
14	40	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
15	45	+	−	+	−	−	+	+	+	−	−	+	−	+	+	−	−	+	−	−
16	47	+	−	+	−	−	+	+	+	−	−	+	−	+	+	−	−	+	−	−

for R_1 positive cells. As no reaction was observed in R_1 negative cells the serum was designated as anti-R_1.

C. Preparation of anti-J

For the preparation of anti-J, 20 J negative animals belonging to the experimental herd of Animal Genetics Division, Indian Veterinary Research Institute, Izatnagar were bled and their sera were separated. These sera were tested against five J positive animals of the same herd, whose blood type was already known(Table 3). The reactive sera were noted and later the strength of J antibodies was then observed by titre test and the animal showing highest titre (animal No. 1317) was bled and its serum was separated (Table 4). To test the unity of this serum, this was tested against 50 cattle with known blood types. when it did not show any non-specific reaction the reagent was labelled as anti-J.

The reagents were prepared and standardized. The typing was done by the reagents as supplied by blood group laboratories of France (R_1) and Norway (E). The reagent J was isolated from Hariana cattle and used throughout the present study.

Table 3

Showing the preparation of anti-J

Cell suspension	Sera of twenty different animals																				Controls	
	948	961	996	998	1036	1061	1164	1169	1183	1187	1212	1219	1240	1317	1355	1393	1407	1448	1532	1573	Saline	Complement
715(J^+)	+	-	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-
1152 (J^+)	±	-	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-
1332(J^+)	+	-	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-
1420(J^+)	±	-	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-
1716(J^+)	±	-	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-

Table 4

Titre testing of anti-J sera obtained from animal No. 1317

Cell suspension	Anti-J serum							Controls	
	1:1	1:2	1:4	1:8	1:16	1:32	1:64	Saline	Complement
715 (J^+)	+	+	+	+	+	+	-	-	-

D. Blood typing

The blood samples for the present study were obtained from 230 cattle belonging to Ongole breed. These cattle were stationed at two farms, one at Chintaladevi and other at Mahanandi in Andhra Pradesh. In addition the blood from 101 animals of Zebu-Jersey crosses (Jersey x non-descript) was obtained from the animals maintained in and around the town of Vishakapatnam in Andhra Pradesh. About 5-10 cc of citrated blood was collected in the skirt type glass vials and was transported in ice-boxes. The blood was directly collected from the jugular vein of the animals. The procedure for washing cells and blood typing was same as described previously. The criterion for noting the haemolysis is described above.

Collection of data and statistical analysis

For studying the association, if any, of blood groups with the economic characters data on following lines was collected from the available records maintained at Government Livestock Farm, Chintaladevi and Government Livestock Farm, Mahanandi in Andhra Pradesh. As the Zebu-Jersey crossbreds were maintained by private owners no

information was available regarding their production.

The investigation for finding an association with blood factors included following production characters.

(1) Birth weight
(2) Age at first calving
(3) Age at first service
(4) Gestation period
(5) First lactation yield
(6) First calving interval
(7) First dry period
(8) Days in yield in first lactation (1st lactation period)
(9) Average yield per day in first lactation
(10) Average yield per day based on total lactations completed
(11) Breeding efficiency

Data so obtained were analysed statistically following the standard procedures given in Snedecor (1961).

Whether an antigen was associated with any particular trait was tested by analysing the variance of the records within and between the two groups viz. the group which had the antigen in question and the one which lacked it. The

calculated F values were then compared with tabulated values at corresponding degrees of freedom to see their significance. But in case of R_1 weighted 't' test (Cochron, 1950) was applied because the variances in the two groups (i.e. with blood group factor and without the blood group factor) were inhomogenous.

The gene frequencies were calculated by taking the square-root of the recessive phenotypes (in blood group studies the individuals negative for a particular factor are considered as homozygous recessives). Standard error of gene frequencies was calculated by the formula

$$S.E. = \sqrt{\frac{1 - q^2}{4G}}$$

Where G = Total number of animals.

Both the phenotypic and gene frequencies in two populations were compared by normal deviate test to know whether there existed any significant difference between two breeds. Breeding efficiency was calculated by the formula given by Wilcox *et al.* (1957).

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Where G = Total number of animals.

Both the phenotypic and gene frequencies in two populations were compared by normal deviate test to know whether there existed any significant difference between two breeds. Breeding efficiency was calculated by the formula given by Wilcox *et al.* (1957).

Haemoglobin typing

Haemoglobin typing was done by starch gel electrophoresis. The procedures followed are explained below.

Purification of starch

For this the procedure described by Smithies (1955) was followed. B.D.H. starch (potato) was hydrolysed by adding acetone-HCl mixture (100 volume reagent grade acetone + 1 volume concentrated HCl) at a rate of 2 ml per gram starch. The mixture was then kept at 38.5°C for 45 minutes. Then M sodium acetate (aquous) was added to the mixture at a rate of 1/2 ml per gm substance. The starch was then filtered off on Buchner's funnel with frequent washing of distilled water. It was resuspended in distilled water and allowed to stand overnight. Next day the starch was filtered and thoroughly dried.

Bridge buffer

The bridge buffer of Gahne et al. (1960) was used in this study. This buffer contained 20.2 gm sigma 7-9 Tris; 2 gm EDTA and 1.5 gm boric acid per litre, with a pH of 9.0. The same buffer diluted to 1:7 with distilled

water was mixed with 10% starch for the preparation of gel. The starch gel was prepared on 10 cm x 15 cm x 0.5 cm polythene plates. The samples were allowed to run for 1½ hour at 250 volts pressure.

Calculation of gene frequency for haemoglobin

The gene frequency was calculated by using the following formula.

$$\text{Frequency of } Hb^A \text{ gene} = \frac{2A + AB}{2N}$$

where N = Total number of animals.

The chi-square test (goodness of fit) was applied to test the law of genetic equilibrium.

RESULTS

The results of blood typing of 230 heads of Ongole and 99 heads of crossbred cattle are in summarised in Table 5(a) and (b).

