Theoretical and Applied Genetics 48, 237-242 (1976)

Polymorphism of Blood Serum Amylase and Leptospirosis of Pigs of Large White Polish Breed

T. Przytulski and D. Porzeczkowska

Department of Genetics and General Animal Breeding, Institute of Animal Breeding and Technology of Animal Production, Agriculture Academy, Szczecin (Poland) and Veterinary Hygiene Research Station, Szczecin (Poland)

Summary. In a population of 1460 head Large White Polish pigs, the authors proved the correlation between the phenotypes and alleles of blood serum amylase (Am) and the level of leptospira antibodies.

With increase of the level of leptospira antibodies, the frequency of Am^A allele also increased, and the frequency of Am^B allele decreased.

Am^B allele seems to be in certain proportion positively connected with natural resistance of Large White Polish pigs to leptospirosis, contrary to Am^A allele.

The results of the investigations indicate the favourable mating of pigs having Am BB phenotypes, for the trait under consideration.

The development of investigations into polymorphic systems of animals blood proteins and enzymes induced a number of authors to search for correlations between those systems and pathological conditions of animals.

From investigations concerning genotypes of cows blood serum transferrins, Malik *et al.* (1970) found differences between the genotypes in the proportion of under quarters with positive Californian Test (CMT) results. They also observed that the greatest percentage of infection caused by *Streptococcus agalactiae* occurred in cows with genotypes Tf D_1D_1. There was no infection by this microbe in cows with genotypes Tf EE.

Studying pigs' blood serum, Imlah (1970) indicated the possible existence of a conjunction between Tf C transferrin locus and an early lethal factor showing its activity.

Investigations of polymorphism of other blood proteins, made by Osterhoff (1971), proved that there was an additional fraction of haemoglobin C in sheep attacked by a disease known as the geldikkop - enzootic icterus syndrome. This fraction did not exist in animals which were tested before falling ill. Only the sheep of A or AB haemoglobin phenotype possessed this fraction, but it did not appear in the sheep with B haemoglobin during the period of disease.

The same author (1974) proved the correlation between the genotype of milk B - lactoglobulins and mastitis in cows.

Rasmusen and Lewis (1973), studying sheep, found greater mortality in young ewes having the L blood system and in young rams with M or L blood systems. There was no such dependence in Targhee or in Suffolk × Targhee lambs.

Ernst *et al.* (1973) investigated the correlation between natural resistance against leukaemia and polymorphic blood characteristics. They showed that the frequence of antigens, B, Y_2, D' and P', in sick animals was 5-12% lower and the frequence of C_1, S, and U antigens was 3-11% higher than the average for the population (n - 2495).

Fomiceva (1973) suggested that the mortality of embryos might be caused by the supremacy of progeny having postalbumins' genotypes inconsistent with their mothers. The progeny descended from Estonian Black and White Lowland cows mated with bulls of unknown genetic postalbumins' type.

Collins and Millson (1975) stated that there was no correlation between the frequency of phenotypes and alleles of blood serum transferrins in two Herdwick sheep lines - susceptible and resistant to scrapie.

The results of the above studies suggest further researches.

The aim of our investigation was to find correlations between the frequencies of phenotypes, homozygotes, heterozygotes and alleles of pigs blood serum amylase according to the result of the serological diagnosis of leptospirosis.

Materials and Methods

1460 pig serum samples had been chosen from the diagnostic materials of the Veterinary Hygiene Research Station to determine the phenotypes of blood serum amylase. In 769 samples the leptospira antibodies were present while the remaining 691 samples were serologically negative.

The pigs of Large White Polish breed were taken from different farms.

The phenotypes of blood serum amylase were determined by starchgel electrophoresis according to Hesselholt (1969).

The serological test method used microscope agglutination according to the orders of the Experts' Committee WHO (1967a, 1967b, 1972). 4-14 day old standard leptospira cultures were used as an antigen: RGA (icterohaemorrhagiae), Moskva V (grippotyphosa), M-84 (sejroe) Perepelicin (tarassovi), Pomona (pomona).

The results were statistically analysed by χ^2 method.

