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Genetic differences in the frequency of the hinge variants of porcine IgA is breed dependent☆

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Abstract

The distribution of the IgA^a and IgA^b alleles of porcine IgA in over 160 randomly-selected animals revealed an abundance of heterozygotes but only two b/b homozygotes. Since the IgA^b allotype is a splice site mutant lacking two-thirds of the hinge, this study tests the hypothesis that pigs with this genotype may be at a selective disadvantage while heterozygous individuals may be at some advantage.

This hypothesis was tested by collecting data on 374 animals of known breed and often parentage. We show here that when breed was not considered, young animals of known parentage had genotypic frequencies identical to that expected for Mendelian alleles but that a/b heterozygotes were overrepresented in adults. However, when analyzed with regard to breed, a very strong association between breed and the frequency of the IgA^a and IgA^b alleles was discovered. Meishan and NIH minipigs were homozygous for IgA while heterozygotes predominated in Berkshire, Chester White, Durocs, Hampshire and Landrace. Animals homozygous for IgA^b were best represented in the White Cross line. We show here that this very strong breed dependency of IgA allotypy in swine can produce a sample bias that can explain why only two b/b homozygotes (1.3%) were found in the 160 randomly-selected samples since the original samples came from primarily Landrace and Yorkshire animals. The expected frequency of b/b homozygotes in these breeds would be <3%. Thus, the data presented here reject the hypothesis that swine homozygous for a trait that results in loss of two-thirds of the IgA hinge, are selected against and that heterozygotes are

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positively selected. Rather, the study shows that IgA^a and IgA^b appear to be simple, breed-dependent allotypic markers.

Keywords: Allotype; Hinge; Mucosal immunity; Swine; Breed

1. Introduction

IgA is the major immunoglobulin associated with mucosal immunity and it is the most abundant immunoglobulin in the entire body; humans synthesize >60 mg/kg/day (Mestecky et al., 1983, 1986). Because of its abundance in secretions, IgA is important in the immunologic defense against local infections in such areas as the respiratory and gastrointestinal tract. It is an efficient antiviral and agglutinating antibody, neutralizing viruses before they can enter the host cells. It may also have bactericidal activity against gram-negative organisms in the presence of lysozyme (Vaerman, 1984). Studies by Lamm et al., utilizing polarized cell monolayers growing on a permeable filter, demonstrated that secretory IgA could neutralize viruses *inside* epithelial cells during transcytosis. This could be an important mechanism in the event that IgA antibodies in the gut lumen fail to neutralize the virus before it enters the cells (Mazanek et al., 1993). The Lamm group also showed that the transport pathway for dimeric IgA through epithelial cells can be used to eliminate potentially damaging immune complexes of viruses-IgA, bacterial antigen-IgA or dietary protein-IgA that are present in the mucosal lamina propria (Mazanek et al., 1993). Removal of such complexes is important since Bogers et al. (1991) have shown that immune complexes containing IgA can activate the alternative complement pathway and potentially produce local tissue damage.

IgA has been identified as the major immunoglobulin in the mucosal tissues of all common mammals and some birds (see reviews Mestecky et al., 1998; Peppard and Russell, 1998). With exception of humans, closely-related primates and the rabbit, IgA is encoded by a single Cα gene. Human IgA occurs in the form of two subclasses; the IgA1 subclass contains a 13 amino acid hinge insertion that includes the site of catalytic cleavage for human bacterial IgA proteases (see below). Rabbits and other lagomorphs have 13 IgA subclasses although, unlike other common mammals, they have only a single Cγ gene (Butler, 1997). Isolation and sequencing of the porcine IgA gene has shown that swine have only one gene coding for IgA and its deduced amino acid sequence is most similar to that of another artiodactyl, i.e. bovine Cα, with homology nearing 75% (Brown et al., 1997). When the gene for swine IgA was isolated and characterized, it was found to occur in two forms, identical except for their hinge regions (Brown et al., 1995). One form (IgA^a) encodes a form of IgA with a six amino acid hinge whereas the other form (IgA^b) encodes an IgA with a two amino acid hinge. This difference arises from a mutation in the gene for IgA^b where a change from 'G' to 'A' occurs at the splice acceptor site in the Cα1–Cα2 intron. As a result, splicing in the 'short-hinged form' occurs at an 'AG' site 12 nucleotides downstream from the normal splice acceptor site in the Cα2 domain. The hinge region of IgA^b is thus shorter by four amino acids than the hinge of IgA^a. Brown et al. (1995) analyzed 16 offspring from the mating of two heterozygous

pigs by PCR-RFLP genotyping (as discussed in Section 2) and was able to determine that the IgA variants behaved as Mendelian allotypes. The heterozygous matings resulted in 4 a/a, 8 a/b and 4 b/b; i.e. expected 1:2:1 ratio.

