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Weaning of Pigs with Special Focus on the Intestinal Health

Lennart Melin

SWEDISH UNIVERSITY OF AGRICULTURAL SCIENCES

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Abstract

Post weaning diarrhoea (PWD) is a disease affecting pigs world wide. PWD is *E. coli*-associated but the background is clearly multifactorial. In this work, weaning of piglets was studied with focus on selected faecal microfloras and selected immune functions. However, the work also focused on health status and a model to induce PWD was developed. The work is divided in five separate studies:

In the first study, a weaning of healthy piglets was monitored. The phagocyting and killing capacity of neutrophils in serum was found sufficient from day 6 post partum and these functions were not affected by weaning. Despite absence of diarrhoea, a disturbance of the balance in the coliform flora was observed during the weeks post weaning.

In the second study, the influence of feed supplementation with 2500 ppm ZnO was monitored in healthy pigs. The disturbance of the coliform flora due to weaning was reduced.

In the third study, a weaning complicated by an exposure via the environment of a single pathogenic strain of *E. coli* was monitored. One pathogenic strain of *E. coli* was not sufficient to induce PWD. However, challenged pigs displayed a longer period with a disturbed coliform flora than control pigs. The leukocyte levels increased due to weaning regardless of bacterial challenge. Some immune responses were decreased due to weaning, but the pigs were found capable to mount relevant immune responses. The levels of these responses were however not validated.

In the fourth study, the influence of several pathogenic strains of *E. coli* was monitored. Further, ACTH was given to some groups simulating weaning under stressful conditions. Three pathogenic strains together induced PWD. PWD was also induced by one serotype in connection to shed of rotavirus. Rotavirus itself did not induce PWD. ACTH was not required to induce PWD, but amplified clinical signs.

In the fifth study, different feed related prophylactic measures to prevent PWD were monitored. ZnO-enrichment of feed, meal feed with lactose and dietary fibres; and a probiotic comprising 60 non-pathogenic *E. coli*-strains all appeared to have a potential to prevent PWD.

Keywords: pig, weaning, diarrhoea, *Escherichia coli*, infection model, faecal microflora, diversity, immunology, probiotics, feed

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Papers I-V

The present thesis is based on the following papers, which will be referred to by their Roman numerals:

I. Melin, L., Jensen-Waern, M., Johannisson, A., Ederoth, M., Katouli, M. & Wallgren, P. 1997.
Development of selected faecal microfloras and of phagocytic and killing capacity of neutrophils in young pigs.
Veterinary Microbiology 54, 287-300.

II. Katouli, M., Melin, L., Jensen-Waern, M., Wallgren, P. & Möllby, R. 1999.
The effect of zinc oxide supplementation on the stability of the intestinal flora with special reference to composition of coliforms in weaned pigs.
Journal of Applied Microbiology 87, 564-573.

III. Melin, L., Katouli, M., Lindberg, Å., Fossum, C. & Wallgren, P. 2000.
Weaning of piglets. Effects of an exposure to a pathogenic strain of *Escherichia coli*.
Journal of Veterinary Medicine B 47, 663-675.

IV. Melin, L., Mattsson, S., Katouli, M. & Wallgren, P. 2001.
Development of post weaning diarrhoea in piglets. Relation to presence of *Escherichia coli*-strains and rotavirus.
Submitted for publication

V. Melin, L. & Wallgren, P. 2001.
Post weaning diarrhoea in piglets. Aspects on the influence of feed related prophylactic measures.
Submitted for Publication

Abbreviations

ACTH	Adrenocorticotropic hormone
APC	Antigen-presenting cell
BPT	Biochemical Phenotype
CFU	Colony forming unit
C. perfringens	Clostridium perfringens
Con A	Concanavalin A
DWG	Daily weight gain
E. coli	Escherichia coli
ETEC	Enterotoxigenic E. coli
Ig	Immunoglobulin
IL	Interleukin
IL-2R	Interleukin-2 receptor
LT	Heat labile toxin
MHC	Major histocompatibility complex
PBMC	Peripheral blood mononuclear cells
PWD	Postweaning diarrhoea
PWM	Poke Weed Mitogen
ST (a and b)	Heat stabile toxin

Introduction

Gastro-intestinal disturbances cause diarrhoea and general discomfort. Some intestinal infections, such as *Salmonella*, may affect man and animals at any age, whereas other types of diarrhoeas are age-related. Neonatal diarrhoeas in piglets may be lethal due to a ceased feed intake and loss of fluids, electrolytes and energy. Diarrhoeas are generally considered the most important disease–complex among young animals, *i.e.* in pigs weighing less than 25 kg, but also elder piglets can be severely affected by intestinal diseases.

In domesticated pigs, the weaning is a moment that increases the risk for development of diarrhoea, partly because the piglet instantly is forced to switch from a diet mainly based on milk to cereals. Indeed, postweaning diarrhoea (PWD) is common and in Danish piglet production a figure of 6% has been documented (Svensmark et al., 1989). In Sweden, a ban for use of growth promoters, *i.e.* routine in-feed medications with low dose antibiotics, was effectuated in 1986. During the subsequent year the age at 25 kg body weight increased with 7 days and the mortality post weaning increased with 1.2% (Robertsson and Lundeheim, 1994). Since then, the situation has improved and the performance of today is better than before the ban, mainly due to improved management systems. Still, PWD is the major problem within piglet production. Today PWD is generally treated with zinc oxide (ZnO) in Sweden and this use corresponds to a treatment incidence of 9% against PWD in the Swedish pig population (Odensvik et al., 1999).

The gastro-intestinal tract is basically located outside the body and inhabited by hundreds of bacterial species, and it is the balance of that flora that commonly is referred to as "intestinal health". The intestinal flora develops through a process of microbial successions in the intestine, and has a tremendous role in the state of health and disease of pigs, not the least during the suckling and post-weaning periods. PWD is commonly associated to *E. coli* infections, but appears to be dependent also on other provoking factors (Madec and Josse, 1983; Spencer et al., 1989; Nabuurs et al., 1993; Hampson, 1994). Other important factors comprise the influence of the intestinal physiology, diet regimes and food compositions, immune status of the pig as well as environmental factors.

The development of the gastrointestinal tract of the young pig

Intestinal physiology

The functional development of the gastrointestinal tract is dependent on an intrinsic interaction between genetics, endogenous regulatory mechanisms and environmental factors (Lebentahl, 1989). After birth, the degradation of feed to absorbable molecules provides the animal with energy and nutrients. In piglets, the stomach weight increase faster than the body weight during the first three

days of life (Xu et al., 1992). The primary site of tissue growth is the mucosa of the fundic region where the major exocrine secretions take place. The secretions mainly comprise hydrochloric acid (HCl) from the parietal cells and various proteolytic enzymes from the chief cells. The capacity to secrete acid is low at birth, but because of the rapid growth it increases up to seven times during the first week of life (Xu and Cranwell, 1990).

All gastric proteolytic enzymes are secreted as zymogens that require hydrogen ions for their conversion into active enzymes (Foltmann, 1966). In newborn piglets, the conditions are adapted to a special situation. Chymosine, which primarily is a milk-clotting enzyme, has a pH optimum at 3-4 while the major proteolytic enzyme, pepsin A, has a pH-optimum of 2. The relatively high gastric pH in neonatal piglets reduces the pepsin A activity, but stimulate the chymosine activity. Chymosine acts specifically against the k-casein in the milk protein, but do not degrade the peptide bonds further. This allows entrance of undegradated peptides and immunoglobulins into the small intestine. During the first day of life, with a maximum within 4-12 h after birth, these macromolecules can be absorbed across the jejunal epithelium into the lymphatic vessels (Holland 1990). This ability declines rapidly and is referred to as "gut closure" (Westrom et al., 1985). The clotting of the milk does also influence the distension of the ventricle and thereby the gastric emptying (Decuypere et al., 1986).

At birth the length of the small intestine of the piglet is 2-4 m, out of which the jejunum comprise 90% and duodenum and ileum 5% each (Nickel et al., 1993). These proportions are kept throughout the life of the pig. Within the first days of life, the area of the intestinal epithelium is enlarged by 60 to 70 % and the length of the intestinal tract is increased by 1 meter (Sangild, 1999). During the first four weeks of life, the length of the small intestine will increase further to about 10 meters. About 25 % of digested proteins are required to allow this growth (Sangild, 1999).

Epithelium-covered finger-shaped villi and crypts constitute the luminal surface. The epithelium is derived from stem cells in the bottom of the crypts. By differentiation, they give rise to secreting and absorptive enterocytes as well as mucin producing goblet cells. The digestive functions of the enterocytes begin when the structural development is completed and cells move towards the villous tip. The absorptive functions start later, above the mid level of the villous. The time from stem cell to the shed from the top of the villi is 2-4 days (Moon, 1971). At birth, the villous are relatively low and sparsely distributed over the surface. During the first 10 days of life the length and diameter of the villi as well as depth of the crypts will increase.

The digestive tract of the newborn piglet is specialised for a diet comprising of milk, and a high lactase activity is present in the small intestine of the piglet (Hampson and Kidder, 1986). Other organs important for the digestive system, such as pancreas (Kelly, 1991a), are quite inactive during intensive suckling. As

piglets start to consume solid feed, other systems become important and the lactase activity rapidly declines during the post-weaning period (Hampson and Kidder, 1986). Instead, production of saliva with a-amylase and pancreatic juice is increased (Kelly et al., 1991a) (Kelly et al., 1991b).

The milk constitutes a compact food, and the intestine (especially the large intestine) is comparably small during the suckling period (Kelly et al., 1991a). At weaning domesticated piglets are offered cereals instead of milk and therefore the stomach and the intestine rapidly increase in size (Kelly et al., 1991). Despite this, the ability to absorb nutrients might decrease due to a reduction in the height of the intestinal villi during the post-weaning period (Hampson, 1986), resulting in a decreased total area of the surface of the intestinal lumen. The diet might also influence the size of the intestine during the subsequent rearing of pigs. Fibre-rich feed sources are correlated to an enlargement of both stomach and large intestine (Anugwa et al., 1989). The latter is rather expected, because fermentation takes place in the large intestine as well as absorption of electrolytes and fluids.

