# THE ETIOLOGY, PATHOLOGY AND EPIDEMIOLOGY OF BACTERIAL GASTRO-ENTERITIS IN CALVES AND PIGLETS INTRODUCTION

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## Session 2

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## INTRODUCTION

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Intestinal pathology is the main cause of morbidity and mortality in young animals. Diarrhea has several etiologies which involve several bacterial strains (*Escherichia coli*, Salmonella, Clostridium, Campylobacter) viruses or parasites such as Cryptosporidia and Coccidia.

Beyond question, the enterotoxigenic *E. coli* (ETEC) is foremost, because of the morbidity and mortality it induces and the extent of studies and research carried out on this strain. Of the two septicemic and enteric syndromes it causes in the new-born, ETEC is the cause of most pathological cases and most economic losses. It results in profuse diarrhea that can be fatal. The virulence of the *E. coli* strains, that bring on these symptoms, has two characteristics: the first consists in the secretion of one or possibly two enterotoxins which leads to a drawing of water and electrolytes into the intestinal lumen, which causes the diarrhea. The second, called the adherence factor, is specific to most animals and to a particular site along the digestive tract. It is made up of a peripheral fibrous cell structure that enables it to adhere to the intestinal mucosa.

Epidemiological research has stressed that most of the animals affected are very young, almost three quarters are under four or five days of age (Martel *et al.*, 1981). However, by lack of available ETEC selective techniques, no progress has been made to detect them in microbial sub-groups until two years ago when their stocks and mode of transmission were investigated. The prevalent path of infection and transmission is no doubt fecal-oral. Some important progress has recently been made owing to the application of original methods (ELISA) (Van Zijderveld *et al.*, 1982) as well as to the use of efficient markers (Moulin *et al.*, 1983; Contrepois and Gouet, 1983) Excretion is now known to last several weeks. Mothers can host large amounts of ETEC, and all young calves are carriers, whether healthy or not. Thus, ETEC infections can persist in a dairy herd for several months because the bacterial agent is recycled to older calves and cows. The pathogenic strain can emerge when another disorder, with another cause appears. Nonetheless, contaminated and infected calves are still the greatest cause of contagion.

The preferred site of ETEC development is located in the anterior segments of the small intestine, where there is a gradient of decreasing anteroposterior sensitivity to enterotoxins. Small intestine epithelium adherence is an important parameter of ETEC virulence. By releasing the toxin when in touch with membrane receptors, its action is considerably reinforced. These adherence antigens are host-specific, to the anterior small intestine and they are immunogenic. The first identified adhesin was isolated from pig ETEC and official nomenclature designated it K88 (Jones and Rutter, 1972). Different identical antigen variants were later identified and named K88ab, K88ac and K88ad. The $K88^-$ ETEC from the pig has also been shown to produce pili called the 987P (Morris *et al.*, 1982) which confers adherent properties. The K99 antigen common to calf and lamb ETEC is also present in the piglet.

More recently in sub-groups O9 and O101 it has been observed in the calf that there is an additional adhesin, the F41 (Contrepois *et al.*, 1982). Last of all, two other structures the F(y), F(92b) have just been revealed, which brings the total number of bovine ETEC structures known to day to four (Con-

trepois *et al.*, 1982). The respective differences between these different pili is currently being studied. The $K99^+Fy^+$ strains have *in vitro* adherence properties that are completely comparable to those of $K99^+Ky^-$ which would mean that Fy has a preponderant role. Besides, the latter can coexist in the same cell as K99 and F41. The Fy adherence is inhibited by the N-acetyl glucosamine in concentrations higher than 0.5 %. It is still not possible to indicate the true frequency of associations of K99 with Fy and F92b, but current observations on pathogenic calves justify investigations.

Adherence antigens are fine protein fibres and their synthesis is generally codified by a plasmid with the exception of 987P which is coded by chromosomes (Y is now being studied). The role of these antigens and their specificity as regards to the host in the colonization of the small intestine was able to be analysed owing to the use of strains with one or several plasmids: K88, K99, etc...

