SHORT COMMUNICATION

A Ten-Year Molecular Survey on Parvoviruses Infecting Carnivores in Bulgaria

C. Filipov[1], C. Desario[2], O. Patouchas[2], P. Eftimov[3], G. Gruichev[4], V. Manov[1], G. Filipov[5], C. Buonavoglia[2] and N. Decaro[2]

[1] Faculty of Veterinary Medicine, University of Forestry, Sofia, Bulgaria
[2] Department of Veterinary Medicine, University of Bari, Valenzano, Italy
[3] Faculty of Biology, Sofia University St. Kliment Ohridski, Sofia, Bulgaria
[4] Faculty of Forestry, University of Forestry, Sofia, Bulgaria
[5] Veterinary Practice, Montana, Bulgaria

Keywords:
parvoviruses; carnivores; molecular survey; Bulgaria; real-time polymerase chain reaction; minor groove binder probe assays

Correspondence:
C. Filipov. Department of Infectious Pathology, Hygiene, Technology and Control of Foodstuffs of Animal Origin, Faculty of Veterinary Medicine, University of Forestry, Sofia, Bulgaria, bld 'Kliment Ohridski', 10 Sofia 1756, Bulgaria.
Tel.: +359899626342; Fax: +35928622830; E-mail: chavdar_filipov@abv.bg

Received for publication August 6, 2014

doi:10.1111/tbed.12285

Summary

Parvoviruses represent the most important infectious agents that are responsible for severe to fatal disease in carnivores. This study reports the results of a 10-year molecular survey conducted on carnivores in Bulgaria (n = 344), including 262 dogs and 19 cats with gastroenteritis, and 57 hunted wild carnivores. Real-time polymerase chain reaction (qPCR), followed by virus characterization by minor groove binder (MGB) probe assays, detected 216 parvovirus positive dogs with a predominance of canine parvovirus type 2a (CPV-2a, 79.17%) over CPV-2b (18.52%) and CPV-2c (2.31%). Rottweilers and German shepherds were the most frequent breeds among CPV-positive pedigree dogs (n = 96). Eighteen cats were found to shed parvoviruses in their faeces, with most strains being characterized as FPLV (n = 17), although a single specimen tested positive for CPV-2a. Only two wild carnivores were parvovirus positive, a wolf (*Canis lupus*) and a red fox (*Vulpes vulpes*), both being infected by CPV-2a strains.

Introduction

Carnivore parvoviruses are a group of very closely related viruses that infect wild and domestic carnivores, inducing a variety of clinical forms. These may range from subclinical infections to severe, often fatal disease characterized by loss of appetite, depression, fever, leukopenia and haemorrhagic gastroenteritis. In addition to feline panleukopenia virus (FPLV) and canine parvovirus type 2 (CPV-2), which have been known since 1920s and late 1970s, respectively, additional parvoviruses have been detected in wild carnivores (Decaro and Buonavoglia, 2012b). Currently, carnivore parvoviruses are members of the genus *Parvovirus* (family *Parvoviridae*, subfamily *Parvovirinae*) and are considered as host variants of a unique viral species, *Feline panleukopenia virus* (http://ictvdb.bio-mirror.cn/Ictv/fs_parvo.htm).

CPV-2 emergence was associated to a cross-species transmission of FPLV to dogs through an intermediate host, possibly an unidentified wild carnivore. However, while the latter virus has retained a certain genetic stability (Decaro et al., 2008), CPV-2 has displayed a more complex evolutionary history, which is quite uncommon for a DNA virus (Decaro et al., 2009). Soon after CPV-2 emergence, two antigenic variants, CPV-2a and CPV-2b, replaced the original type, giving rise to additional mutants in different countries (Truyen, 2006). A further variant, designated as CPV-2c, was first detected in Italy (Buonavoglia et al., 2001) and subsequently spread to other countries (Decaro et al., 2007a, 2011; Hong et al., 2007; Perez et al., 2007; Calderon et al., 2009; Nandi et al., 2009; Decaro and Buonavoglia, 2012a).

In Bulgaria, CPV infection was first reported between 1979 and 1981 with 100% morbidity and 85% mortality

© 2014 Blackwell Verlag GmbH

in puppies up to 2 months old of a shelter housing military German shepherd dogs (Filipov, 1983). To date, apart from a report on CPV detection in dogs with diarrhoea (Filipov et al., 2011), there is no extensive study assessing the epidemiology of parvoviruses in different carnivore species in Bulgaria.

