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Prevalence and Genetic Pattern of Feline Coronaviruses in Urban Cat Populations

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SUMMARY

The prevalence and phylogeny of feline coronaviruses were studied in urban cat populations by sampling of 113 clinically healthy cats. Rectal swab samples were subjected to a nested reverse-transcription polymerase chain reaction, specific for the conservative nucleocapsid region of the virus genome. More than 30% of the sampled animals proved positive for the presence of feline coronaviruses. The nucleotide sequences of amplified 440 bp products were determined, aligned and the phylogenetic analysis revealed noticeable genetic clusters among the prevalent feline coronaviruses in the surveyed geographic area. These findings will hopefully contribute to the elucidation of the epidemiology of feline infectious peritonitis. © 2000 Harcourt Publishers Ltd

KEYWORDS: Feline; coronavirus; RT-PCR; prevalence; phylogeny.

INTRODUCTION

Feline coronaviruses (FCoV) are members of the family Coronaviridae in the newly established Order Nidovirales (de Vries et al., 1997). The family Coronaviridae is divided into three distinct antigenic groups (Siddell et al., 1983; Horsburgh et al., 1992; Motokawa et al., 1996); FCoV belongs to the group which contains a human respiratory coronavirus (HCV 229E), transmissible gastroenteritis virus (TGEV) of swine, porcine respiratory coronavirus (PRCV), porcine epidemic diarrhoea virus (PEDV), and canine coronavirus (CCV). Feline coronaviruses are enveloped viruses with at least 20kb long, single-stranded, positive-sense RNA genomes. In addition to non-structural genes, the genome encodes four structural proteins termed S (spike or peplomer), E (envelope), M (matrix), and N (nucleocapsid; de Groot et al., 1987; Luytjes, 1995).

Two serotypes of FCoV have been discriminated (Hohdatsu et al., 1991a,b) which differ in their in vitro growth characteristics and in their prevalence in the field (Pedersen, 1987b; Hohdatsu et al., 1992). The replication of coronaviruses is characterized by a high frequency of RNA recombination (Lai et al., 1985; Makino et al., 1986; Keck et al., 1988; Baric et al., 1990; Kusters et al., 1990; Kottler et al., 1995; Lai, 1996). Recent findings suggest that type II feline coronaviruses have arisen from a recombination event between CCV and a type I FCoV (Herrewegh et al., 1998).

Based on pathogenicity, FCoV has been divided into two biotypes, termed feline enteric coronavirus and feline infectious peritonitis virus (FECV and FIPV, respectively; Pedersen, 1987a). Feline infectious peritonitis virus (FIPV) is an important pathogen of cats, causing death among young animals, especially those originating from...
The prevalence of asymptomatic FCoV carriers and to investigated the phylogeny of coronaviruses in urban cat populations.

**MATERIALS AND METHODS**

**Cat populations**

The 113 sampled cats lived in urban areas of Eastern Hungary. Approximately 60% of the animals were allowed outside and 40% were kept indoors. The majority of the cats (88 animals) are kept alone in a household. Two breeding stocks were involved in the investigations, comprising 4 and 11 breeding Persians, respectively. The age distribution of the examined animals from either gender varied between 6 months and 10 years. Altogether, 71 mixed-bred (63%), 34 Persian (30%), and eight Siamese (7%) cats were sampled.

**Clinical specimens and sample preparation**

The clinically healthy cats, presented to private veterinary clinics for routine examination, vaccination, or castration were sampled by rectal swabbing as described by Foley et al., (1997b). The RNA extraction was carried out according to Boom et al. (1990) and Cheung et al. (1994), using size-fractionated silica and guanidine thiocyanate based buffers.

**Reverse transcription**

The FCoV cDNA synthesis was performed in 28-μL reaction mixtures. A preliminary mixture containing 5 μL RNA, 5 μL diethyl pyrocarbonate-treated water, and 1 μL random hexamers (0.02 U; Pharmacia Biotech) was incubated at 65°C for 5 min to denature the RNA. Subsequently, tubes were placed on ice, and 17 μL premixture was added containing 2.5 μL of each deoxynucleotide triphosphate (2 mM; Pharmacia), 5 μL of 5 X reaction buffer [0.25 M Tris-HCl (pH 8.3), 0.375 M KCl, 15 mM MgCl2], 1 μL RNAsguard (24 U; Pharmacia), and 1 μL Moloney murine leukaemia virus reverse transcriptase (200 U; Gibco BRL). The reactions were incubated at 37°C for 90 min, followed by incubation at 98°C for 5 min to inactivate the enzyme.