The functional significance of these two allelic forms of porcine IgA in relationship to the functional role of mammalian IgA remains unknown. Since the hinge is effected, resistance to bacterial proteases might be a possible consequence. In the human, studies have shown that the long hinged IgA1 is more susceptible to bacterial proteases than is IgA2 (Plaut, 1983). The extended hinge of human IgA1 contains four proline–serine or proline–threonine bonds that are the catalytic target of the bacterial proteases (Mestecky et al., 1998). Although less dramatic in hinge length difference, the same differences in susceptibility might hold true for the swine IgA allotypes since IgA^a contains one proline–serine bond that is absent in IgA^b (Brown et al., 1995). This is still under study. It is also believed that the hinge plays an important part in the flexibility of immunoglobulins, perhaps affecting their ability to bind or cross-link antigens. IgA^a with its longer hinge, might be more flexible that IgA^b. Empirical evidence that hinge differences between these two porcine IgA allotypes might have biological significance was suggested since only 1.3% of 160 randomly-types swine were homozygous for IgA^b. Therefore, we hypothesized that the hinge deletion mutant of swine IgA was a negative selection trait. This hypothesis formed the basis for the current study in which we compared the gene frequencies of IgA^a and IgA^b in closed herds of known breeds. When tested in this manner, the results show that while the gene frequency of IgA^a and IgA^b is strongly breed dependent, we have found no correlation between piglet mortality and IgA allotype.

2. Materials and methods

2.1. Source of animals and animal material

We tested 187 samples of tail tissue, blood or purified DNA from adult White Cross (1/4 Yorkshire, 1/4 Large white, 1/4 Chester white, 1/4 Landrace) and Meishan females reared at the Meat Animal Research Center (MARC; Clay Center, NE), 156 samples of DNA, tail tissue or blood from both adult and young Duroc, Hampshire, Yorkshire, Landrace and Chester White pigs from animals in experimental herds at Iowa State University and 162 tail samples from adult Berkshire, Hampshire, Duroc, Landrace, Large White, and Pietran pigs provided by PIC USA (Franklin, KY). In addition, we also tested 41 NIH mini pig blood DNA and cell samples representing three different SLA haplotypes. This provided 374 of the animals of known breed, of which the genotype of the parents were known for 79. Both the breed and the parental genotype were known for 31 of the animals at Iowa State University.

2.2. Method of genotyping

Total genomic DNA was extracted using the DNAzol reagent (Molecular Research Center, Cincinnati, OH). Samples of blood, spleen, and liver were processed according to

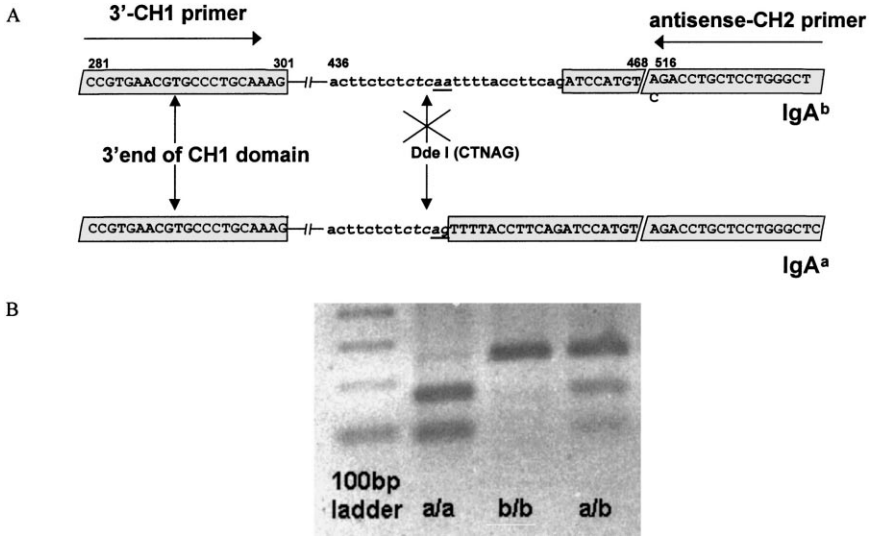

Fig. 1. (A) PCR strategy for amplification of C_H1/C_H2 IgA fragments. Lower case letters indicate the intronic region, while the boxed sequences indicate the exons. (B) RFLP results.