The aim of this study is to report the results of a molecular survey on domestic and wild carnivores from Bulgaria that spans a 10-year period (2004–2014).

Materials and Methods

Samples – animal species, breeds, origin

Canine samples (n = 262) were faeces or anal swabs collected *in vivo* or intestinal contents collected at post-mortem examination from dogs with acute gastroenteritis in 28 sites of 12 towns of Bulgaria (Table 1). The sampling sites were 24 veterinary clinics, 1 kennel and 3 shelters for stray dogs. When possible, the anamnesis and clinical history of the samples dogs were recorded, including geographic origin, age, breed, administered vaccines, clinical signs of the patients, preliminary testing with in-clinic assays and storage of samples. Most dogs, for which there was information about breed, were purebred animals (n = 118) of 36 different breeds, including rottweiler (n = 14), German shepherd (n = 12), spitz (n = 7), pinscher (n = 7), Jack Russell terrier (n = 6), Yorkshire terrier (n = 6), Chihuahua (n = 5), dachshunds (n = 5), Siberian husky (n = 4), Labrador (n = 4), Pomeranian (n = 4), Caucasian shepherd (n = 4), pit bull (n = 4), beagle (n = 3), Bolognese (n = 3), Bulgarian shepherd (n = 3), akitas inu (n = 3), boxer (n = 2), English cocker spaniel (n = 2), Samoyed (n = 2), golden retriever (n = 2), Epagneul breton (n = 2), great dane (n = 1), Corso dog (n = 1), English bulldog (n = 1), Saint Bernard dog (n = 1), Central Asian shepherd dog (n = 1), Bulgarian scenthound (n = 1), American staffordshire terrier (n = 1), Bernese mountain dog (n = 1), fila brasileiro (n = 1), Pekinese (n = 1), bull terrier (n = 1), mop (n = 1), collie (n = 1), drahthaar (n = 1). Mixed-bred dogs were 75, and no data were available for 69 animals. The feline specimens (n = 19) were freshly collected faeces from 1 siamese and 6 European cats; for most cats (n = 12), no data were recorded. All cats were sampled at veterinary practices in two cities (Sofia and Varna).

The samples from the wild carnivores (faeces and/or intestines) were collected from foxes *(Vulpes vulpes*, n = 39), stone martens *(Martes foina*, n = 12), jackals (*Canis aureus, n* = 4), wolves (*Canis lupus, n* = 1) and badgers (*Meles meles, n* = 1) in six different regions of the country (Pernik, Razgrad, Kardzhali, Troyan, Harmanli and Velingrad) during the hunting detours. All specimens from the animals were stored at −20°C and tested at different intervals of time.

Field diagnostics with in-clinic tests

A total of 120 canine and 14 feline faecal samples were tested by the practitioners by in-clinic assays for detection of CPV antigen or both CPV and canine coronavirus antigens (Anigen Rapid CPV Ag Test Kit or Anigen Rapid CPV/CCV Ag Test Kit; BioNote Inc., Seoul, Korea) and for FPLV antigen (Anigen Rapid FPV Ag Test Kit; BioNote Inc.) respectively, according to the producer recommendations (http://www.bionote.co.kr/ANIMAL/ENG/).

Table 1. Regional distribution of CPV in dogs with acute gastroenteritis in Bulgaria (2004–2014) and summarized anamnestic data

				Parvovirus positive samples		CPV type						Parvovirus negative samples	
						2a		2b		2c			
Towns		No. of sampling sites	No. of samples	No.	%	No.	%	No.	%	No.	%	No.	%
1	Sofia	16	163	139	85.28	113	81.29	22	15.83	4	2.87	24	14.72
2	Kozloduy	1	34	32	94.18	14	43.75	18	56.25	–	–	2	5.88
3	Varna	1	25	14	56.00	14	100	–	–	–	–	11	44.00
4	Plovdiv	2	14	8	51.14	8	100	–	–	–	–	6	42.86
5	Dobrich	1	14	13	92.86	13	100	–	–	–	–	1	7.14
6	Stara Zagora	1	5	5	100	5	100	–	–	–	–	–	–
7	Kazanlak	1	2	2	100	2	100	–	–	–	–	–	–
8	Veliko Tarnovo	1	1	1	100	1	100	–	–	–	–	–	–
9	Vratsa	1	1	–	–	–	–	–	–	–	–	1	100
10	Berkovitsa	1	1	1	100	1	100	–	–	–	–	–	–
11	Samokov	1	1	–	–	–	–	–	–	–	–	1	100
12	Blagoevgrad	1	1	1	100	–	–	–	–	1	100	–	100
Total	No./%	28	262	216	82.44	171	79.17	40	18.52	5	2.31	46	17.56

