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Relationship between transferrin and ceruloplasmin polymorphism and the frequency of diarrhoea in piglets

Summary

The relationship between a phenotype of transferrin (Tf) and ceruloplasmin (Cp) and the frequency of diarrhoea occurrence was analysed in 317 piglets. It was observed that diarrhoea caused by haemolytic Escherichia coli strains including K88 ab and K88 ac occurred with greater frequency in piglets with phenotypes Tf AB and Cp AB than in piglets with phenotypes Tf BB and Cp BB. It is believed that polymorphic type of Tf A and Cp A is related with piglets susceptibility to colibacteriosis which is caused by strains E. coli K88 ab and K88 ac.

Key words: diarrhoea, colibacteriosis, ceruloplasmin, transferrin, polymorphism, piglets.

Zusammenfassung

Titel der Arbeit: **Beziehung zwischen dem Polymorphismus von Transferrin und Ceruloplasmin und der Durchfallhäufigkeit bei Ferkeln**

Es wurden die Beziehungen zwischen dem Phänotyp von Transferrin (Tf) und Ceruloplasmin (Cp) bei 317 Hybridferkeln und der Durchfallhäufigkeit analysiert. Dabei konnte festgestellt werden, daß der von hämolythischen Stämmen von Escherichia coli, darunter auch von K88 ab und K88 ac hervorgerufene Durchfall viel häufiger bei Ferkeln mit den Phänotypen Tf AB sowie Cp AB als bei Ferkeln mit den Phänotypen Tf BB und Cp BB auftrat. Es wird geschlußfolgert, daß der polymorphe Typ Tf A und Cp A mit der Anfälligkeit der Ferkel gegen Kolibakteriose zusammenhängt, die von den Stämmen E. coli K88 ab und K88 ac verursacht werden.

Schlüsselwörter: Durchfall, Kolibakteriose, Ceruloplasmin, Transferrin, Polymorphismus, Ferkel

Introduction

Diarrhoea occurring in piglets is mainly caused by pathogenic Escherichia coli strains. E. coli strains having antigen K88, also determined as F_4, play a particularly significant role in their pathogenesis. These strains is the source of disease both in neonatal period and suckling and weaned as well (BERTSCHINGER, 1995).

E. coli K88 is found in three serological antigenic variants: K88 ab, K88ac and K88 ad (ORSKOV et al., 1964; GUINÉE and JANSEN, 1979). The presence of receptors for K88 fimbriae on enterocytes of epithelial cells of the small intestine in piglets enables the adherence of fimbriaic E. coli strains and their growth (MOON et al., 1979). As E. coli colonizes the small intestine and they have an ability to produce toxins they are regarded as the source of disease (SOJKA, 1971). SELLWOOD et al. (1975) showed that the occurrence of receptors for E. coli K88 ac on the surface of the small intestine conditions the susceptibility of piglets to diarrhoea. However, the lack of receptors causes their resistance.

According to many authors the existence of receptors for K88 is coded by an autosomal dominant gene - S, whereas their lack is coded by a recessive allele - s (RUTTER et al., 1975; GIBBONS et al., 1977; SELLWOOD et al., 1975).

As the present research results show it is known that the receptors for antigens K88 ab and K88 ac are determined by dominant genes located in two closely linked loci (BONNEAU et al., 1990; GUÉRIN et al., 1993; EDFORS-LILJA et al., 1995; MARKLUND et al., 1996).

Genetic mechanism of inheritance of the receptor for K88 ad has not been fully explained so far (RAPACZ and HASLER-RAPACZ, 1986). EDFORS-LILJA et al. (1995) informed that the distance between loci of the genes coding the receptors for K88 ab and K88 ac was 1cM. They also stated that there was also a strong link between loci: K88 abR and transferrin and ceruloplasmin, respectively: K88 abR and Tf - 7,4 cM and Tf and Cp - 1,5 cM. The results of their research prove GIBBONS et al. (1977) suggestion who showed the existence of dependence between polymorphic types Tf and piglets susceptibility to diarrhoea caused by E. coli K88. The results obtained by GUÉRIN et al. (1993) correspond to them as well. Loci of the above discussed genes are located in 13 chromosome. The linkage map of this chromosome presented by MARKLUND et al. (1996) shows that the links between loci K88 abR, K88 acR, Tf and Cp are even stronger than those given by EDFORS-LILJA et al. (1995). Passive protection of piglets by antibodies found in colostrum and milk of immunized sows is a commonly used method against colobacteriosis. It does not always bring satisfactory results. Possibility of applying polymorphic types of transferrin and ceruloplasmin as genetic resistance markers seems to be justified.

