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HAEMOGLOBIN POLYMORPHISM IN INDIAN WATER BUFFALOES

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SUMMARY

Three hundred and fifty random blood samples from Indian water buffaloes were studied for their haemoglobin variants by the paper electrophoretic technique. Three sire-families constituting 53 dams and their young ones were investigated to know the possible mode of inheritance of the haemoglobin variants in them. Blood samples from the newborn calves and the cord were also studied to see if there were any differences.

All excepting 3 random samples showed Hb-(I+II,) while the 3 samples had Hb-I devoid of Hb-II.The 53 young ones, without exception, revealed Hb-(I+II) like their parents presenting an impression of *in block* inheritance. The exact mode of inheritance, however, shall be clear either from the study of some rare critical families or Catallo, a hybrid between buffalo and cattle with Hb-BB type.

Blood samples from the newborn calves and the cord revealed Hb-(I+II) identical to those of the adults, a situation like which has been reported in horse but not in ruminants. The Hb-I and II had, however, similar mobilities like the Hb-X and Hb-A of cattle in the present study. This study warrants further work to know the exact evolutionary significance of the haemoglobin variants and their inheritance in buffaloes.

INTRODUCTION

Water buffaloes are still found in wild state, though they were domesticated in India probably as early as cattle, 4,000 years ago. There are 44.91 million buffaloes of which about 21.66 million she-buffaloes kept for breeding and milk production and 5.95 million working buffaloes (MAHAJAN, 1959). Thus the buffalo, like cattle, plays an important role in the rural economy of India which is essentially an agricultural country.

Indian Zebu cattle have so far revealed 4 types of haemoglobins (NAIK et al. 1965; NAIK and SANGHVI, 1964) besides the foetal type. Limited studies in Indian and American buffaloes have shown two haemoglobin variants in all animals (GIRI and PILLAI, 1956; BRAEND and STORMONT, 1963; BALANI and BARNABAS, 1964). So it was envisaged to investigate a large number of buffalo blood samples to establish the haemoglobin variants in them, and this communication deals with the results of the study.

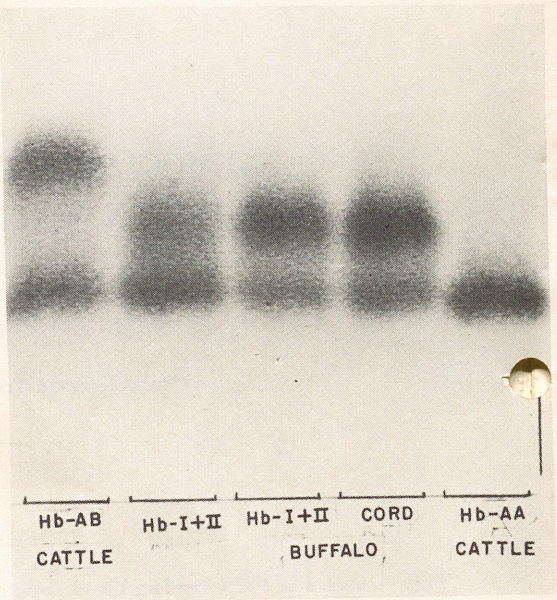

*Fig. 1*a.

Haemoglobin variants in buffaloes (Paper electrophoresis, veronal buffer pH 8.6), with cattle haemoglobins for comparison. Pattern No. 2 from left shows Hb-II more than Hb-I in a buffalo.

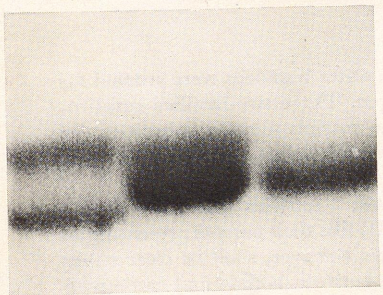

*Fig. 1*b.

Buffalo haemoglobin showing only Hb-I fraction along with Hb-(I + II) and cattle Hb-AB for comparison.