Intestinal Immunology

Several species-specific aspects contribute to the immunological conditions of the young piglet. The specialised epitheliochorial placenta does not allow an intra uterine passage of maternal IgG to the foetuses why piglets are born without any passive immune protection. However, fetal lymphocytes develop already during the first trimester (Sinkora et al., 1998), and these lymphocytes can respond with IgG production to antigen exposure during the second trimester (Salmon, 1984) (Trebichavsky et al., 1996). Still, the immune system of the newborn piglet is anatomically and functionally immature (Stokes and Bourne, 1989) and the survival of the piglet is dependent on an adequate transfer of IgG via colostrum.

During the last month of gestation, a transfer of serum immunoglobulins to the udder occurs (Holland, 1990; Wallgren, et al., 1998). As a result, IgG constitutes 80% and IgA 10% of the total amount of Ig in colostrum (Wingstrand, 1992) (Salmon, 1999). The high content of IgG in colostrum offer the piglets a passive protective immunity. In addition, a homing (see below) of IgA to the udder starts two weeks prior to farrowing and continues throughout the entire suckling period (Evans, et al., 1980). Therefore, IgA constitutes around 60 % of the total amount of immunoglobulins in milk throughout the suckling period (Klobasa and Butler, 1987). This continuous presence of IgA in the milk will enforce the local immunity in the gut of the piglets, for instance by preventing adhesion of bacteria or toxin to epithelial cells, by killing pathogens directly or via antibody dependent cell-mediated cytotoxicity (Kagnoff, 1993). However, this specific active immunity of the porcine intestine does not develop until 4-7 weeks of age (Gaskins and Kelly, 1995).

The half-life of maternal IgG is around 14 days depending on the infectious load (Bourne, 1973) and neonatal piglets are not yet able to compensate for this loss (Klobasa and Butler, 1987). Indeed, the presence of maternal IgG may also obstruct an endogen production of antibodies (Pedersen and Jensen, 1981). Consequently, pigs will experience a dip of circulating IgG when aged 2 to 5 weeks (Bourne, 1973) when they easily succumb to infections.

A bacterial infection/translocation leading to an inflammation will cause a local generation of complement fragments, particularly C5a. In turn, this leads to the expression of adhesion molecules on the endothelial lining of the capillaries of that area (Butcher, 1991). Neutrophils express receptors that bind specifically to these adhesion molecules (Butcher, 1991) and can then penetrate the capillary wall. This enables circulating neutrophils to respond fast and precise. In the inflamed tissue neutrophils will engulf and thereafter kill invading bacteria through release of lysozymes. When at a low level of energy the neutrophil will autolyse, and by the release of lysozyme it will continue the local inflammatory reaction. The inflammation will also attract macrophages that will phagocyte invading bacteria and the cell debris. Neutrophils are present in the circulatory system already when piglets are born, but they increase in number during the first weeks of life (McCauley and Hartmann, 1984). However, a low chemotactic (Stokes et al., 1992) and a low capacity to phagocytose (Hoskinson et al., 1990) during the first three weeks of life have been reported.

The special situation in the gastrointestinal tract with exposure to an abundance of food and microbial antigens demands special qualities from the immune system. To react prompt against everything "foreign" would be self-detrimental, but still a relevant response against pathogenic organisms must be allowed. Anatomically and physiologically, the adaptation can be revealed in the layout of immunologically competent cells for recent review see (Solano-Aguilar et al., 2001).

In the mucosa lymphocytes are found in large numbers in three main regions;

a) Within the epithelial layer
b) Scattered throughout the lamina propria
c) In the Peyers patches

a) The epithelial layer: Several of the pathogenic intestinal microbes are located intracellularilly *e.g.* rotavirus and *Isospora suis*. Antigens from these microbes will therefore be presented by MHC I molecules on the surface of infected epithelial cells. An attack with $CD8^+$ cytotoxic T-cells would be the appropriate response. Indeed, between the villous epithelial cells are mainly $CD8^+$ cytotoxic T-cells (Kagnoff, 1993). These cells have a limited range of specificities that is distinct from most other T cells.

b) The lamina propria: Within the lamina propria, all different types of immune cells are found. Macrophages are the predominant type of APC in the pig but also

dendritic cells are present. Both T- and B-lymphocytes are found from birth but a dramatic increase in cell number is seen over the first 4 weeks (Bianchi et al., 1992). In the first week, this is mainly due to a rise of $CD4^+$ T cells. $CD8^+$ T cells increase more slowly over the first 7 weeks. $CD4^+$ cells will predominantly be found in the centre of the villous and $CD8^+$ T cells near the basement membrane (Stokes and Bourne, 1989). In the adult animal $CD8^+$ T cells will be the predominant type in the whole of lamina propria.

c) The Peyers patches: These foci of lymphoid tissue are situated in well-defined areas just underneath the epithelial layer. There are approximately 30 discrete Peyers patches in the jejunum, one long ridge in the Ileum and approximately 10 in the colon (Nickel et al., 1993). These numbers and positions will remain constant throughout the life of the pigs, but the size will increase due to hypertrophy. The Peyers patches are covered with a specific epithelial layer of so called M-cells.

Intestinal microflora

In the piglet, as in all other species, the gastrointestinal tract is sterile at birth. However, bacteria can be isolated from the gut within three hours after birth (Ducluzeau, 1983) and within 12 h up to 10^9 bacteria per gram can be isolated from faeces (Smith, 1965). The sequence of appearance of different bacterial species in faeces from different animal species is similar but not identical (Smith and Crabb, 1961). In piglets, the initial colonisers include *E. coli*, *Streptococci*, *Clostridia* and *Lactobacilli*. Later anaerobic bacteria such as *Bacteroides*, *Bifidobacteria* and *Peptococci* will dominate (Smith and Crabb, 1961). The source for this colonising is initially the vaginal tract, but it is rapidly dominated by skin, milk and faeces of the sow. However, Katouli et al. (1995, 1997 and 1999) have found differences on day seven post partum between the faecal coliform floras of different piglets within litter, but also between the offspring and the faecal coliform flora of the sow.

The survival and growth of ingested microbes is mainly controlled by the pH in the ventricle. This in turn is chiefly dependent of the secretion of HCl. In the newborn piglet, this capacity is low. The rapid colonisation of lactic acid producing bacteria such as *Lactobacilli* and *Streptococci* may then help to maintain a low pH during the first week of the life of the piglet (Fuller, 1982). Indeed an inoculation with *Lactobacillus fermentum* and *Streptococcus salivarius* were found to reduce the gastric content of E. coli in neonatal piglets (Fuller, 1982). The density of the total gastric population of adherent anaerobic/facultative anaerobic bacteria may be about 10^6 per cm^2 (McGillivery and Cranwell, 1992). Typical species would be *Actinomyces*, *Bifidobacterium*, *Clostridium*, *Lactobaccili*, *Streptococcus*, *Staphylococcus*, *Escherichia* and *Klebsiella*.

Intestinal populations can be described by analysing the way they utilise different substrates, and biochemical fingerprint can be defined (Möllby et al., 1993; Kühn, 1985). By comparing such fingerprints from different pigs it was concluded that the faecal flora of sows and their offspring were very similar three days post partum, which indicates that the sow is an initial source of the intestinal flora in piglets (Katouli et al., 1997). However, the piglets develop microfloras that differ from the sow during the suckling period. Littermates develop very similar intestinal floras during suckling. Penmates will always develop comparable similar floras with time. However, the intestinal population will alter with time due to aging and altered feed habits (Katouli et al., 1997).

Modern pig production

In modern systems, pigs are allowed to suckle for a few weeks and are thereafter referred to as weaners until a weight of around 25 kg. At that weight, they are allocated to fattening units. The pig production of today is under constant development towards higher efficiency. In this way high quality food for human consumption can be delivered at low costs. This is mainly achieved through rationalisations and increased specialisation. The process also involves a development towards fewer but larger production sites. The health status of the pigs is a crucial factor, especially since even mild (sub-clinical) sessions of disease may enforce high costs for the producer (Wallgren, 2000).

This put high demands on the producers to improve attitudes and routines for husbandry. The introduction of age-segregated systems has decreased spread of infections from elder to younger pigs (Clark et al., 1991; Lindahl and Wallgren, 1997). Batch wise rearing of pigs from birth to market weight is now effectuated in around 80% of the Swedish pig herds. The prevalence of herds approved by a quality assurance system demanding All in-All out production ranges from 75 to 87% between regions (G. Johansson, SQM, Stockholm, Sweden, personal communication). Due to increased health status, pig in age-segregated farrow to finish enterprises reach market weight (107 kg body weight) at the age of 180 days. This corresponds to an improved weight gain with 15% compared to pigs in continuous rearing systems that reach market weight at an age of 207 days (Wallgren et al., 1993).

With a strong and sound biosecurity, pigs can be protected from exposure to a number of potential pathogens. In such Specific Pathogen free (SPF) systems, the growth rate has been further improved. These pigs reach market weight at an age of 149 days, corresponding to an increased DWG with 39% when compared to conventional pigs reared in continuous production systems (Wallgren et al., 1993).

Health is not only beneficial from an economical point of view. It also improves animal welfare, and has several positive ecological advantages. As

health improves the weight gain the rearing period will be shorter, and the nitrogen burden on the environment will decrease. The feed protein content is 15%, out of which around 16% is pure nitrogen. Pigs utilize around 75% of the protein consumed. Around 50% of the remaining nitrogen can be utilised in the rotation of crops, while the rest will contribute to the nitrogen overload. As Swedish pigs consume around 6000 tonnes of feed daily, they will contribute to that overload with around 6,570 tonnes of nitrogen annually (Wallgren, 2000). Every shortening of the fattening period with one day will annually reduce that overload with 33 tonnes. Healthy pigs also contribute to a decreased environmental contamination with antibiotics, zinc or other chemical agents used to treat diseases. In this matter its noteworthy that a decreased exposure of antibiotics to microbes will decrease the risk for development and spread of bacterial resistance towards antimicrobals (Aarestrup et al., 2001).