**Primer selection**

Primers were selected from the highly conserved nucleocapsid (N) region of the FIP virus strain 79–1146 genome (Vennema et al., 1991; GenBank Acc. No: X56496) using the OLIGO 4.0 program (National Biosciences Inc.). The sequences of the primers are shown in Table I.
PCR
The PCR assays were carried out in 50-μL volumes. The reaction mixtures contained 5 μL of 10 X GeneAmp PCR buffer II (Perkin-Elmer Cetus), 15 pmol of each primer (OFIP1A and OFIP2A in the first reaction), 2.5 μL of each deoxynucleotide triphosphate (10 mM; Pharmacia), 2.5 mM MgCl₂, AmpliTaq DNA polymerase (1 U; Perkin-Elmer Cetus), and 5 μL of cDNA. Two drops of mineral oil (Sigma) were added to prevent evaporation.

The second (nested) PCR was performed under similar conditions to the first PCR, using 15 pmol of each of the primers OFIP4 and OFIP3 and 2 μL of the first PCR product as target. Distilled water was used as negative control.

During the 35 cycles of the first round, the temperature profile was: 94°C for 45 s for denaturation, 45°C for 60 s for primer annealing, (50°C for 60 s for the first five cycles), and 72°C for 2 min for synthesis. For the second round of amplification 96°C for 45 s, 52°C for 1.5 min, and 72°C for 3 min was applied. This cycle profile was repeated 30 times followed by a final extension step at 72°C for 7 min.

The PCR products were detected by agarose gel electrophoresis and ethidium bromide staining.

Nucleotide sequencing and phylogenetic analysis
A total of 36 nested PCR products were sequenced in both directions with primers OFIP3 and OFIP4, using an ABI PRISM sequencing device, based on the incorporation of fluorescent labelled dideoxynucleotide terminators. The nucleotide sequences were aligned by the MegAlign program of the DNASTAR multiple program package (DNASTAR Inc.) using the Clustal method (Higgins et al., 1992).

RESULTS
PCR positive cats
By running the nested PCR, 36 (31.8%) out of the 113 examined animals were found to be positive. The PCR positivity of the pure-bred and the mixed-bred animals was 45.2 and 23.9%, respectively. The virus was most frequently detected among the Persians (52.9%), and the two Persian breeding catteries showed 100% (all the four animals being positive) and 72.7% (eight positives out of the eleven cats) of prevalence.

The PCR positivity was evenly distributed through the age categories, exemplified by 34.6% positive animals in cats younger than 1 year of age, 31.6% among animals aged 1–5 years, and 35.3% among cats older than 5 years.

Phylogenetic analysis of the amplified sequences
Figure 1 shows the dendrogram prepared from the sequence alignments and reveals the marked clustering of the detected viruses. Furthermore, FCoV originating from the two Persian breeding stocks showed a very close similarity to each other (with one or two nucleotide differences) and displayed separate clusters with some distance (due to four to six nucleotide alterations) from the majority of the examined viruses. In this major group there is an apparent cluster comprising cats Nos. 72, 8, 48, 58, 49, 69P, and 36 (see Fig. 1). The overall difference between the most disparate strains originating from the examined cats remained below 10 out of the examined 440 nucleotides.

