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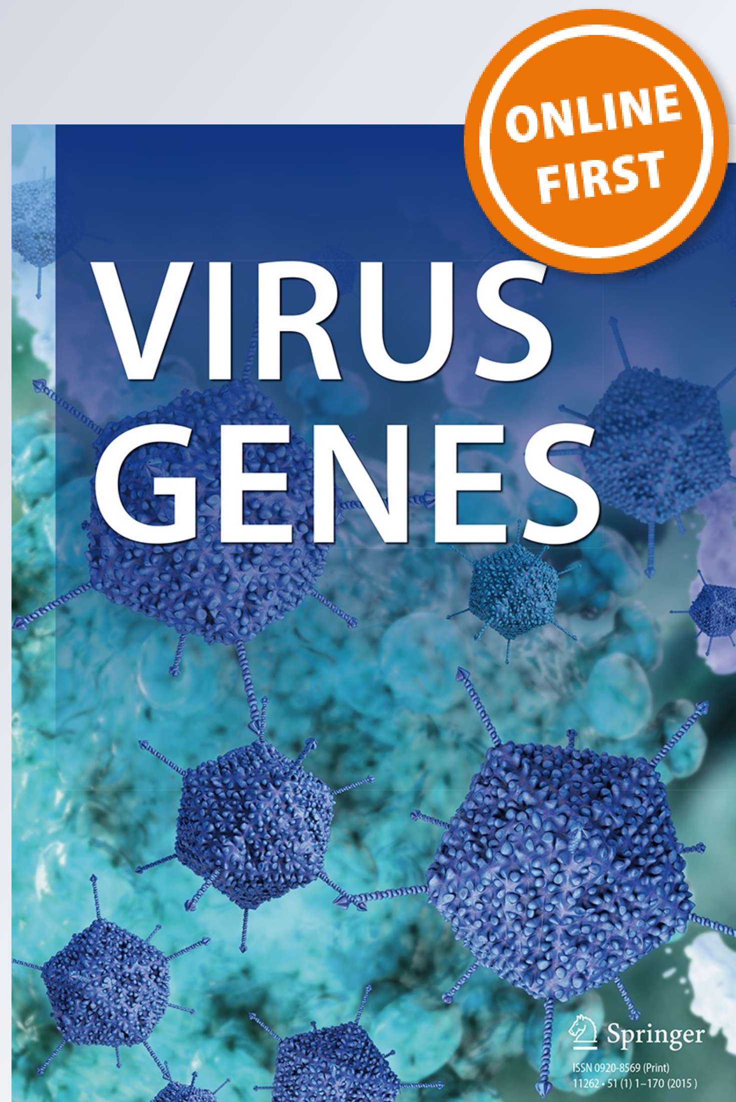
Virus Genes

ISSN 0920-8569

Virus Genes
DOI 10.1007/s11262-015-1232-7



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Discovery of new feline paramyxoviruses in domestic cats with chronic kidney disease

Michael Sieg[1] · Kristin Heenemann[1] · Antje Rückner[1] · Iwan Burgener[2] · Gerhard Oechtering[3] · Thomas W. Vahlenkamp[1]

Received: 16 June 2015 / Accepted: 28 July 2015
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Abstract Paramyxoviruses constitute a large family of enveloped RNA viruses including important pathogens in veterinary and human medicine. Recently, feline paramyxoviruses, genus morbillivirus, were detected in cats from Hong Kong and Japan. Here we describe the discovery of several new feline paramyxoviruses. Infections with these diverse viruses were detected in urine samples from cats suffering from chronic kidney disease (CKD). No viral RNA was found in cats without clinical signs of uropathy highlighting an association between feline paramyxovirus (FPaV) infections and CKD. Phylogenetic analyses of the detected viruses showed that they represent at least two different species, one of them representing the feline morbilliviruses detected previously in Hong Kong and Japan. In addition, a new FPaV was detected sharing only 73 % homology on the nucleotide level of the viral L-gene to currently known paramyxoviral species.

Keywords Cat · FLUTD · Paramyxoviruses · Urine

Edited by William Dundon.

Nucleotide sequences accession numbers: KP159802, KP159803, KP159804, KP159805, KR269598, KR269599, KR269600 and KR269601.

Electronic supplementary material The online version of this article (doi:10.1007/s11262-015-1232-7) contains supplementary material, which is available to authorized users.