Table 5(a)

Results of blood typing in Ongoles

Sl. No.	Animal No.	Antigenic factors			Controls	
		R_1	E	J	Saline	Complement
1	844	R_1	E	J	-	-
2	1016	R_1	E	J	-	-
3	V_{14}	R_1	E	J	-	-
4	V_{34}	-	-	-	-	-
5	V_{80}	-	-	J	-	-
6	V_{81}	-	-	J	-	-
7	V_{82}	-	E	-	-	-
8	V_{87}	-	E	-	-	-
9	615	-	E	-	-	-
10	645	-	-	-	-	-
11	688	-	E	-	-	-
12	760	-	E	-	-	-
13	780	-	E	J	-	-
14	781	-	E	-	-	-
15	812	-	E	-	-	-

Table 5(a) (contd)

Sl. No.	Animal No.	Antigenic factors			Controls	
		R_1	E	J	Saline	Complement
16	826	-	E	-	-	-
17	841	R_1	E	J	-	-
18	845	-	-	-	-	-
19	854	-	E	J	-	-
20	859	R_1	-	J	-	-
21	862	-	-	-	-	-
22	865	-	-	-	-	-
23	866	-	E	-	-	-
24	867	-	E	J	-	-
25	884	R_1	E	-	-	-
26	888	R_1	E	-	-	-
27	895	-	E	-	-	-
28	896	-	-	-	-	-
29	905	-	E	J	-	-
30	930	-	E	J	-	-
31	942	-	E	J	-	-
32	947	-	E	J	-	-
33	948	-	-	-	-	-
34	950	-	E	-	-	-
35	952	-	E	J	-	-
36	959	-	-	-	-	-
37	961	-	-	-	-	-
38	963	-	-	-	-	-

Table 5(a) (contd)

Sl. No.	Animal No.	Antigenic factors			Controls	
		R_1	E	J	Saline	Complement
39	964	-	E	J	-	-
40	970	-	E	-	-	-
41	989	-	-	-	-	-
42	990	-	-	-	-	-
43	995	-	-	J	-	-
44	996	-	E	J	-	-
45	998	-	E	J	-	-
46	999	-	E	-	-	-
47	1004	-	-	-	-	-
48	1006	-	E	J	-	-
49	1010	-	E	-	-	-
50	1011	-	E	J	-	-
51	1018	-	-	-	-	-
52	1020	-	-	-	-	-
53	1021	-	-	-	-	-
54	1023	R_1	-	J	-	-
55	1037	-	E	J	-	-
56	1051	-	-	J	-	-
57	1053	-	E	-	-	-
58	1057	-	E	-	-	-
59	1067	-	-	J	-	-
60	1075	-	E	-	-	-
61	1076	-	-	J	-	-

Table 5(a) (contd)

Sl. No.	Animal No.	Antigenic factors			Controls	
		R_1	E	J	Saline	Complement
62	1078	-	-	-	-	-
63	1082	-	-	-	-	-
64	1084	R_1	E	J	-	-
65	1086	-	-	-	-	-
66	1087	R_1	E	J	-	-
67	1090	-	-	-	-	-
68	1102	-	E	J	-	-
69	1113	-	-	-	-	-
70	1115	-	-	-	-	-
71	1118	-	E	-	-	-
72	1122	-	-	-	-	-
73	1134	-	E	-	-	-
74	1144	-	-	J	-	-
75	1146	-	E	J	-	-
76	1154	-	-	J	-	-
77	1156	R_1	E	-	-	-
78	1157	-	E	-	-	-
79	1160	-	-	-	-	-
80	1162	-	-	-	-	-
81	1163	-	-	-	-	-
82	1165	-	-	J	-	-
83	1166	-	E	J	-	-

Table 5(a) (contd)

Sl. No.	Animal No.	Antigenic factors			Controls	
		R_1	E	J	Saline	Complement
84	1170	-	-	J	-	-
85	1173	R_1	E	-	-	-
86	1174	-	-	-	-	-
87	1176	-	-	J	-	-
88	1177	-	E	J	-	-
89	1180	-	-	-	-	-
90	1181	R_1	E	J	-	-
91	1183	-	E	J	-	-
92	1187	R_1	-	-	-	-
93	1188	-	E	J	-	-
94	1193	-	E	-	-	-
95	1202	-	E	-	-	-
96	1203	-	E	-	-	-
97	1213	R_1	E	J	-	-
98	1228	-	-	-	-	-
99	1247	-	-	J	-	-
100	1253	-	-	-	-	-
101	1255	-	E	J	-	-
102	1264	-	-	-	-	-
103	1269	-	-	-	-	-
104	1298	-	E	-	-	-
105	1300	-	E	J	-	-

Table 5(a) (contd)

Sl. No.	Animal No.	Antigenic factors			Controls	
		R_1	E	J	Saline	Complement
106	1302	-	E	-	-	-
107	1305	-	E	J	-	-
108	1306	R_1	E	-	-	-
109	1307	-	E	J	-	-
110	1445	-	E	-	-	-
111	1454	-	-	-	-	-
112	1457	-	E	-	-	-
113	1465	-	E	J	-	-
114	1474	R_1	-	-	-	-
115	1492	-	-	J	-	-
116	1493	-	E	J	-	-
117	1494	R_1	-	-	-	-
118	1496	-	-	-	-	-
119	1497	R_1	E	J	-	-
120	1499	-	E	-	-	-
121	1502	-	E	J	-	-
122	1503	R_1	E	J	-	-
123	1513	-	-	-	-	-
124	1514	-	-	-	-	-
125	1517	-	-	-	-	-

Table 5(a) (contd)

Sl. No.	Animal No.	Antigenic factors			Controls	
		R_1	E	J	Saline	Complement
126	1519	-	E	-	-	-
127	1520	-	-	-	-	-
128	1522	-	E	-	-	-
129	1526	-	E	-	-	-
130	1528	-	E	J	-	-
131	1531	-	-	-	-	-
132	48	-	-	-	-	-
133	58	-	E	-	-	-
134	59	-	E	-	-	-
135	61	R_1	E	J	-	-
136	62	-	-	-	-	-
137	72	-	E	-	-	-
138	73	-	E	J	-	-
139	86	-	-	-	-	-
140	87	-	E	-	-	-
141	93	-	E	-	-	-
142	97	-	E	-	-	-
143	101	-	-	-	-	-
144	103	-	E	J	-	-
145	104	-	E	J	-	-
146	107	-	E	J	-	-

Table 5(a) (contd)

Sl. No.	Animal No.	Antigenic factors			Controls	
		R_1	E	J	Saline	Complement
147	120	-	E	J	-	-
148	123	-	E	J	-	-
149	128	R_1	E	J	-	-
150	133	-	-	-	-	-
151	134	-	E	J	-	-
152	135	-	E	J	-	-
153	142	-	E	J	-	-
154	143	-	E	-	-	-
155	145	-	E	-	-	-
156	146	-	E	J	-	-
157	316	R_1	E	J	-	-
158	317	R_1	E	J	-	-
159	331	R_1	E	-	-	-
160	342	R_1	E	-	-	-
161	345	R_1	E	-	-	-
162	347	-	E	-	-	-
163	349	-	E	J	-	-
164	351	-	E	-	-	-
165	355	-	E	-	-	-
166	360	-	E	-	-	-

Table 5(a) (contd)

Sl. No.	Animal No.	Antigenic factors			Controls	
		R_1	E	J	Saline	Complement
167	367	-	E	-	-	-
168	370	-	-	J	-	-
169	380	-	E	J	-	-
170	382	-	-	J	-	-
171	388	-	E	-	-	-
172	404	-	E	-	-	-
173	405	R_1	E	-	-	-
174	407	-	-	J	-	-
175	408	-	E	J	-	-
176	414	-	-	-	-	-
177	419	-	E	-	-	-
178	424	-	E	-	-	-
179	427	-	E	J	-	-
180	430	-	E	J	-	-
181	432	-	E	-	-	-
182	435	-	-	-	-	-
183	436	-	E	J	-	-
184	437	-	E	J	-	-
185	438	-	E	-	-	-
186	444	-	E	-	-	-
187	445	-	E	J	-	-