Results

The level of leptospira antibodies in seroreagents was contained within the limits of titre from 1:100 to 1:12800. The height of microscope agglutinations' titre was taken as a criterion and the animals were divided into 4 groups: serologically negative; with low titres - from 1:100 to 1:200; with medium titres - from 1:400 to 1:1600; and with high titres - from 1:3200 to 1:12800 (Table 1).

Table 1. Distribution of the studied pigs into groups in respect of the results of the serological test

Group	Number of animals	The height of titre
I	691	result negative
II	545	1:100 to 1:200
III	134	from 1:400 to 1:1600
IV	90	from 1:3200 to 1:12800

In the studied population of 1460 head of pigs, there were three phenotypes of amylase (Am), Am AA, Am BB, Am AB, determined by two codominant alleles; Am^A and Am^B. The frequencies of genotypes, homozygotes, heterozygotes and alleles of amylase in each group are presented in Table 2.

These results show that the frequency of Am AA phenotype in the group of pigs (serologically negative) was 2,17%. In the II and the IV groups (i.e. in animals whose blood serum showed antibodies at dilutions from 1:100 to 1:200 and from 1:3200 to 1:12800) the frequency of this phenotype was 2,39% and 2,22% respectively. The highest proportional share of Am AA phenotype belonged to the III group of pigs (the group with titres from 1:400 to 1:1600) and was 4,48%.

The frequency of Am BB phenotype appear to decline with the increase of titres in the particular groups of pigs. The frequency of Am BB phenotype in group I was 78,87%, in II - 75,05%, III - 73,88% and IV - 66,67%.

Although the decrease in frequency of the phenotype between the groups was as high as 22,2%, the χ^2 obtained (Table 3) do not indicate any significant differ-

Table 2. Characterization of the studied pigs in respect of the frequencies of phenotypes, homozygotes, heterozygotes and alleles of blood serum amylase

Group	N		Phenotypes of amylase			Homo.	Hetero.	Frequencies of alleles	
			Am AA	Am BB	Am AB			Am^A	Am^B
I	691	n	15	545	131	560	131	0.116	0.884
		%	2.17	78.87	18.96	81.04	18.96		
II	545	n	13	409	123	422	123	0.137	0.863
		%	2.39	75.05	22.57	77.43	22.57		
III	134	n	6	99	29	105	29	0.153	0.847
		%	4.48	73.88	21.64	78.36	21.64		
IV	90	n	2	60	28	62	28	0.178	0.822
		%	2.22	66.67	31.11	68.89	31.11		

Commentary: Homo. - homozygotes
Hetero. - heterozygotes
N - number of elements in groups
n - number of elements of phenotypes, heterozygotes, homozygotes of amylase.

Table 3. Significance of differences in the studied groups of pigs in respect of the frequencies of phenotypes, homozygotes, heterozygotes and alleles of pigs blood serum amylase

		Groups				Significance of difference between the groups - χ^2		
		I	II	III	IV	I-II	I-III	I-IV
The frequency of amylase phenotypes (%)	AA	2.17	2.39	4.48	2.22	0.05	2.37	0.01
	BB	78.87	75.05	73.88	66.67	0.56	0.29	1.49
	AB	18.96	22.57	21.64	31.11	1.93	0.42	5.81[a]
Homozygotes (%)		81.04	77.43	78.36	68.89	0.50	0.06	1.42
Heterozygotes (%)		18.96	22.57	21.64	31.11	1.93	0.42	5.81[a]
The frequency of alleles	Am^A	0.116	0.137	0.153	0.178	1.74	5.10[a]	13.08[b]
	Am^B	0.884	0.863	0.847	0.822	0.26	0.80	2.26

Commentary: [a] - significant difference ($P < 0.05$)
[b] - highly significant difference ($P < 0.01$).

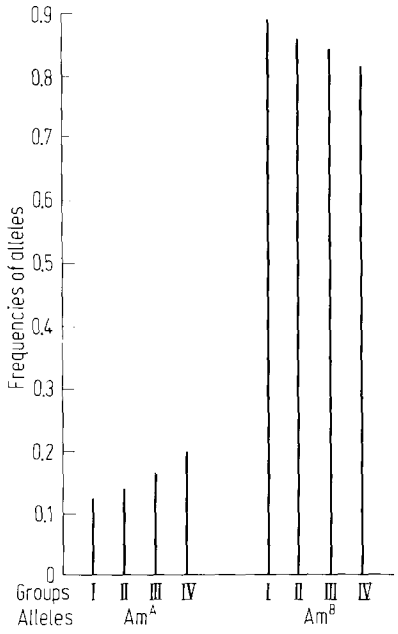

Fig. 1. Correlation between the height of titre and the frequency of Am^A and Am^B alleles

ence between the frequencies of phenotype in particular groups ($P > 0.05$).

