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HAEMOGLOBIN AND ALBUMIN POLYMORPHISMS IN INDIAN WATER BUFFALOES

By

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In recent years much work by use of electrophoretic techniques has been done on genetic variations of blood proteins in different livestock species, while very limited information is available on this aspect in water buffaloes. This species was reported to have two haemoglobin bands (*Giri & Pillai* 1956, *Vella* 1958). *Vella* who investigated haemolysates from buffaloes in Bali also described traces of a third band in one individual. Two haemoglobin bands were further reported in Siamese water buffaloes (*Loypetjra* 1962) and in Indian water buffaloes (*Sen et al.* 1966). *Naik & Sukumaran* (1967) reported the presence of one haemoglobin band in three buffaloes out of 350 tested. The rest had two bands.

Genetically controlled albumin polymorphism has been described in several animal species. *McIndoe* (1962) reported in chicken, *Stormont & Suzuki* (1963), *Brænd* (1964) in horses, *Ashton* (1964), *Ashton & Lampkin* (1965) in cattle, *Efremov & Brænd* (1965a) in sheep.

The Indian water buffaloes are very important dairy, beef and draught animals of the Indian subcontinent, hence the present work.

MATERIAL AND METHODS

A total of 507 blood samples from the Murrah breed of Indian water buffaloes were collected from four buffalo breeding farms located at Izatnagar, Haringhata, Bikaner and Pantnagar. Of these 18 were from animals younger than 2 months. The samples

were collected in an anticoagulant of isotonic sodium citrate. The protein separation was accomplished in horizontal starch gel electrophoresis. Gels were prepared with 10 % potato starch, hydrolysed locally from commercial Norwegian starch. For haemoglobin separation, tris-EDTA-boric acid buffer *(Gahne et al.* 1960) at pH 8.9 was used as electrode buffer. The same buffer diluted ten times was used as gel buffer. The insertions were done on filter paper. Bridges were from filter paper. The voltage output of power supply unit was 400 v for gels of 20 cm × 13 cm × 0.4 cm dimension corresponding to 220 v across the bridges. The gels were bisected horizontally and the lower half stained with benzidine solution. For separation of albumins several buffer systems were tried, but the discontinuous system described by *Brænd & Efremov* (1965) was found to be the most satisfactory. The gels were prepared with five times diluted 0.19 M tris — 0.05 M citric acid buffer at pH 5.8 while 0.64 M boric acid — 0.1 M sodium hydroxide buffer at pH 7.8 was used as bridge buffer. The starting voltage applied was 150 v, raised to 200 v after 15 min. for gels of above mentioned dimensions corresponding to 80 and 120 v respectively between bridges. The staining was done with amido-black. Methanol-acetic acid-water (*Smithies* 1955) was used as destaining and fixative fluid.

Selected Hb samples were examined quantitatively by cellulose acetate electrophoresis (*Brænd* 1967).

RESULTS

A. *Haemoglobin*

Out of 507 individual samples tested 503 were observed with two bands and four samples showed three bands. All the calf samples (younger than two months) showed two bands of the same types as commonly found. The relationships between bands in 501 of the samples were so that the fastest component (which shall be called A_1) made about 67 % whereas the slowest (A_2) was about 33 %. In two of the two band samples the relationship was different, A_2 being considerably weaker.

The three different phenotypes are shown in Figs. 1a and b. In Fig. 1a the most commonly occurring phenotype is shown together with the three band phenotype. The two fastest bands in the last mentioned type correspond in positions to the A_1 and A_2 bands. The A_1 band is, however, considerably weaker