The aim of this research was to check if polymorphic types of transferrin and ceruloplasmin occurring in piglets can be factors differentiating the frequency of diarrhoea including these ones being caused by E. coli K88 ab and K88 ac.

Material and Methods

The research was carried out in the Plant of Swine Fattening. It included 317 piglets from 1 to 4 weeks old. The piglets came from 102 litters of hybrid sows: Polish Large White x Polish Landrace. The following breeds were represented by boars (n=17): Duroc, Pietrain, Zlotnicka Spotted and hybrids of these breeds. Sows were immunized according to an instruction by NEOCOLIPOR vaccine (Rhone Merieux - France), containing E. coli strains with antigenes: K88 ab, K88 ac, K88 ad, K99, 987 P. and F_{41}. In blood serum obtained from piglets the type of transferrin (Tf) and ceruloplasmin (Cp) was determined by the method of electrophoresis in starch gel according to HESSELHOLT, 1969. Faeces (n=221 samples) was taken from healthy piglets (n=74) and from those with diarrhoea (n=243). The samples with faeces underwent routine bacteriological examinations in order to determine E. coli strains (non - haemolytic and haemolytic). The examinations were carried out by Department of Veterinary Hygiene in Olsztyn. Identification of fimbrial antigens K88 ab and K88 ac was done according to the method given by OSEK and TRUSZCZYŃSKI, 1992. These examinations were

carried out by Department of Microbiology at Veterinary Institute in Puławy. The obtained results underwent statistical analysis applying chi^2 test.

Results and Discussion

Table 1 presents piglets characteristics comprising phenotypes in transferrin and ceruloplasmin systems and their state of health.

Table 1
Characteristic of piglets considering transferrin and ceruloplasmin phenotype and their health state (Charakteristik der Ferkel im Bezug auf den Phänotyp von Transferrin (Tf) und Ceruloplasmin (Cp) sowie auf ihren Gesundheitszustand)

Phenotype of piglets	Number		Frequency of occurrence and classification of diarrhoea cases in piglets							Piglets without diarrhoea (bacteriologically negative)
	piglets	samples of faeces	E. coli strains including K88			Citrobacter	Samples negative	Non examined	Total	
			N	H	K88					
Tf:										
AA	48	37	15	12	4	1	6	9	43	5 (3)
AB	152	112	47	38	24	4	17	27	133	19 (6)
AC	3	1	-	1	-	-	-	1	2	1 (-)
BB	108	66	30	9	2	1	15	6	61	47 (11)
BC	6	5	1	-	-	-	3	-	4	2 (1)
Cp:										
AB	67	48	20	23	12	-	3	9	55	12 (2)
BB	250	173	73	37	18	6	38	34	188	62 (19)
Total	317	221	93	60	30	6	41	43	243	74 (21)

N - non-haemolytic ; H - haemolytic

Five phenotypes were observed in Tf system: AA, AB, AC, BB and BC. They were determined by alleles - Tf^A, Tf^B and Tf^C with the following frequency, respectively: Tf^A - 0,396, Tf^B - 0,590 and Tf^C - 0.014. Piglets Tf AB (47,9% of the total number) and Tf BB (34,1%) were the most numerous. Fewer piglets Tf AA (15,1%) and Tf BC (2,0%) were found. Piglets with Tf AC phenotype (0.9%) were observed sporadically. Phenotypes Cp AB and Cp BB occurred in Cp system. Phenotype Cp BB (78,9%) was more popular than Cp AB (21,1%). The frequency of alleles controlling this system was following: Cp^A - 0.106 and Cp^B - 0.894.

KURYŁ et al. (1996) report that in hybrids of generation F_2 obtained from cross-breeding of Polish Large White x Zlotnicka Spotted, phenotype Tf B (67,5%) occurred twice as often as in comparison to Tf AB (31,0%) and relatively rare - phenotype Tf A (1,4%).

