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Escherichia coli and *Salmonella* Diarrhoea in Pigs

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Summary

Diarrhoea due to bacterial infection is a problem mainly in young growing animals, including pigs. Among the bacteria that cause diarrhoea are various strains of *Escherichia coli* and *Salmonella*. Considerable genetic variation in resistance and susceptibility has been found for both neonatal diarrhoea caused by *E. coli* carrying K88 fimbriae and post-weaning diarrhoea and oedema disease due to *E. coli* strains with F18 fimbriae, and the loci for both types of 'receptors' have been mapped. In mice, resistance to *Salmonella* infections is associated with the antimicrobial activity of macrophages and is linked with polymorphism in the *Nramp1* gene. The gene has been identified in several species, including the pig, but data are so far lacking concerning the association between polymorphism in the porcine gene and resistance and susceptibility to *Salmonella* infection.

The rapid development in molecular genetics has given us detailed genome maps and the tools to identify and study individual genes. This means that in the near future we may be able to determine the genotype of individual animals and to study the association between 'disease resistance' genes and production traits. This information is needed before we can include 'disease genes' in breeding programmes.

Introduction

Diarrhoea (scours) is a common problem in animal production, affecting mostly the young growing animal. Despite considerable interherd differences, large field studies have reported that a high average frequency (6–7%) of all litters born are affected with diarrhoea pre-weaning (Svensmark *et al*., 1989a) as well as post-weaning (Svensmark *et al*., 1989b). In another large study the mortality due to scours pre-weaning has been reported as 2.7% of the piglets born, representing 11.9% of the total mortality during that period (Nielsen *et*

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al., 1974). According to Svensmark *et al.* (1989a), piglets that had experienced pre-weaning diarrhoea reached 25 kg liveweight 2 days later than others, corresponding to a decreased productivity of 3%. Further, the risk for developing diarrhoea post-weaning increased if the piglets had experienced gastrointestinal disorders during the suckling period, and the risk of dying before reaching 25 kg body weight was increased fourfold for piglets having post-weaning diarrhoea (Svensmark *et al*., 1989b).

The routine use of feed additives (antibiotics) has been prohibited in Swedish pig production since 1986. Initially this led to an increased frequency of post-weaning diarrhoea and to decreased productivity of the piglets (Robertsson and Lundeheim, 1994). Thus, specific management and hygiene demands were required to prevent disease outbreaks in Sweden. The total mortality among piglets was 14.6% pre-weaning and 3.4% post-weaning in Swedish conventional herds during 1992, figures that were reduced to 7.0% and 0.1%, respectively, in specific pathogen free (SPF) herds (Wallgren, 1993). These differences, and the large interherd differences obtained between the conventional herds, demonstrate a large influence of management and environment on health status and productivity of piglets.

During the neonatal period, scours is generally associated with one pathogen, commonly *E. coli* or *Clostridium perfringens*. Among older piglets, various infectious agents may cause diarrhoea, among them bacteria such as *E. coli* and *Salmonella*. However, viruses and protozoa also contribute to the clinical status. Diarrhoea is thus a multifactorial disease where the outcome of an infection is due to many factors and their interactions. The genetic make-up of the bacteria, determining various virulence factors such as fimbriae enabling adherence to intestinal mucosa and enterotoxin production, is essential for the pathogenity of the bacteria. Although management and housing routines influence the frequency and severity of scours, the genotype of the pig also has a large impact on resistance and susceptibility to clinical infection. This chapter will deal mainly with the genetic resistance of pigs to bacterial infections leading to diarrhoea, focusing on *E. coli* and *Salmonella* infections.

E. coli Diarrhoea

The ability of enteropathogenic (EPEC) or enterotoxigenic (ETEC) *E. coli* to adhere to the brush borders of enterocytes is fundamental for the initiation of the infection. Attachment of pathogenic bacteria to the mucosa of the small intestine is mediated by distinct surface antigens, called pili or fimbriae (Duguid and Anderson, 1967). Several fimbrial adhesins have been identified both in animal and human EPEC/ETEC strains. In the pig, strains expressing fimbriae of F4 (K88), F5 (K99), F6 (987P) or F41 types dominate during the neonatal period (Söderlind *et al*., 1982; Brinton *et al*., 1983; Gonzáles *et al*., 1995), while strains expressing other types of fimbriae such as F18ab (F107), F18ac (2134P) and Av24 are found during the post-weaning period (Bertschinger *et al*., 1990; Nagy *et al*., 1992, 1996; Hide *et al*., 1995; Kennan *et al*., 1995).

