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## SERUM PROTEIN POLYMORPHISM IN SOME YUGOSLAVIAN PIG BREEDS

By

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In recent years starch gel electrophoresis (*Smithies 1955*) of pig serum have shown that various serum proteins are present in serum in different molecular forms, which occur in the individuals in grouped variations. Genetic investigations have shown that the variations are gene-controlled, and the serum components are transmitted as simple Mendelian characters. Each polymorphous protein is genetically controlled by alleles with a co-dominant mode of inheritance. In pig serum polymorphism has been disclosed in transferrin, hemopexin, ceruloplasmin, amylase, slow  $\alpha_2$ -globulin, prealbumin and albumin. Thus 7 serum type systems are known in pigs (for references see *Moustgaard & Hesselholt 1966; Kristjansson 1966*).

Research for gene markers in domestic mammals has been greatly stimulated by a possible existence of an association — either through a pleiotropic effect of genes, heterozygous advantage or through genetic linkage — between inherited blood characters and polygenic, physiological characters of practical-economical importance. Attempts to reveal an association of practical value have failed, but recent studies have clearly elucidated the existence of genetic linkage between serum protein and hemoglobin loci and loci for red cell antigens (*Imlah 1965; Hesselholt et al. 1966; Larsen 1966*).

Due to their well known mechanism of inheritance the above mentioned loci markers can serve as excellent tools for the parentage control and for population genetic studies. Calculations of the frequencies of genes which control the serum protein

variations in pigs can contribute to information on the genetic composition of pig breeds and can also offer ideas about the genetic variability within and between breeds.

The present report deals with studies into the relative distribution of transferrin, hemopexin, ceruloplasmin and amylase alleles among pigs belonging to 3 native Yugoslavian breeds. Similar studies have been performed in British pig breeds (*Imlah*), Czechoslovakian breeds (*Schrøffel* 1965) and in the Danish Landrace (*Graetzer et al.* 1965). In the discussion the results will be compared with the results obtained in these studies.

#### MATERIALS AND METHODS

Serum samples from 60 Resavka, 73 Mangolica and 105 Pfajfer (Black Slovenian) pigs, randomly taken, were subjected to horizontal starch gel electrophoresis in modifications of the discontinuous system of buffers devised by *Poulik* (1957). The serum samples were typed for transferrin (*Hesselholt* 1966), hemopexin (*Hesselholt & Hristiĉ* 1966), ceruloplasmin (*Graetzer et al.* 1965) and amylase (*Hesselholt et al.* 1966).

The iron-binding properties of the variable serum transferrins were demonstrated by autoradiography after the addition of  $\text{Fe}^{59}$  to serum and electrophoresis. 12.7  $\mu\text{g}$  of iron (containing 50  $\mu\text{C}$   $\text{Fe}^{59}$ ) were added, as ferric chloride in 0.005 N-HCl, to each ml of serum prior to electrophoresis. After electrophoresis the gel was bisected horizontally. The upper part was stained for protein with amidoblack 10 B (*Smithies* 1959). The gel was wrapped in nonporous transparent plastic film and kept in contact with Kodirex X-ray film for 24 hrs. After exposure the film was developed for 5 min. at 20°C in an X-ray developer.

#### RESULTS AND DISCUSSION

After electrophoresis the genotypes of the individuals can be read directly from the phenotypical expressions. Gene frequencies were therefore obtained by simple gene counting. Table 1 demonstrates the gene frequencies calculated in the 3 Yugoslavian pig breeds compared with the frequencies earlier found in Danish Landrace pigs.

In tests for genetic equilibrium according to Hardy-Weinbergs law no significant deviation was observed between the observed and expected distribution of phenotypes within the 4 serum type systems under investigation.

Table 1. Gene frequencies of transferrin (Tf)-, hemopexin (Hp)-, ceruloplasmin (Cp)- and amylase (Am) alleles in Yugoslavian breeds compared with Danish Landrace.

Breed	No. of animals	Gene frequencies											
		Tf			Hp				Cp		Am		
		Tf <sup>A</sup>	Tf <sup>B</sup>	Tf <sup>D</sup>	Hp <sup>0</sup>	Hp <sup>1</sup>	Hp <sup>2</sup>	Hp <sup>3</sup>	Cp <sup>1</sup>	Cp <sup>2</sup>	Am <sup>1</sup>	Am <sup>2</sup>	Am <sup>3</sup>
Danish L.	1000	0.0	1.0	0.0	0.04	0.34	0.12	0.50	0.02	0.98	0.14	0.83	0.03
Resavka	60	0.30	0.70	0.0	0.02	0.75	0.19	0.04	0.0	1.0	0.0	0.99	0.01
Mangolica	73	0.0	1.0	0.0	0.0	0.84	0.16	0.0	0.0	1.0	0.0	0.99	0.01
Pfajfer	105	0.11	0.88	0.01	0.0	0.90	0.08	0.02	0.0	1.0	0.01	0.95	0.04

*The transferrin system.* Four electrophoretically different transferrin fractions, Tf A, Tf B, Tf C and Tf D, have been described in pigs. The fractions, which each consists of 3 bands, seem to be attributed to 4 codominant alleles. In opposition to Danish Landrace pigs the Tf<sup>A</sup> allele segregates in Resavka and Pfajfer breeds. In the Pfajfer breed 1 animal exhibited the slow Tf D fraction earlier described on Czechoslovakian Large Whites. The Tf<sup>C</sup> allele was not observed.