DISCUSSION
In the present study a representative group of animals was investigated to estimate the prevalence of asymptomatic carriers and the genetic diversity of feline coronaviruses occurring in urban cat populations. Although other regions of the genome have been amplified by various groups (Li & Scott, 1994; Herrewegh et al., 1995; Gamble et al., 1997; Gunn-Moore et al., 1998), we applied an RT-PCR assay which targeted the highly conserved N region of the FCoV genome in order to detect all possible

| Table I | Primers used for the RT-PCR of feline coronaviruses |
|---------|---------------------------------------------------|
| Primer  | Sequence (5'-3') | 5' positiona |
| OFIP1A(F) | ATTTT GGAAT TTATG TCCGA GAGA | 990 |
| OFIP2A(R) | CTAGC ACCAT AGAAA GTTGT CACA | 1598 |
| OFIP3(F) | CGCTG AGAGG TGGTT CTTTT ACTTC | 1110 |
| OFIP4(R) | CTTCC AGGTG TGTTT GTTGG CATTTC | 1554 |

F, forward primer; R, reverse primer. a, nucleotide positions in the nucleocapsid coding region, according to Vennema et al. (1991).
variants of the virus. Using this assay, we found that approximately 30% of the clinically healthy cats carried FCoV in the monitored area. The seroprevalence has not been determined in this study; however, 80–90% seropositivity against FCoV has been described in catteries and 10–50% of cats in single cat households (Pedersen, 1976; Addie & Jarrett, 1992a,b). In accordance with previous studies (Foley et al., 1997a), there was no significant correlation between positivity and the gender of the animals. However, the pure-bred animals showed higher rate of positivity than the mixed-bred groups, supporting the observation that pure-bred cats are more susceptible to FCoV infections (Foley & Pedersen, 1996). Since the positivity in the examined groups of age was evenly distributed,
the detection of some chronically infected asymptomatic carrier animals is assumed, in agreement with previous reports (Herrewegh et al., 1997).

It is known that FCoV, CCV, and TGEV are antigenically related. However, based on the deduced amino acid sequences of N and M proteins, separation of FCoV from the other members of group I coronaviruses was shown (Motokawa et al., 1996). Considering antigenicity and pathogenicity, FCoV have been divided as two serotypes and two biotypes, respectively (Pedersen, 1987a, b; Hohdatsu et al., 1992). Based on antigenicity, the separation of the two serotypes is clearly demonstrated (Hohdatsu et al., 1992). However, the discrimination between the two biotypes is complicated, although considerable research has been carried out to investigate the hypothesized evolution of FIPV from FECV (Herrewegh et al., 1997; Vennema et al., 1998). For a better understanding of this process, large populations of clinically healthy cats should be screened and the presence of FCoV should be estimated. Subsequently, the genomes of the detected FCoV variants should be analysed in order to investigate the alterations leading to the switch of the biotype.

The present study aimed at the first main task, i.e. to survey populations of healthy urban cats of various breeds to reveal the prevalence of FCoV. Further, we performed a pilot phylogenetic analysis based on N gene sequences in order to investigate the genetic heterogeneity of the coronaviruses detected in the cat populations. We demonstrated an interesting clustering of the feline coronaviruses: the two Persian breeding stocks composed separate clusters different from those of the prevalent viruses (see Fig. 1). Since it was hypothesized that cats infected with FCoV develop resistance against superinfection by other coronaviruses (Herrewegh et al., 1997), the viruses carried by the Persian breeding animals could represent a unique variant of FCoV. Visiting exhibitions and mating abroad must have contributed to the infection of these otherwise closed stocks of Persian cats. Another noteworthy fact is that, since the establishment of the stocks (which was more than 10 years ago), the cats have not been affected seriously by viral diseases and the suspicion of FIP has never arisen. Thus, either the FCoV variants present in these Persian cats lack the ability to evolve into FIP virus, or these particular cats have a unique resistance against the circulating viruses. The investigations of further genomic regions of the viruses (ORF3c, ORF7b; Vennema et al., 1998) will hopefully provide more information to answer these questions. An additional cluster, consisting of seven viruses (cats Nos. 8, 36, 48, 49, 58, and 69P, see Fig. 1.) may also have the same source of infection since these cats are kept in a restricted area.

In conclusion, the nucleocapsid-based nested RT-PCR assay proved to be a useful tool for monitoring clinically healthy cat populations and to detect asymptomatic FCoV shedding. The obtained sequence data allowed the grouping of the viruses, even so, the differences remained below 10 nucleotides. Thus, the approaches of molecular virus detection and genetic characterization provide effective novel means to study the spread of FCoV variants. Hopefully, these assays and the collected genomic data will contribute in the understanding of the molecular events responsible for the development of infectious peritonitis in cat populations.