Real-time PCR assays for CPV detection, quantification and characterization

The specimens were homogenized (10% wt/vol) in Dulbecco's modified Eagle's medium (DMEM) with a subsequent clarification by centrifuging at 1500 ***g*** for 15 min. The DNA from each sample was extracted from the homogenates by boiling 200 μl of the supernatant for 10 min and chilling on ice. To reduce residual inhibitors of DNA polymerase activity to ineffective concentrations, the DNA extracts were diluted 1 : 10 in distilled water (Decaro et al., 2005, 2006a). The extracted DNAs were tested by a TaqMan assay, which is able to recognize all carnivore parvoviruses (Decaro et al., 2005). Real-time PCR was performed using an i-Cycler iQ™ Real-Time Detection System (Bio-Rad Laboratories Srl, Milan, Italy), and the data were analysed with the iCycler IQ detector software (version 3.0). Duplicates of the CPV standard dilutions and DNA templates were simultaneously subjected to real-time analysis. The 25-μl PCR mixture for one reaction contained 12.5 μl of Supermix 600 nM of primer CPV-For (5′-AAA-CAGGAATTAACTATACTAATATATTTA-3′) and CPV-Rev (5′-AAATTTGACCATTTGGATAAACT-3′), 200 nM of probe CPV-Pb (5′-FAM-TGGTCCTTTAACTGCATTAAA TAATGTACC-TAMRA-3′), and 10 μl of DNA. The following thermal cycling protocol was used: activation of DNA polymerase at 95°C for 10 min, 40 cycles consisting of denaturation at 95°C for 15 s, primer annealing at 52°C for 30 s, and extension at 60°C for 1 min. The internal control consisted of exogenous DNA, extracted from *Ovine herpesvirus 2* (Decaro et al., 2004). For parvovirus characterization, a panel of minor groove binder (MGB) probe assays able to discriminate between CPV/FPLV, CPV-2a/2b, CPV-2b/2c and CPV vaccine/field viruses was used. The specificity and sensitivity of all molecular assays used in the study had been previously calculated (Decaro et al., 2005, 2006a,b, 2007b).

Results

In-clinic tests

In-clinic testing gave positive results for 110 of 120 canine specimens (91.67%), and for 12 of 14 tested feline samples (85.71%).

Real-Time PCR assay/MGB probe analysis

By qPCR, 216 of 262 canine samples tested positive for carnivore parvoviruses, which were all characterized as CPV by MGB probe assays discriminating between canine and feline parvoviruses. Type-specific MGB probe analysis determined the CPV variant distribution as follows: 171 strains were type 2a (79.17%), 40 were type 2b (18.52%), and 5 were type 2c (2.31%) CPVs. CPV-2b showed a very restricted distribution, being detected only in two towns, the capital Sofia, where many veterinary clinics are concentrated, and Kozloduy, where the biggest clinic in North West Bulgaria (Region Severozapaden) is located (Table 1).

There was no substantial fluctuation in the variant distribution over the 10 years.

As reported in previous studies (Houston et al., 1996; Gombač et al., 2008), rottweiler (*n* = 12) and German shepherd (*n* = 11) were the most frequent breeds among the CPV-positive purebred dogs (*n* = 96).

Eighteen of the feline samples tested positive by the TaqMan assay; 17 of the detected strains were true FPLVs, while one specimen was found to contain a CPV-2a virus.

Of all 57 samples from wild carnivores, only 2 were parvovirus positive, one from a wolf and one from a red fox, both containing a CPV-2a strain.

Comparison between in-clinic testing and real-time PCR

Three of the 110 canine samples that tested positive by either in-clinic assays for CPV detection were negative when using qPCR, whereas two in-clinic assay negative specimens were qPCR positive. One feline sample, positive by in-clinic test, was negative by real-time PCR.

