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Biochemical Polymorphisms in the Malaysian Water Buffaloes

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Key words: Biochemical polymorphisms; Water buffaloes; Malaysia.

RINGKASAN

Sepuluh enzim dan protin; transferrin, amilase, hemoglobin, esterase D, asid fosfatase sel darah merah superoksid dismutase, fosfoglikolate fosfatase, glukos-6-fosfat dehidrogenase, 6-fosfoglukonate dehidrogenas, dan glutamate oksaloasetate transaminase larut dari serum dan sel darah merah 88 kerbau, Bubalus bubalis, *telah disiasat dengan menggunakan elektroforesis gel kanji atau poliakrilamid. Empat dari mereka; transferrin, amilase, hemoglobin dan esterase D, menunjukkan perbezaan elektroforesis di kadar polimorfik.*

SUMMARY

Ten enzymes and proteins: transferrin, amylase, haemoglobin, esterase D, red cell acid phosphatase, superoxide dismutase, phosphoglycolate phosphatase, glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase and soluble glutamate oxaloacetate transaminase, from the serum and red blood cell of 88 water buffaloes, Bubalus bubalis, *have been investigated by starch gel or polyacrylamide gel electrophoresis. Four of these: transferrin, amylase, haemoglobin and esterase D show electrophoretic variation at polymorphic proportions.*

INTRODUCTION

The Swamp water buffalo, *Bubalus bubalis,* is indigenous to Southeast China, Burma, Assam, Indochina, Thailand, Malaysia, Indonesia and the Philippines (Mason, 1974). It is much closer to the wild type than are the buffaloes of India. They are different from imported Indian breeds not only in appearance, but also in behaviour and use. In Peninsular Malaysia there are no wild water buffaloes and the habitat of the domestic buffalo is swamp or marshland. MacGregor (1941) named them Swamp buffaloes in contrast to the imported Murrah buffaloes from India which he called River buffaloes since their usual habitat is river valleys.

While the River buffalo has been selected to form improved breeds with high milk yield and differing in horn form, the Swamp buffalo has not been divided into special types. It has retained the low milk yield and the primitive horn shape of the wild arni buffalo found in northern India and Indochina (Mason, 1974). Therefore research on the Swamp buffalo is necessary and should prove fruitful.

In Malaysia, there are 210,067 Swamp buffaloes and 2,449 Murrah buffaloes (Devendra and Cameons, 1979). The Swamp buffaloes are mainly utilized as a drought animal and as a source of meat while the Murrah provide milk.

A survey of the literature indicates that only four biochemical markers (Hb, Tf, albumin, amylase) have been studied in the Indian water buffalo (Basavaiah *et al.,* 1977; Khanna, 1969, 1978, 1979; Khanna *et al.,* 1968, 1978) and the Formosan buffalo (Abe *et al.,* 1969). However, there appears to be only a single study on the albumin of the Malaysian water buffalo (Mak, 1973). As little work has been done in the Malaysian taxon, our aim in this study is mainly to determine the extent of biochemical polymorphism in the local animals.

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The present paper reports the results of our survey of Malaysian Swamp buffaloes for the following blood proteins and enzymes; transferrin and amylase (E.C.3.2.1.1.) from the plasma and haemoglobin, esterase D (E.C.3.1.1.1), red cell acid phosphatase (RCAP, E.C.3.1.3.2), superoxide dismutase (SOD, E.C.1.15.1.1), phosphoglycolate phosphatase (PGP, E.C.3.1.3.18), glucose-6-phosphate dehydrogenase (G6PD, E.C.1.1.1.49), 6-phosphogluconate dehydrogenase (6PGD, E.C.1.1.1.44) and soluble glutamate oxaloacetate transaminase (s-GOT, E.C.2.6.1.1) from erythrocytes. A small number of Murrah and Swamp × Murrah hybrids were also typed.

