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Brief Communication

CERULOPLASMIN POLYMORPHISM AND PARENTAGE TEST IN HEREFORD CATTLE

In recent years parentage control by means of blood grouping tests (blood and protein systems) has been required for bulls to be registered in the Danish Hereford Herd Book. Because the Hereford breed shows less variation in the blood and protein systems, the probability of excluding an incorrectly stated bull (or cow) is estimated to be some 15 % lower in Hereford than in Danish dairy breeds (RDM, SDM and Jersey).

Besides employing more recently developed blood group reagents a search for additional protein systems suitable to improve the effectivity of blood grouping tests in the parentage control of Hereford was made. Two protein systems, the serum amylase II (Am II) and the ceruloplasmin (Cp) systems, which can be determined simultaneous with the transferrin (Tf) and the serum amylase I (Am I) systems, were studied. While in the serum amylase II system described by *Mazumder & Spooner* (1970) only homozygotes for the Am II B component were observed among 127 Hereford animals tested, the ceruloplasmin system discovered in 1966 by *Schröffel* (cited by *Schröffel et al.* 1968) revealed variation in Hereford.

Plasma from blood samples received for parentage test was subjected to electrophoresis in 8 mm thick gels containing 11 % partially hydrolysed starch and using the discontinuous buffer system described by *Kristjansson* (1963). The samples were inserted into the gel by means of 8×5 mm filter paper pieces (Whatman no. 3). To avoid heat inactivation of the amylases the voltage gradient was kept at 7.5 v/cm until the buffer front had migrated 3—4 cm from the insertion point and then increased to 12.5 v/cm. The electrophoresis was discontinued, when the front had migrated 7 cm from the insertion point.

The gel was sliced horizontally into three parts, the upper 1—2 mm being discarded and the second part, about 2—3 mm thick, was stained with amido black-nigrosin for the determination of the transferrin components. The lower part, about 4 mm thick, which proved to be the best for the determination of the ceruloplasmin and amylases, was incubated overnight at

37°C in an acetate buffer (pH = 5.7; 0.15 M) containing o-tulidin (100—150 mg per 100 ml). Then the ceruloplasmin components were visible. Following a further incubation at 4°C in 15 % ethanol for about 2 hrs. and immersion in water, the Am I components became visible. A modification of the anticoagulant suggested by *Schröffel* (1973/74) was used (27 g sodium citrate, 10 g glucose, 5 g sulfamethazine sodium, 0.04 g proflavine hemisulfate per l).

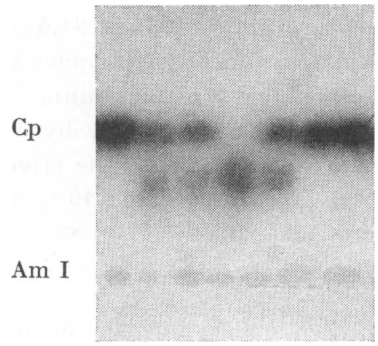

Figure 1. Photograph of starch gel showing the ceruloplasmin (Cp) and amylase (Am I) phenotypes. From left Cp: A/A, A/C, A/C, C/C, A/C, A/A and A/A. Am: B/B, B/C, B/B, B/B, B/B, B/C and B/C.

Three ceruloplasmin phenotypes, corresponding to the genotypes Cp^A/Cp^A, Cp^A/Cp^C and Cp^C/Cp^C, were observed as illustrated in Fig. 1 together with the Am I phenotypes. The Cp B component observed in dairy cattle was not found in the Hereford cattle included in the present study.

The total of 244 bulls and 176 females studied includes no complete family data. However, the distribution of phenotypes among offspring from 100 cows grouped according to ceruloplasmin type was in agreement with the expected for a pair of codominant allele genes. The distribution of genotypes observed among 244 bulls and 176 females and the gene frequencies are given below:

	Genotypes			Frequencies of	
	AA	AC	CC	Cp^A	Cp^C
Bulls	210	30	4	0.92 ± 0.01	0.08 ± 0.01
Females	138	35	3	0.88 ± 0.02	0.12 ± 0.02

The frequencies of the ceruloplasmin genes in the two populations differ significantly, which may be due to the female population being sampled from a limited number of herds. Tested separately the distribution of genotypes in the two popu-

lations are in agreement with the Hardy-Weinberg expectations. Following *Wiener et al.* (1930) and using the over all gene frequencies ($Cp^A = 0.91$ and $Cp^C = 0.09$), the probability of excluding an incorrectly stated bull as possible sire (sire, dam and offspring tested) by means of the ceruloplasmin system in Hereford is 7.8 %. With a probability of excluding an incorrectly stated bull as possible sire by means of 10 blood group systems and three protein systems (Tf, Am I and Ca) of about 80 %, the addition of the information from the ceruloplasmin system will increase that probability by 1.6 percentage units to about 81.6 %.

In a two allele codominant system with allele frequencies of 0.5, the effectivity in parentage test reaches a maximum of 18.8 %, which increases the over all systems probability by 3.8 percentage units above 80. Thus in the Hereford breed the effect of the ceruloplasmin system in parentage tests is some 40 % of the maximum for a two allele system.

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Birgitte Andreasen and *Bent Larsen*
The Department of Physiology, Endocrinology and Bloodgrouping, Royal Veterinary and Agricultural University,
Copenhagen, Denmark.

REFERENCES

Kristjansson, F. K.: Genetic control of two prealbumins in pigs. Genetics 1963, *48*, 1059—1063.

Mazumder, N. K. & R. L. Spooner: Studies on bovine serum amylase; evidence for two loci. Anim. Blood Grps biochem. Genet. 1970, *1*, 145—156.

Schröffel, J.: The ISABR Comparison Test 1973/74: Instructions for reporting the results. Animal Breeding Institute, Hradištko, Czechoslovakia.

Schröffel, J., A. Kúbek & V. Glasnák: Serum ceruloplasmin polymorphism in cattle. Proc. 11. Europ. Conf. Anim. Blood Grps biochem. Polymorphism, Warsaw 1968, 207—210.

Wiener, A. S., M. Lederer & S. H. Polayes: Studies in iso-hemagglutinations. IV. On the chances of proving non-paternity; with special reference to blood groups. J. Immunol. 1930, *19*, 259—282.

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Reprints may be requested from: Bent Larsen, the Department of Physiology, Endocrinology and Bloodgrouping, Royal Veterinary and Agricultural University, Bülowsvej 13, DK-1870 Copenhagen V, Denmark.