Table 5(a) (contd)

Sl. No.	Animal No.	Antigenic factors			Controls	
		R_1	E	J	Saline	Complement
188	447	R_1	E	J	-	-
189	449	-	E	-	-	-
190	450	-	E	J	-	-
191	452	-	E	J	-	-
192	458	-	E	J	-	-
193	460	-	-	-	-	-
194	461	-	E	J	-	-
195	462	-	E	-	-	-
196	463	-	E	-	-	-
197	466	-	E	-	-	-
198	467	-	E	J	-	-
199	469	-	-	J	-	-
200	471	-	E	-	-	-
201	472	-	E	J	-	-
202	482	-	E	J	-	-
203	483	-	-	-	-	-
204	492	-	-	-	-	-
205	671	-	-	J	-	-
206	701	-	E	-	-	-
207	757	-	-	-	-	-
208	768	-	E	-	-	-
209	830	-	-	-	-	-
210	833	-	E	J	-	-

Table 5(a) (contd)

Sl. No.	Animal No.	Antigenic factors			Controls	
		R_1	E	J	Saline	Complement
211	839	-	-	J	-	-
212	874	-	E	J	-	-
213	885	-	-	-	-	-
214	890	-	E	J	-	-
215	894	-	E	J	-	-
216	906	-	E	J	-	-
217	932	-	E	-	-	-
218	975	-	E	J	-	-
219	977	-	E	-	-	-
220	980	-	E	-	-	-
221	986	-	E	-	-	-
222	993	R_1	E	J	-	-
223	1012	-	E	J	-	-
224	1013	-	E	J	-	-
225	1022	-	E	J	-	-
226	1038	-	E	-	-	-
227	1042	-	E	J	-	-
228	1043	R_1	-	J	-	-
229	1047	-	E	J	-	-
230	1054	-	E	J	-	-

Table 5(b)

Results of blood typing in crossbreds

Sl. No.	Animal No.	Antigenic factors			Controls	
		R_1	E	J	Saline	Complement
1	X_1	-	E	-	-	-
2	X_2	-	-	-	-	-
3	X_3	-	E	-	-	-
4	X_4	R_1	E	J	-	-
5	X_5	-	E	J	-	-
6	X_6	R_1	E	-	-	-
7	X_7	-	-	-	-	-
8	X_8	-	E	-	-	-
9	X_9	R_1	E	J	-	-
10	X_{10}	-	-	J	-	-
11	X_{11}	-	E	J	-	-
12	X_{12}	-	E	-	-	-
13	X_{13}	-	E	J	-	-
14	X_{14}	-	E	-	-	-
15	X_{15}	-	E	-	-	-
16	X_{16}	-	E	J	-	-
17	X_{17}	R_1	E	J	-	-
18	X_{18}	-	E	-	-	-
19	X_{19}	R_1	E	J	-	-

Table 5(b) (contd)

Sl. No.	Animal No.	Antigenic factors			Controls	
		R_1	E	J	Saline	Complement
20	X_{20}	-	-	-	-	-
21	X_{21}	-	E	-	-	-
22	X_{22}	-	-	-	-	-
23	X_{23}	-	E	J	-	-
24	X_{24}	-	-	-	-	-
25	X_{25}	-	E	J	-	-
26	X_{26}	-	E	J	-	-
27	X_{27}	-	E	-	-	-
28	X_{28}	-	-	J	-	-
29	X_{29}	-	E	J	-	-
30	X_{30}	-	E	J	-	-
31	X_{31}	-	E	J	-	-
32	X_{32}	-	E	J	-	-
33	X_{33}	R_1	E	-	-	-
34	X_{34}	-	E	-	-	-
35	X_{35}	-	E	-	-	-
36	X_{36}	-	E	J	-	-
37	X_{37}	-	-	-	-	-
38	X_{39}	-	E	-	-	-
39	X_{40}	-	-	-	-	-
40	X_{41}	-	E	-	-	-

Table 5(b) (contd)

Sl. No.	Animal No.	Antigenic factors			Controls	
		R_1	E	J	Saline	Complement
41	X_{42}	-	E	-	-	-
42	X_{43}	-	E	J	-	-
43	X_{44}	-	E	-	-	-
44	X_{45}	-	E	-	-	-
45	X_{46}	R_1	E	-	-	-
46	X_{47}	-	E	-	-	-
47	X_{48}	-	E	-	-	-
48	X_{49}	-	E	-	-	-
49	X_{50}	R_1	E	-	-	-
50	X_{51}	-	-	-	-	-
51	X_{52}	-	E	J	-	-
52	X_{53}	-	E	J	-	-
53	D_1	-	E	-	-	-
54	D_2	-	E	-	-	-
55	D_3	R_1	-	J	-	-
56	D_4	-	E	-	-	-
57	D_5	-	E	J	-	-
58	D_6	-	E	-	-	-
59	D_7	-	E	-	-	-
60	D_8	-	E	-	-	-

Table 5(b) (contd)

Sl. No.	Animal No.	Antigenic factors			Controls	
		R_1	E	J	Saline	Complement
61	D_9	-	-	-	-	-
62	D_{10}	-	E	J	-	-
63	D_{11}	-	E	-	-	-
64	D_{12}	-	E	-	-	-
65	D_{13}	-	-	J	-	-
66	D_{14}	-	E	-	-	-
67	D_{15}	R_1	E	-	-	-
68	D_{16}	-	E	-	-	-
69	D_{17}	R_1	E	-	-	-
70	D_{18}	-	E	-	-	-
71	D_{19}	-	E	-	-	-
72	D_{20}	-	E	-	-	-
73	D_{21}	-	E	-	-	-
74	D_{22}	-	-	-	-	-
75	D_{23}	-	E	-	-	-
76	D_{24}	-	E	J	-	-
77	D_{25}	-	E	J	-	-
78	D_{26}	R_1	E	J	-	-
79	D_{27}	-	E	-	-	-
80	D_{28}	-	E	-	-	-

Table 5(b) (contd)

Sl. No.	Animal No.	Antigenic factors			Controls	
		R_1	E	J	Saline	Complement
81	D_{29}	R_1	E	J	-	-
82	D_{30}	-	E	J	-	-
83	D_{31}	-	E	-	-	-
84	D_{32}	R_1	E	J	-	-
85	D_{33}	-	E	J	-	-
86	D_{34}	-	-	-	-	-
87	D_{35}	-	E	-	-	-
88	D_{36}	R_1	E	-	-	-
89	D_{37}	-	E	-	-	-
90	D_{38}	R_1	E	J	-	-
91	D_{39}	-	E	J	-	-
92	D_{40}	-	E	-	-	-
93	D_{41}	-	E	J	-	-
94	D_{42}	-	E	-	-	-
95	D_{43}	-	E	J	-	-
96	D_{44}	-	E	-	-	-
97	D_{45}	-	E	J	-	-
98	D_{46}	R_1	E	J	-	-
99	D_{47}	-	E	-	-	-

From Table 5(a) and (b) it will be seen that out of 230 Ongoles 31 showed R_1 and 199 did not giving a percentage of incidence 13.48. In case of E 154 showed the antigenic factor and 76 did not giving an incidence of 66.52% for this factor. As regards J 103 showed the factor and 127 did not. The percentage of incidence in this case was 44.78.