In order to ascertain the iron-binding properties of the variable fractions, especially the Tf D fraction, an autoradiographic study with Fe<sup>59</sup> was performed according to the method given above. The results are demonstrated in Fig. 1. It can be seen on the autoradiograph that the radioactivity is confined entirely to the variable fractions, Tf A, Tf B and Tf D. Each transferrin fraction consists of 3 bands. The band with an intermediar electrophoretic mobility apparently binds more Fe<sup>59</sup> than the slower and the faster bands. When stained with amidoblack the bands in each fraction show similar staining intensity with the intermediar band most intensely stained.

The Tf<sup>A</sup> and Tf<sup>B</sup> frequencies obtained in Resavka pigs resemble those observed in British and Czechoslovakian Large White pigs. The relative low Tf<sup>A</sup> frequency in Pfajfer pigs is close to the Tf<sup>A</sup> frequency in Czechoslovakian Presticke pigs.

*The hemopexin system.* This serum type system contains 6 alleles, Hp<sup>0</sup>, Hp<sup>1F</sup>, Hp<sup>1</sup>, Hp<sup>2</sup>, Hp<sup>3F</sup> and Hp<sup>3</sup>. Each allele expresses 1 hematin-binding protein band. The Yugoslavian samples were, however, typed for only Hp<sup>0</sup>, Hp<sup>1</sup>, Hp<sup>2</sup> and Hp<sup>3</sup>. In comparison with Danish Landrace pigs it is noteworthy that the Yugoslavian pig breeds exhibit a high Hp<sup>1</sup> frequency and a relatively low

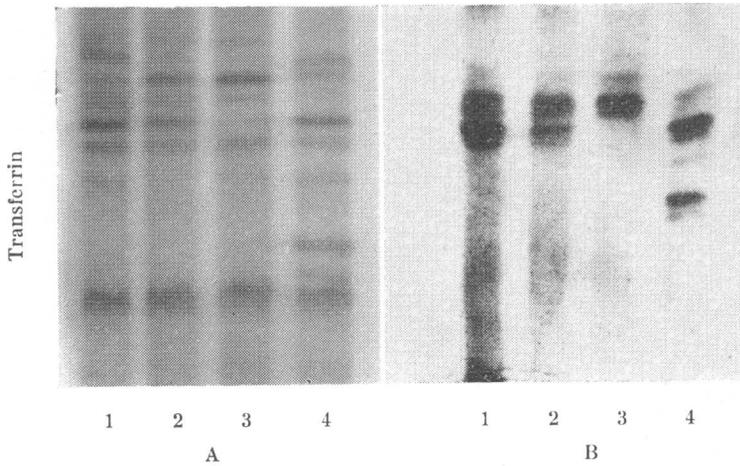


Figure 1. A: Protein stained starch gel demonstrating 4 porcine transferrin phenotypes. 1: Tf BB, 2: Tf AB, 3: Tf AA, 4: Tf BD. B:  $Fe^{59}$  autoradiograph of the same samples as A. For technical details, see text.

frequency of the  $Hp^3$  allele. Nearly the same distribution has been reported in Czechoslovakian and British Large White pigs, in Czechoslovakian Presticke and in Hungarian pigs (own investigations).

*The ceruloplasmin system.* Two copper-binding protein fractions, Cp 1 and Cp 2, have been observed in pig serum by starch gel electrophoresis. The fractions, which each consists of 3 bands, are attributed to 2 codominant alleles,  $Cp^1$  and  $Cp^2$ .

Table 1 demonstrates that the fast ceruloplasmin fraction, Cp 1, was not observed in the Yugoslavian breeds. From these data it is assumed that the  $Cp^1$  allele is very rare or absent in these breeds. This distribution is a common feature of the pig breeds investigated for ceruloplasmin variations. In Danish and British Landrace the  $Cp^1$  allele is very rare and in British Large White pigs the allele seems to be absent.

*The serum amylase system.* Three alleles,  $Am^1$ ,  $Am^2$  and  $Am^3$ , at the serum amylase locus are known. Each allele expresses 1 enzyme band.

Table 1 indicates that an extremely low frequency or absence of  $Am^1$  was observed in the Yugoslavian breeds. An equivalent  $Am^1$  frequency has been reported in British Large White pigs. The  $Am^3$  allele which occurs in the Yugoslavian breeds and in Danish Landrace pigs with a low frequency has also been ob-

served in Czechoslovakian Large Whites with an extremely low frequency. This allele seems to be absent in British Large White.