More progress is also being made in studying the biosynthesis of adhesins. With the exception of Fy, none of these adhesins is synthetized when the bacteria are grown at 18 °C. The culture medium composition has an important role in the biosynthesis of some adhesins. With K99 the use of a semi minimum medium (Guinee *et al.*, 1977) is essential, to ensure the growth of all strains. Some $K99^+$ strains require glucose to express adhesins (glucose-dependent strains) whilst others do not (glucose-constitutive) (Girardeau *et al.*, 1982). Furthermore, adding 10 mM AMPc has no effect on the biosynthesis of K99. Depending on whether the serological type of the K99 strains involved was O or K, the production of K99 was more or less pronounced. Alanine amino-acid repressed K99 and F41 biosynthesis (but not Y biosynthesis) which partially explains the difficulty in finding *E. coli* $K99^+$ strains again in some media, and the absolute necessity of using a specific medium (Girardeau *et al.*, 1981).

Finally, great progress has been made in characterizing the different adhesins: the amino-acid composition of different subunits, nucleotide sequence coding or protein subunits of K88ab and K88ac, as well as amino-acid sequence of the N end of K88, K99, CFAI and F41. The genetic map of K88 and K99 determinants was set up and DNA fragments that coded K99 and K88 were cloned and transferred.

As enterocyte adherence constitutes the initial stage of the pathogenicity process, the inhibition of this adherence makes it possible to predict ETEC establishment in the small intestine, which thus ensures the prevention of enteritis which they determine. In the sow, the ewe and the cow, the immunogenic action of pili was also taken advantage of to induce the appearance of specific antibodies which, when concentrated in the mammary gland during the first few days after birth, are transmitted to the new-born in colostrum. When absorbed in sufficient amounts right after birth, these antibodies prevent the colonization in the small intestine of strains that carry these corresponding factors of adherence specificity and protect the young animal against a natural or induced infection (Isaacson *et al.*, 1980; Desmettre *et al.*, 1982).

The production of a thermolabile immunogenic enterotoxin by bacterial strains from the pig made it possible to vaccinate using one or the other of these inactivated toxins, associated with or without the specific adherence factors of pig strains. When administered to the sow in gestation, such vaccinations induce antibody synthesis which neutralize the activity of the enterotoxin that may be produced in the enterocytes, when transmitted to the piglet in the colostrum.

ETEC is indisputably one of the best-known bacteria, as much for its physiology as for its pathogenicity factors and it is an exemplary model for studying the mechanisms of bacterial virulence whilst at the same time, it provides a mean of specific prevention of its effects.

Compared to colibacillosis, salmonellosis is less frequent; it affects older animals and in particular meat-producing animals. It involves animals in industrialized rearing systems which often undergo many types of stressful situations: transportation, feeding, antibiotics, which disturb the digestive microflora that favour the emergence of the pathogenic bacteria present in the digestive tract, but which are repressed by host defenses. Nevertheless, Salmonella are becoming a problem with the development of intensive calf husbandry systems. In the calf, infection most often evolves as acute septicemia that is concomitant with diarrhea and frequent pulmonary localization. In most cases, two serotypes are the cause of these disturbances: *S. dublin*, the specific bovine serotype and *S. typhimurium* a ubiquitous serotype, which is pathogenic to many animal species and man. These bacteria are characterized by their ability to resist many antibiotics, which explains the difficulties in choosing a suitable treatment and the dangers of antibio-prevention. Thus, the intestinal flora of animals, especially monogastrics reared intensively, can build up a considerable stock of antibio-resistant plasmids that evolve intricately as a function of the contamination of other animals and selection pressure.

Feed is sometimes a major path to infection, especially meat flour, which tends to put plasmids that were probably selected by farm animals back into circulation. In calves, extensive use of antibiotics, either for therapy or prophylaxis, has largely

contributed to multi-resistant strains. Thus, the antibio-resistance of animal salmonella is still of concern, and above all, should focus on preventive means such as improved transportation and better sanitary conditions, which are imperative.