Discussion

The present study represents the first 10-year molecular survey for carnivore parvoviruses conducted on domestic and wild carnivores in Bulgaria. The obtained findings, indicating a higher frequency of CPV-2a in Bulgarian dogs, are in agreement with the results from recent studies in other countries of the Balkan peninsula, such as Albania and Greece (Ntafis et al., 2010; Cavalli et al., 2014). Only five samples were positive for CPV-2c (one for the 2004–2010 period), despite the widespread distribution of this virus in western Europe (Decaro and Buonavoglia, 2012a, b) and the continuous import of dogs in Bulgaria from this area.

As for the feline samples, it is noteworthy that 1 cat with clinical feline panleukopenia was not infected by an FPLV strain, but by CPV-2a. Cases of feline panleukopenia caused by the CPV antigenic variants have been increasingly reported worldwide and, recently, CPV-2a and CPV-2c infections in cats have occurred in Italy (Decaro and Buonavoglia, 2012a).

Surprisingly, there was a good correlation between in-clinic and molecular testing, which is in contrast with the results of previous studies, showing a lack of sensitivity of the rapid assays (Desario et al., 2005; Schmitz et al., 2009; Decaro et al., 2010, 2013). However, it could not be ruled

 © 2014 Blackwell Verlag GmbH

out that most practitioners sent us only the in-clinic assay positive samples, which may have also overestimated the real prevalence of parvovirus infections in dogs and cats with acute gastroenteritis.

In addition, few samples that had tested positive by the in-clinic assay were not confirmed by real-time PCR, accounting for lower specificity of rapid testing in comparison with molecular assays rather than for a poor sensitivity of the latter methods. PCR inhibition was also ruled out as specimens that tested negative by real-time PCR were positive for the internal control.

Another interesting finding of the present report is that the only two parvovirus strains detected in wildlife were both CPV-2a. While FPLV and all the three CPV variants have been detected in wild carnivores in the USA (Allison et al., 2013), in Europe, only FPLV and CPV-2b have been proven to circulate in wildlife (Battilani et al., 2001; Steinel et al., 2001; Barlow et al., 2012; Duarte et al., 2013). In some instances, the lack of sequence analyses of the VP2 protein gene or of the sophisticated molecular tools, such as MGB probe assays, prevented any definitive characterization of the parvoviruses circulating in wild carnivores (Martinello et al., 1997; Burtscher and Url, 2007; Duarte et al., 2012).

The present study provides a useful contribution to the understanding of the carnivore parvovirus evolution and epidemiology in the European continent, thus helping define the most appropriate vaccination strategies in the different carnivore species.

Acknowledgements

The authors acknowledge the Project BG051PO001-3.3.06-0056 'Support for the development of young people in University of Forestry', granted by Human Resources Development Operational Programme of the European Social Fund, European Union (University of Forestry, Sofia, Bulgaria). Special thanks to Donato Narcisi, Arturo Gentile and Carlo Armenise for their technical assistance in the laboratory work and to all veterinary practitioners in Bulgaria who contributed to this study.

References

Allison, A. B., D. J. Kohler, K. A. Fox, J. D. Brown, R. W. Gerhold, V. I. Shearn-Bochsler, E. J. Dubovi, C. R. Parrish, and E. C. Holmes, 2013: Frequent cross-species transmission of Parvoviruses among diverse carnivore hosts. *J. Virol.* 87, 2342–2347.

Barlow, A. M., A. Schock, J. Bradshaw, E. Mullineaux, A. Dastjerdi, D. J. Everest, S. McGowan, F. Steinbach, and S. Cowen, 2012: Parvovirus enteritis in Eurasian badgers (*Meles meles*). *Vet. Rec.* 170, 416.

Battilani, M., A. Scagliarini, E. Tisato, C. Turilli, I. Jacoboni, R. Casadio, and S. Prosperi, 2001: Analysis of canine parvovirus sequences from wolves and dogs isolated in Italy. *J. Gen. Virol.* 82, 1555–1560.

Buonavoglia, C., V. Martella, A. Pratelli, M. Tempesta, A. Cavalli, D. Buonavoglia, G. Bozzo, G. Elia, N. Decaro, and L. E. Carmichael, 2001: Evidence for evolution of canine parvovirus type-2 in Italy. *J. Gen. Virol.* 82, 3021–3025.

Burtscher, H., and A. Url, 2007: Evidence of canine distemper and suggestion of preceding parvovirus-myocarditis in a Eurasian badger (*Meles meles*). *J. Zoo Wildl. Med.* 38, 139–142.