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Paramyxoviruses are enveloped, single-stranded RNA viruses which are associated with emerging infectious diseases in humans and animals. In the recent years, many new paramyxoviruses from wild-living and domestic animals were discovered, and presumably most of them have originated due to crossing species barriers [1]. Recently, a feline morbillivirus (FmoPV) was described to be associated with tubulointerstitial nephritis in domestic cats (*Felis catus*) in Hong Kong [2]. In addition, these viruses were also detected by two other research groups in Japan [3, 4]. Associations between paramyxovirus infections and kidney diseases due to renal tissue damage were shown for humans [5], rodents [6, 7] and bats [8]. So far, cross species transmission of paramyxoviruses to cats has only been described for canine distemper virus [9].

Systematic investigations of cat populations for paramyxoviruses and their possible association with chronic and acute kidney diseases are not yet available. Chronic kidney disease (CKD) is one of the most common diseases, especially in older individuals, with a reported prevalence of 1.6–20 % in domestic cats [10, 11]. In many cases, the progressive and irreversible characters of these illnesses finally result in the death of the animals.

In this study, urine samples from domestic cats with (diseased group) and without (control group) kidney diseases (Table 1) were collected over a period of 2 years as part of the clinical examination by performing a cystocentesis or urinary tract catheterization during the cat's stay

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Table 1 Characteristics of investigated cats

Characteristics	Diseased group	Control group
Number of tested urines samples	120	86
Clinical findings	FLUTD, nephritis, haematuria, urolithiasis, urostase, cystitis, chronic kidney failure, chronic nephropathy, proteinuria, bacteriuria	No clinical and laboratory (urine status) signs of urotract diseases
Sex (female/male)	42/79	36/52
Mean age (years)	8 (1–18)	10 (2–21)
RT-PCR positive	8 (∼6.7 %)	0
Cole's coefficient of interspecific association	$\Phi_{corr.} = 1.0$	

and treatment at the Department of Small Animal Medicine. The sample collection was performed under consideration of animal welfare and ethical aspects.

RNA isolation was performed using the QIAamp Viral RNA Mini Kit (Qiagen, Hilden) following the manufacturer's instructions with the exception that 300 µl of urine was applied for each sample. For screening purposes, extracted RNA was used for the amplification of a small portion of the paramyxoviral L-gene as described previously [12] with the minor modification that the 'SuperScript III One-Step RT-PCR System with Platinum Taq High Fidelity' (Life Technologies) was used for amplification. PCR products were visualized by agarose gel electrophoresis with 1× Tris-acetate-EDTA buffer (40 mM tris-acetate, 1 mM EDTA, pH 8.3) containing 0.2 µg/ml of ethidium bromide. Specific PCR fragments were cut out of the gel and purified using a Gel/PCR DNA Fragments Extraction Kit (Geneaid, Taiwan). Sequencing was done by Sanger's dideoxy termination method in duplicates. The resulting nucleotide sequences were screened at the NCBI website using the Basic Local Alignment Search Tool (BLAST). For phylogenetic analysis, we calculated genetic distances employing the Tamura-Nei model at the nucleotide level. Dendrograms were built by the maximum likelihood method with 1000 bootstrap replicates [13].

Using this approach, we detected eight paramyxoviral sequences from 120 urine samples belonging to the diseased group. In contrast, none of the 86 urine samples of the control group was positive for paramyxoviral RNA. The obtained nucleotide sequences have been submitted to the GenBank nucleotide database, and the accession numbers are the following: KP159802, KP159803, KP159804, KP159805, KR269598, KR269599, KR269600 and KR269601 (summarized in Supplementary Table S2).

Phylogenetic analyses revealed that these sequences represent at least two diverse paramyxoviral species (Fig. 1): one of them corresponds to the previously isolated strains from Hong Kong and Japan (FmoPV). Overall homology on nucleotide level was found to be between 94 and 99 % indicating that these viruses are also present in Germany (Fig. 1, arch A, sample 90673 [accession number KP159802] was not included in the phylogenetic tree due to the fact that sequence information was only available from a different region of viral polymerase). The samples were taken from seven-, eight- and a 18-year-old male cats with a long-term feline lower urinary tract disease (FLUTD), urostasis, urolithiasis and cystitis suggesting a possible association between these viruses and CKD as shown previously [2, 3].

