Chapter 2: Objectives

NoVs are a major cause of acute gastroenteritis and are responsible of large outbreaks with important economic consequences in industrialised countries. In Belgium, NoV was found to constitute the first cause of foodborne gastroenteritis before *Salmonella* spp and *Bacillus cereus*. Genetically related NoVs have been identified in domestic animals including pigs, calves and dogs; yet the clinical significance of these infections remains to be defined. Furthermore, HuNoVs were able to experimentally infect gnotobiotic pigs and calves. Legitimately, the detection of NoVs in humans and animals raised questions on zoonotic transmissions or the existence of an animal reservoir. As far, NoVs are considered to be species-specific and previous studies have not been able to confirm a transmission between humans and animals.

NoVs are extremely swift viruses with exceedingly high genetic flexibility due to two major evolutionary forces: point mutation and recombination. These mechanisms could enhance viral host switching and viral host adaptations. Thus, the study of these phenomena is crucial for the understanding of NoV evolution. Molecular epidemiological studies have shown the circulation of highly diverse NoV strains and phylogenetic studies have suggested the existence of recombinant NoVs in all five NoV genogroups. For long, the experimental study of NoV recombination has been hampered by the absence of cell culture systems or small animal models. The discovery of the cultivable MNV has offered new insights for the research on NoV biology.

The aim of this thesis was to evaluate the importance and the potential implications of NoV recombination on the evolution of NoV in natural conditions and in experimental conditions.

By monitoring the genetic nature of NoVs circulating among humans in a country representing the epidemiological situation of Western Europe like Belgium, the importance of recombinant NoVs will be evaluated. Furthermore, an experimental model was set up to investigate the potential of recombination by MNV by coinfecting RAW cells with two distinguishable wild-type viruses. Finally, the consequences of recombination were evaluated by assessing the biological properties of the recombinant virus in comparison with the parental ones both in cell culture and after per oral inoculation in immunocompetent mice.

In the first study, a systematic genotyping procedure was implemented for the analysis of all NoV-confirmed cases of suspected foodborne gastroenteritis reported to the Scientific Institute of Public Health in Belgium during a 4-year period (December 2006 until December 2010). Phylogenetic analysis of partial sequences of the polymerase and the capsid gene were conducted in order to identify potential incongruent genetic clustering between these two parts of the genome. Recombination breakpoints were accurately localised for NoVs suspected to be recombinant by the amplification of sequences covering the junction between ORF1 and ORF2.

The second part of this thesis was dedicated to the *in vitro* generation of recombinant NoVs and to the investigation of the effects of recombination on the viral characteristics. A first objective was the elaboration in our laboratory of an experimental model for the study of NoV recombination using the murine NoV. PCR-based tools allowing the discrimination at different locations in the genome between two wild-type parental viruses were developed to allow the identification of recombinant viruses among the progeny viruses after cell coinfections. Furthermore, the biological properties of the recombinant virus were assessed in cell culture in comparison of the parental viruses in order to determine the impact of recombination on the viral fitness. Subsequently, the effects of recombination were further evaluated in the natural host of MNVs by orally inoculating wild-type mice with the recombinant and the parental viruses.

Altogether, these studies were conducted in order to assess the role of recombination in the evolution of a major pathogen in humans by means of a robust homologous model.