the manufacturer's published protocol. Another source of DNA, the tails from newborn piglets, was also used. Because the tail of pigs is difficult to homogenize, extraction of DNA necessitated some modification of the DNAzol procedure. Specifically, DNA preparation consisted of crushing ≈100 mg of tail tissue in liquid nitrogen, resuspending it in 1 ml of the DNAzol reagent, adding 20 μl of proteinase K (20 mg/ml), and incubating this mixture overnight at 37°C. The samples were then treated as specified in the manufacturer's protocol. All DNA samples were resuspended in 8 mM NaOH to a concentration of ca. 100 ng/μl. In some cases, small fleshy tissue fragments (ca. 2 mm across) were cut from tail samples and not crushed in liquid nitrogen but incubated with 20 μg of proteinase K at 55°C for 2 h and then for 15 min at 95°C. The samples were then ethanol-precipitated and resuspended in dH_2O.

Animals were genotyped by PCR-RFLP. Synthetic oligonucleotide primers corresponding to regions in the first and second domains of porcine IgA were used to amplify part of the α gene (Fig. 1). The first oligonucleotide, 5′-CCGTGAACGTGCCCTG-CAAAG-3′, corresponds to positions 281–301 in the first domain of the IgA gene. The second oligonucleotide, 5′-GAGCCCAGGAGCAGGTCT-3′, corresponds to the antisense of nucleotides 516–533 in the second domain. The reaction mixture was prepared by adding 100 ng of the total genomic DNA, 150 pmol of primers, 67 μm of each dNTP, 1 mM $MgCl_2$, and 1 U of Taq polymerase in a 30 μl volume. Reaction conditions for a Coy Temp cycler II (Coy, Grass Lake, MI) were: 94°C for 45 s, followed by 35 cycles of a program of denaturation at 94°C for 1 s, annealing at 62°C for 15 s, and extension at 72°C for 5 min. When used in a MJ PTC-200 cycler, the reaction conditions were: 94°C for 3 min, followed by 35 cycles of denaturation at 94°C for 5 s, annealing at 62°C for 30 s, and extension at 72°C for 5 s. A final extension cycle was performed at 72°C for 5 min.

After amplifying the desired regions of the IgA gene by PCR, the presence of an appropriate-sized product was confirmed by electrophoretic analysis in 1% agarose gel. One-third of the unpurified product was then incubated for 2 h at 37°C with one unit of Dde I endonuclease, 5 μg of BSA, and 2.5 μl of New England Nuclear #3 digestion buffer, in a final volume of 25 μl. The resulting fragments were analyzed after electrophoretic separation in a 1.5% agarose gel.

The 'a' allotype contains the sequence 'CTCAG' at the 3′ end of the first intron. This is cleaved by Dde I, which recognizes the sequence 'CTNAG'. The 'b' allotype has 'CTCAA' instead, so the enzyme is unable to cleave the PCR product encoded by the 'b' allele (Fig. 1). The result of this RFLP is a pattern of bands that allows the identification of an animal as either heterozygous or homozygous for IgA^a and IgA^b (Fig. 1).

2.3. Statistical analysis

Results obtained were analyzed by Chi-square (χ^2) to determine if the probability of finding a certain genotype distribution depends upon the breed or line of pig being tested (Lyman, 1993). In addition, the observed frequency of a/a, a/b and b/b individuals within each breed/line was tested according to the Hardy–Weinberg equilibrium. Observed and expected values were then tested for significance by χ^2.

3. Results

3.1. Frequency of IgA^a and IgA^b in offspring of known matings irrespective of breed

The analysis of the allelic frequency of IgA^a and IgA^b among *all* progeny of known matings but irrespective of breed, confirmed the previous results of Brown et al. (1995) for young animals. Namely, matings of heterozygotes yield the expected 1:2:1 ratio (Fig. 2A). However, when animals of known matings were tested as adults, the distribution of genotypes was skewed toward a/b animals (Fig. 2B).