Among crossbreds out of 99 animals tested in case of antigenic factor R_1, 17 showed the factor and 82 did not giving an incidence of 17.17%. In case of E there were 84 cases which had the factor and 15 cases which did not. The incidence of occurrence of this factor was thus 84.84%. As regards J 38 had the factor and 61 did not. The incidence of this factor among crossbreds was thus 38.38%.

When we compared the incidence of 3 antigenic factors in the two classes of animals we found that as regards R_1, it occurred more frequently in crossbreds but the difference was not statistically significant. In case of factor E there was highly significant difference in its occurrence in two classes of animals. Its occurrence was also more in crossbreds than Ongole. The antigenic factor J occurred more frequently in Ongole but the difference was not statistically significant.

Results of haemoglobin typing was carried out only in crossbred cattle. The results are presented in Table 6.

Table 6

Results of haemoglobin typing in crossbreds

Sl.No.	Animal No.	Haemoglobin type
1	X_1	BB
2	X_2	BB
3	X_3	AB
4	X_4	AB
5	X_5	AA
6	X_6	AB
7	X_7	AB
8	X_8	AA
9	X_9	AB
10	X_{10}	BB
11	X_{11}	AB
12	X_{12}	AB
13	X_{13}	AB
14	X_{14}	BB
15	X_{15}	AA
16	X_{16}	BB
17	X_{17}	AA
18	X_{18}	AA

Table 6 (contd)

Sl.No.	Animal No.	Haemoglobin type
19	X_{19}	BB
20	X_{20}	BB
21	X_{21}	BB
22	X_{22}	BB
23	X_{23}	AB
24	X_{24}	AB
25	X_{25}	BB
26	X_{26}	BB
27	X_{27}	AB
28	X_{28}	AB
29	X_{29}	AB
30	X_{30}	AB
31	X_{31}	BB
32	X_{32}	AA
33	X_{33}	AA
34	X_{34}	AA
35	X_{35}	BB
36	X_{36}	AA
37	X_{37}	BB
38	X_{38}	BB
39	X_{39}	BB
40	X_{40}	BB
41	X_{41}	AA

Table 6 (contd)

Sl.No.	Animal No.	Haemoglobin type
19	X_{19}	BB
20	X_{20}	BB
21	X_{21}	BB
22	X_{22}	BB
23	X_{23}	AB
24	X_{24}	AB
25	X_{25}	BB
26	X_{26}	BB
27	X_{27}	AB
28	X_{28}	AB
29	X_{29}	AB
30	X_{30}	AB
31	X_{31}	BB
32	X_{32}	AA
33	X_{33}	AA
34	X_{34}	AA
35	X_{35}	BB
36	X_{36}	AA
37	X_{37}	BB
38	X_{38}	BB
39	X_{39}	BB
40	X_{40}	BB
41	X_{41}	AA

Table 6 (contd)

Sl.No.	Animal No.	Haemoglobin type
42	X_{42}	BB
43	X_{43}	AB
44	X_{44}	AB
45	X_{45}	BB
46	X_{46}	AA
47	X_{47}	AB
48	X_{48}	AA
49	X_{49}	AB
50	X_{50}	AA
51	X_{51}	AB
52	X_{52}	AB
53	X_{53}	AB
54	D_1	AB
55	D_2	BB
56	D_3	AB
57	D_4	AB
58	D_5	BB
59	D_6	AA
60	D_7	AA
61	D_8	AA
62	D_9	BB
63	D_{10}	AB
64	D_{11}	AB

Table 6 (contd)

Sl.No.	Animal No.	Haemoglobin type
65	D_{12}	BB
66	D_{13}	AB
67	D_{14}	AA
68	D_{15}	AA
69	D_{17}	AB
70	D_{18}	AA
71	D_{19}	AB
72	D_{20}	BB
73	D_{21}	AB
74	D_{22}	AA
75	D_{23}	AA
76	D_{24}	AA
77	D_{25}	AB
78	D_{26}	AB
79	D_{27}	AA
80	D_{28}	AB
81	D_{29}	AB
82	D_{30}	AB
83	D_{31}	AB
84	D_{32}	AB
85	D_{33}	AB

Table 6 (contd)

Sl.No.	Animal No.	Haemoglobin type
86	D_{34}	AA
87	D_{35}	AA
88	D_{36}	AB
89	D_{37}	AA
90	D_{38}	AB
91	D_{39}	AB
92	D_{40}	AA
93	D_{41}	AB
94	D_{42}	AA
95	D_{43}	AB
96	D_{44}	AB
97	D_{45}	AB
98	D_{46}	AB
99	D_{47}	AB
100	D_{48}	AB
101	D_{49}	AA

Out of 101 animals typed for haemoglobin 24 had BB, 48 AB and 29 AA giving the respective incidences of 23.76%, 47.52% and 28.71%.

Gene frequencies of R_1, E and J observed in two classes of animals are summarised in Table 7.

Table 7

Gene frequencies

Blood group antigens	Ongole (a)	Crossbreds (b)	Difference (a-b)
R_1	0.0699 ± 0.0100	0.0999 ± 0.0223	-0.0300
E	0.4214 ± 0.0264	0.6107 ± 0.0141	-0.1893**
J	0.2569 ± 0.0200	0.2160 ± 0.0300	0.0409

** Significant at 0.01 level

From Table 7 it will be seen that there was statistically no significant difference in the frequencies of R_1 and J, while in case of E highly significant difference was noted. The crossbreds had a higher frequency than Ongoles.

As information was available for more than one farm in case of Ongoles farmwise data were also analysed. The results are brought out in Table 8.

Table 8

Gene frequencies

Blood group antigens	Chintaladevi (a)	Mahanandi (b)	Difference (a-b)
R_1	0.0800 ± 0.0200	0.0572 ± 0.0100	0.0228
E	0.3400 ± 0.0100	0.5505 ± 0.0100	-0.2105**
J	0.2551 ± 0.0283	0.3323 ± 0.0100	-0.0772

** Significant at 0.01 level

From Table 8 it will be seen that there was statistically no significant difference in the frequencies of R_1 and J, while in case of E highly significant difference was noted. The Mahanandi farm had a higher frequency of E than Chintaladevi.

