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INHERITANCE OF AN ABNORMAL HAEMOGLOBIN HAPLOTYPE IN HORSES AND ITS POSSIBLE INFLUENCE ON BLOOD VALUES

By

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BRAEND, M., J. B. CLEGG and A. STORSET: *Inheritance of an abnormal haemoglobin haplotype in horses and its possible influence on blood values.* Acta vet. scand. 1983, *24*, 384—391. — In a breeding experiment a stallion of the native Norwegian Trotter breed with an abnormal Hb haplotype (N) and with the Hb type BI/N, sired 6 offspring. The abnormal haplotype controls one α-chain only, having lysine at position 60 and phenylalanine at position 24. Three of the offspring received the N haplotype from the sire and the BII haplotype from the dam, whereas the other 3 offspring received BI from the sire. The BII/N horses have two Hb components after alkaline electrophoresis or isoelectric focusing with the ratio between the fast and the slow band (anodal, cathodal) being approximately 30:70. Blood value determinations did not give any definite conclusions as regards differences between horses with the abnormal Hb type and normal horses of similar age within the same breed.

mutant Hb haplotype; horses; inheritance; influence on blood values; normal blood values; Norwegian Trotter; Standardbred; stallions.

During the routine pedigree control of Norwegian trotter horses a male foal was found who had an atypical Hb type (*Lie* 1973). This type differed from the normal 60:40 and 80:20 two-band Hb types (*Braend* 1967), having a ratio of approximately 30:70 between the fast and the slow band in alkaline electrophoresis. Both the dam and the sire of the foal had 60:40 ratios. Furthermore, 33 dam-offspring pairs in the family of the sire had the three normal Hb types, 60:40, 80:20 and 100:0, when diagnosed by electrophoresis (*Braend* 1967, *Braend & Clegg*

1973). It was, therefore, concluded that a mutation had taken place in a germ cell of the foal's sire.

Kilmartin & Clegg (1967) using chromatographic techniques, found that the difference between the fast and slow Hb components in the 60:40 and 80:20 horses was due to the substitution of the lysine residue at position 60 in the slow Hb α-chain by glutamine in the fast Hb α-chain. The beta chains of both Hb components were identical. But in a number of 60:40 horses position no. 24 in the alpha chain was occupied by both tyrosine and phenylalanine, whereas other horses had either tyrosine or phenylalanine alone. *Clegg* (1970) explained the occurrence of these 4 different α-chains as being controlled by 2 closely linked α-chain genes, of which one is responsible for 60 % and the other 40 % of the total amount of the haplotype haemoglobin. Furthermore, the chromatographic techniques enabled *Clegg* (1970) to differentiate between 6 commonly occurring Hb types instead of the 3 found by electrophoresis (*Braend* 1967).

In the present article results from a breeding experiment involving the horse with the abnormal Hb type are reported together with investigations of blood values of the atypical and of normal horses.

MATERIAL AND METHODS

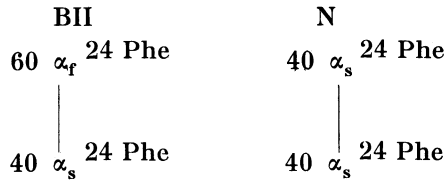
The sire ('Tollef') with the "abnormal" Hb type served a total of 19 mares in the spring and summer of 1975, when he was 5 years old. These were Døle and Norwegian Trotter horses. Blood samples (heparin) from 6 foals and their dams were investigated in Oslo for Hb type using cellulose acetate electrophoresis (*Braend* 1967). In Oxford the amino acid residues at position 24 in the α-chain were determined by the use of chromatographic techniques (*Clegg* 1970). Two of the offspring were also examined with the LKB Multiphor System of isoelectric focusing on PAG plates (5.5—8.5) according to LKB Instruction Note 1804, with application point in the middle of the gel and using 10 % lysates of washed red cells without any other pretreatment.