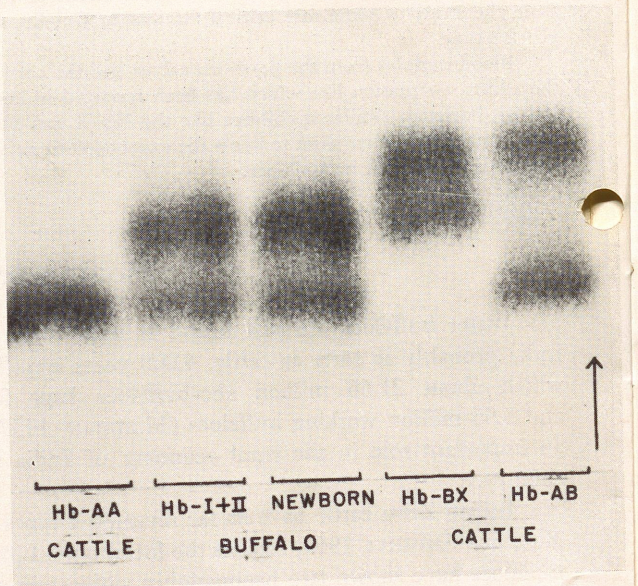

Fig. 2.

Haemoglobin variants in buffalo in comparison with those of cattle (Paper electrophoresis, veronal buffer pH 8.6).

MATERIAL AND METHODS

Oxalated blood samples were aseptically collected from the external jugular vein of 350 random animals at Bandra Slaughter House. Three sire families constituting 53 dams and their young ones were also investigated from Aadarsha Dugdhalaya, Malad,

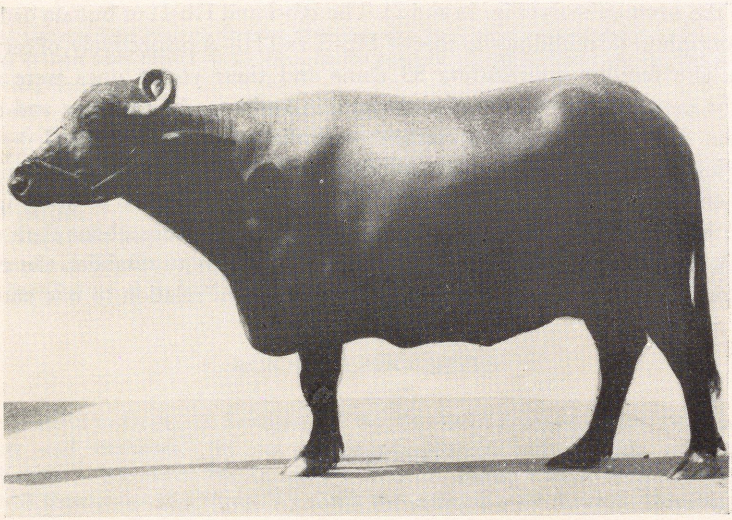

Fig. 3.
Indian water buffalo.

to study the mode of inheritance of haemoglobin variants in them. Blood samples from the cord and the newborn ones were also studied for foetal fraction.

The cells were washed free of plasma with normal saline. Haemoglobin solution was prepared by the addition of an equal volume of distilled water to the packed cells. To this haemolysate was added one fourth volume of toluene and mixed thoroughly. The tubes were left over night in the deep freeze, then they were thawed and centrifuged at 3 000/4 000 r.p.m. for half an hour, the next day. Haemolysate was separated free from toluene and precipitated proteins. The haemoglobin solution was then adjusted to 10 g % with distilled water and preserved as carboxyhaemoglobin.

Paper electrophoretic technique described by SMITH and CONLEY (1953) was used to study the haemoglobin variants. Electrophoretic runs were made at 20-25° C for 10-12 hours at 200 volts and 3 mA per strip (8.7 cm × 36.5 cm) of filter paper (Whatman No. 3) in veronal buffer of pH 8.6 with an ionic strength of 0.05 M. Since the haemobin is a coloured protein the patterns were read directly after drying.