The above frequency studies indicate that breed differences are pronounced. Generally, the breeds are not sharply distinguished, for in no case has an allele been found in all members of one breed and entirely lacking in another. When the number of animals typed is considered it must be concluded that only the Tf<sup>B</sup> allele segregates in the Danish Landrace. Other alleles at the transferrin locus seem to be absent. Table 1 shows an apparent absence of some serum type alleles in the Yugoslavian breeds, but a definite confirmation necessitates studies of an increased number of animals.

When the relative distribution of alleles is considered it is noticed that in one system the differences are pronounced, in another they are minimal. A marked difference in allele frequencies between Danish Landrace pigs and Yugoslavian pigs is present in the hemopexin system. The causal mechanism of these breed differences is unknown.

The main purpose of the research for loci markers in domestic animals, such as red cell antigens and polymorphous serum proteins, has been the application of these blood characters in parentage control, population genetic studies and investigations into an association between these characters and other hereditary traits, including polygenic, physiological characters of practical value. Attempts to reveal an association of this kind have hitherto failed. Blood groups and serum types are, however, widely used in the parentage control. Data from large scale investigations of human red cell antigens and serum proteins have been applied in physical anthropology in many years. In cattle, where the research for hereditary blood characters is most advanced compared with other domestic mammals, these traits have been used for studies on differences between breeds and breed structure. As mentioned in the introduction only a few papers on breed differences in serum protein alleles in pigs have been published, probably due to the fact that a systematic mapping of the porcine chromosomes by means of hereditary blood traits was initiated only 10 years ago.

The above frequency studies in 3 indigenous Yugoslavian pig breeds were performed at the present time, because it is expected that these breeds will disappear from Yugoslavian pig breeding

and be substituted by new and more productive breeds. The present report might serve as a contribution to future investigations into breed differences and breed structure in pigs.

#### ACKNOWLEDGMENT

The present work was undertaken on the initiative of Professor, dr. med. vet. Johs. Moustgaard, whose valuable help and continuous interest during planning and carrying out these studies are very much appreciated.

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## SUMMARY

Serum samples from 3 Yugoslavian pig breeds, Resavka, Mangolica and Pfajfer, have been typed for transferrin, hemopexin, ceruloplasmin and amylase by means of starch gel electrophoresis.

The iron-binding properties of the variable serum transferrins were ascertained in an autoradiographic study with Fe<sup>59</sup>.

The gene frequencies obtained are discussed and compared with those found in other swine breeds, including the Danish Landrace. Even though the number of samples typed was relatively low, and additional data are required, the frequencies found in the Yugoslavian breeds showed a marked difference from those obtained in the Danish Landrace. The different distribution of alleles was especially pronounced in the transferrin and hemopexin systems.

## ZUSAMMENFASSUNG

*Serumproteinpolymorphie in einigen jugoslavischen Schweinerassen.*

Serumproben von folgenden drei jugoslavischen Schweinerassen, Resavka, Mangolica und Pfajfer, wurden mit Hilfe von Stärkegelelektrophorese auf Transferrin, Hemopexin, Ceruloplasmin und Serumamylase gruppenbestimmt.

Die Fähigkeit der Serumtransferrinvarianten Eisen zu binden wurde mit Hilfe von Autoradiographie wobei Fe<sup>59</sup> benutzt wurde festgestellt.

Die Genfrequenzen werden diskutiert und mit den Frequenzen bei anderen Rassen, hierunter die Dänische Landrasse, verglichen. Obgleich die Zahl der Gruppenbestimmungen nur verhältnismässig gering war und es weiteren Untersuchungen bedarf, unterschieden die gefundenen Genfrequenzen sich deutlich von den Genfrequenzen der Dänischen Landrasse. Der Unterschied in der Verteilung der Serumgruppenallele war besonders gross in dem Transferrin- und Hemopexinsystem.

## SAMMENDRAG

*Serumproteinpolymorfi i nogle jugoslaviske svineracer.*

Serumprøver fra 3 jugoslaviske svineracer, Resavka, Mangolica og Pfajfer, blev typebestemt for transferrin, hemopexin, ceruloplasmin og serumamylase ved hjælp af stivelsegelelektroforese.

Serumtransferrinvarianternes evne til at binde jern blev konstateret ved hjælp af autoradiografi under anvendelse af Fe<sup>59</sup>.

Genfrekvenserne diskuteres og sammenlignes med frekvenserne i andre racer, herunder Dansk Landrace. Selvom antallet af typebestemmelser var relativt lavt, og yderligere undersøgelser er nødvendige, viste de fundne genfrekvenser en tydelig forskel fra frekvenserne i Dansk Landrace. Forskellen i fordelingen af serumtypealleler var især udtalt i transferrin- og hemopexinsystemet.

(Received September 14, 1966).