In addition, two other samples from a 15- and an 18-year-old cat with CKD also gave a positive result for FmoPV although the overall homology only reached 86 % compared to already known feline morbilliviruses (Fig. 1, arch B). Taking into account that a sequence homology larger than 85 % is accepted to define a new viral species [14], it is possible that these samples characterize a new paramyxovirus although it cannot be excluded that these isolates represent a different genotype of FmoPV. The described diversity within these feline morbilliviruses is supported by recently published data [4] and is advanced by observed reassortment events between different strains of FmoPV [15].

Apart from the detected FmoPV sequences, we identified paramyxoviral RNA in three samples from three-, eight- and a nine-year-old cats which did not fit to any currently known paramyxoviruses. All cats suffered from an episode of FLUTD and nephropathy at the time point of sample collection. To amplify a larger region of the paramyxoviral L-gene from positive samples, we applied previously described primer combinations [12] as well as newly developed primer pairs (Supplementary Table S1) using the 'SuperScript III One-Step RT-PCR System with Platinum Taq High Fidelity' as stated above. Detailed phylogenetic analyses revealed a 72 % nucleotide homology to bat paramyxoviruses and 74 % homology to rodent paramyxoviruses (Fig. 1, arch C) based on the nucleotide

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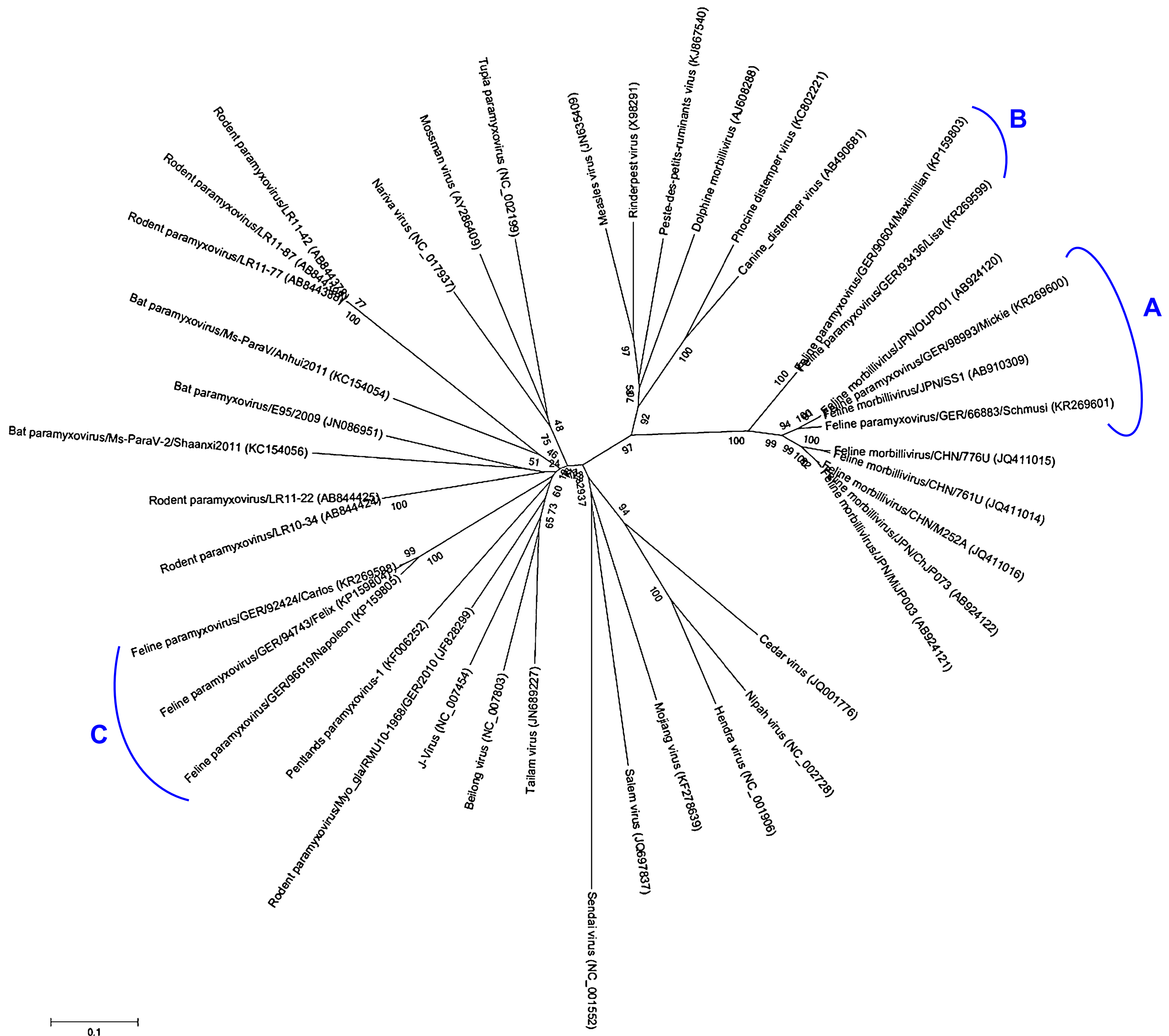