Haemoglobin values were determined for 'Tollef' and his 3 offspring with the mutant type. For comparative purposes, corresponding blood values were determined for a total of 24 Norwegian Trotter stallions, age 4 years or more, 18 two years old Norwegian Trotter horses (10 females, 8 stallions) and 18 Warmblood Trotter (Standardbred) stallions, 4 years or older. All were

investigated in the month of March. Hb concentration (g/dl) was determined by the cyanmethaemoglobin method using the Hemometer (*Linson* Instrument, Lars Ljungberg & Co, Stockholm, Sweden). PCV (% packed red cells) was examined using microhaematocrit centrifuge (Hemokrit 4, Ljungberg & Co) after 5 min of 12 000 G. Red cell numbers (mill/mm^3) were counted in Celloscope 101 (Ljungberg & Co).

RESULTS

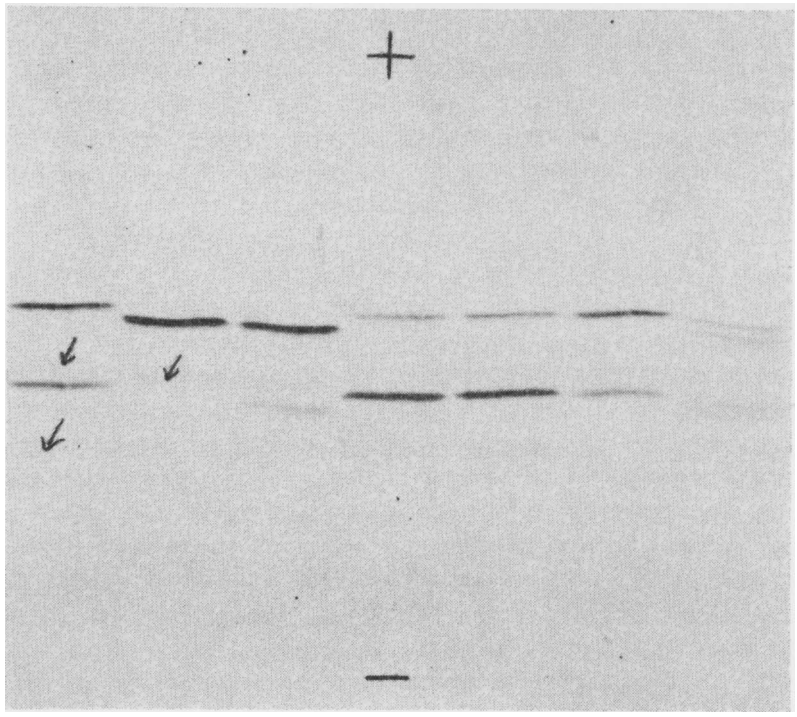
Of the 19 mares 8 became pregnant. Two of the foals died immediately after birth, one of unknown causes since the owner did not inform us until several days afterwards. The other foal was subjected to post mortem and the cause of death given as inguinal hernia. Another of the foals also had inguinal hernia, but this did not give any serious effects. Accordingly, 6 living foals were obtained. Their Hb types together with those of 'Tollef' and the dams are shown in Table 1. The sire's mutant Hb haplotype, named N (for Norway) was transmitted to 3 of the 6 offspring. But they were all 3 in combination with the BII haplotype. According to theory (*Clegg* 1974) this Hb type can be written as follows:



This should result in 10 % less Hb than normal, since the N haplotype would control 20 % less than normal. Furthermore, the ratio between the fast and slow component should be approximately 30:70 (*Clegg* 1974).

Table 1. Haemoglobin types in the family of the atypical stallion.