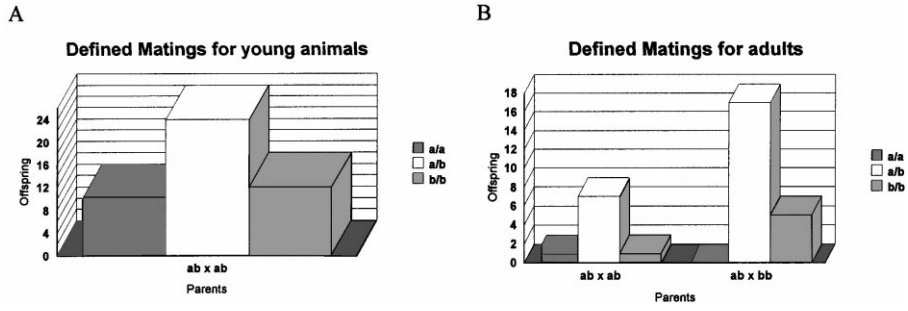

Fig. 2. Genotype frequency among progeny from parents of known genotype. (A) Genotype of newborn piglets from heterozygous matings between Durocs. (B) Allotype distribution among older animals of known progeny.

Table 1
Allelic distribution among breeds[a]

	a/a	a/b	b/b	Total	Hardy–Weinberg significance
Berkshire	2	11	0	13	χ^2=6.99, p<0.05*
	4.66	*6.61*	*1.73*		
Duroc	24	35	12	71	χ^2=0.06, p<0.5
	25.45	*36.12*	*9.44*		
Hampshire	14	45	7	66	χ^2=9.48, p<0.01**
	23.65	*33.57*	*8.77*		
Landrace	33	39	6	78	χ^2=1.44, p<0.25
	27.95	*39.68*	*10.37*		
Meishan	13	0	0	13	χ^2=0.00, p<0.5
	4.66	*6.61*	*1.73*		
NIH Mini pig	41	0	0	41	χ^2=0.00, p<0.5
	4.66	*6.61*	*1.73*		
Pietrain	6	3	0	9	χ^2=1.15, p<0.5
	3.23	*4.58*	*1.2*		
White Cross	8	27	18	53	χ^2=0.23, p<0.5
	18.99	*26.96*	*7.05*		
Yorkshire	13	14	3	30	χ^2=0.074, p<0.05
	10.75	*15.26*	*3.99*		
Total	154	174	46	374	
χ^2 =83.11, F=16	p-value=0.0005				

[a] Italicized rows indicate the expected counts from χ^2-test for breed association. The p-value of 0.0005 shows that the null hypothesis (the column classifications are independent) is false, since there is dependency between allotype distribution and breed. The extreme right hand column tests whether the allelic frequency within each breed fits the expected H-W distribution when tested by χ^2.

* Significant at 0.05; **significant at 0.01.

3.2. Frequency of IgA^a and IgA^b in young and adult pigs of the same breed

When the genotype frequency in both adult and young animals was analyzed according to breed, differences in genotypic distribution were statistically significant (Table 1). When adult animals were examined, some breeds, such as Hampshire, had a higher proportion of the 'a/b' genotype than did others. All Meishan pigs tested were 'a/a', while 'b/b' animals outnumbered 'a/a' animals 3:1 among the White Cross breed. With this exception, 'b/b' animals were outnumbered by 'a/a' animals at least 2:1 when *all* animals tested were considered. Chi-square analysis of the genotype distribution data also showed that there was a dependency between breed and genotype (Table 1). Furthermore, only two breeds differ from H–W equilibrium expectation.

When both young animals and adults of the same breed were compared, the allotypic distribution was similar (Fig. 3). Although the sample size was small, χ^2 analysis validates the results, with p-values well above the α-value of 0.05.

4. Discussion

The results presented in this report test a hypothesis that is based on the distribution of IgA^a and IgA^b in 160 randomly-sampled swine. This unpublished, preliminary study