Calderon, M. G., N. Mattino, D. Bucafusco, F. Fogel, P. Remorini, and J. La Torre, 2009: Molecular characterization of canine parvovirus strains in Argentina: detection of the pathogenic variant CPV2c in vaccinated dogs. *J. Virol. Methods* 159, 141–145.

Cavalli, A., C. Desario, I. Kusi, V. Mari, E. Lorusso, F. Cirone, I. Kumbe, M. L. Colaianni, D. Buonavoglia, and N. Decaro, 2014: Detection and genetic characterization of Canine parvovirus and Canine coronavirus strains circulating in district of Tirana in Albania. *J. Vet. Diagn. Invest.* 26, 563–566.

Decaro, N., and C. Buonavoglia, 2012a: Canine parvovirus-A review of epidemiological and diagnostic aspects, with emphasis on type 2c. *Vet. Microbiol.* 155, 1–12.

Decaro, N., and C. Buonavoglia, 2012b: Canine parvoviral enteritis. In: Gavier-Widen, D., J. P. Duff, and A. Meredith (eds), Infectious Diseases of Wild Mammals and Birds in Europe, pp. 182–184. Wiley-Blackwell, Somerset, NJ.

Decaro, N., A. Tinelli, A. Pratelli, V. Martella, M. Tempesta, and C. Buonavoglia, 2004: First two confirmed cases of malignant catarrhal fever in Italy. *New Microbiol.* 26, 339–344.

Decaro, N., G. Elia, V. Martella, C. Desario, M. Campolo, L. D. Trani, E. Tarsitano, M. Tempesta, and C. Buonavoglia, 2005: A real-time PCR assay for rapid detection and quantitation of canine parvovirus type 2 DNA in the feces of dogs. *Vet. Microbiol.* 105, 19–28.

Decaro, N., G. Elia, V. Martella, M. Campolo, C. Desario, M. Camero, F. Cirone, E. Lorusso, M. S. Lucente, D. Narcisi, P. Scalia, and C. Buonavoglia, 2006a: Characterisation of the canine parvovirus type 2 variants using minor groove binder probe technology. *J. Virol. Methods* 133, 92–99.

Decaro, N., V. Martella, G. Elia, C. Desario, M. Campolo, D. Buonavoglia, A. L. Bellacicco, M. Tempesta, and C. Buonavoglia, 2006b: Diagnostic tools based on minor groove binder probe technology for rapid identification of vaccinal and field strains of canine parvovirus type 2b. *J. Virol. Methods* 138, 10–16.

Decaro, N., C. Desario, D. D. Addie, V. Martella, M. J. Vieira, G. Elia, A. Zicola, C. Davis, G. Thompson, E. Thiry, U. Truyen, and C. Buonavoglia, 2007a: The study molecular epidemiology of canine parvovirus, Europe. *Emerg. Infect. Dis.* 13, 1222–1224.

Decaro, N., C. Desario, G. Elia, M. Campolo, A. Lorusso, V. Mari, V. Martella, and C. Buonavoglia, 2007b: Occurrence of severe gastroenteritis in pups after canine parvovirus vaccine

administration: a clinical and laboratory diagnostic dilemma. *Vaccine* 25, 1161–1166.

Decaro, N., C. Desario, A. Miccolupo, M. Campolo, A. Parisi, V. Martella, F. Amorisco, M. S. Lucente, A. Lavazza, and C. Buonavoglia, 2008: Genetic analysis of feline panleukopenia viruses from cats with gastroenteritis. *J. Gen. Virol.* 89, 2290–2298.

Decaro, N., C. Desario, A. Parisi, V. Martella, A. Lorusso, A. Miccolupo, V. Mari, M. L. Colaianni, A. Cavalli, L. D. Trani, and C. Buonavoglia, 2009: Genetic analysis of canine parvovirus type 2c. *Virology* 385, 5–10.

Decaro, N., C. Desario, M. J. Beall, A. Cavalli, M. Campolo, A. A. Dimarco, F. Amorisco, M. L. Colaianni, and C. Buonavoglia, 2010: Detection of canine parvovirus type 2c by a commercially available in-house rapid test. *Vet. J.* 184, 373–375.