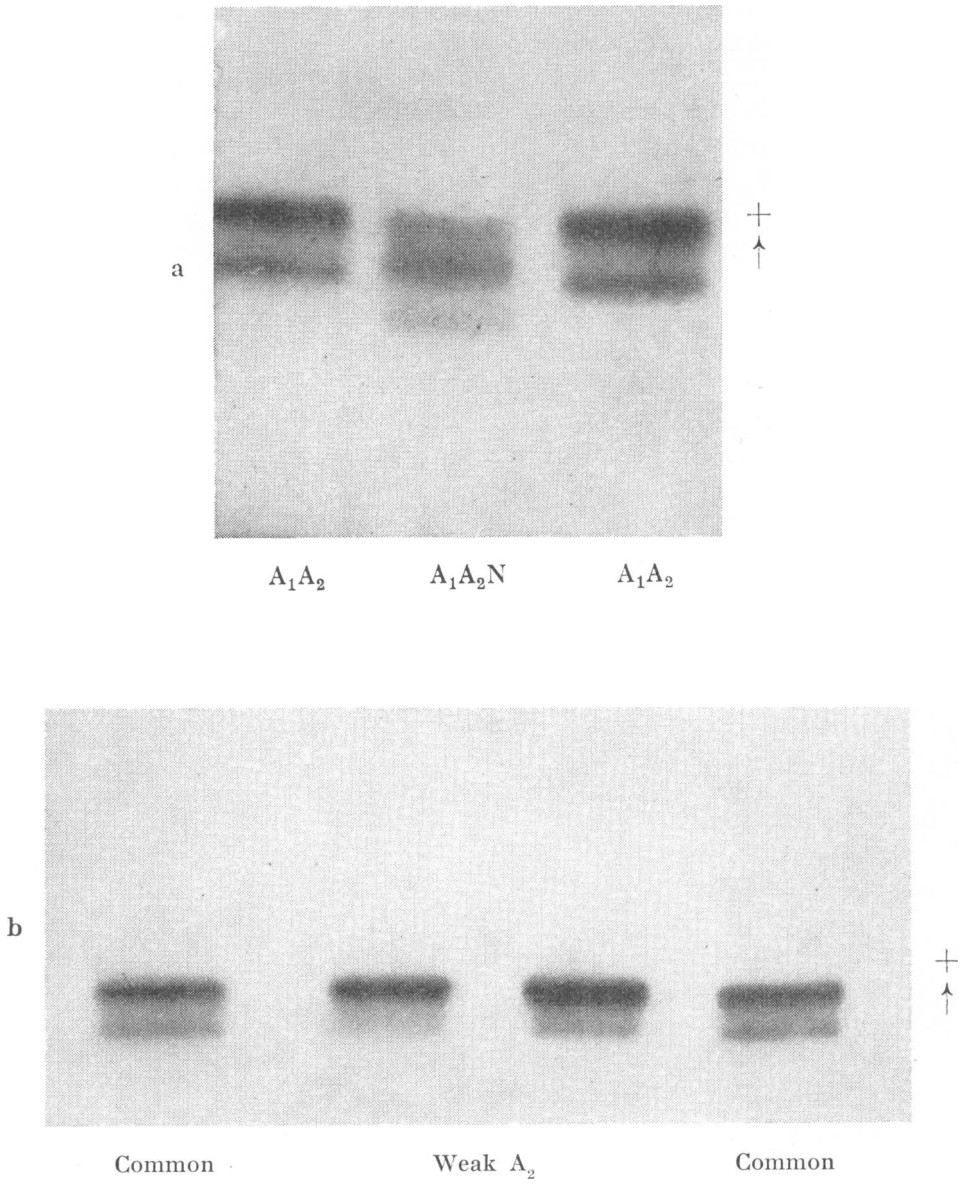

Figure 1. Photographs of stained starch gels showing (a) haemoglobin phenotypes, (b) variability in density of A_1 and A_2 haemoglobin bands in Indian water buffaloes.

than the common A_1 band, and the third band which we tentatively shall call N is weaker than the common A_2 band.

The animals having three bands (A_1A_2N) were of both sexes of varying ages and apparently healthy looking. Due to the low frequency of this phenotype limited family data were available. It can be mentioned, however, that the mother of one A_1A_2N animal also was A_1A_2N whereas the sire was A_1A_2. In another family the offspring of an A_1A_2N cow was A_1A_2 after having been mated to an A_1A_2 bull.

The haemoglobin bands of the two animals having another relationship between A_1 and A_2 are shown in Fig. 1b. The concentrations vary, but compared with the common A_1A_2 type and judged by the unaided eye the A_2 band seems to be of about half the strength of what is found for the common A_1A_2 type.

These results may have any of several explanations. The occurrence of the same two band pattern A_1A_2 in the majority of animals indicates the existence of three polypeptide chains of which one is common for A_1 and A_2. Genetically, these may be governed by two structural genes only, but so that a mistranslation or an ambiguity causes the synthesis of two different chains. *Balani & Barnabas* (1964) reported the common chain to be β, while the α chains of the two components differed. An amino acid substitution in the β chain might therefore explain the retarded electrophoretic mobilities and the appearance of two other haemoglobin molecules. If the three common polypeptide chains tentatively are called α^{A_1}, α^{A_2} and β and the mutated one β^X we should expect the four haemoglobin molecules: $\alpha_2^{A_1}\beta_2$, $\alpha_2^{A_2}\beta_2$, $\alpha_2^{A_1}\beta_2^X$, $\alpha_2^{A_2}\beta_2^X$. The occurrence of three components only may be due to the same rate of migration of the $\alpha_2^{A_2}\beta_2$ and $\alpha_2^{A_1}\beta_2^X$ molecules and thereby overlapping.