Weaning in modern pig production

In undomesticated pig populations the piglets are weaned at an approximate age of 16 weeks (Jensen and Recèn, 1985). The sow generally initiate suckling by grunts during the first week after farrowing, but from the second week of life suckling is generally initiated by the piglets (Algers, 1993). Therefore, free-living sows continuously reduce the contact with their litter from the second week post partum. Domesticated sows share pen with their offspring and must be protected from to intensive suckling. In practise this leads to weaning at an earlier age. In modern agriculture, systems weaning often takes place at the age of two to six weeks. In Sweden, the animal welfare law prohibits weaning of piglets younger than 4 weeks, and most of the farms effectuate the weaning at a mean age of around 5 weeks.

The free-living pig is fairly well developed with respect to immune functions as it is finally weaned, and it is well equipped to meet challenges from the environment (Joling et al., 1994). It is however not only the age that differs the weaning of free-living and domesticated pigs. The non-attendance of the dam forces the free-living piglets to search for supplementary food and therefore weaning of free-living pigs is a gradual process in which the piglet slowly will be adapted to feed of non-milk origin. There is no practical way to imitate the gradual weaning that free-living piglets experience (Kunavongkrit et al., 1985). The domesticated piglets have constant access to the dam and as they prefer milk they only consume minor amounts of creep feed during the suckling period. When the sow is taken away at weaning the switch from milk to cereals is instant for the domesticated piglets. Further, the piglets will experience an immediate loss of the local intestinal protection provided by the IgA present in the milk of the sow.

It could be questioned how mature the farmed piglet is with respect to immune functions at weaning, because the percentages of $CD2^+$, $CD4^+$, and $CD8^+$

lymphocytes still increase (Bianchi et al., 1992; Joling et al., 1994) and the in vitro ability of lymphocytes to proliferate (Wattrang et al., 1998) and to produce antibodies (Wallgren et al., 1998) are low. Further, weaning itself has been suggested to be immunosuppressive (Bailey et al., 1992; Hessing et al., 1995; Wattrang et al., 1998). Piglets are capable to mount an immune response to antigens and feed components already at the age of three weeks (Welch et al., 1988; Bailey et al., 1992). However, tolerance to common fed proteins is not fully achieved until the piglet is aged 8 weeks. Further, the magnitude of primary responses towards novel dietary components decrease with age (Miller et al., 1984; Wilson et al., 1989; Miller et al., 1994). Indeed failure to down-regulate immune responses towards harmless dietary proteins or commensal bacteria has been suggested to contribute to outbreaks of PWD (Miller et al., 1984; Li et al., 1991).

Diarrhoea in young pigs

Diseases are a matter of great concern within pig production and for the young piglet gastrointestinal disturbances constitute the major problem (Svendsen et al., 1975; Svensmark et al., 1989; Cutler et al., 1999). These diseases can be classified according to the causative agent. Many microorganisms have been isolated in connection with outbreaks of diarrhoea in young pigs. In a global perspective *E. coli*, *C. perfringens*, *Isospora suis*, rotavirus, Transmissible Gastroenteritis virus (TGEV) and *Salmonella* spp are among the most commonly isolated microbes (Cutler et al., 1999). However, TGEV is not present in Sweden (Elvander et al., 1997) and *Salmonella* is only rarely observed in Swedish pigs (Anonymous, 2000). Consequently, TGEV and *Salmonella* are not discussed further in this manuscript.

In common practice diarrhoeas are classified according to the age of the affected animal. Neonatal diarrhoeas occur during the first week of life, suckling diarrhoeas from the age of one week to weaning and postweaning diarrhoeas (PWD) generally take place during the first two weeks post weaning. Below the diarrhoeas are described according to the age of the affected piglets and the causative agens.

Neonatal diarrhoeas

Neonatal diarrhoeas are generally of a unifactorial origin, most commonly induced by either *E. coli* (Alexander, 1994) or *C. perfringens* type C (Morin et al., 1983). Great interest has also been paid to infections with *C. perfringens* type A (Nabuurs et al., 1983) (Jestin et al., 1985).

E. coli

Pathogenic strains of *E. coli* are often referred to as enterotoxigenic *E. coli* (ETEC). They adhere to the intestinal wall via one or more fimbria and by release of one or more enterotoxins they induce diarrhoea. The fimbrial adhesines consist of structural proteins supporting the adhesive protein on the top of the fimbrie. More than 30 different types are described and classified by serologic reactivity according to a system proposed by (Orskov and Orskov, 1983). The fimbria F165 has occasionally been associated to neonatal diarrhoea induced by *E. coli* O8 (Fairbrother et al., 1989). F 165 is not specified in vaccines available in Sweden and occasionally neonatal *E. coli* O8 diarrhoea is recorded in spite of proper vaccination routines (unpublished data).

The adhesines F4 (K88), F5 (K99), F6 (987P) and F41 are considered the most important adhesines in ETEC-associated diarrhoea in pigs. The production of F41 is controlled from chromosomal genes while F4, F5 and F6 are controlled by genes on plasmids (Bertschinger and Fairbrother, 1999). Therefore, the latter genes are more transmissible to other bacterial cells. The fimbriae adhere to specific receptors on the epithelial cells of the small intestines. F4-positive ETEC tends to colonize the whole length of both jejunum and ileum while ETEC with F5, F6 and/or F41 colonise the distal part of jejunum and the whole of ileum (Bertschinger and Fairbrother, 1999). However, the susceptibility to F5 positive ETEC declines from the age of two weeks, possibly due to a reduction of the number of receptors. (Runnels et al., 1980).

Some piglets lack the F4-receptor. This is recessively inherited and the loci for F4ab and 4ac are closely linked to chromosome 13 (Edfors-Lilja et al., 1995). Also an inherited resistance towards verotoxin producing strains of *E. coli* that induce oedema disease has been discussed, because the receptor for the F18-fimbriae is linked to chromosome 6 (Bertschinger et al., 1993).

To induce diarrhoea ETEC that adhere to the epithelial must also produce enterotoxins. These could be divided into heat labile toxins (LT) or heat stabile toxins (STa + STb) (Smith and Gyles, 1970). LT is a complex toxin with high molecular weight. It binds to a ganglioside receptor and activate adenylat cyclase that increase the production of adenosine monophosphate (cAMP). This results in a secretion of Cl, Na, HCO_3 and water into the lumen (Gill et al., 1981). STa on the other hand is a small protein that stimulates guanylyl cyclase, which stimulates the production of cyclic guanosine monophosphate (cGMP). This reduces the absorption of electrolytes and water from the intestine (Dreyfus et al., 1984). STa is most active in pigs younger than two weeks (Cohen et al., 1988), and the effect of STa declines by aging of the piglets. STb is a small protein unrelated to STa. The mechanism behind the stimulation of secretion is not yet totally clear but the function appears to be mediated by prostaglandin E2 (Harville and Dreyfus 1995). Strains that only produce STb have been proven

able to induce diarrhoea (Fairbrother et al., 1989) and generate some villous atrophy (Rose et al., 1987)

Table 1. Distribution of *E. coli* serotypes isolated from Swedish piglets aged less than one week and expressing signs of neonatal colibacillosis

Serotype		1964-67* (n=133)	1969-70** (n=521)	1983-84¤ (n=252)	1994# (n=62)	2000# (n=113)
O8	%	17	7	5	8	5
O9	%	1	-	11	2	2
O20	%	3	-	5	2	4
O64	%	2	-	9	0	1
O101	%	3	-	22	15	17
O139	%	2	-	-	0	0
O141	%	9	-	4	0	1
O147	%	13	-	-	0	0
O149	%	7	44	20	5	4
Other	%	23	-	18	3	7
O?	%	20	-	6	65	59

* = post mortem, piglets with intestinal disorders (Söderlind, 1971)
** = serotype present in herds with neonatal diarrhoea (Söderlind 1971)
¤ = faecal samples from piglets with diarrhoea (Söderlind et al., 1988)
= faecal samples from piglets with diarrhoea (Melin et al., unpublished)

E. coli induced diarrhoeas in neonatal piglets are treated with antibiotics, and the sensitivity towards antimicrobals among coliforms isolated from Swedish pigs is favourable (Melin et al., 1996) (Melin et al., 2000). In severe cases, fluid therapy with an electrolyte replacement containing glucose could be considered.

For prevention, a good husbandry including age-segregated production with good hygienic standards are of importance. This will decrease the load of pathogenic microorganisms on herd bases and the piglets can be provided with optimal environmental conditions. Vaccination of sows against *E. coli* has successfully prevented this type of diarrhoea (Moon and Bunn 1993). With a strategic vaccination prior to farrowing piglets can receive a protective passive immunity (Holland, 1990).

Clostridium perfringens type C

Clostridium Perfringens type C is an encapsulating sporforming grampositive bacillus that infects the newborn animals orally within minutes after birth. It multiplies attached to the apexes of the villi of jejunal epithelium. After

desquamation of that epithelium the microbe will proliferate along the basement membrane inducing a complete necrosis of the lamina propria. The bacteria will then continue to proliferate in injured tissue and in the protein-rich intra luminal debris of blood and destroyed cells.

C. perfringens type C produce an alpha-toxin and a necrotising beta-toxin, out of which the latter is often lethal. The beta-toxin will spread from the intestine via the blood to other organs and induce tissue damage. This is mainly seen as haemorrhages and emphysema in the mesenteric lymph nodes and in the peritoneum. However, death is primarily believed to be a result of the intestinal damage and to a lesser extent from the extra intestinal effects of the toxin (Taylor and Bergeland 1999). The lesions found at autopsy will be tissue damage and haemorrhages caused of the beta toxin. Their graveness will be dependent on the course of the disease.

The onset of clinical signs normally occurs within the first 2-3 days after birth, and the clinical signs vary according to the age and immune status of the affected piglet (Taylor and Bergeland 1999). The disease is generally classified as peracute, acute, subacute or chronic. In the peracute form piglets will seem normal for the first 10 hours of life, but thereafter rapidly become weak, moribund and sometimes develop hemorrhagic diarrhoea. Typically they will be found dead within 36 hours after birth. In the acute variant, piglets will survive for a couple of days shedding reddish-brown liquid faeces containing grey necrotic debris. However, piglets will gradually loose condition and generally die within 3 to 4 days of age. In the subacute form, the animals will have persistent diarrhoea without haemorrhage for five to seven days. During that time, the pigs will be fairly alert and have a fair appetite. Still, they will progressively become thin, dehydrated and often die. In the chronic form, piglets may display intermittent or persistent non-hemorrhagic diarrhoea for a week or more. Affected piglets may die after as much as 10 days, but can also survive. In these cases, the growth rate will be retarded.