Cryptosporidia protozoa, parasites related to coccidia are a major cause of diarrhea in the young. Moreover, the unicity of the cryptosporidium makes the same strain pathogenic to the calf, the lamb, the piglet and man. It appears that immuno-depressed subjects are more likely to contract the illness. Cases of cryptosporidiosis are frequently associated with other known pathogenic agents: ETEC, rotavirus and coronavirus (Morin *et al.*, 1979). Contagiousness is relatively high, and calves remain receptive until one month after birth. Diarrhea occurs around the seventh day, even before, if associated with another pathogenic agent. The complete cycle of this parasite is not known, but it does include a sexuated phase and an asexuated phase. Furthermore, bovine cryptosporidia do not appears to need an intermediary host, and they are directly infectious (Moon and Bemrick, 1981). Cryptosporidia as opposed to coccidia are extra-cellular parasites that get attached to the microvilli of the epithelial cell, mainly in the lower gut, as the ileum is the most frequent site of development. Diagnosis is often difficult because of the simultaneous and frequent presence of *E. coli*, rota and coronavirus, and also because it involves a small-sized parasite that is difficult to see. The parasite often goes unnoticed.

In addition to the main types of pathology that involve known or strongly suspected infectious agents whether bacterial, parasitic, or viral, a good one-third of diarrhea in the young cannot be explained. These cases should be studied further, with strains that have been investigated, and better still, in association with several more resistant pathogenic strains found in older animals, as already mentioned. Still other cases can be caused by the association of many opportunist germs that are very difficult to detect, because they only show their pathogenicity in specific, well-defined ecological conditions which either we do not know or which we still have not attempted to reproduce. Some are found in the digestive tract which is a particularly intricate site, owing to the physiology of the host itself, and to the complexity of the microflora and the many interactions that occur between them. What is most difficult, is to reproduce true conditions, since they are required to show the pathogenicity of the agents.

Among the factors likely to be favorable, diet and the use of antibiotics are probably the most important. Both feedstuffs and antibiotics result in the emergence of the germs most frequently subdominant, except for the bacterial strains which repress them. What follows is the appearance of totally different microflora which strict anaerobes usually repress, and this allows such strains as *Proteus, Pseudomonas, E. coli* to predominate. *Clostridium* can then be associated with these strains. This has been well demonstrated with *C. difficile*, the cause of colites, associated with antibiotics in man and guinea pigs. The pathogenesis of *Clostridium* even appears to be present exclusively in subjects receiving antibacterial agents. No research has been conducted on this strain in the calf and pig from this new point. However, the *C. perfringens* which is investigated and isolated very frequently in healthy as well as in diarrheic animals, is always under study. By contrast, although the toxins of *C. perfringens* have been identified and are understood, no work has been carried out on the adherence factors of these strains to the intestinal epithelium, which is a determining factor for all pathogenic bacterial strains.

Parasites must also be taken into consideration. Parasites have hardly been studied until now. Coccidia can be assumed to aid the emergence of potentially pathogenic strains in the intestinal lumen. Finally, respiratory pathology, the main pathology in veal calves, should not be overlooked because it probably is involved in many cases and facilitates the appearance of gastro-intestinal disorders.

## Conclusions

Great progress has been made over the last few years in the digestive pathology of the new-born.

Colibacillary diarrhea in calf prophylaxy which have been widely recognized for their efficiency are now available along with vaccination against Ag K99 and other adhesins. Associated with rehydration therapy, colibacillary diarrhea in the calf should become much less frequent in the near future and permanently. In the piglet, despite the dual vaccination against the TL enterotoxin and K88 and 987P antigens, less progress has been made. It will not be long before they are definitive.

Aside from these important positive results, more dependent research on the ETEC, which provides a wonderful instrument for studying the biosynthesis of biochemical, physiological and genetic mechanisms, would be useful. Investigation of parasites, especially Cryptosporidia and Coccidia must necessarily be furthered, both because of their own virulence and their interactions with other pathogens or potentially pathogenic agents. The epidemiology of *Salmonella* must be continued, given the dangers it involves for man and animals. Studies to find new strains of *Clostridium* must be undertaken, and the role of *Campylobacter* should be determined. Lastly, it is

now essential to perfect models to investigate the influence of stress on the infectious pathology of the young, because all germs can emerge as a result of insufficient immunological reactions associated with imbalances in intestinal microflora which are provoked by all types of stress.

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