For studying the mode of inheritance of the 3 antigenic factors, the data were rearranged according to sires, mates and their progeny. Results are summarised in Table 9(a), (b) and (c).

Table 9(a)

Sirewise incidence of R_1

Sl.No. of sires	Sire	Mates	Progeny	
			R_1 positive	R_1 negative
1	R_1	R_1 (3)	1	2
	R_1	- (18)	2	16
2	R_1	- (7)	2	5
3	-	R_1 (1)	-	1
	-	- (12)	1*	11

*The occurrence of a R_1 positive offspring, when both of its parents were negative for that factor was suggestive of discrepancy in recording its parentage.

Table 9(b)

Sirewise incidence of E

Sl.No. of sires	Sire	Mates	Progeny	
			E positive	E negative
1	E	E (10)	5	5
	E	- (11)	6	5

Table 9(b) (contd)

Sl.No. of sires	Sire	Mates	Progeny	
			E positive	E negative
2	E	E (5)	3	2
	E	- (2)	-	2
3	E	E (7)	6	1
	E	- (6)	5	1

Table 9(c)

Sirewise incidence of J

Sl.No. of sires	Sire	Mates	Progeny	
			J positive	J negative
1	J	J (6)	2	4
	J	- (15)	2	13
2	J	J (3)	3	-
	J	- (4)	2	2
3	J	J (5)	4	1
	J	- (8)	5	3

Frequencies of haemoglobin A and B types as found in crossbreds was worked out and are given in Table 10.

Table 10

Phenotypic and gene frequencies of haemoglobin

	Phenotypes			Chi-square (d.f.=2)	Gene frequencies	
	AA	AB	BB		Hb^A	Hb^B
Observed	29	48	24	0.36	0.5247	0.4753
Expected	27.53	49.86	22.59			

It will be seen from the table that the crossbreds were in genetic equilibrium as there was no statistically significant deviation of observed phenotypes from the expected ones.

Relationship between 3 antigenic factors and certain economic traits were studied. Results are summarised in Table 11(a), (b) and (c). (vide Appendices I and II).

Table 11(a)

Relationship between R1 and 9 economic traits in Ongole

Sl. No.	Trait	Mean values of animals		Difference (a-b)
		With blood group factors (a)	Without blood group factors (b)	
1	Birth weight (kg)	23.68±2.84 (18)	25.72±2.52 (63)	-2.04
2	Age at 1st calving (days)	1198.01±200.40 (13)	1228.27±279.03 (90)	-30.26
3	Age at 1st service (days)	875.83±115.81 (6)	933.33±61.21 (65)	-57.50
4	Gestation period (days)	271.66±16.28 (6)	287.18±8.32 (66)	-15.52
5	1st lactation yield (kg)	1014.10±347.10 (9)	1276.47±597.60 (76)	-262.37
6	1st lactation period (days)	303.77±81.50 (9)	314.97±77.80 (76)	-11.20
7	1st dry period (days)	221.37±101.90 (8)	178.94±120.04 (69)	42.43
8	Average yield per day in 1st lactation (kg)	2.05±0.65 (9)	2.50±1.40 (70)	-0.45
9	1st calving interval (days)	548.62±87.30 (8)	496.04±121.50 (69)	52.58

Figures in parenthesis indicate the number of animals

Table 11(b)

Relationship between E and 11 economic traits in Ongole

Sl. No.	Trait	Mean values of animals		Difference (a-b)
		With blood group factors (a)	Without blood group factors (b)	
1	Birth weight (kg)	24.69±3.04 (77)	25.14±2.13 (44)	-0.45
2	Age at 1st service (days)	949.19±24.31 (42)	898.48±15.42 (29)	50.71
3	Gestation period (days)	285.34±4.05 (43)	286.68±3.00 (29)	-1.34
4	Age at 1st calving (days)	1230.55±316.30 (63)	1214.82±175.20 (40)	15.73
5	1st lactation yield (kg)	1141.95±555.70 (52)	1339.21±598.20 (33)	-197.26
6	1st lactation period (days)	316.01±81.10 (52)	310.27±91.80 (33)	5.74
7	1st dry period (days)	143.40±82.30 (47)	245.93±141.70 (30)	-102.53**
8	1st calving interval (days)	525.38±121.20 (47)	464.10±106.30 (30)	61.28*
9	Average daily milk yield in 1st lactation (kg)	2.20±1.07 (48)	3.01±1.50 (31)	-0.81**
10	Average daily milk based on total lactations completed (kg)	2.50±1.10 (21)	2.60±0.76 (14)	-0.10
11	Breeding efficiency (%)	76.80±13.20 (18)	81.20±11.40 (12)	-4.40

*Significant at 0.05 level
**Significant at 0.01 level
Figures in parenthesis indicate the number of animals

Table 11(c)