In a global perspective this disease is less important than ETEC diarrhoea. However, within herds the disease may be very grave. Due to the intestinal damage present already at the onset of clinical signs there is a poor prognosis of treatment (Hogh, 1967). In affected herds, healthy piglets can be treated daily with oral antibiotics for the first three days of life to prevent disease. However, thorough vaccination of pregnant sows that provide piglets with a passive immunity will be preferable (Ripley and Gush 1983).

Clostridium perfringens Type A

Clostridium perfringens type A is ubiquitous and present in porcine gut content, faeces and soil. Finishing pigs and sows generally have antibodies to the bacteria (Estrada Correa and Taylor, 1989). Therefore, it has been considered a part of the

normal flora (Mansson and Smith, 1962). However, *C. perfringens* type A has increasingly been connected to enteric disease in both neonatal and weaned pigs (Nabuurs et al., 1983; Jestin et al., 1985). No detailed studies have yet determined whether diseases are induced by specific strains, but there are indications that common bacterial characteristics are shared between outbreaks at different farms (Taylor and Bergeland, 1999).

The epidemiology is not yet known but the major source of the organism is porcine excrements. Piglets may be infected within hours after birth and the bacteria can be demonstrated in the first faecal sample following the meconium. In non-immune animals, the digesta from jejunum to rectum may harbour levels of 10^8-10^9 *C. perfringens* type A per gram content (Taylor and Bergeland, 1999).

No epithelial attachment or invasion of the pathogenic strains has been seen. However, *C. perfringens* type A produce a powerful enterotoxin on sporulation that adhere to the epithelial cells. This enterotoxin causes widespread villous necrosis giving an extensive outpouring of fluid to the intestinal lumen of the small intestines and a decreased colonic capacity to resorb water. A production of alpha toxin together with other toxins may also occur. These toxins can also cause necrosis of the intestinal epithelium (Taylor and Bergeland, 1999).

Affected piglets get dehydrated, lose condition and develop a creamy non-hemorrhagic diarrhoea within 48 hours after infection. The clinical signs remain for 4-7 days both in neonatal and weaned piglets. Affected animals do not develop fever and the mortality is low, but the DWG may be affected. In other occasions the diarrhoea have been watery and transient, lasting for only 24-48 hours (Taylor and Bergeland, 1999).

At necropsy the small intestine is thick-walled and has a frothy or pasty content with no blood. The mucosa shows a mild inflammation and a villous atrophy with superficial necrosis and accumulation of fibrin. The large intestines are distended with the same type of content but display a normal mucosa.

The disease can be treated with antibiotics and prevented by vaccination. Vaccinations of sows have been proven successful, but the ubiquitous presence of the microbe is most probably sufficient to induce an adequate concentration of IgG and IgA in colostrum and IgA in the milk. In many herds, antibodies to these toxins are present in colostrum. They will protect the neonatal pigs but in such herds disease may be seen after the disappearance of maternal antibodies i.e. in weaned piglets (Estrada Correa, 1986). However, in weaned pigs both symptoms and lesions tend to be milder.

Diarrhoeas in suckling piglets

Intestinal disturbances in suckling piglets older than one week are more complex compared to neonatal diarrhoeas. Other microbes are involved, and synergistic effects between these agens may develop. Different strains of *C. perfringens*, especially type A, may still be of interest, but they are discussed above. *E. coli* still plays an important role and is therefore shortly mentioned below. In addition, some other microbes associated to diarrhoeas in suckling piglets, rotavirus and *Isospora suis*, are presented.

E. coli

ETEC-diarrhoeas in piglets are thoroughly discussed in the part that describes neonatal diarrhoeas. However, some complementary information is given here. Table 2 shows the distribution of different *E. coli* serotypes isolated from suckling pigs aged more than one week in Swedish piglets. The specimens comprise samples sent to the National Veterinary Institute due to intestinal disturbances within the scrutinised herds. The samples range from the mid-sixties with a weaning age of 6-8 weeks until today, when weaning generally is effectuated as the piglet is aged 5 weeks.

Table 2. Distribution of *E. coli* serotypes isolated from suckling piglets in Sweden over time. The piglets were aged one week or more and expressed signs of colibacillosis

Serotype		1964-67* (n=66)	1983-84¤ (n=302)	1994# (n=97)	2000# (n=145)
O8	%	8	6	4	5
O9	%	1	7	4	3
O20	%	3	7	3	5
O64	%	3	4	-	0
O101	%	0	11	2	5
O139	%	9	-	6	3
O141	%	15	2	0	1
O147	%	9	-	0	0
O149	%	6	42	8	28
Other	%	29	19	5	7
O?	%	17	2	68	43

* = post mortem, piglets with intestinal disorders (Söderlind, 1971)
¤ = faecal samples from piglets with diarrhoea (Söderlind et al., 1988)
= faecal samples from piglets with diarrhoea (Melin et al., unpublished)

Rotavirus

Rotavirus was first demonstrated in pigs by Wood and Bridger (1975). Since then, four (A, B, C and E) of the seven described serogroups have been found in pigs (Chasey et al., 1986; Pedley et al., 1986). Of these, group A is considered the most important.

Rotavirus is a ubiquitous gastrointestinal virus with the ability to produce severe enteritis with villous atrophy in pigs and many other animal species.
The spread is worldwide, more than 60 % of herds are infected (Svensmark et al., 1989) and within infected herds nearly 100% of the animals become seropositive (Svensmark, 1983). Thereby almost all piglets are defended through IgG plus IgA in colostrum and IgA in the milk from seropositive sows. The incidence of rotavirus infection among piglets will reach nearly 100 % but mostly they will stay subclinical. Outbreaks of diarrhoea will occur when the dose of virus exceeds the capacity of the local immunity, often when the passive maternal immunity declines (at 3-5 weeks) or after weaning when IgA ceases (Tzipori et al., 1980). Gilt litters may be affected at younger age and may display more sever clinical signs (Paul and Stevenson, 1999). In sensitive or non-immune animals, the infective dose can be as low as 90 rotavirus particles (Payment and Morin, 1990).

The replication of the virus occurs in villous epithelium of the small intestine (Crouch and Woode, 1978) and will start within 4 to 24 hours after infection. Almost all apical epithelial cells of the small intestine will be affected at the onset of diarrhoea (Crouch and Woode, 1978). The disruption and desquamation of the villous epithelium leads to a release of large amounts of virus and cell debris into the digesta (Crouch and Woode, 1978). In this way virus can infect more caudal parts of the intestine and the shedding of virus in faeces will become profuse. If one piglet in a litter/group develops diarrhoea this amplification may lead to shedding of up to 10^{11} virus particles per gram faeces (Fenner et al., 1993). Consequently, the environment-load of rotavirus increase, which may contribute, to spread of the disease, as the pathogen load may beat the local immunity of other pigs.

The viral infection will lead to a shortening of villi and the crypts will be lengthened and fused. The damaged epithelium will be replaced by immature flat cubodial cells with incomplete microvillar border. These cells are incapable to produce several degrading enzymes such as lactase and thereby osmotic diarrhoea due to malabsorption will be induced.

Clearance of the infection probably occurs by several mechanisms: 1) the immature epithelial cells are refractive to virus infection. 2) Gut perstalsis is increased. 3) A leukocyte infiltration of the damaged tissue by mononuclear and polymorphonuclear cells occurs. In this way viral antigen will be presented to the

immune system resulting in both humoral and cell mediated interactions (Offit, 1996).

Unless affected pigs are secondarily infected the diarrhoea ends within 3-5 days, and infected piglets generally recover within 1-10 days (Lecce and King, 1978). This recovery is correlated to regeneration of the villi (Crouch and Woode, 1978). Morbidity is often very high and mortality may reach 50 % in very young piglets. In piglets older than 14 days death is uncommon, but the animals display diarrhoea dehydration, weight loss, depression and occasionally vomiting. In association with other enteric pathogens such as *E. coli* a synergistic effect with more severe diarrhoea and a greater number of fatalities have been noted (Tzipori et al., 1980; Lecce et al., 1982).

No known therapeutic treatment is available. However, a supportive treatment including electrolyte solutions with glucose, minimizing of draft, and preservation of a suitable temperature reduce the mortality. Antibiotic treatment to reduce losses from secondary bacterial infections may also be used (Paul and Stevenson, 1999). An increased concentration of virus in the environment has been shown to cause more severe disease at a younger age (Lecce and King, 1978).

A good hygiene and the use of age-segregated production with All in-All out management may decrease the virus load in the herd. However, rotavirus is extremely stable and resistant to various environmental conditions. In a faecal preparation it will survive for 30 minutes at 60°C and can stay infective for 7-9 months at 18-20°C (Paul and Stevenson, 1999). It is stable at pH 3-9 and can withstand several chemicals and disinfectants. Vaccines are commercially available, but their efficacy has been questioned (Alenius, 1992).

Isospora suis

Coccidiosis is the most important protozoal disease in pigs. The causative agent *Isospora suis* was first described in 1934, but was not connected to coccidiosis in piglets until 1978 (Stuart et al., 1978). The coccidial lifecycle consists of three phases: sporogony, excystation and endogenous development. Sporogony is the development from the unsporulated oocyst to the infective agent. For *I. Suis* this will happen within 12 hours at temperatures between 20-37 C, and the parasite is very resistant to disinfectants. Excytation is the development from oocyst to invasive sporozoite in the intestine. The endogenous stages occur in the enterocytes of the small intestine where the parasite is performing intracellular multiplication resulting in destruction of the cell followed by consecutive infection of new cells.

Clinical signs will typically be observed in piglets aged 7 and 14 days (Morin et al., 1983). Faeces are yellowish to grey with a pasty to liquid consistence and a

rancid odour of sour milk. Affected piglets develop a rough hair coat, become dehydrated and have depressed weight gains (Lindsay et al., 1985). Morbidity is usually high but the mortality is moderate if not complicated by a concurrent infection with another enteropathogenic agent. Occasionally *I. Suis* have been isolated from piglets with postweaning diarrhoea (Nilsson, 1988). However, the causative role of *I. Suis* in PWD is disputed.