MATERIALS AND METHODS

Blood samples from 88 adult buffaloes found at Puchong Farm, Universiti Pertanian Malaysia, (74 Swamp, 11 Murrah and 3 Swamp × Murrah) were collected into venoject tubes with heparin as anticoagulant. The red cells were separated from the plasma by centrifugation at 3,000 rpm for 15 minutes. They were then stored at −20°C after the red cells had been mixed with an equal volume of preservative (Vande Berg and Johnston, 1977). Samples were usually used within a month of collection though it had been claimed that under the conditions used for storage, samples were usable up to five years (Vande Berg and Johnston, 1977).

All electrophoretic separations were made on horizontal flat slab starch gels except for G6PD and haemoglobin where 5% thin layer flat slab polyacrylamide gels were used. Undiluted plasma and red blood cell hemolysates were imbibed onto suitable grades of Whatman filter paper strips and placed into slits near the cathodal end of the gel since all the proteins analysed migrated towards the anode.

The buffers and electrophoretic separation conditions used were as follows:- Amylase was typed as in Khanna (1978), esterase D as in Harris and Hopkinson (1976) and PGP as in Baker and Hopkinson (1978). Transferrin was separated as in Khanna (1969) and stained with Commassie Brilliant Blue G 250 as in Gahne *et al.* (1977). Haemoglobin was separated as in Gahne *et al.* (1960). The red haemoglobin bands were clearly observed on the polyacrylamide gel after separation so no staining was found to be necessary. Samples were typed for RCAP twice, once as in Anderson and Giblett (1975) and once as in Karp and Sutton (1967) and stained using 4-methyl umbelliferyl phosphate as substrate as in Harris and Hopkinson (1976). G6PD was separated as in Shaw and Prasad (1970) and stained as in Steiner and Johnston (1973). 6PGD was separated using the same buffer system as for RCAP (Anderson and Giblett, 1975) and stained as in Harris and Hopkinson (1976). SOD can be observed by exposing G6PD or 6PGD gels to light after they have been read. Reincubating at 37°C may be necessary.

Apart from transferrin, amylase and haemoglobin in which the systems used had been adapted from those used to phenotype Murrah buffaloes, all the other systems used had been adapted from the procedures originally developed for studying humans and other animal species.

RESULTS AND DISCUSSION

No band was detected when haemolysates were run and stained for soluble-glutamate oxaloacetate transaminase (s-GOT) following the procedure of Chen and Giblett (1971) for humans.

The results obtained for the biochemical markers that show variation in the sample of

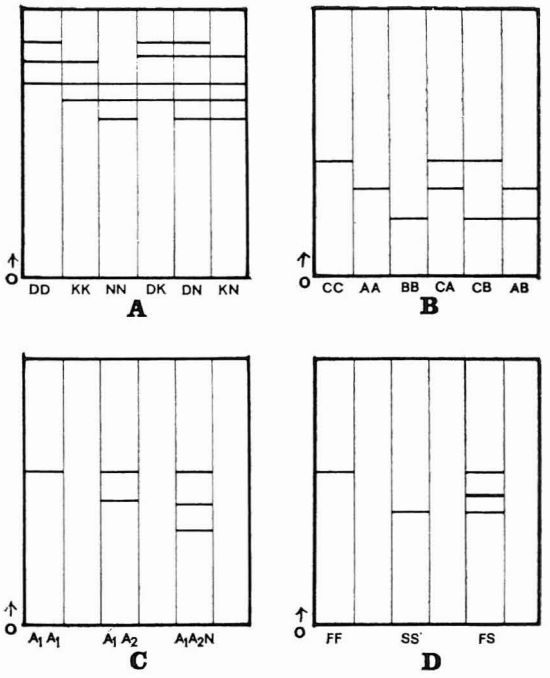

Fig. 1. Diagramatic representation of the (A) transferrin, (B) amylase, (C) haemoglobin, (D) esterase D phenotypes in water buffaloes. O is the origin.

TABLE 1

Enzymes or proteins that show variation in Malaysian water buffaloes.