Relationship between J and 11 economic traits in Ongole

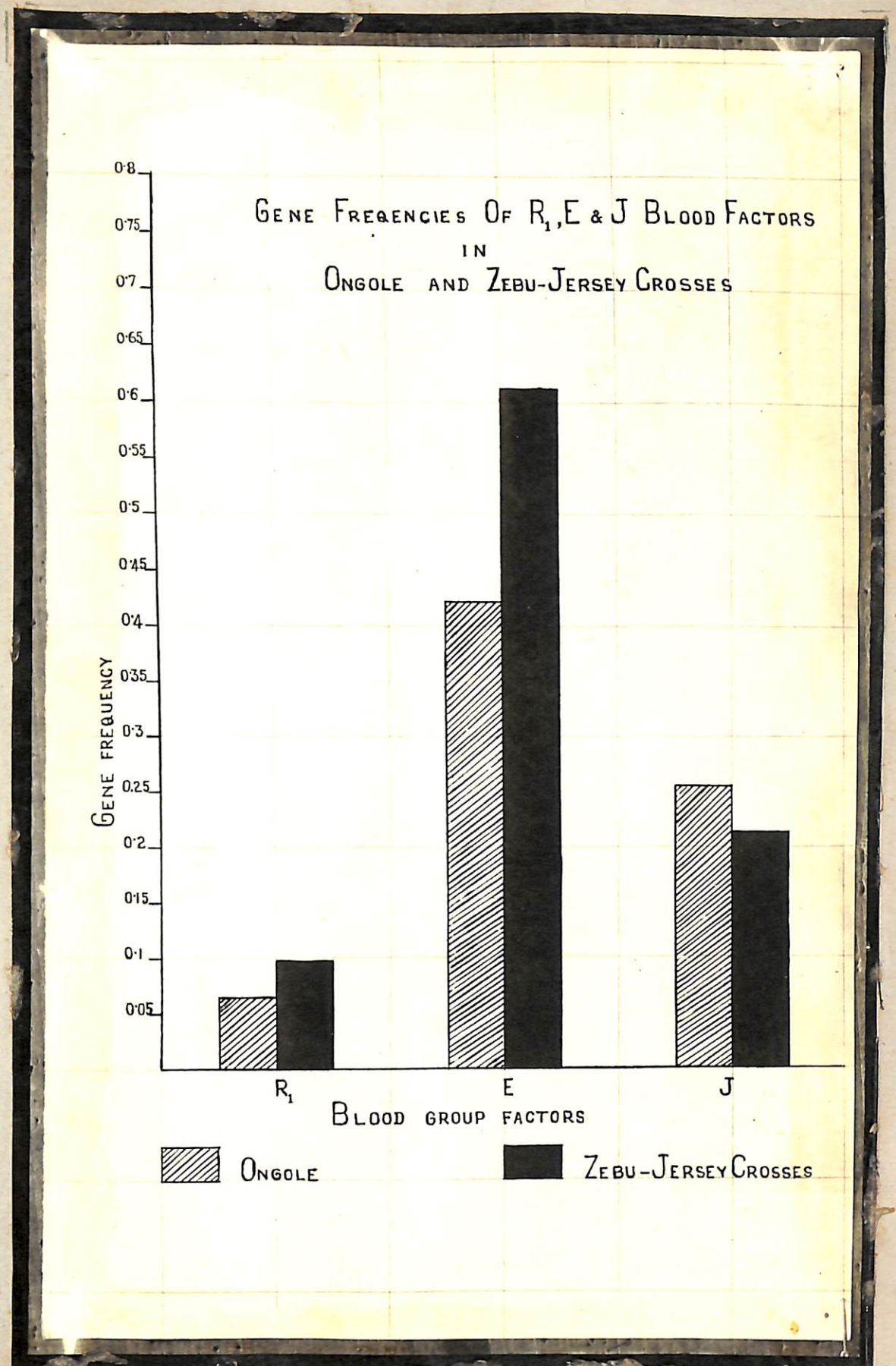
Sl. No.	Trait	Mean values of animals		Difference (a-b)
		With blood group factors (a)	Without blood group factors (b)	
1	Birth weight (kg)	24.86±2.78 (56)	24.84±2.74 (65)	0.02
2	Age at 1st service (days)	908.08±164.29 (24)	938.89±199.32 (47)	-30.81
3	Gestation period (days)	284.29±8.35 (24)	296.68±11.06 (48)	-12.39
4	Age at 1st calving (days)	1257.04±193.80 (44)	1201.35±313.60 (59)	55.69
5	1st lactation yield (kg)	1182.46±561.60 (36)	1245.05±592.80 (49)	-62.59
6	1st lactation period (days)	306.66±84.20 (36)	319.02±86.05 (49)	-12.36
7	1st dry period (days)	234.06±138.02 (32)	147.29±86.90 (45)	86.77**
8	1st calving interval (days)	547.25±125.60 (32)	468.98±103.30 (45)	78.27**
9	Average daily milk yield in 1st lactation (kg)	2.30±1.07 (34)	2.60±1.50 (45)	-0.30
10	Average daily milk yield based on total lactations completed (kg)	2.60±0.34 (14)	2.50±1.04 (21)	0.10
11	Breeding efficiency (%)	75.40±14.50 (12)	80.70±24.70 (18)	-5.30

** Significant at 0.01 level
Figures in parenthesis indicate the number of animals

It will be seen from the table that the antigenic factor R_1 did not show any relationship with the characters studied. The factor E was highly significantly related with 1st dry period and average daily milk yield in 1st lactation. It also showed a significant relation with 1st calving interval, while the antigenic factor J showed a highly significant relation with 1st dry period and 1st calving interval.

The gene frequencies of R_1, E and J antigenic factors in two classes of animals are diagrametically represented in Fig. 1.

Fig. 1


Gene Freqencies Of R1, E & J Blood Factors
IN
Ongole and Zebu-Jersey Crosses
Gene frequency
0·8
0·75
0·7
0·65
0·6
0·55
0·5
0·45
0·4
0·35
0·3
0·25
0·2
0·15
0·1
0·05
R1
E
J
Blood group factors
Ongole
Zebu-Jersey Crosses

DISCUSSION

As a result of investigations begun nearly three decades ago, it has been established beyond doubt that each of the numerous bovine blood group factors, A, B, C, ... X, Y, Z; A', B', $E'_1E'_2$ ..... etc., is inherited as a single Mendelian dominant in contrast with its absence. In other words each bovine blood factor makes its appearance on the red cells of an individual only when present on the red cells of one or both of that individual's parents (Ferguson, 1941; Ferguson et al., 1942; Stormont, 1950 and Stormont, 1967). In our present investigation, an attempt was made to study the inheritance of R_1, E and J antigenic factors in Ongole. It was observed that the three factors under investigation were following the general rule of Mendelian inheritance, and their presence was dominant to absence. The three bulls investigated were heterozygous at these loci, except the sire No. 3 which was homozygous recessive at R1 locus. During this study one interesting discrepency was noted, that is an offspring positive for R_1 was found (Table 9a) when none of its parents had that particular antigen on their red cells. This was suggestive of an illegitimacy in recording its pedigree. The importance of accurate recording of pedigree cannot be under-estimated in any breeding programme and as such the information

regarding the blood type of all animals will be useful in determining correct parentage of individuals.

The difference between breeds are the result for the most part, of differences in large number of genes with individually inconspicous effects, while an occasional gene with a distinct effect shows a difference in frequency in the breeds being compared. These differences in the frequency range from cases in which a gene is virtually fixed in one breed, while an allele is fixed in another; the subtle contrasts detected only by statistical tests. Differences in genes with small effects may also involve variations in frequency of the gene concerned rather than the fixation of distinct alleles in different populations (Owen et al., 1947). The blood group genes have not been subjected to any direct selection. Significant differences with regard to the frequency of several of these genes between two populations would therefore suggest the existance also of other genetic differences.

The present study, though not directly dealing with the genetic differences but the antigenic frequencies do reflect the nature of possible differences. Chet Ram and Khanna (1961) published their first report on Indian cattle blood groups, and their findings were that when comparison

was made between Hariana and Kumauni with two foreign breeds i.e. Holstein and Guernsey, Kumauni and Guernsey breed had more similarity in themselves than Holstein-Friesian. This was significant since both the breeds were developed on hilly tracts. They also reported that factors L and A were very common while R and M were rare in their occurrence. Singh (1965) studied the frequency of occurrence of 15 antigenic factors in Hariana and Sindhi-Jersey crossbreds. His study revealed that four antigens (Z, R, J, M) were significantly more frequent in Hariana. An interesting fact was that the factor R (C-system) was completely absent in crossbreds while it had a frequency of 0.700 in Hariana. In our study the factor R_1 (C system) was found to occur in Ongole and Zebu-Jersey crosses with a frequency of 0.1348 and 0.1717 respectively and the difference was statistically insignificant. The low frequency of R_1 is in agreement with the findings of Chet Ram and Khanna (1961) who reported that antigens R and M occurred rarely in Hariana and Kumauni Hill cattle. The frequency (phenotypic) of J factor in Hariana and Sindhi-Jersey crosses was reported to be 0.3375 and 0.0412 respectively (Singh, 1965). Khanna, Chet Ram and Prabhu (unpublished) have studied the frequency of J in 11 breeds of Indian cattle and found that frequency ranged from 0.057 to 0.750. The frequency in this study for J was

found to be 0.2160 for Ongoles and 0.2569 for Zebu-Jersey crossbreds. The factor E was found to occur with a frequency of 0.6652 and 0.8484 in Ongole and crossbreds respectively, and the difference was significant at $P < 0.05$ level. Fiorentini and Rognoni (1960) reported percentage phenotypic frequencies of 10 and 73 for R_1 and E factors respectively, and the gene frequency of J was 0.197 in Italian breeds. Our results are in agreement with the above findings. We have also compared the frequency of occurrence of three blood group factors in two samples of the same breed (Ongole) stationed at two different farms. It could be seen that difference in case of factor E was highly significant. Probably, this might be due to the extensive use of a popular bull with homozygous E locus in that particular farm, which resulted in the high frequency of E.