It may be observed that, with increase of the titre, the proportional frequency of heterozygotes increased too. Their frequencies were: I - 18,96%, II - 22,57%, III - 21,64%, IV - 31,11%. The obtained values of χ^2 (Table 3) show a significant difference between the frequencies of Am heterozygotes for pigs of the I and the IV groups ($P < 0.05$).

It is worth noticing that, with the increase of titre, the frequency of Am^A allele increased from 0,116 in the I group, 0,137 in II and 0,153 in III, to 0,178 in the IV group (Tables 2, 3). The obtained values of χ^2 (Table 3) indicate significant differences between the frequencies of Am^A allele in the I and the III groups of pigs ($P < 0.05$), and a highly significant difference between the frequencies of the Am^A allele when comparing pigs from the I and the IV groups ($P < 0.01$).

On the other hand, the frequency of Am^B allele decreased with the increase of titre and was equal in the I-IV groups: 0,884, 0,863, 0,847 and 0,822 (Tables 2, 3). However there was no statistically significant difference between the particular groups of pigs in the frequencies of Am^B allele ($P > 0.05$).

The correlation between the height of titre and the frequencies of Am^A and Am^B alleles in the studied material is additionally illustrated by a graph (Fig. 1).

Discussion

In the specification of the results of our serological studies, the leptospira serotypes, with which the animals reacted positively, have been left out of consideration, because the aim of this work was to show a general connection between positive serological reaction and polymorphism of pigs blood serum amylase.

According to many authors (WHO: 1967a, 1967b, Michna 1967), the reaction of microscope agglutination with living antigens is highly sensitive and specific, as little as 1:100 titre being taken up as an indicator of infection. The distribution of pigs into groups,

Table 4. Frequency of genes which determine the phenotypes of blood serum amylase in pigs of various breeds

Breed	N	Alleles				Reference	Country
		Am^A	Am^{BF}	Am^B	Am^C		
Large White	131	0.023	-	0.977	0 -	Meyer (1972)	South Africa
Large White	236	0.250	-	0.737	0.013	Gavalier *et al.*(1966)	Czechoslovakia
Large White	161	0.05	-	0.95	-	Imlah (1964)	Scotland
Slovakian Large White	195	0.256	-	0.744	-	Meyer (1973)	Czechoslovakia
Landrace	578	0.178	0.010	0.809	0.003	Meyer (1972)	South Africa
Danish Landrace	716	0.147	-	0.828	0.026	Hesselholt (1969)	Denmark
Landrace	174	0.158	-	0.833	0.009	Gavalier *et al.*(1966)	Czechoslovakia
Danish Landrace	175	0.13	-	0.84	0.03	Graetzer *et al.*(1964)	Denmark
Landrace	95	0.20	-	0.80	0 -	Imlah (1964)	Scotland
Dutch Landrace	77	0.097	-	0.903	-	Hesselholt (1969)	Holland
German Landrace	225	0.113	0.024	0.845	0.018	Dinklage (1968)	West Germany
German Landrace	873	0.08	-	0.90	0.02	Willer, Neuffer (1970)	East Germany
German Landrace	986	0.040	0.025	0.928	0.007	Schmid (1968)	West Germany
Mangalica	73	-	-	0.99	0.01	Hristič *et al.*(1967)	Yugoslavia
Hungarian Mangalica	360	-	-	1.000	-	Fésüs (1968)	Hungary
Duroc	2877	-	-	1.00	-	Smith *et al.*(1968)	USA
Duroc	248	-	-	1.00	-	Baker (1968)	USA
Wessex Saddleback	19	0.553	-	0.421	0.026	Meyer (1972)	South Africa
Hampshire	2351	0.09	-	0.90	0.01	Smith *et al.*(1968)	USA
Hampshire	243	0.09	-	0.91	-	Baker (1968)	USA
German Edelschwein	157	0.13	-	0.85	0.02	Willer, Neuffer (1970)	East Germany
Bohemian Edelschwein	327	0.310	-	0.687	0.003	Schröffel (1967)	Czechoslovakia
Black and White Prestice	177	0.014	-	0.972	0.014	Gavalier *et al.*(1966)	Czechoslovakia
Black and White Prestice	133	0.015	-	0.978	0.007	Schröffel (1967)	Czechoslovakia
Cornwall	86	-	-	1.000	-	Schröffel (1967)	Czechoslovakia
Cornwall	150	-	-	1.000	-	Gavalier *et al.*(1966)	Czechoslovakia
Large Black	83	0.072	-	0.928	-	Meyer (1972)	South Africa
Yorkshire	76	0.355	-	0.645	-	Hesselholt (1969)	Holland
Pietrain	49	--	-	1.000	-	Hesselholt (1969)	Holland
Pfajfer (Black Slovenian)	105	0.01	-	0.95	0.04	Hristič *et al.*(1967)	Yugoslavia
Resavka	60	-	-	0.99	0.01	Hristič *et al.*(1967)	Yugoslavia
Ukrainian White Steppe	387	0.151	-	0.787	0.062	Plakhotnikov (1974)	USSR
Ukrainian Spotted Steppe	245	0.112	-	0.829	0.059	Plakhotnikov (1974)	USSR
Zlotnicka	219	0.0089	0.1667	0.8244	-	Tomaszewska - Guszkiewicz *et al.*(1972)	Poland
Minnesota No. 1	78	0.006	0.006	0.974	0.013	Meyer (1972)	South Africa
Minnesota × Vietnamese	119	0.003	-	0.445	0.552	Schleger, Dworak (1972)	
Bantu pig	230	0.035	0.102	0.715	0.148	Meyer (1972)	South Africa
Kolbroek	13	-	0.154	0.692	0.154	Meyer (1972)	South Africa
Göttingen Miniature		0.078	0.039	0.296	0.587	Gruhn, Dinklage (1971)	West Germany