Neonatal diarrhoea

Much neonatal diarrhoea is due to infections with *E. coli* strains possessing fimbriae of the F4 (K88) type. The frequency of K88 amongst enterotoxigenic strains isolated in various countries differs somewhat, but these strains mostly dominate (Söderlind and Möllby, 1978; Söderlind *et al.*, 1982, 1988; Brinton *et al.*, 1983).

Three antigenic variants, *ab*, *ac* and *ad*, have been identified for the K88 fimbriae, all containing a common a-type antigen (Ørskov *et al.*, 1964; Guiné and Jansen, 1979). The K88 fimbriae adhere to specific receptors on the intestinal cell brush borders. Early studies showed that the K88ac receptor contained a variety of sugar molecules, such as D-galactoside (Kearns and Gibbons, 1979; Sellwood, 1980), *N*-acetylglucoseamine, *N*-acetylgalactoseamine and D-galactoseamine (Sellwood, 1984). A later study has described the K88ac receptor as a mucin-type sialoglycoprotein (Erickson *et al.*, 1994). Glycoproteins of 210 and 240 kDa binding K88ab and K88ac, but not K88ad, fimbriae have been identified (Seignole *et al.*, 1994; Billey *et al.*, 1998). Another glycoprotein (74 kDa) belonging to the transferrin family that binds *in vitro* to K88ab fimbriae has been detected (Grange and Mouricout, 1996). However, K88ac and K88ad fimbriae did not bind to this intestinal transferrin.

Detection of receptor phenotype

Identification of the receptor phenotype of pigs can be performed by examining the adhesion of *E. coli* K88-positive bacteria to intestinal cell brush borders *in vitro* (Sellwood *et al.*, 1975). A variant of the assay, in which whole enterocytes instead of brush borders are used, has also been described (Rapacz and Hasler-Rapacz, 1986). Mostly, the intestinal specimens have been sampled after slaughter and the enterocytes have been collected by gently rubbing or scraping a segment of the intestine. The technique has also been performed on specimens from intestinal biopsies (Snodgrass *et al.*, 1981). To shorten the assay time, an enzyme immunoassay and an ELISA (enzyme-linked immunosorbent assay) have been developed (Chandler *et al.*, 1986; Valpotic *et al.*, 1989).

Testing potential breeding animals by intestinal biopsies or test matings are costly and cumbersome. Nor can any of the described assays differentiate between pigs carrying one or two copies of the receptor allele, i.e. distinguish between heterozygous and homozygous animals. Identification of the gene coding for the receptor structure will make direct typing of breeding animals possible and the genotype of individual animals can then be determined. Several laboratories are currently performing research towards this goal.

Inheritance of receptor phenotype

A genetic influence on resistance to ETEC was described as long as 30 years ago (Sweeney, 1968) and a Mendelian inheritance with a dominant receptor allele was later found for *E. coli* K88ac (Sellwood *et al.*, 1975; Gibbons *et al.*, 1977). One locus coding for both the K88ab and K88ac receptors was first suggested (Rapacz and Hasler-Rapacz, 1986; Bijlsma and Bouw, 1987), but later studies suggested two closely linked loci (Guérin *et al.*, 1993; Edfors-Lilja *et al.*, 1995).

The inheritance of the receptor for K88ad has been less clear and in several studies a weak adherence phenotype has been identified (Rapacz and Hasler-Rapacz, 1986; Bijlsma and Bouw, 1987; Hu, 1988). Studies by Hu *et al.* (1993) suggest that there are two receptors for K88ad, a high-affinity and a low-affinity receptor, both allelic to the K88ab and K88ac receptor(s).

Strong linkage disequilibrium between the *K88abR* and *K88acR* loci, with very few pigs positive for *K88abR* and negative for *K88acR*, has been found in most breeds studied so far (Bijlsma *et al.*, 1982; Edfors-Lilja *et al.*, 1986, 1995; Rapacz and Hasler-Rapacz, 1986; Hu *et al.*, 1993; Baker *et al.*, 1997). However, in one recent study a somewhat higher frequency of the K88abR$^+$–K88ac$^-$ phenotype was found in the Hampshire breed, i.e. four of 24 tested pigs (Baker *et al.*, 1997). With a recombination distance of 1–2%, one would expect to find the recombinant haplotypes *K88abR*$^+$–*K88acR*$^-$ and *K88abR*$^-$–*K88acR*$^+$ occurring at high frequency in some breeds. As this is not the case, there are two possible explanations for the strong association: (i) that haplotypes either positive for both *K88abR* and *K88acR* or negative for both are favoured by selection; or (ii) that the recombination frequency was overestimated in studies that suggest two loci, by typing errors or incomplete penetrance. The K88ab and K88ac proteins differ only slightly in amino acid composition (Gaastra *et al.*, 1979). The finding that the antibody response is not variant specific (Bijlsma *et al.*, 1987) might explain the linkage disequilibrium, as discussed by Ollivier and Renjifo (1991).