Tollef	BI/N		
Dam 1	A/BII	Dam 4	A/BI or A/BII
Off. 1	BI/BII	Off. 4	A/BI
Dam 2	A/BI	Dam 5	BI/BII
Off. 2	BI/BI	Off. 5	BII/N
Dam 3	BI/BII	Dam 6	BI/BII
Off. 3	BII/N	Off. 6	BII/N



BII/BII A/A A/BI BII/N BII/N BII/BII BI/BII

Figure 1. Photograph showing the appearance of Hb types after isoelectric focusing.
The normal haplotype A has two closely linked genes but gives only one α-chain which has glutamine at position 60 and tyrosine at position 24.
The normal haplotype BI gives two α-chains, the one having glutamine at position 60 and tyrosine at position 24, the other with lysine at position 60 and tyrosine at position 24.
The normal haplotype BII gives two α-chains, the one having glutamine at position 60 and phenylalanine at position 24, the other with lysine at position 60 and phenylalanine at position 24.
The abnormal haplotype N, from two different horses, is shown in combination with BII.
Approximate relationships between Hb components are:
A/BI, 80:20, BII/N, 30:70, BII/BII, 60:40, BI/BII, 30:30:20:20.
Extra minor bands are marked with arrows.

A photograph showing the appearence of the BII/N type after isoelectric focusing is given in Fig. 1 together with some normal Hb types. The 2 samples in Fig. 1 are from the 2 atypical horses still living, a mare and a gelding. The 2 samples show an ap-

Table 2. Blood values of the atypical stallion and his mutant family compared with those of normal horses belonging to Norwegian trotter breeds.

	RBC (10^6/mm^3)	Hb (g/dl)	PCV (%)	MCV (fl)	MCH (pg)	MCHC (g/dl)
Tollef (5 years)	7.39	12.5	38	51.42	16.90	32.89
Offsp. 3 (2 years) ♀	7.94	14.6	46	50.37	18.39	36.50
Offsp. 5 „ ♂	7.77	12.0	33	42.47	15.44	36.36
Offsp. 6 „ ♂	7.98	11.6	31	38.85	14.54	37.42
Norw. Trotter stallions > 4 years (n = 24)	8.06±0.74[1] (9.47—6.89)[2]	14.60±1.08 (16.7 —12.8)	40.79±2.78 (46—37)	50.78±2.40	18.17±0.88	35.78±0.77
Norw. Trotter 2 years (n = 18)	8.07±0.67 (9.46—6.76)	12.72±1.02 (14.9—10.6)	36.05±2.60 (41—31)	44.76±2.31	15.78±0.81	35.28±0.93
Warmblood Tr. stallions > 4 years (n = 18)	9.14±0.75 (10.08—7.70)	17.14±1.30 (19.2—13.5)	48.22±3.93 (54—37)	52.88±3.49	18.79±0.98	35.57±0.67

PCV = packed cell volume, MCV = mean corpuscular volume, in femotoliters (10^{-15}), MCH = mean corpuscular Hb content, in picograms (10^{-12}), MCHC = mean corpuscular Hb concentration, in g/dl.

[1] mean ± standard deviation.

[2] highest — lowest values.

proximate relationship between the 2 components of 30:70, which was found by the use of chromatographic techniques.

In Table 2 blood values are presented. Three of the mutant haplotype horses seem to be on the low side as regards Hb concentration and PCV, when compared with normal horses of the same age groups. However, there is a rather large variation between horses in the 3 normal groups. Thus, there are normal horses which show about the same low values as 'Tollef' and 2 of his offspring with the abnormal Hb type.

The Warmblood Trotter stallions were investigated for the purpose of showing breed differences. Hb concentration and PCV are significantly higher in Warmblood Trotter stallions than in Norwegian Trotter stallions ($P < 0.001$). The mean corpuscular volume (MCV), in femtoliters, and the mean corpuscular haemoglobin content (MCH), in picograms, differed slightly between the stallions of the 2 groups ($0.05 > P > 0.02$). Mean corpuscular haemoglobin concentration MCHC (g/dl), however, was the same for all the three normal groups.

DISCUSSION

The result from the breeding experiment is rather limited. It would have been more informative with a higher number of offspring, particularly those with other combinations between the N and other normal haplotypes. An F_2 generation giving a homozygous N animal would have been of particular interest. However, there were many problems connected with the breeding experiment.