RESULTS

Most of the buffaloes exhibited two haemoglobin variants called Hb-I and Hb-II, the Hb-I being faster than Hb-II (Fig. 1a). From 350 healthy adult buffaloes only 3 showed Hb-I devoid of Hb-II (Fig. 1 b), while all the rest had Hb-(I + II). No animal was found

homozygous for Hb-II. Unlike in heterozygous cattle where Hb-A and Hb-B were found almost in equal proportion, in buffaloes the Hb-I was found always about 60-70 % while Hb-II was 30-40 % in concentration excepting in one instance where it was reverse (Fig. 1a). No foetal fraction could be demonstrated when cord blood and blood from new born calves were subjected to either electrophoresis or to alkali-denaturation technique. They too had two fractions identical in mobility and concentration to those of the adult animals (Fig. 1a and 2). The Hb-I and Hb-II of buffalo had, however, similar electrophoretic mobilities to that of Hb-X and Hb-A respectively, of cattle (Fig. 2).

Three sire families constituting 53 dams and their young ones were studied to establish the mode of inheritance of haemoglobin variants. All the sires and dams were heterozygous and the young ones without exception also showed Hb-(I + II), giving an impression that these variants are inherited *in block*. The three adult animals with only Hb-I encountered during this study could not be investigated to know more about inheritance of the haemoglobins due to the lack of informations about their parentage. During this study we did not come across suitable families to establish the exact mode of inheritance of the Hb-I and Hb-II individually and in relation to one another.

DISCUSSION

Cattle and buffaloes differ from one another in their morphology and physiology. They were therefore taxonomically classified under *Bos indicus* and *Bos bubalis* (Fig. 3) respectively.

Haemoglobin polymorphism is known to be common among the ruminants. The two common haemoglobin variants, which are determined by two allelic codominant genes in cattle are known to occur in three phenotypes, Hb-A, Hb-AB and Hb-B. In buffaloes, the two variants were commonly found together except in three animals as revealed in the present study, which showed Hb-I devoid of Hb-II. Unlike in cattle this was also observed in 53 young ones resulting from an equal number of matings between different heterozygous parents. So the genetics of haemoglobin variants in buffaloes seems to be similar to that of the haptoglobins or transferrins where the phenotypes are expressed by one or more bands. The dilemma of the inheritance of haemoglobin variants, however, shall be solved after the study of some rare and suitable families or from the study of the haemoglobin of Catallo, a cross between a buffalo and a cattle with Hb-BB type. It is still not clear whether the excess of heterozygotes over the homozygotes and the absence of individuals with Hb-II alone, as seen in the present series, are of any evolutionary significance.

It is also interesting to note that the haemoglobin variants from the new born animals and from the cord blood samples showed no difference with the adult haemoglobin variants in their limited physico-chemical properties studied by us. This finding therefore warrants further work on buffalo embryos to establish whether foetal fraction is present in this species or not. This preliminary study has therefore opened up a vast field for experimental work to characterize the individual haemoglobin variants and their evolutionary significance and to establish their exact mode of inheritance in buffaloes.

RÉSUMÉ

Les hémoglobines de 350 échantillons de sang de buffles indiens, récoltés au hasard, ont été analysées par électrophorèse sur papier. Trois familles paternelles, composées de 53 femelles et de leurs petits ont été étudiées pour déterminer le mode de transmission des variants d'hémoglobine. On a également étudié les hémoglobines des sangs de veaux nouveaux-nés et de sangs du cordon ombilical.

Tous les échantillons étaient de type Hb-(I + II), sauf trois qui présentaient Hb-I sans Hb-II. Les 53 petits étaient tous Hb-(I + II) comme les parents, ce qui suggère une transmission en bloc des deux hémoglobines.

Néanmoins, le mode exact de transmission sera élucidé par l'analyse des quelques rares familles critiques, ou du Catallo, hybride entre le buffle et les bovins (de type Hb-BB).

Les échantillons de sang cordal ou de sang de nouveau-né sont identiques à ceux des parents présentant le type Hb-(I + II), ce qui est comparable à ce qui est signalé chez le cheval mais non chez les bovins.

Hb-I et Hb-II ont les mobilités de Hb-X et Hb-A bovines.

Cette étude exige d'être prolongée pour déterminer la véritable signification évolutive des variants d'hémoglobine et de leur transmission chez le buffle.

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