Among examined piglets (Table 1) phenotype Tf AB dominated, however piglets Tf BC and Tf AC occurred as well. Participation of these phenotypes was insignificant (AC - 0,9 and BC - 2,0%) in comparison to the observed ones by ŻURKOWSKI et al. (1974) in Zlotnicka Spotted pigs (Tf AC - 7,4 and Tf BC - 17,9%). The authors also report that high frequency of alleles Tf^C (0,1280) is a particular feature of this breed

which does not occur in Polish Large White and Polish Landrace and is very seldom in other breeds (ŻURKOWSKI et al., 1975).

Monomorphism is observed in the system Cp in the majority of breeds (HESSELHOLT, 1969). Zlotnicka Spotted breeds is contradiction because in this system polymorphism was found (ŻURKOWSKI et al., 1974; KURYŁ et al., 1996). KURYŁ et al. (1996) point out that phenotypes Cp F (8,3%), Cp FS (30,6%) and Cp S (61,1%) are observed in Zlotnicka Spotted, in hybrids of Polish Large White x Zlotnicka Spotted three phenotypes occurred as well. The above presented results (Table 1) show that two phenotypes Cp AB and Cp BB were reported among the examined piglets. Allele Cp^A could have been inherited from boars of Zlotnicka Spotted because as ŻURKOWSKI et al. (1975) report this allele does not occur in Polish Large White, Polish Landrace pigs and very seldom in other breeds.

The analysis of piglets health state shows that diarrhoea (in 76,5%) was mainly caused by E. coli strains (Table 1). In case of 200 piglets with diarrhoea which faeces was examined bacteriologically, in 93 cases non-haemolytic E. coli strains caused diarrhoea, in 60 cases - haemolytic strains. Fimbriae K88 ab or K88 ac were identified in 20% E. coli strains. Despite diarrhoea symptoms, the faeces samples taken from 41 piglets were bacteriologically negative, however the samples from 6 other piglets showed Citrobacter sp.

Table 2
Influence of transferrin and ceruloplasmin phenotype in piglets on their health state (Einfluß des Phänotyps von Transferrin und Ceruloplasmin der Ferkel auf ihren Gesundheitszustand)

Piglets phenotypes	Number of piglets	Observed and expected number of piglets		Value chi^2
		with diarrhoea	healthy	
Tf:				
AA	48	43 (36,8)	5 (11,2)	4,47^{x}
AB	152	133 (116,5)	19 (35,5)	10,01^{xx}
AC	3	2 (2,3)	1 (0,7)	0,16
BB	108	61 (82,8)	47 (25,2)	24,59^{xx}
BC	6	4 (4,6)	2 (1,4)	0,33
Total	317	243	74	39,56^{xx}
Cp:				
AB	67	55 (51,4)	12 (15,6)	1,08
BB	250	188 (191,6)	62 (58,4)	0,29
Total	317	243	74	1,37

^{x} $P \leq 0,05$; ^{xx} $P \leq 0,01$

The influence of phenotypes of piglets in system Tf and Cp on their susceptibility to diarrhoea was present in Table 2. The obtained results show that phenotype Tf of piglets influenced in a significant way their state of health. Diarrhoea was observed at higher frequency in piglets with phenotype Tf AB and Tf AA, whereas among healthy piglets there were found the ones with phenotype Tf BB. Differences in frequency of occurrence of diarrhoea cases in individuals being different of type Tf were statistically significant.

There was no significant dependence between piglets phenotype in Cp a system and

their susceptibility to diarrhoea. Although more phenotype Cp BB were observed among healthy piglets and Cp AB among ill ones.

Table 3
Characteristics of E. coli strains decomposition in the faeces of piglets differentiated by Tf and Cp type (Charakteristik der Verteilung von E. coli - Stämmen, die im Exkrement der nach dem Typ von Tf und Cp differenzierten Ferkel vorhanden sind)

Piglets phenotype	Percentage of examined samples of faeces	Observed and expected number of diarrhoea caused by E. coli strains:		
		N	H	K88
Tf:				
AA	17,2	15 (15,8)	12 (10,2)	4 (5,2)
AB	52,1	47 (47,9)	38 (30,7)	24 (15,6)
BB	30,7	30 (28,3)	9 (18,1)	2 (9,2)
Total	100,0	92	59	30
Value x^2		0,07	$6,67^{x}$	$11,03^{xx}$
Cp:				
AB	21,7	20 (20,2)	23 (13,0)	12 (6,5)
BB	78,3	73 (72,8)	37 (47,0)	18 (23,5)
Total	100,0	93	60	30
Value x^2		0,02	$9,29^{x}$	$5,38^{x}$