Now with the full elucidation of modes of inheritance of the antigenic factors, much better picture of differences and similarities between breeds can be obtained by studying the actual gene frequencies instead of the occurrence of individual factors. Stormont (1952a) found significant genic differences between Holstein-Friesians and Guernsey with regard to F-V and Z systems, and further reported that both were in genetic equilibrium. Stormont (1952b) reported striking differences between the occurrence of different B

alleles. Similar differences for Danish and Sweedish breeds have been worked out by Neimann-Sorensen (1950a, b) and Rendel (1958) respectively. Studies of gene frequencies, in this study, also revealed significant differences between Ongole and crossbreds with respect to factor E.

An association between any two particular characters reveals a tendency, over the whole population, for the associated features to occur together more often than would be expected, if the features were occurring independently of each other. In cattle previous investigations by several workers have tried to relate the blood group substances with economic traits. It has been observed that selective forces influence the number of homo and heterozygote animals for A system in Dutch cattle breeds (Osterlee, 1965). This indicates that at least a part of the genes controlling the blood groups are of neutral value and are possibly related to physiological functions.

Meclure (1952) observed that the frequency of A factor is higher in Jersey than Friesian. It seemed that gene governing factor A had some effect on fat content because Jerseys had higher fat content than Friesians. Several other workers viz. Dunlop (1951), Nair et al. (1955), Nair (1957), Laben and Stormont (1958), Stormont (1959), Rendel

(1959), Metscherlich et al. (1959) and Tolle (1960) reported relationship of blood group factor and production character.

Salerno (1963) found significant relationship between A and F alleles and age at first calving. J allele was reported to have influence on service period. Salerno (1964) found a significant effect of V and S_1 genes on birth weight of female calves. Hogrevee (1965) reported an unfavourable effect of E and a favourable effect of U2 on lifetime production in Black Pied Lowland cattle. Khanna et al. (1965a, b) also reported significant relation between factors Iz_{15}, BF_2 with milk yield. V and Iz_9 with age at first calving. Singh (1965) reported highly significant association of age at first calving with M and Iz_{35} factors in Hariana. The factor M seemed to decrease the age at first calving while Iz_{35} increased it. Antigens R and W were significantly associated with milk yield in first lactation, while in our study no relation could be found between R_1 and milk yield in first lactation. In crossbreds he reported a significant relationship between factor A and calving interval which increased the calving interval. Similarly A and Iz_{39} were significantly associated with birth weight in crossbreds. Our study failed to reveal any relationship either of age at first calving or birth weight in respect of

the three antigens (R_1, E and J).

Gupta (1965) reported significant relationship between Z' and M factor and gestation period, while in our study gestation period was not found to be associated with any one of the three factors. He also reported the relationship of R and V factors and number of services per pregnancy and Iz_{30} and Iz_{36} and dry period. Out of 31 association studies carried out during the period of this investigation, only 4 significant associations could be found. Highly significant association was found between E and length of dry period and average daily milk yield in first lactation. In both the cases, the presence of E had a negative effect i.e. the cows with E factor had the dry period lesser by 102.53 days on an average than those without E, and the cows with E factor produced 1.81 kg less milk on an average per day than those without E. A significant association was noticed between E and intercalving period, where the presence of E increased the 1st intercalving period by 61.28 days. A highly significant association was found between J and first calving interval and 1st dry period where the presence of J increased the calving interval and as well as 1st dry period. Mitcherlich et al. (1959) reported that the factors R (C system) was associated with decreased milk yield but in our study the factor R_1 (C system) failed to reveal any

association with milk yield or anyone of other 8 characters studied.

Until now, only one instance of a consistantly significant association has been found between the allele $B^{BO_1Y_2D'}$ of B system and fat percentage (Rendel, 1961; Neimann-Sorensen and Robertson, 1961; Coneally, 1962; Coneally and Stone, 1965). Except this, none of the other findings reported by the different workers agreed with each other and a conclusive evidence on the problem was still awaited. Hence until now our knowledge of the association between blood groups and other genetically determined characters might be said to be still limited.

Apart from the blood group studies, haemoglobin typing was also done in 101 cattle of Zebu-Jersey crosses. Three distinct phenotypes of haemoglobin were recorded in these animals viz. HbAA, HbAB and HbBB. Vella (1958) reported a third haemoglobin HbC in Bos indicus. The existence of HbC was confirmed by Crockett et al. (1963), Naik and Sanghvi (1965) and Osterhoff and VanHerdeen (1965) in cattle of Indian and African origin. HbC was not detected in any one of the cattle studied by us. Lehman (1959) reported an excess of heterozygotes in Indian Gir cattle. Sen et al. (1966) investigated 6 Indian breeds of cattle including

Desi cattle and found an excess of AB individuals in all of them. From this it appears that selection favours heterozygotes in Indian breeds. On the other hand, African Zebu populations had shown little deviation from the equilibrium law (Bangham and Blumberg, 1958; Lehman and Ross, 1961; Rollison, 1963). The present study revealed that the crossbreds were in genetic equilibrium as no significant deviation was found between the observed and expected genotypes.

Sen et al. (1966) also reported that average frequency (0.35) of the Hb^B gene was higher than the figure (0.29) given for African Zebus' (Bangham and Blumberg, 1958; Lehman and Rollison, 1958; Lehman and Ross, 1961; Rollison, 1963). These values however exceeded the estimates of less than 0.21 obtained for European breeds, except from the Jersey breed, in various parts of the world(Bangham, 1957; Grimes et al., 1957; Salisbury, 1959; Cabannes and Serain, 1963). The frequencies obtained for Jersey were of the order of 0.50. Our study revealed a much higher value for Hb^B gene (0.47) than the Indian and African Zebu cattle (0.35 and 0.29), but it was less than the value for Jerseys (0.50). From this it is evident that the introduction of Jersey blood has definitely influenced the gene frequency of Hb^B in Zebu-Jersey crossbred cattle studied. Naik et al. (1965) also reported

a higher frequency for Hb^B (0.43 to 0.50) in five Indian breeds viz. Malvi, Khillari, Kankrej, Dangi and Gir. The frequency of gene A (0.52) in crossbreds was less than the figure (0.64) given by Sen et al. (1966). Balakrishnan and Nair (1966) also reported a higher value for gene A in their study on three Indian breeds (Red Sindhi, Sahiwal and Tharparkar). The frequency for gene A in males of the 3 breeds was 0.700, 0.707 and 0.897 respectively and in females 0.625, 0.671 and 0.894 respectively. These values are much higher than the figures of gene Hb^A in Zebu-Jersey crosses (0.53) studied by us.