The receptor(s) for K88ab and K88ac have been determined in newborn as well as adult pigs. In contrast, it has been found that the weak adhesion phenotype for K88ad cannot be detected in pigs after the age of approximately 16 weeks (Hu *et al.*, 1993). A similar age influence has also been found for the adhesion of *E. coli* carrying K99 (Runnels *et al.*, 1980) and 987P fimbriae (Dean-Nystrom, 1995). A variation in amount of K88 receptor along the length of the small intestine has been reported (Chandler *et al.*, 1994).

Chromosomal localization and candidate genes

In humans, the P blood group constitutes the adhesion factor for urinary tract infections with pathogenic *E. coli* (Källenius *et al.*, 1980; Svensson *et al.*, 1983). Linkage studies between blood group loci and the K88 receptor have also been performed, and a weak linkage between the L blood group locus and the K88 receptor was found (Vögeli *et al.*, 1992). However, linkage had also been suggested between the transferrin locus and the K88ac receptor (Gibbons *et al.*, 1977; Guérin *et al.*, 1993). The *TF* and *EAL* loci have been assigned conclusively to two different chromosomes, the q31 band on chromosome 13 (Chowdhary *et al.*, 1993) and the q arm of chromosome 4 (Marklund *et al.*, 1993). The establishment of detailed linkage maps in the pig (Rohrer *et al.*, 1994; Archibald *et al.*, 1995; Marklund *et al.*, 1996) has improved the opportunities to map the receptor. The localization of the gene for the K88ac receptor to chromosome 13 has been confirmed in this way, and the locus for the receptor has been localized 7.4 cM proximal to the transferrin locus (Edfors-Lilja *et al.*, 1995). This chromosomal region is homologous to human chromosome 3 and, using comparative mapping, research is in progress to

map candidate genes in this region. Ten human chromosome 3 genes have been assigned to porcine chromosome 13 (Van Pouke *et al*., 1997; Peelman, 1998a). Further mapping indicates that the K88ac receptor locus is localized terminal to the transferrin locus, and one marker showing no recombination with the K88ac receptor locus has been mapped (Peelman, 1998b). Radiation hybrid mapping (Alexander *et al*., 1998) and intestinal cDNA libraries (Winterø *et al*., 1996) are other tools currently used to identify markers close to the receptor gene and possibly the causative gene itself.

Selection for the receptor phenotype – performance of sows and fattening pigs

The newborn pig is dependent on the mothering capacity of the sow, which includes the provision of antibodies in the colostrum and milk. Sows lacking the receptor produce low levels of antibodies to K88 after natural exposure or oral vaccination (Sellwood, 1979, 1982; Bijlsma *et al*., 1987). A small but significantly higher IgG response has been found in receptor-positive pigs 3 weeks after intramuscular immunization, suggesting that the immunization acted as a booster dose in receptor-positive pigs (Edfors-Lilja *et al*., 1995). This confirms earlier observations where pigs possessing receptors for K88ab and K88ac had a more pronounced IgG response to K88 after subcutaneous immunization than did pigs lacking the receptors (Edfors-Lilja *et al*., unpublished observations).

Although the receptors mediate increased susceptibility to neonatal *E. coli* diarrhoea, the function and significance of the receptors on a more basic level is not known. A low frequency of pigs possessing the receptor has been identified in breeds not selected for increased growth. No receptor phenotype pigs were identified in the Chinese Meishan breed (Chappuis *et al*., 1984; Michaels *et al*., 1994), while a low frequency of the receptor phenotype was found in the Chinese Minzu breed (Michaels *et al*., 1994). In another study, weak adhesion to intestinal cells, but with no correlation with virulence, was found for Chinese Meishan pigs (Bertin and Duchet-Suchaux, 1991). Both European wild boars that were used as parents in a reference pedigree for gene mapping lacked the receptor (Edfors-Lilja *et al*., 1995).