^{x} $P \leq 0,05$; ^{xx} $P \leq 0,01$; N - non-haemolytic; H - haemolytic

Analysing the cases of colibacteriosis the piglets representing the most numerous phenotypes were taken into consideration: Tf AA, Tf AB and Tf BB, neglecting less numerous Tf AC and Tf BC. The data presented in Table 3 show that diarrhoea caused by non-haemolytic E. coli strains with similar frequency occurred among piglets with a different phenotype determined both in system Tf as well in system Cp. The cases of its occurrence were proportional to the number of samples of faeces taken from piglets Tf: AA, AB, BB and Cp AB and Cp BB. Diarrhoea which was caused by haemolytic E. coli strains was mostly determined by piglets phenotypes. More cases of diarrhoea were found in piglets with phenotypes Tf AB and Tf AA, whereas in piglets with phenotype Tf BB it occurred more seldom than it had been expected. These deviations were statistically significant.

Statistically significant deviations occurred too between the number of the observed cases of diarrhoea among piglets having different Cp type and the expected number. Piglets Cp BB where diarrhoea was caused by haemolytic E. coli strains were very few but in piglets with Cp AB phenotype diarrhoea occurred more frequently.

The analysis of cases of diarrhoea caused by fimbrial E. coli showed that in 22 piglets it was caused by fimbriae K88 ac whereas in 8 by K88 ab. Registered number of piglets with diarrhoea which differed by the Tf and Cp types showed significant deviations with the expected number (resp. statistically highly significant and significant). Significantly more piglets with Tf AB and Cp AB phenotypes showed greater susceptibility to this disease factor in comparison to piglets Tf BB and Cp BB. The obtained results partly correspond to the results presented dy GIBBONS et al. (1977). They stated that susceptibility of piglets to diarrhoea caused by E. coli strains

K88 should be related to Tf AB phenotype, resistance with Tf BB phenotype. This feature was suggested as existence of the link between allele Tf^A and gene S coding the occurrence of receptors for E. coli K88 on the surface of the small intestine. The presented data (Table 3) show that diarrhoea caused by fimbrial E. coli strains mostly occur in piglets Tf AB but significantly less frequently in piglets Tf BB. Probably in the examined piglets both allele Tf^A and Cp^A were linked with genes conditioning the existence of receptors. The observed relationship between allele Tf^A and in smaller degree Cp^A (because of its lack or lower frequency in pigs in majority of breeds) and genetically conditioned predisposition to the occurrence of diarrhoea caused by E.coli K88 may have a practical application. Polymorphism of transferrin may serve as an index used while choosing couples for reproduction. It is proved by the fact that a susceptible offspring, resistant sows and susceptible boars according to SELLWOOD (1979) is particularly susceptible to diarrhoea. Colostrum of resistant sows even after their immunization shows a low level of antibodies for E. coli K88 and it does not protect their susceptible piglets against infection (SELLWOOD, 1979). Colostrum of susceptible sows by higher antiadhesive and opsonic activities than resistant sows inhibits the binding antigen K88 to brush borders of the intestine in piglets (SELLWOOD, 1982).

JOVANOVIC et al., 1996 state that genetic polymorphic conditioning the resistance to colibacteriosis K88 occur among the majority of breeds. It causes the necessity for carrying out selection in order to decrease the cases of this disease. BAKER et al. (1997) observed that the occurrence of non-adhesive phenotypes for E. coli K88 in pigs, characteristic for resistant individuals, showed different frequency depending on the breed.

According to JOVANOVIC et al. (1996) the existence of both susceptible and resistant pigs to colibacteriosis in herd causes necessity of identification of resistant genotypes which can be carried out by using biochemical markers. This identification should concern first of all boars especially those which are used for industrial cross-breeding.

Conclusion

The obtained results help in formulating the following statements:

Phenotype differentation in piglets occurring in transferrin and ceruloplasmin systems was related to their susceptibility to diarrhoea. This regularity was observed especially in case of diarrhoea caused by haemolytic E. coli strains including K88 ab and K88 ac. Diarrhoea occurred in greater number of piglets with phenotypes Tf AB and Cp AB than in piglets with phenotypes Tf BB and Cp BB. It is believed that polymorphic type TfA and Cp A predisposes piglets to susceptibility to diarrhoea which is caused by E. coli K88ab and K88 ac.

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