Tollef's father had been proven by blood typing to have wrong sire in the pedigree records. Consequently, the regulations did not permit 'Tollef' to be used for breeding and mated to Norwegian Trotter mares. This in spite of the fact that 'Tollef' did very well on the race track with 23 first prices over a 5 year period. Fortunately though, the state advisor in charge of horse breeding policy allowed us to have 'Tollef' serve a limited number of mares, with the conditions though that the offspring could not be registered. There were also difficulties in finding owners willing to have their mares served by 'Tollef' (since we only had available three mares owned by state institutions) with the result that we were not able to assemble a normal group of breeding mares. Several were rather odd horses which had never been used for breeding. As a consequence, a low number of pregnant mares was the result.

The number of horses with the mutant Hb haplotype is insufficient for a meaningful investigation of blood values, since there are so many variable factors with influence on blood composition. Such factors are age, breed, nutrition and feeds and excitement (*Schalm et al.* 1975, *Persson* 1967) to mention some. It could therefore, be argued that it would be pointless to look for a difference in Hb amount between such a small group of mutant horses and comparable normal horses. On the other hand, the variation might have been quite marked and thus significant. Even though the number of mutant horses turned out to be too low for finding significant differences from normal horses we may draw some conclusions from these comparisons. Thus the highly significant differences in Hb conc. and PCV between the Warmblood Trotter horses and the Norwegian Trotter horses (which are socalled coldblood) suggest that there are also genetically determined within-breed differences, with regard to blood values. Since we do not know the genetically determined Hb potentials for the dams of the three mutant horses it is not possible

to say whether these three offspring have 10 % less Hb than they would have had with another sire. For that conclusion to be made many more offspring from each mare would have been necessary. Obviously, this was not possible — one of the limitations in experimental breeding research with such an animal as the horse.

The results from the investigations of blood values in normal horses need comments since in the 2 groups of adult stallions the Hb conc. as well as PCV were higher than given as normal values for comparable horses in the literature (*Schalm et al.* 1975, *Paulsson & Åberg* 1965). However, they compare well with the material assembled by *Aas Hansen* (unpublished) and which are used as standard normal values in the teaching of students in their clinical training at Department of Internal Medicine I. The reason for the higher Hb concentration may be due to the cyanmethaemoglobin method which is the most precise one and which also measures haemoglobin derivatives (*Schalm et al.* 1975). The higher values of PCV could in the opinion of *Aas Hansen* (personal communication) be due to the fact that the stallions of the 2 breeds were in good training or working conditions when their blood was sampled at their showing at the yearly fair for trotter horses.

The nature of the mutation in a germ cell of Tollef's sire is as yet not known. A back mutation of a fast to a slow α-chain (24 phenylalanine) gene, a deletion giving in each case a chromosome with a single slow α chain or a non-homologous cross over (*Braend & Clegg* 1973, *Clegg* 1974) have been suggested as possibilities. Presently, studies are underway in an attempt to elucidate this matter. These will be reported separately at a later stage.

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SAMMENDRAG

Nedarving av en unormal hemoglobin (Hb) haplotype hos hest og dens mulige innvirkning på blodverdier.

En Norsk Traver hingst med en unormal Hb haplotype (N) og med Hb type BI/N fikk i et avlsforsøk seks avkom. Den unormale haplotype kontrollerer en α-kjede. Denne har lysin i posisjon nr. 60 og fenylalanin i posisjon nr. 24. Tre avkom fikk N haplotypen fra hingsten og BII fra hoppen, mens de tre andre avkom fikk BI fra hingsten. Hestene med Hb typen BII/N viser to Hb komponenter etter elektroforese i alkalisk miljø eller isoelektrisk fokusering. Forholdet mellom den hurtigste og den saktere komponent (anode—katode) er omtrent 30:70. Bestemmelser av blodverdier viste ingen sikre forskjeller mellom de hester som har den unormale Hb type og normale hester av tilsvarende alder og rase.

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