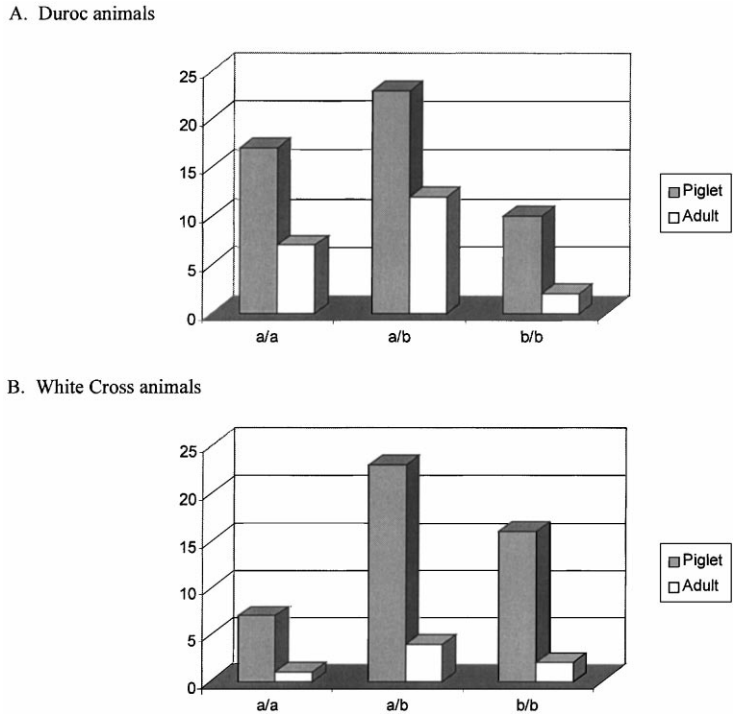

Fig. 3. Distribution of allotypes in young and adult animals of the same breed. Chi-square analysis gives a *p*-value for Duroc animals of 0.513, for White Cross it is 0.935, using a α-value of 0.05.

identified only two animals homozygous for IgA^b and the only female was identified in The Netherlands. The study reported here tests the hypothesis that IgA^b could be a negative selection trait in pigs prior to adulthood but with no effect on fetal or newborn piglets. An analysis of 374 purebred animals that were genotyped, including both adult and young pigs, indicated there were more than twice as many a/a and a/b animals as b/b animals (Table 1). However, we observed no unexpected shortage of b/b animals as we had observed in randomly-selected samples (Table 1). Although the number of progeny typed from the mating of animals of known genotype was small, the findings corroborate the results of Brown et al. for newborn piglets indicating that homozygous IgA^b piglets do not possess a lethal genotype (Brown et al., 1995). Nevertheless, if one assumes that the allotypic distribution among the original adult animals tested resembles that of the known matings, then b/b adults are found at a lower than expected number and heterozygotes are over represented. We also observed this effect in the 374 purebred animals studied (Fig. 2B). This suggested that b/b animals may have a problem to reach adulthood, possibly due to loss from infectious disease. This might indicate that the mutant IgA^b molecule is unable to perform effectively in protection against pathogenic infection. Using the same reasoning, since a/a animals are also under-represented, heterosis may offer the greatest advantage, perhaps because the hinge of IgA^a does display a single site for IgA protease attack that is absent in IgA^b (Mestecky et al., 1998; Brown et al., 1995).

Before reaching such a conclusion, it is important to compare the allelic and genotypic distribution in adult and young pigs of the same breeds. Compared in this manner, the allotypic distribution is essentially identical between adults and young pigs and no animals are seemingly lost from the populations as they grow to adulthood (Fig. 3). Survival data on a herd of White Cross animals shows that all five of the b/b animals observed reached adult age. Furthermore, the expected allelic frequency within breed calculated from the Hardy–Weinberg equation seldom differed from the observed allelic distribution. It therefore appears that the results of our initial typing studies, i.e. 160 random samples, and those presented in Fig. 2B, are the result of sampling bias. The unexpectedly low number of b/b animal and over-representation of a/b animals, resulted from pooling the data without regard to breed-dependent differences in allotype frequency. Analyzed according to breed, we show that allotype distribution is highly breed-dependent (Table 1). Duroc animals have a higher frequency of a/a animals than b/b, while the opposite is true for White Cross animals. Although all of the young pigs in this study were Durocs, NIH mini pigs or of White Cross breed, the allotypic distribution in the young animals is similar to that in adults of the same breed (Fig. 3). When random samples were studied, the majority of the 160 samples studied were obtained from PIC and the National Animal Disease Laboratory in Ames, Iowa and the pigs were primarily a mixture of Yorkshire and Landrace animals. Since we now know these breeds have a low frequency of b/b and an abundance of a/a and a/b (Table 1), random mating of animals from these breed would result in <3% b/b animals which is similar to the 1.3% of b/b animals among the original 160 animals tested.