Fig. 1 Phylogenetic tree of paramyxoviruses constructed from partial (438 nucleotides) viral polymerase sequences. Strains identified in this study were highlighted with *blue arches*. *Scale bar* indicates 0.1 nucleotide substitutions per site. Accession numbers of paramxoviral strains included in phylogenetic analysis are shown in *brackets*

level. The three sequences shared 95 % homology to each other, and so it is likely that they represent different isolates of the same viral species, hereinafter referred to as feline paramyxovirus (FPaV). Whether the origin of FPaV is a rodent or bat cannot yet be finally concluded, but it is probably that they evolved from an interspecies transmission because such events are well documented for many other animal paramyxoviruses [1]. Further amplification attempts of the viral genome were hampered due to limited sample amounts from the cats.

For improving the detection of these new FPaV isolates, a highly specific and sensitive (detection limit: 10 copies of RNA per reaction) quantitative real-time PCR was developed. In brief, 10 µl of isolated RNA (max. 1 µg) was mixed with 12.5 µl 2× OneStep SYBR Green Master Mix (Quanta Biosciences), 0.5 µl primer Diag-FPaV-For (10 µM), 0.5 µl primer Diag-FPaV-Rev (10 µM) and 0.5 µl qScript One-Step RT (Quanta Biosciences) to make up a final volume of 25 µl. Analyses were done using the Rotor-Gene Q (Qiagen) device with the following two-step cycling program: 5 min. at 50 °C, 2 min. at 95 °C, followed by 40 cycles at 95 °C for 5 s., 30 s. at 60 °C, and data collection at 78 °C. A positive control was generated using RNA from sample 94743 as template in combination with the primers RES-MOR-HEN-R/F1 as described above. The resulting PCR fragment was cloned into pJET1.2/blunt vector

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(Thermo Scientific) according to the manufacturer's instructions, and then RNA was transcribed using the 'T7 Scribe Standard RNA IVT Kit' (Biozym). In vitro-transcribed RNA (Supplementary Figure 1) was purified with the 'peqGOLD Total RNA Kit' (peqlab) and stored at −80 °C until employment as real-time-PCR positive control.

By using this approach, FPaV was easily detected even if the urine samples had a high background of bacterial RNA (Supplementary Figure 1) from common agents of urinary tract infections like *Escherichia coli* and *Enterococcus faecalis* [16]. Applying this procedure to the FPaV-positive individuals, all three samples determined a comparable high viral load in the urine (copy numbers between 8×10^6 and 5×10^5 ml^{-1}). Moreover, these paramyxoviruses persisted for several weeks (>4 weeks for all three cats, in one cat for more than 11 weeks) in the urine after initial diagnosis was made, suggesting that these viral agents might provoke a long lasting or even chronic infection. In contrast, no viral RNA was detected in the blood and in the serum of urine-positive cats accounting for the fact that there is no or only a short period of systemic infection.

To elucidate the possibility of a correlation between the detected FmoPV and FPaV with CKD our data were analysed using the equation from Cole [17]. This resulted in a coefficient of interspecific association of $\Phi_{\mathrm{corr.}} = 1.0$ meaning that there is a strong positive association between paramyxovirus infections and CKD in domestic cats. Seven out of eight paramyxoviral positive cats were male suggesting that there might be a higher risk for male cats in suffering from FmoPV and FPaV infections. However, it must be noted that a general overrepresentation of male individuals within the study group exists. Further research is needed to clarify whether paramyxoviral infections are causatively involved in feline CKD or just benefit from inflamed tissues of the urotract as the basis for their infection. Future serological testing of healthy and diseased cat populations will give a more precise insight into virus distribution and the association between feline paramyxoviruses and different kidney diseases in cats.