Post-weaning diarrhoea

Diarrhoea in the older pig is often associated with strains other than those causing neonatal diarrhoea. The change in diet at weaning and some nutritional components are thought to predispose to diarrhoea and oedema disease. The frequency of these problems differs largely between countries and populations, but breed differences have also been observed. In Switzerland, oedema disease and post-weaning diarrhoea are responsible for considerable economic losses (Bertschinger *et al*., 1992) and can also be a problem in adult pigs (Sydler *et al*., 1996). Oedema disease is rarely seen in pigs in Australia, but a majority of strains isolated from pigs with post-weaning diarrhoea were positive for F107 fimbriae (Hide *et al*., 1995). Similar results have been found in Denmark (Ojeniyi *et al*., 1992), where several outbreaks of oedema disease have

been reported recently (Jorsal *et al*., 1996). In Sweden, oedema disease has a low frequency, but almost 50% of *E. coli* strains collected from pigs with post-weaning diarrhoea contained the gene for a major subunit of the F107 fimbriae (Kennan *et al*., 1995).

The F107 fimbriae belong to a group of related adhesins named F18 (Imberechts *et al*., 1994). Like the K88 fimbriae, the F18 possesses a common antigenic variant *a*, and two variant-specific determinants, *b* and *c* (Rippinger *et al*., 1995). Another adhesion group is the F17 family (Bertin *et al*., 1996) which includes *E. coli* strains associated with bovine diarrhoea and human urinary tract infections (Martin *et al*., 1997).

Detection and inheritance of receptor phenotype

A genetic influence on the frequency of post-weaning diarrhoea and oedema disease was described 30 years ago (Smith and Halls, 1968) and has since been confirmed (Bertschinger *et al*., 1986). After the development of the adherence assay for identification of K88 receptor phenotype pigs, similar studies were performed to identify pigs resistant to oedema disease and post-weaning diarrhoea. Genetic studies have shown that susceptibility to colonization by F18ab-positive *E. coli* is dominantly inherited (Bertschinger *et al*., 1993). Further studies have mapped the locus for the F18ab receptor to chromosome 6, close to the genes for blood group system S and the calcium release channel, CRC (Vögeli *et al*., 1996). Two α (1, 2)-fucosyltransferase genes (*FUT1* and *FUT2*) are closely linked to the S and F18 receptor loci and a polymorphism in *FUT1* co-segregates with *E. coli* F18 adhesion (Meijerink *et al*., 1997).

Salmonella Diarrhoea

Salmonella infections are an important human health problem in many countries. Swine, poultry, cattle and seafood are important carriers (Wilcock and Schwartz, 1992). There are over 2 million cases of meat and poultry food-borne disease in humans in the USA per year, at a cost approaching US$1.4 billion. Most of this disease is attributed to *Salmonella* and *Campylobacter* infections (Menning, 1988). The most frequently reported *Salmonella* serotype is *S. typhimurium*. In addition to the economic impact of salmonellosis on the human population, it is also an economic disease of swine resulting in lost income to the pork industry. Data by Fedorka-Cray *et al.* (1994) suggest that two types of disease syndromes appear to occur after infection; clinical disease within 48 hours and a subclinical syndrome that may be important in establishing a carrier state.

In Sweden, an official control programme with respect to *Salmonella* spp. has been running since 1961 and was last revised in 1995 (Anonymous, 1995). Infected farms are subject to restrictions including a total ban of movements of animals, with the exception for transportation to sanitary slaughter. Regular controls are performed at the abattoirs, aiming to detect a prevalence of infection at 5% with a confidence level of 95%. The control is based on bacteriological examinations from five ileo-caecal lymph nodes per animal and from

surface swabs of approximately 1400 cm^2 per animal. Further, feed plants are controlled with respect to *Salmonella*. Regardless of the source, all isolations of *Salmonella* made have to be reported (Eld *et al*., 1991; Malmqvist *et al*., 1995). Together these measurements have accomplished low prevalences of animals positive to *Salmonella* at slaughter, i.e. well below 1% among broilers (Wierup *et al*., 1992) as well as among ruminants and pigs (Wahlström *et al*., 1997).

