# Summary

The group of *E. coli* strains is a highly heterogeneous group of strains, including pathogenic and non-pathogenic strains. The classification of these strains is made upon specific virulence factors of these bacteria. Some of these pathogenic strains are not capable to cross over the intestinal border and are responsible for intestinal disorders associated with diarrhoea. Other strains can cross the intestinal border and produce septicaemia and other complications depending on the infected organs. These pathogenic strains of *E. coli* interact with the host in a typical manner and produce specific lesions. These specific interactions are observed after the colonisation of the gut by pathogenic bacteria and are the results of the presence of specific virulence factors. The colonisation of the gut is mediated by specific adhesins, which are specific for each group of pathogenic *E. coli*. It seems that these adhesins are not only responsible to initiate the interaction between the pathogen and the host but are also responsible for the host specificity shown by some pathogenic strains. It's for that that a better understanding of these adhesins would permit a better understanding of the host specificity and a better evaluation of the zoonotic potential of these strains.

The aim of this work was to identify bacterial structures involved in the intestinal adhesion step and in the intestinal colonisation of the enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC) and verotoxigenic *E. coli* (VTEC) bovine isolates, focusing mainly (but not exclusively) on strains of serogroup O26.

In order to achieve this objective, two different approaches have been followed: i) the immunologic approach and ii) the genetic approach. The immunologic approach is using the benefits of the 2F3 monoclonal antibody (MAb), which was obtained against an outer membrane protein extract from a human O26 EHEC strain. The work on this approach focused on the study of this MAb as epidemiological tool and on the identification of the epitope recognised by this antibody and of its genetic basis.

The work on the genetic approach involves the screening by PCR and by colony hybridization of a collection of EHEC, VTEC and EPEC of human and bovine isolates for the presence of homologues for gene clusters coding for fimbriae of type I, II, III and IV: (i) putative adhesins of human EPEC and EHEC strains like LifA, Iha, LpfA, BfpA; (ii) fimbriaires adhesins (CS31A et F17) and afimbriaire adhesins of Afa family (Afa III, Afa VII, Afa VIII and F1845) described for other groups of bovine pathogenic *E. coli*.

The accomplished work allowed, first of all, to confirm that the 2F3 MAb is specific for the O26 EPEC and O26 EHEC strains without being capable to distinguish between human and animal isolates, that means without being capable to distinguish them depending on theirs host.

Moreover, it has been shown that: i) the epitope recognised by the 2F3 MAb is component of the O antigen from the O26 EPEC and O26 EHEC strains; ii) the genetic basis of this epitope is located inside the O antigen gene cluster of O26 EPEC and O26 EHEC strains; and iii) the 2F3 + and O26 + characters are two characters that can be dissociated by random insertional mutagenesis in a bovine EHEC strain. Nevertheless, the results of this work not allowed us to explain the specificity of the 2F3 MAb for the O26 EPEC and O26 EHEC strains. Actually, by sequencing the O-antigen gene cluster (the *rfb* locus) of an O26 non-EPEC and non-EHEC strain and comparing the obtained sequence with the already published sequence of the O-antigen gene cluster of an O26 EHEC strain no major differences (which could explain the specificity of the 2F3 MAb for the O26 EPEC or EHEC strains) were observed.

The obtained results in the genetic approach have been shown that: i) all O145 and O157 EPEC and EHEC strains were positive for the LpfA probe (*lpfA* gene is coding for the type IV pili) and all other human and bovine strains belonging to other serogroups (O5, O26, O103, O111 and O118) have no homologue sequences for this probe; ii) a big majority of EPEC and EHEC strains belonging to O5, O26, O103, O111 or O118 serogroups were positives for the LifA probe (the *lifA* gene is involved in the synthesis of adhesins not very well characterised) but none of those belonging to the O145 et O157 serogroups; iii) different proportions of these strains belonging to various serogroups were positives for the Iha probe (the *iha* gene is involved in the synthesis of an adhesin not very well characterised); and iv) ten out of twelve bovine O26 EPEC strains were positive for the ClpE probe and all other strains (human EPEC and EHEC strains and bovines EHEC strains) were negative for the ClpE probe. The *clpE* gene is involved in the synthesis of the chaperon protein of the CS31A fimbria, which was described for the enterotoxigenic and septicaemic bovine and porcine *E. coli* isolates.

Since the O145 and O157 EHEC strains show the same pathotype, it looks like that the presence of the *lpfA* gene and the absence of the *lifA* gene is linked more to a specific pathotype than to a specific serotype. Concerning the results obtained results with the ClpE probe for the O26 EPEC strains, it is possible that these strains have not an entire *clp* gene cluster since all the strains positive for the ClpE probe were negative for the ClpG (*clpG* gene

is involved in the biosynthesis of the major unit of the CS31A fimbriae). Nevertheless, it is possible that the *clpE*-like gene of these strains belongs to an entire gene cluster for which the major unit is different from the ClpG.

Finally, the screening of a collection of O8 and O20 VTEC bovine isolates for the presence of sequences homologues to the genes involved in the biosynthesis of adhesins of the Afa family (Afa III, Afa VII, Afa VIII and F1845), adhésines produced mainly by human uropathogenic isolates and by some septicaemic strains isolated from animals, allowed the identification of two O8 VTEC bovine strains harbouring the *afa-VIII D/afa-VIII E* genes and expressing the Afa VIII E adhesin.

During this work, a lot of progresses, that could be useful for a better understanding of the pathogenesis of the EPEC, EHEC and VTEC strains, have been done. Despite that, the general objective of this project – description of new factors involved in the initial adhesion stage and/or in the intestinal colonisation by the EPEC, EHEC and VTEC bovine strains in order to allow an easy and reliable diagnostic of these strains – have been not accomplished.

In perspective of this work, complementary works should focus: i) to identify the epitope recognised by the 2F3 MAb; and ii) to investigate which is the role of the adhesins, for which homologues sequences have been described in the human or bovine EPEC and EHEC isolates of different serogroups, in the adhesion to eukaryotic cells like bovine and human enterocytes.