Acknowledgments The authors thank Ines Müller and Grit Müller for excellent assistance in collecting urine samples and in providing urinalysis data from the cats investigated in this study.

Author contributions T W. Vahlenkamp designed and supervised the study and revised the manuscript, M. Sieg performed experimental work, interpreted all of the data and wrote the manuscript draft. Antje Rueckner and Kristin Heenemann assisted in virus isolation attempts and in the preparation of primary cells. I. Burgener and G. Oechtering supervised sample collection and provided clinical data of diseased animals.

References

1. J.F. Drexler, V.M. Corman, M.A. Müller, G.D. Maganga, P. Vallo, T. Binger, F. Gloza-Rausch, V.M. Cottontail, A. Rasche, S. Yordanov, A. Seebens, M. Knörnschild, S. Oppong, Y.A. Sarkodie, C. Pongombo, A.N. Lukashev, J. Schmidt-Chanasit et al., Nat. Commun. **3**, 796 (2012)
2. P.C.Y. Woo, S.K.P. Laua, B.H.L. Wong, R.Y.Y. Fan, A.Y.P. Wong, A.J.X. Zhang, Y. Wu, G.K.Y. Choi, K.S.M. Li, J. Hui, M. Wang, B.-J. Zheng, K.H. Chan, K.-Y. Yuen, Proc. Natl. Acad. Sci. USA **109**, 5435–5440 (2012)
3. T. Furuya, Y. Sassa, T. Omatsu, M. Nagai, R. Fukushima, M. Shibutani, T. Yamaguchi, Y. Uematsu, K. Shirota, T. Mizutani, Arch. Virol. **159**, 371–373 (2014)
4. S. Sakaguchi, S. Nakagawa, R. Yoshikawa, C. Kuwahara, H. Hagiwara, K. Asai, K. Kawakami, Y. Yamamoto, M. Ogawa, T. Miyazawa, J. Gen. Virol. **95**, 1464–1468 (2014)
5. C.F. Basler, A. García-Sastre, P. Palese, Emerg. Infect. Dis. **11**, 108–112 (2005)
6. F. Brooks, A.R. Wood, J. Thomson, D. Deane, D.J. Everest, C.J. McInnes, Vet. Microbiol. **170**, 391–397 (2014)
7. P.C. Woo, S.K. Lau, B.H. Wong, A.Y. Wong, R.W. Poon, K.Y. Yuen, J. Virol. **85**, 13473–13474 (2011)
8. A. Kurth, C. Kohl, A. Brinkmann, A. Ebinger, J.A. Harper, L.F. Wang, K. Mühldorfer, G. Wibbelt, PLoS ONE **7**, e38688 (2012)
9. C. Green, *Infectious Diseases of the Dog and Cat*, 4th edn. (Saunders Elsevier, Philadelphia, 2012)
10. E.M. Lund, P.J. Armstrong, C.A. Kirk, K.M. Kolar, J.S. Klausner, J. Am. Vet. Med. Assoc. **214**, 1336–1341 (1999)
11. A. Watson, Aust. Vet. Pract. **31**, 54–58 (2001)
12. S. Tsong, S.W. Chern, Y. Li, M.A. Pallansch, L.J. Anderson, J. Clin. Microbiol. **46**, 2652–2658 (2008)
13. K. Tamura, D. Peterson, N. Peterson, G. Stecher, M. Nei, S. Kumar, Mol. Biol. Evol. **28**, 2731–2739 (2011)
14. G. Kuno, G.J.J. Chang, K.R. Tsuchiya, N. Karabatsos, C.B. Cropp, J. Virol. **72**(1), 73–83 (1998)
15. E.S. Park, M. Suzuki, M. Kimura, K. Maruyama, H. Mizutani, R. Saito, N. Kubota, T. Furuya, T. Mizutani, K. Imaoka, S. Morikawa, Virology **468**, 524–531 (2014)
16. A.L. Litster, S.M. Moss, M. Honnery, R. Rees, D.J. Trott, Vet. Microbiol. **121**, 182–188 (2007)
17. M.J. Warrens, Psychometrika **73**, 777–789 (2008)