N - number of individuals examined.

done in an arbitrary way according to the height of titre, may awaken some doubt. The height of titre may depend, among other factors, upon the period passed from the moment of infection, which was not taken into account in this work, and upon the individual immunological reactivity of pigs in contact with an infecting factor.

However, it is intriguing that, with the increase of titre, the percentage of blood serum Am heterozygotes and of Am^A alleles increases also (Table 3, Fig.1).

For Am heterozygotes, there is no significant difference in proportional share between the serologically negative group and the group with 1:100 to 1:200 titre, but between the negative group and the group with high titre - from 1:3200 to 1:12800 - there is a statistically significant difference ($P < 0.05$).

On the other hand, for the frequency of Am^A allele, significant ($P < 0.05$) and highly significant ($P < 0.01$) differences occurred between the serologically negative group and the groups whose titres were 1:400 and higher (Table 3).

No statistically significant differences have been deserved in the frequency of Am^A allele between the serologically negative group of pigs and the group with low titres (from 1:100 to 1:200), which is a difficult phenomenon to interpret.

In the studied population of pigs, the Am^A allele exists in the homozygote form at very low frequency (2,17-4,48%), but in the heterozygote state it is found within the limits of 18,96-31,11% (Tables 2, 3).

It is evident from observation of the frequencies of Am^A and Am^B alleles, that the frequency of the Am^A allele increases in the particular groups proportionally to the increase of their titre, while the Am^B allele acts in a quite different way, for its frequency decreases with increase of titre (Tables 2, 3, Fig. 1).

From the list of literature (Table 4), it is evident that the Am^A allele is probably forced out from some pig breeds, because this trait is not an object of selection. In certain breeds the frequency of Am^A allele is very small or the allele does not exist at all.

A few studies suggest that the Am^A allele may be negatively connected, in a direct or an indirect way, with some productive, breeding or sanitary parameters of pigs.

Andresen (1966), for example, stated that the Am^A allele is connected with the I_b blood system. However Kennedy *et al.* (1973) showed that the I_b system is not connected with a variety of productive and breeding parameters of pigs.

It is evident from Table 4 that in pig breeding Am^B allele is preferred.

Bezenko *et al.* (1971) proved that pigs with genotypes Am BB and Am CC have good slaughter traits, especially high slaughter efficiency.