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REFERENCES

Addie, D.D. & Jarrett, J.O. (1992a). A study of naturally occurring feline coronavirus infections in kittens. Veterinary Record 130, 133–7.

Addie, D.D. & Jarrett, J.O. (1992b). Feline coronavirus antibodies in cats. Veterinary Record 131, 202–3.

Baric, R.S., Fu, K.F., Schaad, M.C. & Stohlman, S.A. (1990). Establishing a genetic recombination map of murine coronavirus strain A59 complementation groups. Virology 177, 646–56.

Boom, R., Sol, C.J.A., Salimans, M.M.M., Jansen, C.L., Wertheim-van Dillen, P.M.E. & Van Der Noordaa, J. (1990). Rapid and simple method for purification of nucleic acid. Journal of Clinical Microbiology 28, 495–503.

Cheung, R.C., Matsui, S. & Greenberg, H. (1994). Rapid and sensitive method for detection of hepatitis C virus RNA by using silica particles. Journal of Clinical Microbiology 32, 2593–7.
DE GROOT, R.J., TER HAAR, R.J., HORZINEK, M.C. & VAN DER ZEIJST, B.A. (1987). Intracellular RNAs of the feline infectious peritonitis virus strain 79-1146. Journal of General Virology 68, 995–1002.

DE GROOT, R.J. & HORZINEK, M.C. (1995). Feline infectious peritonitis. In The Coronaviridae, ed. Siddell, S.G., pp. 283–315. New York: Plenum Press.

DE VRIES, A.A.F., HORZINEK, M.C., ROTTIER, P.J.M. & DE GROOT, R. (1997). The genome organization of the Nidovirales similarities and differences between Arteri-, Toro-, and Coronaviruses. Seminars in Virology 8, 33–47.

EVERMANN, J.F., MCKEIRNAN, A.J. & OTT, R.L. (1992). Perspectives on the epizootiology of feline enteric coronavirus and the pathogenesis of feline infectious peritonitis. Veterinary Microbiology 28, 243–55.

FEHR, D., HOLZNAGEL, E., BOLLA, S., HAUSER, B., HERREWEGH, A.A., HORZINEK, M.C. & LUTZ, H. (1997). Placebo-controlled evaluation of a modified live virus vaccine against feline infectious peritonitis: safety and efficacy under field conditions. Vaccine 15, 1101–109.

FOLEY, J.F. & PEDERSEN, N.C. (1996). The inheritance of susceptibility to feline infectious peritonitis in pure-bred catteries. Feline Practice 24, 14–22.

FOLEY, J.F., POLAND, A., CARLSON, J. & PEDERSEN, N.C. (1997a). Risk factors for feline infectious peritonitis among cats in multiple-cat environments with endemic feline enteric coronavirus. Journal of American Veterinary Medical Association 210, 1313–8.

FOLEY, J.F., POLAND, A., CARLSON, J. & PEDERSEN, N.C. (1997b). Patterns of feline coronavirus infection and fecal shedding from cats in multiple-cat environments. Journal of American Veterinary Medical Association 210, 1307–12.

GAMBLE, D.A., LOBBIANI, A., GRAMEGNA, M., MOORE, L.E. & COLUCCI, G. (1997). Development of a nested PCR assay for detection of feline infectious peritonitis virus in clinical specimens. Journal of Clinical Microbiology 35, 673–5.

GUNN-MOORE, D.A., GRUFFYDD-JONES, T.J. & HARBOUR, D.A. (1998). Detection of feline coronaviruses by culture and reverse transcriptase-polymerase chain reaction of blood samples from healthy cats and cats with clinical feline infectious peritonitis. Veterinary Microbiology 62, 193–205.

HARVEY, C.J., LOPEZ, J.W., HENDRICK, M.J. (1996). An uncommon intestinal manifestation of feline infectious peritonitis: 26 cases (1986–1993). Journal of American Veterinary Medical Association 209, 1117–20.

HERREWEGH, A.A.P.M., DE GROOT, R., CEPICA, A., EGERBINK, H.F., HORZINEK, M.C. & ROTTIER, P.J.M. (1995). Detection of feline coronavirus RNA in feces, tissues, and body fluids of naturally infected cats by reverse transcriptase PCR. Journal of Clinical Microbiology 33, 684–9.