SUMMARY

1. Blood typing of 230 heads of Ongole and 99 heads of Zebu-Jersey crosses was done with R_1, E and J reagents.

2. The mode of inheritance of these three antigenic factors was studied. The parentage study revealed its value in parentage determination.

3. The frequency of occurrence of 3 antigens in Ongole and Zebu-Jersey crossbreds showed highly significant difference for antigen E. The gene frequencies were calculated separately for Ongole and crossbreds, which also revealed highly significant difference in the frequency of E.

4. The relationships between these blood group factors and 11 economic characters viz. birth weight, age at 1st calving, 1st lactation yield, 1st calving interval, 1st lactation period, 1st dry period, average daily milk yield in 1st lactation, average daily milk yield based on total lactations completed, breeding efficiency, age at first service and gestation period in Ongole were studied.

5. Calving interval showed a significant association with

E and J. Antigen E also indicated a highly significant association with dry period and average daily milk yield in first lactation. Dry period following 1st lactation was found to be highly significantly associated with J. Antigen R_1 did not indicate any association with any one of the 11 characters studied.

6. Haemoglobin typing of 101 Zebu-Jersey crosses was also done. The gene frequencies for Hb^A and Hb^B were calculated which revealed that the crossbreds were in genetic equilibrium at the haemoglobin locus.

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APPENDIX I

Analysis of variance tables for relationship between E and 11 economic characters

1. Birth weight

Source of variation	D.F.	S.S.	Mean square	F
Total	120	920.87		
Between groups	1	5.62	5.62	0.73
Within groups	119	915.25	7.69	

2. Age at 1st service

Source of variation	D.F.	S.S.	Mean square	F
Total	70	2534210		
Between groups	1	44113	44113	1.22
Within groups	69	2490097	36088	

3. Gestation period

Source of variation	D.F.	S.S.	Mean square	F
Total	71	7650		
Between groups	1	31	31	0.28
Within groups	70	7619	108.83	

APPENDIX I (contd)

4. Age at 1st calving

Source of variation	D.F.	S.S.	Mean square	F
Total	102	7540056.5		
Between groups	1	6054.1	6054.1	0.08
Within groups	101	7534002.4	74594.0	

5. 1st lactation yield

Source of variation	D.F.	S.S.	Mean square	F
Total	84	28657339.2		
Between groups	1	788077.7	788077.7	2.34
Within groups	83	27869261.5	335774.2	

6. 1st lactation period

Source of variation	D.F.	S.S.	Mean square	F
Total	84	621335.19		
Between groups	1	666.65	666.65	0.089
Within groups	83	620668.54	7477.93	

APPENDIX I (contd)

7. 1st dry period

Source of variation	D.F.	S.S.	Mean square	F
Total	76	1090293.54		
Between groups	1	192496.35	192496.35	16.08**
Within groups	75	897797.19	11970.62	

**Significant at 1% level

8. 1st calving interval

Source of variation	D.F.	S.S.	Mean square	F
Total	76	1100873.30		
Between groups	1	68771.49	68771.49	4.99*
Within groups	75	1032101.81	13761.35	

* Significant at 5% level

9. Average daily milk yield in first lactation

Source of variation	D.F.	S.S.	Mean square	F
Total	78	142.22		
Between groups	1	11.84	11.84	7.4**
Within groups	77	130.38	1.60	

** Significant at 1% level

APPENDIX I (contd)

10. Average daily milk yield based on total lactations completed

Source of variation	D.F.	S.S.	Mean square	F
Total	34	34.10		
Between groups	1	0.17	0.17	0.16
Within groups	33	33.93	1.02	

11. Breeding efficiency (percentage)

Source of variation	D.F.	S.S.	Mean square	F
Total	29	4869.2		
Between groups	1	140.4	140.4	0.83
Within groups	28	4728.8	168.8	

APPENDIX II

Analysis of variance for the relationship between J and 11 economic characters

1. Birth weight

Source of variation	D.F.	S.S.	Mean square	F
Total	120	920.87		
Between groups	1	0.01	0.01	0.001
Within groups	119	920.86	7.73	

2. Age at 1st service

Source of variation	D.F.	S.S.	Mean square	F
Total	70	2534210		
Between groups	1	15085	15085	0.28
Within groups	69	2579125	53598	

3. Gestation period

Source of variation	D.F.	S.S.	Mean square	F
Total	71	7650		
Between groups	1	92	92.0	0.85
Within groups	70	7558	107.9	

APPENDIX II (contd)

4. Age at 1st calving

Source of variation	D.F.	S.S.	Mean square	F
Total	102	7540056.5		
Between groups	1	81628.5	81628.5	1.1
Within groups	101	7458428.0	73845.8	

5. 1st lactation yield

Source of variation	D.F.	S.S.	Mean square	F
Total	84	28657339.19		
Between groups	1	80580.10	80580.1	0.23
Within groups	83	28576759.10	344298.3	

6. 1st lactation period

Source of variation	D.F.	S.S.	Mean square	F
Total	84	621335.19		
Between groups	1	3167.21	3167.21	0.42
Within groups	83	618167.98	7447.80	

APPENDIX II (contd)

7. Ist dry period

Source of variation	D.F.	S.S.	Mean square	F
Total	76	1090293.54		
Between groups	1	140814.41	140814.41	11.1**
Within groups	75	949479.13	12659.72	

** Significant at 1% level

8. Ist calving interval

Source of variation	D.F.	S.S.	Mean square	F
Total	76	1100873.30		
Between groups	1	114574.32	114574.65	8.7**
Within groups	75	986298.98	13150.65	

** Significant at 1% level

9. Average daily milk yield in 1st lactation

Source of variation	D.F.	S.S.	Mean square	F
Total	78	142.22		
Between groups	1	2.72	2.72	1.5
Within groups	77	139.50	1.81	

APPENDIX II (contd)

10. Average daily milk yield based on total lactations completed

Source of variation	D.F.	S.S.	Mean square	F
Total	34	34.10		
Between groups	1	0.17	0.17	0.16
Within groups	33	33.93	1.02	

11. Breeding efficiency (percentage)

Source of variation	D.F.	S.S.	Mean square	F
Total	29	4869.2		
Between groups	1	202.6	202.6	1.21
Within groups	28	4666.6	166.6	