Breed/ Marker	No. tested	Phenotype						Gene frequency		
Transferrin		DD	KK	NN	DK	DN	KN	Tf^D	Tf^K	Tf^N
Swamp(S)	73	4	49	2	13	2	3	0.16 ± 0.03	0.78 ± 0.03	0.06 ± 0.02
		(1.81)	(44.50)	(0.28)	(17.95)	(1.42)	(7.02)	$\chi^2_2 = 17.57$	$P < 0.001$	
Murrah(M)	11		5	5		1				
S×M	3		1	2						
Amylase		CC	AA	BB	CA	CB	AB	Am^C	Am^A	Am^B
Swamp	74	5	57	5	3	0	4	0.09 ± 0.02	0.82 ± 0.03	0.09 ± 0.02
		(0.56)	(50.43)	(0.65)	(10.66)	(1.2)	(11.48)	$\chi^2_2 = 76.75$	$P < 0.001$	
Murrah	11	1	10							
S×M	3		2				1			
Haemoglobin		A_1A_1		A_1A_2		A_1A_2 N				
Swamp	74	20(27.02%)		53(71.62%)		1(1.35%)				
Murrah	11			10		1				
S×M	3	1		2						
Esterase D		FF		FS		SS		ESD^F		ESD^S
Swamp	73	65(62.42)		5(10.17)		3(0.41)		0.92 ± 0.02		0.08 ± 0.02
								$\chi^2_1 = 19.10$		$P < 0.001$
Murrah	11	7		2		2				
S×M	3	1		2						

Numbers in parentheses are expected equilibrium values except for haemoglobin where they represent percentage frequency of each type.

water buffaloes are presented in Table 1. Gene frequencies were calculated by the method of gene counting as the mode of inheritance of each of the systems that do show variation is that of codominant alleles at an autosomal locus. The gene frequency for haemoglobin was not calculated as its mode of inheritance in the water buffalo is still unclear (Khanna, 1979) but the percentage of each type was calculated.

The water buffalo transferrin types were named according to the nomenclature suggested by Braend (1965) and used by Khanna (1969) in his study of Indian water buffaloes *(Fig. 1)*.

The water buffalo transferrin phenotypes are due to an autosomal locus with three codominant alleles, Tf^D, Tf^K and Tf^N (Khanna, 1969, Khanna and Singh, 1978). The gene frequencies obtained in the sample are within the range of those observed in various herds of Indian Murrah buffaloes (Khanna and Singh, 1978). Abe *et al.*, (1969) typed 100 Formosan water buffaloes but used a different system of naming them from that used by Khanna (1969). A comparison of the photographs and textual descriptions of these papers, showed that the phenotype named AA by Abe *et al.*, (1969) was similar to that termed DD by Khanna (1969); and that termed DD by Abe *et al.*, (1969) was called KK by Khanna (1969). By changing the nomenclature to that of Khanna (1969), the allele frequencies in Formosan water buffaloes are $Tf^D = 0.155$ and $Tf^K = 0.845$. These are quite similar to the allelic frequencies in Malaysian water buffaloes of $Tf^D = 0.16$, $Tf^K = 0.78$. However, no Tf^N was reported by Abe *et al.*, (1969). The Chi-square test showed that the Swamp buffaloes in

this survey were not in the Hardy-Weinberg equilibrium for the transferrin phenotypes. This is to be expected because of the widespread practice of artificial insemination in producing the herd of buffaloes at the Puchong Farm. Therefore siblings were present in the sample. Also, there were only six males in the sample of 88 buffaloes. No new variant was observed in the current study.

Water buffalo amylase phenotypes are controlled by an autosomal locus with three codominant alleles, Am^A, Am^B, Am^C (Khanna, 1978) In the present study five phenotypes were observed *(Fig. 1)*. The gene frequencies obtained were within the range of those obtained for various herds of Indian Murrah buffaloes (Khanna, 1978) except that the highest frequency of Am^C observed by Khanna was 0.03 whereas the frequency for Am^C observed in this study is 0.09. As expected, the population was not in the Hardy-Weinberg equilibrium for the amylase phenotypes. No new variant was observed.