HERREWEGH, A.A.P.M., MAHLER, M., HEDRICH, H.J., HAAGMANS, B.L., EGERBINK, H.F., HORZINEK, M.C., ROTTIER, P.J.M. & DE GROOT, R. (1997). Persistence and evolution of feline coronavirus in a closed cattled breeding colony. Virology 234, 349–63.

HERREWEGH, A.A.P.M., Smeerki, I., HORZINEK, M.C., ROTTIER, P.J. M. & DE GROOT, R. (1998). Feline coronavirus type II strains 79-1683 and 79-1146 originate from a double recombination between feline coronavirus type I and canine coronavirus. Journal of Virology 72, 4508–14.

HIGGINS, D.G., BLEASBY, A.J. & FUCHS, R. (1992). Clustal V: Improved software for multiple sequence alignment. CABIOS 8, 189–91.

HODATSU, T., OKADA, S. & KOMAI, H. (1991a). Characterization of monoclonal antibodies against feline infectious peritonitis virus type II and antigenic relationship between feline, porcine, and canine coronaviruses. Archives of Virology 117, 85–95.

HODATSU, T., SASAMOTO, T., OKADA, S. & KOMAI, H. (1991b). Antigenic analysis of feline coronaviruses with monoclonal antibodies (Mabs) which discriminate between FIPV strain 79-1146 and FECV strain 79-1683. Veterinary Microbiology 28, 13–24.

HODATSU, T., OKADA, S., ISHIZUKA, Y., YAMADA, H. & KOMAI, H. (1992). The prevalence of types I and II feline coronavirus infections in cats. Journal of Veterinary Medical Science 54, 557–62.

HORSBURGH, B.C., BRIERLEY, I. & BROWN, T.D. (1992). Analysis of a 9.6 kb sequence from the 3' end of canine coronavirus genomic RNA. Journal of General Virology 73, 2849–62.

KECK, J.G., MATSUSHIMA, G.K., MAKINO, S., FLEMING, S., VANNIER, D.M., STOHLMAN, S.A. & LAI, M.M.C. (1988). In vivo RNA-RNA recombination of coronavirus in mouse brain. Journal of Virology 62, 1810–3.

KOTTIER, S.A., CAVANAGH, D. & BRITTON, P. (1995). Experimental evidence of recombination in feline infectious bronchitis virus. Virology 213, 569–80.

KUSTERS, J.G., JAGER, E.J., NIESTERS, B.G.M. & ZEIST, B.A.M. (1990). Sequence evidence for RNA recombination in feline infectious bronchitis virus. Vaccine 8, 605–8.

LAI, M.M.C., BARIC, R.C., MAKINO, S., KECK, J.G., ENGBERGT, J., LEIBOWITZ, J.L. & STOHLMAN, S.A. (1985). Recombination between nonsegmented RNA genomes of murine coronaviruses. Journal of Virology 56, 449–56.

LAI, M.M.C. (1996). Recombination in large RNA viruses: coronaviruses. Seminars in Virology 7, 381–8.

LI, X. & SCOTT, F.W. (1994). Detection of feline coronaviruses in cell cultures and in fixed feline tissues using polymerase chain reaction. Veterinary Microbiology 42, 65–77.

LUNTJE, W. (1995). Coronavirus gene expression: genome organization and protein expression. In The Coronavirus, ed. S.G. Siddell, New York: Plenum Press. pp. 33–49.

MAKINO, S., KECK, J.G., STOHLMAN, S.A. & LAI, M.M.C. (1986). High-frequency RNA recombination of murine coronavirus. Journal of Virology 57, 729–39.

MOTOYAMA, K., HODATSU, T., HASHIMOTO, H. & KOMAI, H. (1996). Comparison of the amino acid sequence and phylogenetic analysis of the plomper, integral membrane and nucleocapsid proteins of feline, canine and porcine coronaviruses. Microbiology and Immunology 40, 425–33.