Another possible explanation is the assumption of three structural genes of which two are closely linked, each structural gene governing one polypeptide chain. This should theoretically result in the same electrophoretic picture as with the ambiguity theory.

The existence of animals with a different quantitative relationship between A_1 and A_2 bands than the common one resembles the situation in some horse breeds (*Brænd & Efremov* 1965, *Schmid* 1965, *Brænd* 1967) where three phenotypes exist. One has one band only, A_1. In the two others there are differences in quantitative relationship with an average of 38 % A_2 in the one

phenotype and 19 % A_2 in the other. *Brænd* (1967) proposed a modulating locus, Hb^m, being responsible for this variation either by working through inhibition of the synthesis of one of the polypeptide chains of A_2 or by eventually hindering a mistranslation to take place if we accept the ambiguity theory. The reported findings in buffaloes might be explained in the same way. In this connection it should be mentioned that *Naik & Sukumaran* (1967) reported finding of water buffaloes with one band only.

As conclusion we therefore assume two different loci to be engaged in the control of the observed variation of water buffalo haemoglobin.

B. *Albumin*

Three types of phenotypes were observed for albumin when subjected to electrophoresis in acid pH. The relative positions of these phenotypes are shown in Fig. 2.

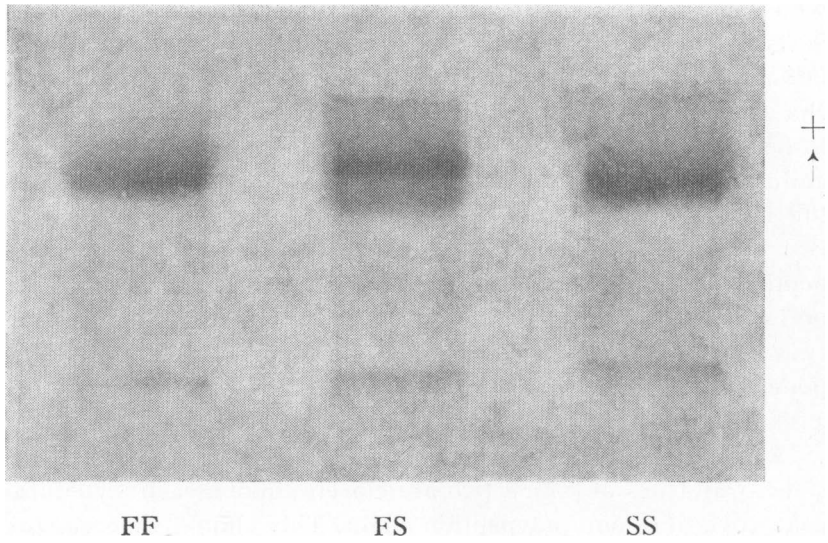

Figure 2. Photograph of stained starch gel showing different albumin phenotypes in water buffaloes.

The fastest single band towards the anode was designated as FF, while the slowest single band was denominated as SS. The intermediate type having two bands, i.e. one band having the mobility of FF and the other having mobility of SS, was termed as FS. The frequency of occurrence of these phenotypes is pre-

Table 1. The frequency of different albumin phenotypes at four different buffalo breeding farms.

Location of farms	Albumin phenotypes			
	FF	FS	SS	total
Izatnagar	3	37	166	206
Haringhata	0	18	121	139
Bikaner	0	14	29	43**
Pantnagar	8	52	59	119**

** Significant at 1 % level.

sented in Table 1. An analysis was conducted to find out whether these frequencies differ significantly at different farms. The results showed that a significant difference existed between Pantnagar and the rest of the farms, and between Haringhata and Bikaner, while there was no significant deviation between Izatnagar and Haringhata farms. Therefore, the data from Izatnagar and Haringhata farms were pooled, and further analysis was conducted jointly. The data from Pantnagar was analyzed separately and Bikaner was excluded.

The data were further divided according to (i) sex and (ii) different age groups, viz. 0 to 1 year, 1 to 2 years, 2 to 3 years, and above 3 years and were subjected to χ^2-test with a view to testing an eventual effect of these two characters on the frequency of different albumin phenotypes. The results are presented in Tables 2 and 3.