Genetic resistance

In chickens, differences between inbred strains in resistance to various serotypes of *Salmonella*, including *S. typhimurium* and *S. enteritidis,* have been described (Bumstead and Barrow, 1988, 1993). In these studies, birds were challenged orally, intramuscularly and intravenously, suggesting that resistance is not a function of adherence to epithelial cells. It was also shown that the resistance was inherited as a dominant autosomal trait and that it was not linked to the major histocompatibility complex (MHC). Further studies with *S. enteritidis* have confirmed these chicken strain differences in resistance and susceptibility (Guillot *et al*., 1995; Protais *et al*., 1996). In a recent linkage study, a region on chicken chromosome 5 has been identified that accounts for more than 50% of the difference in resistance between two chicken lines (Mariani *et al*., 1998). This region corresponds to part of mouse chromosome 12 and human chromosome 14, regions that so far contain no mapped genes likely to contribute to resistance.

In mice, a high level of resistance to *S. typhimurium* infection and other facultative intracellular bacteria is determined primarily by the *Ity/Lsh/Bcg* gene (Skamene *et al*., 1982; Lissner *et al*., 1983). This gene was later identified as *Nramp*, natural resistance-associated macrophage protein gene (Vidal *et al*., 1993). Nramp is a family of integral membrane proteins that have been identified in several species, including *Drosophila*, plants and yeast (Cellier *et al*., 1995). The function is not known, but it has been suggested that the Nramp polypeptides are part of a group of transporters or channels spanning the plasma membrane. The Nramp1 protein seems to have many effects in regulating macrophage activation, including respiratory burst activity, synthesis of nitric oxide synthase, antigen processing, MHC class II molecule expression and regulation of production and release of the cytokines TNF-α and IL-1β (reviewed by Blackwell, 1996).

The *Nramp1* gene has been identified and assigned in mice and humans to chromosomes 1 and 2, in chickens to chromosome 7 (Girard-Santosuosso *et al*., 1997) and in sheep to chromosome 2 (Pitel *et al*., 1994, 1995). A second gene, *Nramp2*, has been identified in humans and mice and localized to chromosomes 15 and 12, respectively (Gruenheid *et al*., 1995). In the pig, a full-length cDNA of *Nramp1* was recently sequenced (Tuggle *et al*., 1997). The gene has been assigned to chromosome 15 and a small population study revealed large allele frequency differences among breeds (Sun *et al*., 1998). Association between polymorphism and resistance or susceptibility to *Salmonella* infection is rather well documented in mice (Blackwell, 1996). In humans, data suggest

an association between polymorphism in the *Nramp1* promoter region and susceptibility to rheumatoid arthritis. Linkage studies to determine whether this polymorphism also contributes to infectious disease susceptibility are in progress (Blackwell, 1996).

Conclusions

Diarrhoea due to bacterial infections is a problem in pig production, both with regard to the loss in productivity and also from a human health view, as the pig can act as a carrier of human infections. Genetic resistance to neonatal diarrhoea caused by *E. coli* carrying K88 fimbriae has been known for many years. Also, genetic resistance to post-weaning diarrhoea or oedema disease due to *E. coli* with F18 fimbriae has been identified, and the loci for both types of 'receptors' have been mapped. The receptor for *E. coli* K88, as well as the receptor for uropathogenic *E. coli*, contains various carbohydrate molecules. The nature of the F18 receptor is not yet known, but a close linkage with two α-fucosyltransferase genes has been found.

Resistance to *Salmonella* infections is not associated with 'receptor' molecules, but with the antimicrobial activity of macrophages. In mice, this resistance is linked with polymorphism in the *Nramp1* gene. The gene has been identified in several species including the pig, but data are so far lacking for any association with resistance or susceptibility to *Salmonella* infection. The rapid development in molecular genetics has given us detailed genome maps and the tools to identify and study individual genes. This means that, in the near future we may be able to select breeding animals of preferred genotype. How to decide which genotype to select? Some data suggest that pig populations not selected for growth have a low frequency of the K88 receptor, but we do not yet have enough results to know whether receptor-phenotype pigs grow faster. Even fewer studies concerning the influence of the F18 receptor on production traits have been reported. As regards *Salmonella* infections, it has been suggested that good resistance might increase the frequency of autoimmune diseases.

In conclusion, we know far more about genetic resistance to bacteria caused diarrhoea than we did some years ago. The development of DNA-based tests will enable us to determine the genotype of individual animals, and hence it will also be possible to study association between 'disease genes' and production traits. Thus, in the near future we will have the knowledge to identify and select the preferred genotypes.

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