The results of the investigations show that Am^B allele may be to some extent a genetic marker of natural resistance to leptospirosis. It is found in serologically negative pigs to have a high frequency (0,884), decreasing with increase of titre (0,837).

The confirmation of such researches in other pig breeds, and on precisely epizootically diagnosed populations, may have great importance for selection of pairs to be mated for those traits.

The supplement to our results would be a study of those pigs of Large White Polish breed in which leptospirosis had been proved in a clinical and bacteriological way.

On the basis of the presented results, it would seem most appropriate to mate pigs of Am BB phenotype (Am BB × Am BB). On the other hand, mating Am BB × Am AB, Am AA × Am BB or Am AB × Am AB types, for a natural resistance to leptospirosis, seems unprofitable.

Conclusions

The results of the investigations allow for the following conclusions:

1. The following phenotypes of pigs blood serum amylase were established in the studied population of pigs: Am AA, Am BB, Am Ab.

2. With increasing levels of leptospira antibodies, the frequency of Am^A allele also increased ($P < 0.05$ and $P < 0.01$), and the frequency of Am^B allele decreased in particular groups of animals.

3. Am^B allele seems to be in some proportion positively connected with the natural resistance of Large White Polish pigs to leptospirosis, contrary to the Am^A allele.

4. With increase of titre, an increase in the proportional frequency of heterozygotes was established, confirming to some degree the negative influence of Am^A allele on natural resistance against this disease.

5. It seems appropriate to mate pigs of phenotypes Am BB × Am BB, but it is not profitable to mate those of Am BB × Am AB, Am AA × Am BB, or Am AB × Am AB.

Literature

Andresen, E.: Blood groups of the I system in pigs: association with variants of serum amylase. Science 153, 1660-1661 (1966)

Baker, L.N.: Serum protein variation in Duroc and Hamshire pigs. Vox Sang. 15, 154-158 (1968)

Bezenko, S.P.; Terentiyeva, A.S.; Kabanov, V.D.; Czernuschenko, V.K.; Budnikov, A.V.: Genetical characteristics of the Large White swine breed pertaining to erythrocytal antigens and serum proteins and their relation to certain economically valuable characters (Russian). Genetika 7, 88-94 (1971)

Collins, S.C.; Millson, G.C.: Transferrin polymorphism in Herdwick sheep. Animal Blood Grps. Biochem. Genet. 6, 117-120 (1975)

Dinklage, H.: Das Serum - Amylase - System des Schweines und seine Bedeutung für den Elternschaftsnachweis beim Deutschen veredelten Landschwein. Züchtungskunde 40, 228-231 (1968)

Ernst, L.K.; Klabukov, P.G.; Goldman, I.L.; Sorokovol, P.F.; Smirnov, O.K.; Karlikov, D.V.; Kyoune, K.Ya.: Investigation of the genetic re-