The epidemiology of *I. Suis* is not yet clear. In several studies presence of the parasite in faeces, colostrum and placenta from sows have been investigated and found very low. Still, piglets may develop neonatal coccidiosis with diarrhoea and profuse shedding of I. suis. These observations indicate an influence of environment contamination when piglets are affected (Stuart and Lindsay, 1986).

Improved sanitation in terms of cleaning and disinfections with steam or ammonia has been the most successful method to reduce losses (Lindsay et al., 1999). Effective coccidiostatica have not been available (Lindsay et al., 1999) until recently, when toltrazuril have been tested with a positive effect in preventing coccidiosis in nursing piglets (Driesen et al., 1995).

Postweaning diarrhoea

PWD was first described by Richards and Fraser (1963). They associated PWD with occurrence of β-haemolytic strains of *E. coli*. Later research has shown that haemolysin per se is not an essential virulence factor (Kenworthy and Crabb, 1963; Hampson, et al., 1987) but the adhesines and toxins of all ETEC-strains are. Forty years later PWD is still a major cause to disease and economical losses in pig production.

PWD generally debut within a week post weaning and affected pigs display a yellow or grey fluid diarrhoea. The duration range from a few days up to several weeks and may lead to depression, reduced appetite, dehydration, inappetence weight loss and even emaciation.

During the suckling period the piglets harbour a highly diversified intestinal flora that is reliant on a spectrum of environmental, nutritional, physiological, and immunological factors (Howe et al., 1976; Hinton et al., 1985; Nagy et al., 1990). These conditions are abruptly altered at weaning, which calls for a multifactorial genesis to development of PWD as also suggested by several authors (Spencer et al., 1989; Hampson, 1994). Still, there is a strong relation between development of PWD and presence of pathogenic strains of *E. coli* (Svendsen et al., 1977; Hinton et al., 1985).

Other microbes may also contribute to the development of PWD. They include the previously described *C. perfringens* type A, rotavirus and *Isospora suis*. During the post weaning period also the influence of other microbes, such as

Brachyspira spp and *Lawsonia intracellularis*, will increase and these infections are briefly described below. Further, as PWD is strongly associated to *E. coli*, this specie is again discussed.

E. coli

At PWD a heavy growth of haemolytic *E. coli* can be recovered from jejunum to rectum (Svendsen et al., 1977). These strains isolated from pigs with PWD are rarely isolated from suckling piglets or from mature animals, not even in herds with problems with PWD (Hampson, 1994). Further, pathogenic strains of *E. coli* have been introduced to never before used weaner units by healthy piglets (Hampson et al., 1987). Taken together, these findings indicates that a raised number of pathogenic *E. coli* present post weaning may reflect an overgrowth of strains from the normal flora as well as an exogenous infection.

The number of different serotypes isolated from piglets with PWD is limited. In a global perspective the main O groups are O8, O141 and O149 and to a lesser extent also O138, O139, O147, and O157 (Hampson, 1994). The incidence of different serotypes isolated from Swedish piglets post weaning from 1964 to 2000 is shown in Table 3.

Table 3. Distribution of *E. coli* serotypes isolated from recently weaned piglets in Sweden with diarrhoea. The mean weaning age was around 6-8 weeks during the 1960ies and 5 weeks during 2000

Serotype		1964-67* (n=131)	1983-84¤ (n=80)	1994# (n=193)	2000# (n=600)
O8	%	8	2	4	6
O9	%	2	3	4	1
O20	%	2	3	1	1
O64	%	0	4	0	0
O101	%	0	1	0	2
O139	%	9	-	15	4
O141	%	37	16	0	3
O147	%	6	-	8	5
O149	%	1	43	17	12
Other	%	25	24	6	10
O?	%	10	4	45	56

* = post mortem, piglets with intestinal disorders (Söderlind, 1971)
¤ = faecal samples from piglets with diarrhoea (Söderlind et al., 1988)
= faecal samples from piglets with diarrhoea (Melin et al., unpublished)

In PWD not complicated by other infections, the small intestine of affected animals is dilated and thin walled with a typical and distinct pattern of dilated veins. The mucosa is intact, hyperaemic but not hemorrhagic. In addition, the mesenteric veins of the small intestines are dilated. The intestinal content is profuse, watery or sometimes yellowish. All this findings mirror the pathogenesis of an ETEC-diarrhoea consisting of adherent but not invading *E. coli*, inducing diarrhoea by trigging an increased secretion of water to the intestinal lumen (Guerrant et al., 1985).

Swine dysentery

Swine dysentery is a severe muco-hemorrhagic diarreal disease caused by *Brachyspira hyodysenterie*, a gram-negative, motile, loosely coiled spirochete. The bacteria invade the epithelium of the large intestines. The haemolysin produced by *B. hyodysenterie* has been proven toxic for several types of cell cultures and is considered a major virulence factor in the disease (Hyatt, 1994). Still, the whole mechanism for induction of disease is not yet known.

Swine dysentery primarily affects growing/finishing pigs. However, it also occurs in adults and occasionally in suckling piglets. Swine dysentery was first described in 1921 by Whiting et al. and the disease was first described in Sweden by (Ronéus, 1960). However, the ethological agens was not established until 1971 (Taylor and Alexander, 1971).

Generally, there will be a slow spread of the diarrhoea within an affected herd. The diarrhoea it self will develop from yellow to grey loose faeces over large amounts of mucous diarrhoea with flecks of blood to a dark, red or brown diarrhoea. The animal will be dehydrated, weak, emaciated and eventually die from dehydration, acidosis and hyperkalemia. The diarrhoea is caused by a malabsorption of fluid in colon due to a disturbed transportation of ions in the epithelium. As extra cellular fluid normally is secreted into the small intestine and absorbed by the large intestine, a failure in this function is sufficient to explain the dehydration and death of the disease (Taylor and Bergeland, 1999).

B. hyodysenterie, have been reported prevalent in 11% of herds in the United States (Mapother, 1993) and in 33 % of herds in Western Australia (Mhoma et al., 1992). *B. hyodysenterie* is fairly spread in Sweden (Fellstrom et al., 1996), and the use of antimicrobal drugs directed towards *Brachyspira* spp indicate that around 10% of the fatteners in Sweden experience swine dysentery or dysentery like diseases (Non published data).

Affected animals can be treated with suitable antibiotics, and the antimicrobal susceptibility in Swedish isolates of *B. hyodysenterie* is favourable (Karlsson and Franklin, 2000). As diseased animals consume little feed it should be administrated through the water or by injections. The disease can be eradicated

from a herd through a combination of treatment with antibiotics and a cleaning and disinfection of the premises.

Spirochetal diarrhoea

Spirochetal diarrhoeas are induced by *Brachyspira* pilosicoli, a less pathogenic member of the *Brachyspira* family. Spirochetal diarrhoea is milder than Swine dysentery. Infected weaners usually develop watery and mucoid diarrhoeas with occasional flecks of blood. Affected piglets appear ill-thrifty and sometimes febrile, but the diarrhoea is often self-limiting within 2 – 14 days. Spirochetal diarrhoea uncomplicated by other diseases may lead to a reduced weight gain, but mortality is generally not the feature in this disease.

There are also several other species in the *Brachyspira* family. These species are very common in conventional pig herds (Kinyon and Harris, 1979), but generally considered non-pathogenic.

Proliferative enteritis

Lawsonia intracellularis is an ubiquitous obligate intracellular bacterium first identified by (McOrist et al., 1993) that is strongly associated to Proliferative enteropathies in pigs. The bacteria will associate with the cell membrane of dividing crypt cells in the distal part of the small intestine, preferably the ileum. *L. intracellularis* multiply in the cytoplasm of infected cells. These cells fail to mature, but they continue to proliferate, which results in development of hyperplastic crypts.

Affected pigs generally are aged 6 to 20 weeks and may express minor or no signs of disease. Frequently a decreased growth rate despite a normal feed intake are the only signs of disease, but some pigs may show anorexia or diarrhoea. In uncomplicated cases, affected animals will recover within 4 – 10 weeks.

However, the basic epithelial proliferation may aggravate, leading to Regional Ileitis or Necrotic Enteritis in which the affected parts of the posterior small intestine becomes thickened and rigid. In more severe cases the Proliferative enteritis may also affect colon and induce hemorrhagia. During these conditions the faeces will be black and tarry due to the hemorrhagia, and pigs might die.

Aims of the study

The general aim of this work was to study weaning of pigs in modern agricultural systems from the aspects summarised above. That includes searching for possible causes that may contribute to development of intestinal disturbances post weaning. Such disturbances are commonly referred to as post weaning diarrhoea (PWD). Consequently, some general aims were defined.

These aims included to

a) Monitor the influence of weaning in healthy piglets

To be able to

b) Develop a model inducing PWD
c) Monitor the course of PWD
d) Study the influence of factors that could contribute to PWD
e) Study the efficacy of different preventive measures towards PWD

Comments on material and methods

General aspects on the material and methods used in this thesis are addressed here. Details concerning each study are given in **I - V**.

Animals and husbandry

The animals used were conventional pigs, but with a defined and high health status. All animals originated from one conventional herd free from diseases according to the A-list of International office of epizootics, and several other diseases. Of particular interest for the present study was the absence of haemolysing strains of *E. coli* in suckling piglets, which permitted an easy recognition of the challenge strains used.

All experiments were carried out in previously emptied, cleaned and disinfected experimental facilities. When groups with different health status were created, these groups were always housed in different rooms A "split litter design" with an even distribution of littermates into different groups was used, aiming to minimize the effects related to litter of origin (**I - V**).

Dry conventional pig food without antibiotics or any other feed additive was offered ad lib to the piglets. In **II**, one group was given a feed supplemented with 2500 ppm zinc oxide, and in **V** several different feed compositions or probiotic bacteria were given to the pigs (for details see **V**)

Infection model

In **III – V** all but the control pigs were exposed to pathogenic strains of *E. coli* via the environment. A broth of the infection strain(s) was spread on the floor of empty and previously disinfected pens. A sterile BHI-broth was spread in the pen of the control groups. This was done one hour prior to the arrival of the animals. One hour after the arrival of the piglets, the pens were bedded with sawdust and the animals were given access to feed and water. Three days post weaning some groups was exposed a second time via the same route (**IV** and **V**). In order to simulate a weaning complicated by severe stress some pigs in **IV** were given ACTH for six days.