It may be seen from Table 2 that the χ^2-values are not significant. It may be inferred, therefore, that sex has no detectable

Table 2. The effect of sex on the occurrence of albumin phenotypes in buffaloes.

Albumin phenotypes	Pantnagar		Pooled	
	males	females	males	females
FF	0	8	1	2
FS	1	51	9	46
SS	3	56	66	221
Total	4	115	76	269
χ^2	1.15		1.41	
Probability	$.50 < P < .70$		$.30 < P < .50$	

Table 3. The effect of different age groups on the occurrence of albumin phenotypes in buffaloes.

Age groups	Pantnagar				Pooled			
	FF	FS	SS	total	FF	FS	SS	total
0 to 1 year	1	7	8	16	1	11	60	72
1 to 2 years	2	3	8	13	0	7	68	75
2 to 3 years	0	1	2	3	1	7	39	47
Above 3 years	5	41	41	87	1	30	120	151
Total	8	52	59	119	3	55	287	345
χ^2	4.02				6.11			
Probability	$.50 < P < .70$				$.30 < P < .50$			

effect on the frequencies of albumin phenotypes in any of the two populations.

The results presented in Table 3 indicate that significant differences do not exist between different age groups as to frequencies of albumin phenotypes.

In an attempt to study the mode of inheritance of albumin phenotypes all animals whose family records were available were picked out. The results indicate that two codominant autosomal alleles, viz. Al^F and Al^S are involved in the control of the albumin variation in water buffaloes. The observed phenotypes of the offspring were in consistency with the assumed hypothesis. It was, however, observed that under the mating FS $\times$ SS a higher number SS individuals than expected was observed, with an excess of offspring having genotype as that of the dam.

Table 4. The observed and expected phenotypes and gene frequencies of albumin polymorphism in Murrah breed of Indian buffaloes.

Population	Albumin phenotypes						χ^2	Probability	Frequencies
	FF		FS		SS				
	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.			Al^F
Pantnagar	8	10.0	52	49.0	59	60.0	0.60	$.30 < P < .50$	0.29
Pooled	3	2.8	55	56.5	287	285.7	0.06	$.80 < P < .90$	0.09

Very good agreement between observed and expected values of different albumin phenotypes was observed. It may therefore be said that both populations are in genic equilibrium for the albumin genes. The gene frequencies for Al^F and Al^S were found

to be 0.29 and 0.71 respectively at Pantnagar and 0.09 and 0.91 respectively for the pooled population. The difference in the two populations may be due to herd to herd variations.

DISCUSSION

The commonly occurring two band Hb phenotype in water buffalo is very similar in appearance on starch gels to the most common two band Hb phenotype in horses (*Brænd* 1967) and the Hb phenotype of European bison (*Brænd & Gasparski* 1967). As a genetic explanation for the two Hb bands in horses, *Kilmartin & Clegg* (1967) proposed an ambiguity in the translation of an RNA codon. This theory was based upon their findings of heterogeneity at two different places, positions 24 and 60 in the α chains, making a total of four different α chains, while the β chains were identical. Ambiguity or mistranslation has also been proposed as an explanation for multiple α chains in the rabbit (*Ehrenstein* 1966) and in the mouse (*Rifkin et al.* 1966, *Popp* 1967). Within the two last species the β chains were also identical. This is in contrast to the "normal" haemoglobins of man (see *Ingram* 1963), cattle (*Efremov and Brænd* 1965b, *Schroeder et al.* 1967) and sheep (*Huisman* 1967) where the α chains are identical.

The observed three band variant shows a starch gel phenotype very similar in appearance to that of the European bison-cattle hybrid (*Brænd & Gasparski*). For the occurrence of the three bands in the hybrid a probable explanation (*Brænd,* unpublished) is heterozygosity at a modulating locus. Whether such a modulating locus in the hybrid is working through inhibition of the synthesis of one of the polypeptide chains of the slowest component A_2 or whether it works on an eventual ambiguity or mistranslation mechanism is another question. Within the species of water buffalo, however, heterozygosity at a modulating locus cannot explain the three band phenotype, hence the theory of the variant structural gene for the β chain.

The difference in quantitative relationship between A_1 and A_2 which was observed in a few animals can in our opinion best be explained by heterozygosity at a modulating locus in the same way as for horses (*Brænd* 1967). The lack of the one band phenotype in our material is according to expectation due to the low frequency of the heterozygote. The one band type is, however, reported by *Naik & Sukumaran* (1967).