sistance of cattle to leucosis (Russian). Dokl. Vsesoyuznoj Adad. Selskokhozyaistvennykh Nauk 8, 29-30 (1973)
Fésüs, L.: Blood groups and serum proteins in the Hungarian Mangalica pig. XIth Europ. Conf. Animal Blood Grps. Biochem. Polymorph., Warsaw 275-277 (1968)
Fomiceva, I.I.: Niekotoryje aspekty izucenija geneticeskogo polimorfizma transferrinov i postalbuminov u krupnogo rogatogo skota. Genetika 9, 59-66 (1973)
Gavalier, M.; Hojny, J.; Hradecky, J.; Linhart, J.; Schröffel, J.: Blood groups and serum proteins in pigs. Proc. Xth Europ. Anim. Blood Grp. Conf., Paris 159-164 (1966)
Graetzer, M.A.; Hesselholt, M.; Moustgaard, J.; Thymann, M.: Studies on protein polymorphism in pigs, horses and cattle. Proc. IXth Europ. Animal Blood Group Conf., Prague, 279-293 (1964)
Gruhn, R.; Dinklage, H.: Blutgruppen - und Proteinpolymorphismus beim Götinger Miniaturschwein. Z. Versuchstierk. 13, 179-187 (1971)
Hesselholt, M.: (1966) - after Hesselholt (1969)
Hesselholt, M.: Serum protein polymorphisms in swine. Copenhagen: Munksgaard 1969
Hristič, V.; Hesselholt, M.; Stosič, D.: Serum protein polymorphism in some Yugoslavian pig breeds. Acta Vet. Scand. 8, 43-49 (1967)
Imlah, P.: A study of blood groups in pigs. Proc. IXth Europ. Animal Blood Group Conf., Prague 279-293 (1964)
Imlah, P.: Evidence for the Tf locus being associated with an early lethal factor in a strain of pigs. Animal Blood Grps. Biochem. Genet. 1, 5-13 (1970)
Joint FAO/WHO Expert Comittee on Zooneses: Third Report Wld. Hlth. Org. Techn. Rep. Ser. 387 (1967a) - see also WHO
Kennedy, B.W.; Moxley, J.E.; Saison, Ruth: Some relationships between blood group factors and economic traits in swine. Can. J. Anim. Sci. 53, 387-397 (1973)
Malik, S.S.; Moxley, J.E.; Gibbs, H.C.; MacRae, H.F.: Blood serum transferrin polymorphism and mastitis. Can. J. Anim. Sci. 50, 535-546 (1970)
Meyer, E.H.H.: Serum protein polymorphism in pig breeds in South Africa. XIIth Europ. Conf. Animal Blood Grps. Biochem. Polymorph., Budapest 315-322 (1972)
Meyer, J.N.: Blood group and serum protein polymorphism in the Slovakian Large - White pig. Anim. Blood Grps. Biochem. Genet. 4, 63-64 (1973)
Michna, S.W.: Animal leptospirosis in the British Isles - a serological survey. Vet. Rec. 80, 394-401 (1967)
Osterhoff, D.R.: Haemoglobin types and the geeldikkop - enzootic icterus disease complex in sheep. Animal Blood Grps. Biochem. Genet. 2, 181-184 (1971)
Osterhoff, D.R.; Giesecke, W.H.: Milk protein polymorphism and its relationship to mastitis in Friesland cows. 1st World Congr. Genet. Applied Livestock Prod., Madrid, 283-288 (1974)
Plakhotnikov, A.G.: Genetic polymorphism of amylase and ceruloplasmins in two pig breeds reared in the South of the Ukraine (Russian). Cytology and Genetics 8, 546-547 (1974)
Rasmusen, B.A.; Lewis, J.M.: The M-L blood group system and survival of Suffolk and Targhee lambs. Animal Blood Grps. Biochem. Genet. 4, 55-57 (1973)
Schleger, W.; Dworak, E.: Biochemical polymorphism in Minnesota and Minnesota × Vietnamese pig. XIIth Europ. Conf. Anim. Blood Grps. Biochem. Polymorph., Budapest, 337-341 (1972)
Schmid, D.O.: Über einen neuen Serum - Amylase - Typ beim Schwein. Zbl. Vet. Med. 15, B, 990-994 (1968)
Schröffel, J.; (1967) after Schmid, D.O. (1968)
Smith, C.; Jensen, E.L.; Baker, L.N.; Cox, D.F.: Quantitative studies on blood group and serum protein systems in pigs. J. Anim. Sci. 27, 848-855 (1968)
Tomaszewska - Guszkiewicz, K.; Kluciński, W.; Zurkowski, M.: Serum protein polymorphism in Zlotnicka pigs. Anim. Blood Grps. Biochem. Genet. Supp. 1, 52-53 (1972)
WHO Expert Group Wld. Hlth. Org.: Current problems in leptospirosis. Techn. Rep. ser. 380 (1967b)
WHO Expert Group Bull. Wld. Hlth. Org.: Research needs in leptospirosis. 47, 113-122 (1972)
Willer, S.; Neuffer, K.: Phänotyp - und Genfrequenzen polymorpher Serumproteine beim Schwein. Arch. Exp. Vet. Med. 24, 1071-1076 (1970)

Received March 30, 1976
Communicated by S. Barbacki

mgr Tadeusz Przytulski
Agriculture Academy
Institute of Animal Breeding and Technology of Animal Production,
Broniewskiego 1, blok 34
460-71 Szczecin (Poland)
lek. wet. Danisława Porzeczkowska
Veterinary Hygiene Research Station
Ostrawicka 1
71-337 Szczecin (Poland)