Detection of the challenge strains

β-Haemolytic *E. coli* on blood agar were denoted as potential isolates of the challenge strains (**III, IV** and **V**). They were estimated as percentage of the total number of coliforms, and six colonies per pig and day were tested for presence of capsule antigens specific for the challenge strains. If positive (K85 = O141; K89 = O147; K91 = O149), they were considered as a reisolated challenge strain.

Consistency and dry matter content of faeces

The health status of the animals was inspected at an individual level, paying special attention to faecal consistency. In **I - III** the consistency of faecal samples was estimated on a scale from 1 (diarrhoea) to 5 (very firm). In **III** the dry matter of a representative fraction of each faecal sample was defined and correlated to the consistency scale. In **IV** and **V** it was stated that if the consistency of the faeces allowed the sample to adapt to a spoon it was characterised as diarrhoea.

Quantification of *E. coli*, enterococci and *C. perfringens* in faeces

A weighed part of each faecal sample was homogenised and dispersed in PBS in consecutive tenfold dilutions (**I, II** and **III**). The log10 counts of *E. coli*, enterococci and *C. perfringens* per gram faeces were then defined after incubation on selective media

Methods to detect other pathogenic microorganisms than *E. coli*

Rotavirus was detected by an ELISA demonstrating group A rotavirus antigen in faecal samples and the presence of *Brachyspira* spp was determined by incubation under anaerobic conditions (**I - V**). *Isospora suis* was analysed using a modified version of a flotation/McMaster technique in **III, IV** and **V**.

Biochemical fingerprinting

Faecal samples were spread on MacConkey agar and incubated for 18 h at 37°C. From each sample, 24 colonies of coliforms were picked randomly and inoculated on PhP-RS plates. The absorption values (A_{650}) were measured with a photometer after 16, 40 and 64 hours of incubation at 37°C and the ability to utilise the various substrates was compared.

The fingerprinting identified biochemical phenotypes (BPTs) that allowed calculations concerning;

a) The phenotypic diversity of the coliform populations within pigs, measured as Simpson's index of diversity (**II** - **V**).

b) The homogeneity between coliform floras of different piglets at each sampling occasion, defined as the mean similarity coefficient between bacterial groups (**I**).

c) The population similarity (SP-value) between different coliform populations (**II**, **III**, **IV** and **V**).

d) The fermentative capacity (FC) of the total faecal flora (**II**).

All values calculated in a, b, c and d varies between 0 and 1, in which 1 represents a high value.

Immune functions

The immune functions of the piglets were studied by determination of the following parameters;

a) The total number of leukocytes in blood and differential leukocyte counts (**III**).

b) The phagocytic and killing capacity of neutrophils (**I**).

c) Proliferation of PBMC in vitro, stimulated by Con A, PWM or a heat-inactivated bacterial suspension of the O149 *E. coli* strain used for challenge (**III**).

d) IL-2 production of PBMC in vitro, stimulated by Con A or PWM (**III**).

e) Defining the proportion of IL-2R+ PBMC in medium and after stimulation with Con A (**III**).

f) Defining subpopulations of PBMC (**III**).

Detection of serum antibodies to *E. coli*

A preliminary indirect ELISA system detecting antibodies to *E. coli* in general was developed (**IV**). Aiming to detect a serological response to *E. coli* regardless of serotype the following serotypes of *E. coli* were included in the antigen preparation; O8 (F4, LT, STa, STb), O64 (F6, STa, STb), O101 (F41, F5, STa), O141 (STb, VT2), O147 (STb), O149 (F4, LT, STa, STb).

The strains were grown on Sheep Blood Agar at 37°C for 18 h and harvested into 2 ml PBS without Ca and Mg. The bacterial suspensions were merged and homogenised. Fractions of 8 ml were sonicated for five minutes in an ultrasonic disintegrator (MSE - 60 watt, Measuring scientific Equipment Ltd, London, United Kingdom). The sonicated solution was centrifuged at 12,000g for 20 minutes at 4°C (RC2B, Sorvall, Newtown, USA) and the liquid was used as antigen.

The antigen was diluted 1 to 20,000 in PBS and wells in microtiter plates (Mi29A, Greiner, Frickenhausen, Germany) were coated with 100 ml over night at 20°C. After washing three times with PBS-T, serum diluted 1/1000 in PBS was added to duplicate wells and incubated for 2 h at 20°C. The plates were again washed and a conjugate (Swine Ig, DAKO, Copenhagen, Denmark) diluted 1/5,000 in PBS was added to each well and incubated for 1 h at 37°C. The plates were washed again and 100 ml substrate (TMB, Svanova, Uppsala, Sweden) was added to each well. The reaction was stopped by adding 50 ml 2M H2SO4 and the reaction was read at 450 nm (Titertek Multiscan MCC/340, Lab systems OY, Helsinki, Finland). A standard porcine serum was included on each plate and adjusted to 1.0 absorbance units. The measured values of other sera were adjusted the same way.

Statistical analyses

The significance of differences between groups or litters, respectively, was calculated with the Mann-Whitney U test and the significance of differences within groups or litters over time was determined by the Wilcoxon signed-rank test. The significance of differences in clinical signs between groups or litters, respectively, was calculated by $\chi 2$-tests (**I – V**).

Results

Neutrophil functions (I)

The phagocyting and killing capacity of the neutrophils collected from healthy piglets appeared to be developed already as the pigs were aged one week. These functions were not affected by weaning. No significant change with age was observed.

Leukocytes and subpopulations of PBMC (III)

The mean number of leukocytes increased from 8 x 10^9 leukocytes per ml blood at weaning to 13 x 10^9 two weeks later ($p<0.01$). No difference was recorded between the control group and the group challenged with *E. coli* O149 via the environment. Both these experimental groups expressed similar proportions of cells belonging to the various subpopulations of PBMC determined why the merged results of the two groups are presented below;

The proportion of IL-2R+ cells was low at weaning and was further decreased on day 3 post weaning ($p<0.05$). However, a large amount of the PBMC became IL-$2R^+$ after stimulation with Con A, a figure that increased ($p<0.01$) after weaning.

The proportion of $CD2^+$ T cells gradually decreased ($p<0.05$) from weaning until 14 days later. The proportions of $CD4^+$ and $CD8^+$ T cells were equal on these days. However, their proportions were somewhat decreased ($p<0.001$) on day 3 post weaning. No effects of weaning were observed with respect to the proportion of cells positive to MHCII or IgM, while the proportion of N1c+ cells steadily increased ($p<0.01$) during the observation period.

PBMC activities (III)

The PWM induced IL-2 activity decreased ($p<0.001$) in both groups during the week following weaning and thereafter remained at that level. When assessed with Con A as mitogen, the IL-2 activity was decreased during the first week post weaning in the control group ($p<0.05$). In contrast, no alteration in IL-2 activity was seen over time in the infected group.

On day 3 post weaning a decrease ($p<0.05$) in the Con A induced proliferation was recorded among PBMC collected from the control pigs, resulting in a difference compared to the mean values obtained in the infected group. In contrast, no differences were obtained when the proliferation was induced by PWM, neither over time nor between groups.

Cell proliferation was also induced by a heat-inactivated preparation of the challenge strain. The results were comparable for the two experimental groups at weaning, but 3 and 7 days later the proliferative response of PBMC collected from the challenged pigs was higher ($p<0.05$) than that of the control pigs.

Serum antibodies to *E. coli* (IV)

Serum antibodies to *E. coli* were measured at the termination of **IV:set 2**, i.e. two weeks post weaning. The mean absorbance values in the groups challenged with one serotype of *E. coli* was comparable to the value of the control group. In contrast, higher absorbance values were observed in the groups challenged with three serotypes of *E. coli* (Figure 1).

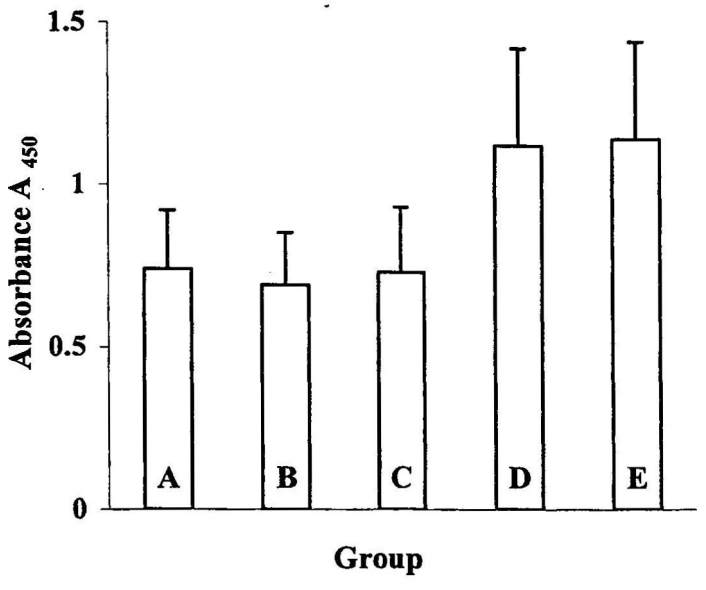

Figure 1. Levels of antibodies to *E. coli* in serum of pigs two weeks post weaning. Pigs in groups B, C, D and E were challenged with *E. coli* O147 via the environment at weaning. Pigs in groups D and E were also challenged with serotypes O141 and O149 three days later. Pigs in groups C and E were given ACTH, aiming to simulate a weaning under extra stressful conditions. Group A was a healthy control group.

Faecal bacterial counts (I, II and III)

During the first 14 days of life, the faeces contained about 10^8 CFU per g each of *E. coli* and enterococci. These figures gradually decreased ($p<0.05$) to a level of about 10^6 CFU per g faeces at weaning and 10^5 CFU per g faeces on day 63.
The density of faecal *C. perfringens* was high (circa 10^4 per gram) during the first 14 days of life. From the age of 21 days onwards, *C. perfringens* could not be detected with a detection level of 10^2 CFU per gram faeces.