OLSEN, C.W. (1993). A review of feline infectious peritonitis virus: molecular biology, immunopathogenesis, clinical aspects, and vaccination. Veterinary Microbiology 36, 1–37.
Paltrinieri, S., Parodi Cammarata, M., Cammarata G. & Comazzi, S. (1998). Some aspects of humoral and cellular immunity in naturally occurring feline infectious peritonitis. Veterinary Immunology and Immunopathology 65, 205–20.

Pedersen, N.C. (1976). Serologic studies of naturally occurring feline infectious peritonitis. American Journal of Veterinary Research 37, 1449–53.

Pedersen, N.C. (1987a). Virologic and immunologic aspects of feline infectious peritonitis virus infection. Advances in Experimental Medicine and Biology 218, 529–50.

Pedersen, N.C. (1987b). An overview of feline enteric coronavirus and infectious peritonitis virus infections. Feline Practice 23, 7–22.

Poland, A.M., Vennema, H., Foley, J.E. & Pedersen, N.C. (1996). Two related strains of feline infectious peritonitis virus isolated from immunocompromised cats infected with a feline enteric coronavirus. Journal of Clinical Microbiology 34, 3180–4.

 Siddell, S., Wege, H. & Ter Meulen, V. (1983). The biology of coronaviruses. Journal of General Virology 64, 761–76.

Vennema, H., de Groot, R., Harbour, D.A., Dalderup, M., Gruffydd-Jones, T., Horzinek, M.C. & Spaan, W.J.M. (1990). Early death after feline infectious peritonitis virus challenge due to recombinant vaccinia virus immunization. Journal of Virology 64, 1407–9.

Vennema, H., de Groot, R.J., Harbour, D.A., Horzinek, M.C. & Spaan, W.J. (1991). Primary structure of the membrane and nucleocapsid protein genes of feline infectious peritonitis virus and immunogenicity of recombinant vaccinia viruses in kittens. Virology 181, 327–35.

Vennema, H., Poland, A., Foley, J. & Pedersen, N.C. (1998). Feline infectious peritonitis viruses arise by mutation from endemic feline enteric coronaviruses. Virology 243, 150–7.

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Book Review

Companion Animal Death
Stewart, M.F., Oxford. Butterworth-Heinemann, 1999. 188pp. £14.99 (soft) ISBN 0750640766

‘A unique new book for the veterinary surgeon and other members of the veterinary practice team ...’ is how this title is described on the back cover. It is interesting that, while euthanasia of pets is a constant and common part of practice, it is only recently that much has been written and discussed about the subject. Mary Stewart brings a warm and instructive approach to this rather sad event.

The book observes the whole subject of euthanasia and death of a pet, but also looks further to thebereavement that follows. As veterinary students, we are taught how to euthanase animals competently; what is often lacking, however, is the skill to deal with owners at a time when they may be unbearably distressed. Many vets do not fully understand the depth of feeling that some owners have for their animals. Mary Stewart sets out to educate both the veterinary practice staff and students by discussing the nature of the human/companion animal bond, which is the basis for the very powerful emotion that can overcome owners at the death of a pet.

She discusses in detail the process of bereavement which, once understood, will give veterinary staff a much better chance of handling the situation competently and with compassion. Information on how to break bad news and helping clients to come to a decision (to euthanase) is well presented by the use of notes and bullet points. The procedures that follow euthanasia (support for the owner, options for disposal of the body and practice sympathy correspondence) are clearly laid out and may give new ideas to help the practice achieve a caring attitude. There is also acknowledgement, and discussion, of the stress and sadness felt by the veterinary team. Many owners forget, in the throes of their own grief, that the staff may be upset to lose an old practice ‘friend’. In the section ‘Stress in veterinary practice’ there is detailed information on the factors (unrelated to euthanasia) that can cause anxiety in practice, and some excellent advice on how to avoid becoming stressed, which should be read by all veterinarians.

The emphasis of the book throughout is on communication, sensitivity and attitude. The consequences of poor client management at the time of death or euthanasia of a pet are stated unequivocally as causing troublesome, unhappy clients who will ‘spread the word’ and may want to ‘take the matter further’. This leads to vets who feel guilty, angry and misunderstood, thereby creating general tension and low morale within the practice. I would encourage all veterinary practices to study this immensely readable book and to adopt some of Mary’s sound advice.

F. Scott-Park