ELSEVIER

Veterinary Microbiology 69 (1999) 139–141

veterinary microbiology

www.elsevier.com/locate/vetmic

Genetic drift and genetic shift during feline coronavirus evolution

Harry Vennema[a,b,*]

[a]*Virology Division, Faculty of Veterinary Medicine, Utrecht University, Utrecht 3584, The Netherlands*
[b]*Center for Companion Animal Health, School of Veterinary Medicine, University of California at Davis, Davis, CA, USA*

Feline coronaviruses (FCoVs) can be divided in strains that cause feline infectious peritonitis (FIP) and strains that cause a subclinical to mild enteric infection, feline enteric coronavirus (FECV). Furthermore, tissue culture adapted FIPV strains exist, that have lost the ability to cause disease. This phenomenon is accompanied by (but not necessarily caused by) deletions in the 7b gene (Herrewegh et al., 1995). The currently available vaccine virus is an example of the attenuated strains. FIPV and FECV cannot be distinguished serologically or morphologically and were therefore called biotypes of the feline coronaviruses; the two viruses differ only biologically. The object of the research was to demonstrate that the biotypes are genetically different and to find out where the (important) differences are located.

A genetic comparison was made of an FECV strain and an FIPV strain from one focal outbreak. The biotypes of these strains were established by experimental infection (Hickman et al., 1995). The genetic comparison revealed that strains of different phenotypes can be genetically more closely related than strains with the same phenotype from other geographic backgrounds (Vennema et al., 1995, 1998). This observation and the fact that the FIP cases followed a widespread infection with FECV, suggested that the FIPV emerged as a variant of FECV (Hickman et al., 1995). To find evidence for this hypothesis we infected 19 cats with the same strain of FECV and looked for clinical signs of FIP which would be indicative of the appearance of a mutant (Poland et al., 1996). The animals were immunosuppressed through feline immunodeficiency virus (FIV) infection to increase the chances of finding a mutant in a relatively small number of cats. It was established that FIV positive cats had 10 to 100-fold higher FCoV viral titers in their faeces, shed virus for a longer period of time and produced lower levels of antibodies after a longer delay than the control cats. After 8 and 10 weeks two of the cats came down

* Present address: Faculty of Veterinary Medicine, Utrecht University, Yalelaan 1, 3584 CL Utrecht, The Netherlands. Tel.: +31-30-2532463; fax: +31-30-2536723
E-mail address: h.vennema@vet.uu.nl (H. Vennema)

0378-1135/99/$ – see front matter
PII: S0378-1135(99)00102-9

with FIP. In a control group of 20 FIV-negative cats which were also infected with FECV, no cases of FIP appeared, indicating that indeed some form of immunosuppression is required for this type of mutant to occur and or become apparent. Viral isolates from the two FIP cats' tissues induced FIP in normal cats after intraperitoneal inoculation. This proves that the FECV had changed genetically into an FIPV in two independent cases. Comparison of more than 8 kb of nucleotide sequence, comprising the 3' one third of the genome, of these FIPV strains with the parent FECV showed a 99.5% homology. In both FIPV strains different small deletion mutations were found, which demonstrate the order of descent. In addition, several point mutations were found. Which of these mutations cause the dramatic changes in phenotype is currently under investigation. Naturally occurring cases of FIP are probably also caused by mutants of the endemic FECV. Evidence for this was found by comparing the sequences of tissue derived FIPV strains from two kittens with a strain from the faeces of a third healthy littermate in a shelter. The three phenotypically different strains were genetically more closely related to each other than to strains from other sources. The two FIPV strains both had a deletion in the 3c gene. The deletions were not identical and the deleted part in either strain was still present in the other strain. Therefore, the two strains were independently derived from a common ancestor, indicating that horizontal spread did not occur and that the FIP causing mutants arose in the individual kitten in which they caused the disease.