Decaro, N., C. Desario, M. Billi, V. Mari, G. Elia, A. Cavalli, V. Martella, and C. Buonavoglia, 2011: Western European epidemiological survey for parvovirus and coronavirus infections in dogs. *Vet. J.* 187, 195–199.

Decaro, N., L. E. Carmichael, and C. Buonavoglia, 2012: Viral reproductive pathogens of dogs and cats. *Vet. Clin. North Am. Small Anim. Pract.* 42, 583–598.

Decaro, N., C. Desario, M. Billi, E. Lorusso, M. L. Colaianni, V. Colao, G. Elia, G. Ventrella, I. Kusi, S. Bo, and C. Buonavoglia, 2013: Evaluation of an in-clinic assay for the diagnosis of canine parvovirus. *Vet. J.* 198, 504–507.

Desario, C., N. Decaro, M. Campolo, A. Cavalli, F. Cirone, G. Elia, V. Martella, E. Lorusso, M. Camero, and C. Buonavoglia, 2005: Canine parvovirus infection: which diagnostic test for virus? *J. Virol. Methods* 121, 179–185.

Duarte, A., M. Fernandes, N. Santos, and L. Tavares, 2012: Virological Survey in free-ranging wildcats (*Felis silvestris*) and feral domestic cats in Portugal. *Vet. Microbiol.* 158, 400–404.

Duarte, M. D., A. M. Henriques, S. C. Barros, T. Fagulha, P. Mendonça, P. Carvalho, M. Monteiro, M. Fevereiro, M. P. Basto, L. M. Rosalino, T. Barros, V. Bandeira, C. Fonseca, and M. V. Cunha, 2013: Snapshot of viral infections in wild carnivores reveals ubiquity of parvovirus and susceptibility of Egyptian mongoose to feline panleukopenia virus. *PLoS ONE* 8, e59399.

Filipov, K., 1983: Parvovirosis in dogs. *Vet. Sbirka* 3, 44–46.

Filipov, C., N. Decaro, C. Desario, F. Amorisco, R. Sciarretta, and C. Buonavoglia, 2011: Canine parvovirus epidemiology in Bulgaria. *J. Vet. Diagn. Invest.* 23, 152–154.

Gombač, M., T. Švara, M. Tadić, and M. Pogačnik, 2008: Retrospective study of canine parvovirosis in Slovenia. *Slov. Vet. Res.* 45, 73–78.

Hong, C., N. Decaro, C. Desario, P. Tanner, M. C. Pardo, S. Sanchez, C. Buonavoglia, and J. T. Saliki, 2007: Occurrence of canine parvovirus type 2c in the United States. *J. Vet. Diagn. Invest.* 19, 535–539.

Houston, D. M., C. S. Ribble, and L. L. Head, 1996: Risk factors associated with parvovirus enteritis in dogs: 283 cases (1982-1991). *J. Am. Vet. Med. Assoc.* 208, 542–546.

Martinello, F., F. Galuppo, F. Ostanello, V. Guberti, and S. Prosperi, 1997: Detection of canine parvovirus in wolves from Italy. *J. Wildl. Dis.* 33, 628–631.

Nandi, S., S. Chidri, M. Kumar, and R. S. Chauhan, 2009: Occurrence of canine parvovirus type 2c in the dogs with haemorrhagic enteritis in India. *Res. Vet. Sci.* 88, 169–171.

Ntafis, V., E. Xylouri, I. Kalli, C. Desario, V. Mari, N. Decaro, and C. Buonavoglia, 2010: Characterization of Canine parvovirus 2 variants circulating in Greece. *J. Vet. Diagn. Invest.* 22, 737–740.

Perez, R., L. Francia, V. Romero, L. Maya, I. López, and M. Hernández, 2007: First detection of canine parvovirus type 2c in South America. *Vet. Microbiol.* 124, 147–152.

Schmitz, S., C. Coenen, M. König, H. J. Thiel, and R. Neiger, 2009: Comparison of three rapid commercial Canine parvovirus antigen detection tests with electron microscopy and polymerase chain reaction. *J. Vet. Diagn. Invest.* 21, 344–345.

Steinel, A., C. R. Parrish, M. E. Bloom, and U. Truyen, 2001: Parvovirus infections in wild carnivores. *J. Wildl. Dis.* 37, 594–607.

Truyen, U., 2006: Evolution of canine parvovirus—a need for new vaccines? *Vet. Microbiol.* 117, 9–13.