For the study of haemoglobin, three types of patterns were observed *(Fig. 1)*. The common A_1 A_2 type showed two bands of equal intensities, one fast and one slow, the variant type A_1 A_2 N showed three bands, two intense bands corresponding to the usual fast and slow bands and a third weak slowest band, and the variant type A_1A_1 showed only a fast intense band. Khanna and Braend (1968), Basavaiah *et al.*, (1977) and Khanna (1979) observed that in Indian buffaloes, mostly of the Murrah breed, there were also three haemoglobin types, A_1A_2 with two intense bands, one fast and one slow, A_1A_2N with two intense bands corresponding to the usual fast and slow bands, and a weak slowest band and A_1A_1 (weak) where the fast band was intense and the slow band was weak. It is likely that the A_1A_1 type in this study corresponds with the A_1A_2 (weak) observed by the Indian workers. Khanna (1979) reported the frequencies of types A_1A_2, A_1A_2N and A_1A_2 (weak) to be 99.08%, 0.29% and 0.63% respectively.

In 100 Formosan water buffaloes, 97 had two clear haemoglobin bands, one fast and one slow, whereas three had only the fast band clear but the slow band was faint (Abe *et al.*, 1969). Abe *et al.*, (1969) named these phenotypes FS and F respectively, but they fitted the descriptions for the A_1A_1 and A_1A_2 (weak) phenotypes reported for Indian Murrah buffaloes.

Esterase D (ESD) has not been reported in water buffaloes before. This esterase activity was not observed when α-napthyl acetate or β-napthyl acetate was used in this study as the substrate for gel staining following the procedure given in Harris and Hopkinson (1976) and it is therefore unlikely to be esterase A, B or C and carbonic anhydrase which do utilise these napthyl acetates as substrates. (Harris and Hopkinson, 1976; Santore *et al.*, 1969).

However, since the enzyme carbonic anhydrase also utilises 4-methyl-umbelliferyl acetate as a substrate, it cannot be claimed positively at this stage that the enzyme obtained here was ESD. It is suggested that inhibition tests (Hg^{++} as inhibitor for ESD and Diamox as inhibitor for carbonic anhydrases) should be carried out in future work to confirm the identification of this enzyme.

At present, it can only be tentatively assumed that this enzyme may be Esterase D.

Esterase D is polymorphic in man and sheep but monomorphic in baboons, monkeys and seal (McDermid *et al.*, 1975). In the Swamp buffaloes, 73 animals showed the presence of a fast band which was called ESD FF; five samples showed a slow band typed as ESD SS and nine samples showed three bands, one corresponding to the fast band of ESD FF, one corresponding to the slow band of ESD SS and the third band intermediate in mobility to the other bands *(Fig. 1)*. The 3-band phenotype was called ESD FS.

In humans, when esterase D was typed using the same conditions as that used for typing buffaloes, three phenotypes similar to those seen in buffaloes were observed (Hopkinson *et al.*, 1973). Homozygosity for allele ESD^1 at an autosomal locus showed a fast band while homozygosity for allele ESD^2 showed a slow band. The heterozygote ESD^1/ESD^2 showed three bands. Therefore, by analogy, it was hypothesised that the buffalo type ESD FF is due to homozygosity at an autosomal locus for allele ESD^F, type ESD SS is due to homozygosity for allele ESD^S while type ESD SF represents the heterozygote. The hypothesis that the locus is autosomal is supported by the fact that one of the animals showing type ESD FS is a male. Assuming this hypothesis, gene frequencies were calculated by gene counting. As expected, the population sampled was not in the Hardy-Weinberg equilibrium. This hypothesis of genetic control for esterase D in buffaloes can be tested by family studies.

When the method of Giblett and Anderson (1975) was used to type RCAP, all the buffalo

samples showed a single band but two bands per sample (an intense slow band and a weaker fast band) were observed when the method of Karp and Sutton (1967) was used. PGP, G6PD, 6PGD and SOD all showed the presence of a single band per sample for all 88 samples. All these red cell enzymes had not been reported in water buffaloes before. It is possible that variants for them may be detected in other breeds of buffaloes or even in larger samples of Swamp buffaloes.

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