In addition to the division in biotypes, FCoVs can be divided in serotype I and II on the basis of cross-reactivity with canine coronavirus (CCV) in virus neutralization assays. In contrast to type II viruses, type I viruses show hardly any or no neutralization with anti-CCV sera. Like CCV, type II FCoVs are relatively easy to culture, whereas type I FCoVs are not. For this reason, acquisition of genetic data for type I viruses has been lagging behind that of type II viruses. Thanks to the advent of PCR technology this gap has been largely filled with sequence data for type I viruses both from tissue culture adapted strains as well as from clinical materials. Comparison of the sequences of type I and II FCoVs and CCV revealed that type II viruses have spike genes that are much more closely related to CCV than to type I FCoVs. This explains the cross-reactivity patterns in neutralization assays mentioned above, since the spike protein is the target for neutralizing antibodies. However, sequences from the 7b gene located at the 3' end of the FCoV and CCV genomes showed that type I and II FCoVs form a homogenous group, distinct from CCV (Herrewegh et al., 1995). The two observations combined, suggested that the type II viruses are derived from recombination of type I FCoV and CCV. Four different type II strains FIPV 79-1146, FIPV Wellcome, FIPV UCD6 and FECV 79-1683 were analyzed and all were found to have unique 3' recombination sites. This means that all recombination events were independent. From multiple sequence alignments the recombination sites could be mapped to within 10 to 30 nucleotides. They are located two each in the E and the M gene.

The major and consistent difference between the two serotypes is the spike protein. This was employed to design a diagnostic procedure that can distinguish between infection with a serotype I or II FCoV. A fragment of the spike gene of FECV UCD was cloned in a bacterial expression vector. The selected fragment shows a high degree of conservation among type I FCoVs and a low degree of similarity to serotype II FCoVs, CCV and TGEV. The expression product was partially purified and used to setup an

indirect ELISA. All serum samples from cats experimentally infected with type I FCoVs reacted positively in the ELISA and none from type II infected cats. Remarkably, type I FECV infected cat sera also did not react. The latter observation suggests that antibodies to this fragment may be formed only during an FIPV infection. The test was also used to establish the prevalence of type I FCoV infection among seropositive cats in the Netherlands. This showed that only 12 of 127 serum samples that were positive in the immunofluorescence assay (IFA) were negative in the ELISA. Of these 12, six had reproducibly high IFA titers. These six serum samples were subsequently tested in a neutralisation assay with a type II FCoV (FIPV 79-1146). In this test they were also negative. The most likely explanation is that these six sera were from cats infected with a type I FCoV that did not respond to the selected fragment of the spike protein. These data indicate that if serotype II FCoVs are present in the Netherlands they form a small minority.

References

Herrewegh, A.A., Vennema, H., Horzinek, M.C., Rottier, P.J., de Groot, R.J., 1995. The molecular genetics of feline coronaviruses: comparative sequence analysis of the ORF7aI7b transcription unit of different biotypes. Virology 212(2), 622–631.

Hickman, M.A., Morris, J.G., Rogers, Q.R., Pedersen, N.C., 1995. Elimination of feline coronavirus infection from a large experimental specific pathogen-free cat breeding colony by serologic testing and isolation. Feline Pract. 23(3), 96–102.

Poland, A.M., Vennema, H., Foley, J.E., Pedersen, N.C., 1996. Two related strains of 101 feline infectious peritonitis virus isolated from immunocompromised cats infected with a feline enteric coronavirus. J. Clin. Microbiol. 34(12), 3180–3184.

Vennema, H., Poland, A., Floyd Hawkins, K., Pedersen, N.C., 1995. A comparison of the genomes of FECVs and FIPVs and what they tell us about the relationships between feline coronaviruses and their evolution. Feline Pract. 23(3), 40–44.

Vennema, H., Poland, A., Foley, J., Pedersen, N.C., 1998. Feline infectious peritonitis viruses arise by mutation from endemic feline enteric coronaviruses. Virology 243(1), 150–157.