The genes encoding MHC and Igs are linked on chromosome 7 (Rothschild and Ruvinsky, 1998). Since NIH Mini pigs representing three different SLA haplotypes were tested, our finding of complete homozygosity for the IgA^a allotype in this breed suggests that these pigs were derived from founders homozygous for IgA^a. Since there were only two founder pigs for all the NIH minipigs and since the IgA^a allotype is so common, this result is not unexpected. The homozygosity of the Meishan probably reflects their separate development from European pigs.

The results presented in this report do not support the view that IgA^b molecules which lack 2/3 of the normal hinge, predispose animals to a higher risk of postnatal mortality. Although IgA^a and IgA^b have not yet been molecularly modeled, 'survival data' among the offspring of at least Duroc and White Cross animals offer no evidence that the 'hingeless variant of porcine IgA' is any less effective in mucosal immunity. Thus, there is no obvious selective pressure to eliminate b/b animal and the breed related association of IgA allotypes reported here may be merely a consequence of the founder animals upon which the breeds were based. If both IgA^a and IgA^b offered a selective advantage, e.g. IgA^a is more flexible and IgA^b is resistant to IgA proteases, then one might expect heterozygous animals to be overrepresented in all breeds. Since this was not observed when each breed was individually examined, our findings collectively indicate that the splice site mutation in IgA^b does not effect the health of young piglets. Based on the known or presumed function of the antibody hinge, our findings are unexpected and therefore significant since they suggest that at least two-thirds of the hinge of IgA can be lost with no negative survival effect on animals bearing this trait. Our results do indicate that hinge allotypy in swine IgA can be a useful, breed- or founder-related genetic trait.

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References

Mestecky, J., Russell, M.W., Jackson, S., Brown, T.A., 1986. The human IgA system: a reassessment. Clin Immunol. Immunopath. 40, 105–114.

Mestecky, J., Kutteh, W.H., Brown, T.A., Russell, M.W., Phillips, J.O., Moldoveanu, Z., Moro, I., Crago, S.S., 1983. Function and biosynthesis of polymeric IgA. Ann. N.Y. Acad. Sci. 409, 292–306.

Vaerman, J.P., 1984. Effector mechanisms of IgA. Annales de Biologie Clinique. 42, 61–70.

Mazanek, M.B., Nedrud, J.G., Kaetzel, C.S., Lamm, M.E., 1993. A three-tiered view of the role of IgA in mucosal defense. Immunol Today 14, 430–435.

Bogers, W.M.J.M., Stad, R.K., Van Es, L.A., Daha, M.R., 1991. Immunoglobulin A: interaction with complement, phagocytic cells and endothelial cells. Complement Inflammation 8, 347–358.

Mestecky, J., Moro, I., Underdown, B.J., 1998. Mucosal immunoglobulins. In: Ogra, P.L., Mestecky, J., Lamm, M.E., Strober, W., Bienenstock, J., McGhee, J.R. (Eds.), Mucosal Immunity. Academic Press, NY, pp. 133–152.

Peppard, J.V., Russell, M.W., 1998. Phylogenetic development and comparative physiology of IgA. In: Ogra, P.L., Mestecky, J., Lamm, M.E., Strober, W., Bienenstock, J., McGhee, J.R. (Eds.), Mucosal Immunology. Academic Press, NY, pp. 163–179.

Butler, J.E., 1997. Immunoglobulin gene organization and the mechanism of repertoire development. Scand. J. Immunol. 45, 455–462.

Brown, W.R., Rabbani, H.H., Butler, J.E., Hammerström, L., 1997. Characterization of a bovine Cα gene. Immunology (British) 91, 1–6.

Brown, W.R., Kacvsckovics, I., Amendt, B.A., Blackmore, N.B., Rothschild, M., Shinde, R., Butler, J.E., 1995. The hinge deletion allelic variant of porcine IgA results from a mutation at the splice acceptor site in the first Cα intron. J. Immunol. 154, 3836–3842.

Plaut, G., 1983. The IgA proteases of pathogenic bacteria. Ann. Rev. Microbiol. 37, 603–622.

Lyman, R., 1993. An introduction to statistical methods and data analysis. In: Lozyaniak, K., Krikorian, S. (Eds.). Duxburry Press, pp. 357–361.

Rothschild, M.F., Ruvinsky, A., 1998. The genetics of the pig. CAB International. Appendix B.