No significant differences were found between litters, or between control groups and groups exposed to pathogenic strains of *E. coli* at weaning (**I, II** and **III**).

Microbial challenge with pathogenic *E. coli* and reisolation of challenge strains (III, IV, V)

All piglets challenged with pathogenic strains of *E. coli* via the environment at weaning became infected (**III, IV** and **V**). In the groups challenged with a single strain (n=5) this strain was reisolated for 6 days in two groups (**III** and **IV:gr12**), for 11 days in one group (**IV:gr13**) and for the entire study period (14 days) in two groups (**IV:gr22 and 23**).

In the eight groups that were challenged with three pathogenic strains of *E. coli* (**III, IV** and **V**) the challenge strains used were generally reisolated until the end of the studies, i.e. for 14 days. In these groups the dominating serotype of *E. coli* could vary from day to day (**IV** and **V**).

Biochemical fingerprinting, diversity within populations (I - V)

In **II** and **III**, the diversity of the faecal coliform populations was high throughout the whole suckling period, but this diversity decreased in all control groups during the first week post weaning (**I – V**). This pattern was also seen in all challenged groups, with exception of one group (**IV:gr24**). No decrease in microbial diversity was seen in the faecal coliform flora of that group on day 3 post weaning.

A high, diversity of the faecal coliform populations was regained 14 days post weaning in all groups but the control group in **II** and the challenged group in **III**. In these groups, the diversity was re-established on day 21 post weaning.

In **IV**, piglets that developed diarrhoea due to bacterial challenge displayed a lower diversity in their faecal coliform flora at weaning than challenged pigs that remained healthy. The lowest diversity obtained during the entire study was revealed on day 3 post weaning in pigs expressing diarrhoea on that day. These pigs had only been exposed to one pathogenic strain of *E. coli*.

The diversity of the faecal coliform flora in pigs given feed supplemented with 2,500 ppm ZnO post weaning was less affected by the weaning than the flora of pigs given standard feed (**II**). None of these groups was challenged with pathogenic strains of *E. coli*.

Biochemical fingerprinting, similarity between populations (I - V)

The similarity between faecal coliform populations of penmates increased during the first living week and remained at a high level until weaning (**I, II** and **III**). This similarity was decreased day three post weaning in all control groups (**I** -

V). The similarity between piglets was also decreased post weaning in all groups that were challenged with one pathogenic strain of *E. coli* (**I, II, III** and **IV**). In contrast, a slightly increased similarity between pen mates was observed after challenge with three pathogenic strains of *E. coli* (**IV** and **V**).

The similarity between the faecal coliform populations of pen mates was less affected during the early post weaning period in the group that was given zinc-supplemented feed, when compared to pigs in the control group (**II**). None of these groups was challenged with pathogenic strains of *E. coli*.

Clinical signs (I - V)

Diarrhoea was only recorded among animals exposed to pathogenic strains of *E. coli*. Exposure to three pathogenic strains of *E. coli* was repeatedly proven able to induce PWD (**IV** and **V**). In groups exposed to a single strain of pathogenic *E. coli* diarrhoea was detected only when coinciding with an excretion of rotavirus (**IV:2**) or occasionally in piglets injected with ACTH (**IV:1**). ACTH was not required to induce PWD, but clinical signs of PWD were graver in the ATCH-injected groups than in the comparable groups not given ACTH.

Influence of litter origin (I - V)

No differences between litters were found according to the immune functions investigated (**I** and **III**), or in the quantification of different bacterial species (**I, II** and **III**). However, diarrhoea was more frequently observed in pigs from certain litters, as will be discussed below.

Effects of different measures to prevent PWD in absence of *E. coli* (II)

A number of differences regarding the faecal flora in healthy pigs given a standard feed and a feed supplemented with 2,500 ppm ZnO were observed (**II**).

The number of coliform BPTs decreased more in the control group ($p<0.05$) and the diversity of the coliform flora among controls was more decreased ($p<0.001$). Further, the homogeneity decreased more among the control animals post weaning, while it increased among zinc oxide treated pigs. Finally, the fermentative capacity of the total flora decreased in the control-group during the first week post weaning (**II**). That decrease was less obvious in the group given ZnO-supplemented feed.

The disturbances of weaning remained until day 7 post weaning in the ZnO-group, but lasted until day 14 post weaning among the control animals. The DWG among zinc oxide treated pigs was 1.6 times higher compared to the control group during the second week post weaning. In contrast, no difference in DWG was recorded during the first, third and fourth weeks post weaning (**II**).

Effects of different measures to prevent PWD in presence of *E. coli* (V)

All the feed related measures monitored appeared to have a potential to reduce the effect of pathogenic strains of *E. coli* (V).

One group was given a defined mixture of 60 non-pathogenic *E. coli* as a probiotic before being exposed to the pathogenic strains of *E. coli* (V). In this group, the onset of diarrhoea was slower and the clinical signs milder compared to other groups. The reisolation of *E. coli* O147 was also lower ($p<0.001$) and the daily feed intake and DWG higher, though not statistically significant. The diversity of the faecal coliform populations in this group was low 7 days post weaning and remained low till the end of the study period, possibly indicating an intestinal colonisation of one or more of the probiotic strains.

Another group was given a meal feed enriched with lactose and dietary fibres (V). In this group the decrease in diversity of the coliform flora post weaning was less pronounced with no accentuated dip on day seven. Further, the similarity between the faecal coliform floras of different piglets did not increase as much after exposure to the challenge strains as in the other challenged groups, indicating a minor influence on the intestinal flora by the challenge strains. An increased resistance to pathogenic strains of *E. coli* was indicated, as the incidence of diarrhoea and the mortality was lower than in the infected control group.

Also the third group, given feed supplemented with 2,500 ppm ZnO (V), resisted the challenge with pathogenic strains of *E. coli* better than the infected control group

Discussion

PWD was first clearly described by Richards and Fraser (1963) and was then associated with a proliferation of β-haemolytic *E. coli* in faeces. Later a proliferation of ETEC has been considered to play a crucial role in the development of PWD (Kenworthy and Crabb, 1963; Svendsen et al., 1977; Hinton et al., 1985; Hampson, 1994). However, a multifactorial background to the syndrome has also been suggested (Lecce et al., 1979; Madec and Josse, 1983; Spencer et al., 1989; Nabuurs et al., 1993; Hampson 1994), not the least because piglets of today experience a number of negative stressors at weaning (Spencer et al., 1989; Madec et al., 1998). In the present thesis, the danger of weaning itself was studied. Further, several factors with a possible influence on the development of PWD were studied, using an experimental model in which

piglets were exposed to various strains of *E. coli* at weaning and transfered to a new environment.

Weaning of healthy pigs (I, II)

All pigs in **I** and **II** were weaned without exposure to exogenous pathogenic microorganisms, and remained apparently healthy during the post weaning period. The densities of endogenous bacteria in faeces were not affected by weaning as such, which is in accordance with earlier longitudinal investigations of the intestinal flora (Smith and Crabb, 1961; Kenworthy and Crabb, 1963). Despite that the present studies were performed 40 years later, the results concerning the development of the faecal density of bacteria in terms of coliforms, enterococci and *C. perfringens* corresponded well to the earlier studies. Consequently, these findings suggest a minor influence of alterations in porcine genotypes, husbandry regimes or diet compositions over time on the establishments of theses bacterial species.

However, when scrutinising the faecal populations with biochemical fingerprinting (Möllby et al., 1993; Kühn 1985) a decreased diversity of the faecal coliform populations was demonstrated post weaning, as also seen in the control groups in **III**, **IV** and **V**. A decreased diversity has previously been shown in apparently healthy piglets following weaning or allocation to new facilities (Kühn et al., 1993; Katouli et al., 1995; Kühn, 1995). As microbial populations with a high diversity reflect stabile communities with a high colonisation resistance (Atlas, 1984), the recorded disturbances in the faecal floras ought to reflect populations with a reduced colonisation resistance. Animals with a low intestinal microbiological diversity ought to be more susceptible to an overgrowth of potentially pathogenic microbes of external or internal origin.

As the faecal density of microbes remains constant post weaning (Smith and Crabb, 1961; Kenworthy and Crabb, 1963), **I**, **II**, **III**) it is likely that the decreased microbial diversity was caused by proliferation of certain clones of bacteria on behalf of others. If identical clones had proliferated in different pigs, the homogeneity of penmates ought to have increased. Instead, the similarity between pigs decreased dramatically after weaning, indicating that different clones of bacteria did proliferate in different pigs. This points to an even increased danger, because different strains obviously dominate in different pigs and potential pathogenic microbes shed by one pig more easily could colonise the intestine of other pigs.

Weaning and immune functions (I, III)

As discussed above also apparently healthy piglets experience an altered intestinal microflora post weaning. It is reasonable to assume that the abrupt change of diet at weaning contributes to this phenomenon. However, the piglets

also experience several other stressors at weaning, and several authors have reported decreased immune functions in young pigs due to weaning (Bailey et al., 1992; Blecha et al., 1983; Wattrang et al., 1998).

In the present study (**III**), a reduced IL-2 activity of PBMC in vitro was obtained when induced by Con A or PWM. This concurred earlier results and indicated a negative effect of weaning itself (Wattrang et al., 1998). However, when PBMC collected from pigs exposed to pathogenic *E. coli* at weaning were stimulated with PWM (but not with Con A) no decrease in IL-2 activity was recorded. Further, a heat-inactivated antigen of the challenge strain induced an increased proliferation of PBMC collected from the challenged pigs in vitro. As an *E. coli* challenge certainly stimulate B cells, it is of interest that PWM is suggested to act on both B and T cells, whereas Con A is believed to act mainly on T cells (Sharon, 1983). Consequently, it could be suggested that the *E. coli* challenge actually did initiate a B cell response in the challenged pigs. This assumption was further supported by increasing amounts of serum antibodies to *E. coli* in pigs challenged with three pathogenic strains of *E. coli*. However, the challenge of O147 alone was not enough to mount a serological response.