Genetically determined albumin variation has now been reported for many species. In man, European cattle and sheep most individuals are homozygous for the common type. The number of different variants, however, increase with the extent of the investigations. Thus in man (*Weitkamp et al.* 1967) at least four variants exist, in African cattle also at least four (*Ashton & Lampkin* 1965, *Carr* 1967). In sheep two variants are so far reported (*Efremov & Brænd* 1965a, *Tucker* 1968). In horses two alleles have been reported both at high frequencies (*Stormont & Suzuki* 1963, *Brænd & Efremov* 1965, *Gahne* 1966). In water buffaloes the rare allele Al^F occurred at a rather high frequency, and as in horses the polymorphism can be considered a true polymorphism according to *Ford* (1945). Why we have such differences between species is not understood at the moment, but on the other hand they are more the rule than the exception for many serum protein systems. Perhaps results from comparative investigations might help us to a better understanding of the forces involved in the maintenance of polymorphisms thereby putting us in a better position in the matter of improving our livestock species.

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SUMMARY

A total of 507 samples from the Murrah breed of Indian water buffalo were examined for haemoglobin and albumin polymorphism using starch gel electrophoresis. The majority of samples showed the same two band haemoglobin phenotype, A_1A_2, where the fastest band A_1 was about 67 % of total haemoglobin. Two rare phenotypic haemoglobin variants were observed. The one, found in four animals, had three bands and is explained by mutation in the β chain. The other, found in two animals, showed another relationship between the A_1 and A_2 bands than the common type and is assumed to be caused through heterozygosity at a modulating locus. Three albumin phenotypes were observed. Family data were in agreement with these being controlled by two codominant alleles, called Al^F and Al^S. Frequency of Al^F was 0.09 and 0.29 in two different populations. Age and sex did not have any effect on distribution of albumin phenotypes.

ZUSAMMENFASSUNG

Hämoglobin- und Albumin-Polymorphie bei indischem Wasserbüffel.

Insgesamt sind 507 Proben von indischem Wasserbüffel, Murrah Rasse, auf Hämoglobin- und Albumin-Polymorphie mit Hilfe von Stärkegel-Elektrophorese untersucht worden. Der grösste Teil der Proben zeigte denselben zwei-Band-Hämoglobinphänotyp, A_1A_2, in dem das schnellste Band, A_1, 67 % der gesamten Hämoglobinmenge ausmachte. Es wurden zwei seltene phänotypische Hämoglobinvarianten gefunden. Der eine, der bei vier Tieren festgestellt wurde, hatte drei Bänder und wird durch eine Mutation in der β-Kette erklärt. Der andere, der bei zwei Tieren festgestellt wurde, zeigte ein anderes Verhältnis zwischen den A_1- und A_2-Bändern als der gewöhnliche Typ. Es wird angenommen, dass dieses Verhältnis von einer Heterozygotie in einem modulierenden Lokus verursacht wird. Drei Albumin-Phänotypen wurden festgestellt. Familien-Untersuchungen deuteten darauf hin, dass diese allem Auschein nach von zwei kodominanten Allelen, Al^F und Al^S genannt, kontrolliert werden. Die Häufigkeit von Al^F war in zwei verschiedenen Populationen 0,09 bzw. 0,29. Alter und Geschlecht zeigten keinen Effekt in bezug auf die Verteilung von Albumin-Phänotypen.

SAMMENDRAG

Hemoglobin- og albumin-polymorfisme hos indisk vannbøffel.

I alt 507 prøver fra indisk vannbøffel, Murrah rase, er blitt undersøkt for hemoglobin- og albumin-polymorfisme ved hjelp av stivelsesgel — elektroforese. Storparten av prøvene viste den samme to bånd hemoglobinfenotype, A_1A_2, hvor det hurtigste bånd, A_1, utgjorde omtrent 67 % av totalt hemoglobin. Det ble funnet to sjeldne fenotypiske hemoglobin varianter. Den ene som ble påvist hos fire dyr hadde tre bånd og er forklart ved mutasjon i β-kjeden. Den andre som ble funnet hos to dyr viste et annet forhold mellom A_1 og A_2 båndene enn i den vanlige type og er antatt å være forårsaket ved heterosygoti i et modulerende lokus. Tre albumin-fenotyper ble funnet. Familie-undersøkelser viste overensstemmelse med at disse er kontrollert av to kodominante alleler, kalt Al^F og Al^S. Hyppighet av Al^F var 0,09 og 0,29 i to forskjellige populasjoner. Alder og kjønn viste ingen effekt med hensyn til distribuering av albumin fenotyper.

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