Despite that contradicting findings are described in the literature concerning the functional capacity of neutrophils (Morris et al., 1987; Hoskinson et al., 1990; Coignoul et al., 1984), the phagocyting and killing capacity of neutrophils appeared to be well developed already at birth and they were not affected by the weaning (**I**). Further, the number of leukocytes actually increased post weaning, indicating an inflammatory response and an overall increased ability of the neutrophil capacity during the post weaning period. However, the increase in leukocyte numbers was equal in both the challenged group and in the control group, possibly indicating a response to endogenous bacteria activated by the weaning in the control group. In addition, alterations in several subpopulations of PBMC were observed post weaning (**III**). However, these alterations mirrored earlier studies well (Joling et al., 1994; Wattrang et al., 1998), and were mainly believed to reflect the ageing of the piglets.

Weaning with exposure to pathogenic of *E. coli* without development of diarrhoea (III, IV)

In **III** the weaning was followed by increased levels of circulating leukocytes in both the control group and the group infected with *E. coli* O149. The bacterial challenge might explain that increase in the latter group, but not in the control group. Consequently, a response activated by the weaning could be suspected as previously suggested (Hampson, 1994).

A certain resistance towards pathogenic strains of *E. coli* was indicated. No pig developed PWD despite that the pathogenic strain of *E. coli* used for infection could comprise up to 60% of the total coliform flora in challenged pigs for 6 days

(**III, IV**). Indeed oral dosing with single strains of ETEC has failed to induce diarrhoea in earlier studies (Smith and Jones, 1963; Kenworthy and Allen, 1966; Armstrong and Cline 1977), and when diarrhoeic and healthy pigs were compared Smith, (1960) and Smith and Jones (1963) were unable to demonstrate significant quantitative differences of *E. coli* in faecal samples. Further, haemolyse itself is a poor indicator for pathogenicity as scouring animals may excrete no or very low numbers of haemolytic *E. coli*, while haemolytic *E. coli* populations can be isolated from healthy pigs (Kenworthy and Crabb, 1963).

Weaning with exposure to pathogenic strains of *E. coli* and development of diarrhoea (IV and V)

PWD was developed in all groups where the piglets were exposed to three pathogenic strains of *E. coli*. However, PWD was also observed in piglets exposed to one pathogenic strain of *E. coli* if they contemporaneously shed rotavirus (**IV**). As the rooms were thoroughly disinfected on arrival of the pigs the rotavirus was of endogenous origin and because rotavirus not was seen after day 6 post weaning (**V, VI**) the shedding was probably induced by the provocations of weaning.

Taken together, these observations strengthen previous suggestions that more than one provoker are required to induce PWD (Smith and Jones, 1963; Hampson et al., 1985; Wathes et al., 1989 Nabuurs et al., 1993; Madec et al., 1998; Madec, 2000). These provokers do not necessarily have to be of infective origin, as indicated by the development of PWD in some piglets that only were exposed to one pathogenic strain of *E. coli* but also given ACTH aiming to simulate weaning under extra stressful conditions. Indeed ACTH did aggravate the signs of PWD but was not essential to provoke PWD (**IV**).

The observation that the dominating serotype of *E. coli* could differ between consecutive sampling days also agrees with earlier results, revealing that a specific serotype not necessarily was demonstrated in faeces for consecutive days in diarrhoeic piglets (Nabuurs et al., 1993). This concur observations stipulating that many strains of *E. coli* could be regarded as intestinal transients (Kühn et al., 1993; Nabuurs et al., 1993; Katouli et al., 1995; Katouli et al., 1997). However, the density of the challenge strains in faecal samples from *E. coli* exposed pigs was considered high (**IV, V**), which might indicate a true colonisation of the challenge strains and not only the result of a faecal-oral recycling.

Effects of different preventive measures (II, V)

A multifactorial background to PWD has been stressed above. Despite the fact that PWD was possible to induce by bacterial challenge the results obtained are not contradictory to the suggestions that PWD more likely is dependent on an overgrowth of endogenous bacteria rather than being the result of an infection

(Hampson, 1994). Indeed, situations when apparently healthy pigs risk to develop PWD have repeatedly been demonstrated in terms of low colonisation resistance and diverging intestinal floras between penmates during the week post weaning (Kühn et al., 1993; Katouli et al., 1995; Katouli et al., 1999); **I-V**).

Taken together, this render possibilities to prevent PWD by obstructing proliferation of certain potentially pathogenic clones of bacteria post weaning. The efficacy of a few prophylactic strategies that currently are applied were tested.

ZnO is effective against PWD under practical conditions (Holm, 1988; Poulsen, 1995; Holmgren, 1994), and did indeed stabilise the intestinal flora in piglets not exposed to pathogenic microorganisms post weaning (**II**). As the effect of ZnO only is achieved when administered orally (Schell and Kornegay, 1994), the effect is probably obtained by local toxicity, which reduce the speed of cell proliferations. In the intestine that may prevent an increased proliferation of certain clones, which then not reduce the density of other clones by competition.

The stabilising effect of ZnO was less clear when pigs were exposed to three pathogenic strains of *E. coli* (**V**). Indeed, the diversity of the faecal coliforms post weaning/challenge was initially (day 3 post weaning) more decreased in the ZnO-group than in the infected-untreated control group. In the latter group the challenge dose that comprised three pathogenic strains of *E. coli* might have obstructed an initial proliferation of endogenous coliforms at that time. However, thereafter the diversity of the coliforms decreased rapidly, and on day 7 post weaning similar diversity levels were obtained in these groups. Still, a preserving effect of ZnO was indicated. When the coliform populations within group were compared to the population at weaning in **V**, the highest similarity was always obtained in the ZnO-group. The protective effect of ZnO was further indicated by a lower incidence of diarrhoea and mortality than in the infected control group. However, this protection was partly hidden as the groups were small and one control pig died in PWD, ironically that decreased the incidence of diarrhoea with respect to days at risk in that group.

Feed composition may affect the intestinal flora as mentioned above (Katouli et al., 1997), and may consequently contribute to presence/absence of intestinal disorders. Non-heated meal feed has prolonged passage times through the stomach when compared to heat-processed feed (Johansen and Bach Knudsen, 1994). Consequently, physiologic disease-preventing systems of the pig, such as saliva, HCl and pepsin, are favoured. In the present study (**V**), the intestinal passage of a meal feed was further prolonged by addition of dietary fibres. In addition, by inclusion of lactose the feed comprised the major source of energy prior to weaning, aiming to reduce the effects of the abrupt switch in diet at weaning. Indeed, the coliform flora was least affected by weaning/challenge in this group (**V**). The diversity of the coliform flora expressed no accentuated dip post weaning and the challenge strains were not capable to induce an increased

similarity between the faecal coliform populations of the group members. Yet, the comparison with the flora obtained at weaning indicated that pigs in this group undramatically altered their intestinal flora post weaning in a way earlier shown in healthy pigs (Katouli, 1997). In the other groups, dominated by either the challenge or the probiotic strains this switch was hidden. Finally, a disease resistant effect of the fibre and lactose enriched meal feed was indicated by a lower incidence of diarrhoea and mortality than in the infected control group. However, as regards the ZnO-group, this protection was partly hidden by the death of a piglet due to PWD in the infected control group.

The use of probiotics in terms of non-pathogenic bacteria have in several studies been proven useful (Barrow and Page, 2000; Methner et al., 1999; Winberg et al., 1993). However, results obtained with probiotics are conflicting, as reviewed by Jonsson and Conway (1992). As ETEC are dependant on receptor specific adhesions (Ofek and Sharon, 1990) a competitive inhibition of these sites by non-pathogenic bacteria could in theory prevent or reduce the effects of ETEC. In **V**, 10^8 CFU of each of 60 different apathogenic strains of *E. coli* were given in that purpose. They were administered orally 15 minutes prior to challenge with pathogenic strains of *E. coli* via the environment. Indeed, the incidence of PWD was significantly lower in this group compared to all other challenged groups, including those given ZnO and meal feed. The potential of "good bacteria" was demonstrated by a remarkably low diversity of the faecal coliform flora maintained during the entire study period. This suggests that some of the non-pathogenic strains offered were good intestinal colonisers that managed to drive others strains out by competition. Despite the low diversity obtained, the piglets resisted diarrhoea as the dominating clones were non-pathogenic

In conclusion, all preventing measures undertaken in **V** appeared to have a potential to be of help in preventing PWD. The supportive role of proper management should however not be neglected in practical pig production. The fundamental importance of relevant hygiene and animal flows that prevent spread of infection can not be overestimated. These tools may however certainly be supported by results obtained in this study, like for instance the relevance of taking the age relation to immune functions into account when weaning pigs and the potential of designing pig feed adapted to the requirements of certain ages appears enormous.

Concluding remarks

These studies support theories suggesting that PWD is a syndrome of multifactorial origin. For instance, the negative impact of stress at weaning was elucidated by development of graver PWD in piglets given ACTH post weaning. Still the results obtained also highlight the importance of pathogenic strains of *E. coli* as potential infective agens in the pathogenesis of PWD. For example, none of the uninfected piglets developed diarrhoea but 38 out of 56 piglets infected with several pathogenic strains of *E. coli* did.

- The phagocyting and killing capacity of neutrophils in serum was found sufficient from day 6 post partum and these functions were not affected by weaning.

- The leukocyte levels increased due to weaning regardless of bacterial challenge.

- Piglets are not mature with respect to immune functions at weaning. Still, weaning pigs were found capable to mount relevant immune responses, but the levels of these responses were not validated. Some immune responses decreased due to weaning.

- A disturbance of the balance in the coliform flora was always observed during the weeks post weaning, regardless of the clinical health status of the pigs.

- Feed supplementation with 2500 ppm ZnO at weaning reduced the negative impact of weaning on the intestinal coliform flora.

- PWD was not induced by an exposure via the environment with a single pathogenic strain of *E. coli*, despite that piglets were truly infected. However, challenged pigs displayed a longer period with a disturbed coliform flora than control pigs.

- Three pathogenic strains together induced PWD. PWD was also induced by one serotype in connection to shed of rotavirus. Rotavirus itself did not induce PWD. ACTH was not required to induce PWD, but amplified clinical signs.

- ZnO-enrichment of feed, meal feed with lactose and dietary fibres; and a probiotic comprising 60 non-pathogenic *E. coli*-strains all appeared to have a potential to prevent PWD.

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