Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Western European epidemiological survey for parvovirus and coronavirus infections in dogs

Nicola Decaro a,*, Costantina Desario a, Monica Billib, Viviana Mari a, Gabriella Elia a, Alessandra Cavalli a, Vito Martella a, Canio Buonavoglia a

aDepartment of Veterinary Public Health, Faculty of Veterinary Medicine, Strada per Casamassima Km 3, 70010 Valenzano (Bari), Italy
bFort Dodge Animal Health, Via Amendola 8, 40121 Bologna, Italy

Article info
Article history:
Accepted 23 October 2009

Keywords:
Canine
Parvovirus
Coronavirus
Variants
Genotype

Abstract
An epidemiological survey for canine parvovirus (CPV) and canine coronavirus (CCoV) infections was conducted in Western Europe. A total of 156 faecal samples were collected from dogs with diarrhoea in Spain (n = 47), Italy (n = 39), France (n = 26), Germany (n = 21), the United Kingdom (n = 8), Belgium (n = 10), and the Netherlands (n = 5). Using molecular assays for virus detection and characterisation, CPV and CCoV were found to be widespread in European dog populations, either alone or in mixed infections. In agreement with previous reports, the original type CPV-2 was shown not to circulate in European dogs. The recently identified virus variant CPV-2c was predominant in Italy and Germany and present at high rates in Spain and France but was not detected in the UK or Belgium. Except for the UK, CCoV genotype I was identified in all European countries involved in the survey, albeit at a lower prevalence rates than CCoV genotype II.

Introduction
Canine parvovirus type 2 (CPV-2) and canine coronavirus (CCoV) are considered the main pathogens responsible for acute gastroenteritis in dogs (Greene and Decaro, in press). After its first emergence in the late 1970s, CPV-2 underwent a rapid evolution and the original type was replaced in the field by two antigenic variants, CPV-2a and CPV-2b, which suddenly spread to the canine population worldwide (Truyen, 2006). A third variant, CPV-2c, was identified in 2000 in Italy (Buonavoglia et al., 2001) and was subsequently found in other European countries, as well as in Asia, America and Africa (Greene and Decaro, in press).

Clinical signs of CPV infections include haemorrhagic diarrhoea, vomiting, fever, lymphopenia and sometimes death (Greene and Decaro, in press). In-house tests are available for in-practice diagnosis of CPV infection, but those methods have been shown to be poorly sensitive when compared to methods based on nucleic acid amplification (Desario et al., 2005; Schmitz et al., 2009). In addition, ELISA-based assays can detect all CPV variants without remarkable differences (Decaro et al., 2010). Increasing concerns have been expressed on the actual efficacy of vaccines based on the old CPV-2 type against field strains, chiefly against the new variant 2c (Pratelli et al., 2001a; Cavalli et al., 2008; Decaro et al., 2008), as several CPV outbreaks are being observed worldwide in dogs vaccinated regularly (Decaro et al., 2008, 2009; Calderon et al., 2009).

Whereas CPV can cause a severe, often fatal, disease, CCoV is generally recognised as an aetiological agent of mild, self-limiting enteritis followed by rapid recovery (Decaro and Buonavoglia, 2008). However, hypervirulent CCoV strains have been identified and are associated with haemorrhagic gastroenteritis and death, as well as with systemic disease under natural and experimental conditions (Decaro and Buonavoglia, 2008).

Two different genotypes of CCoV are known, namely type I (CCoV-I) and II (CCoV-II), which are often detected in mixed infections (Decaro et al., 2005b). In the genome of CCoV-I an additional open reading frame (ORF), ORF3, is present. Remnants of ORF3 have been detected in CCoV-II and in the closely related transmissible gastroenteritis virus of swine (TGEV), suggesting that CCoV-I may be an ancestor of both CCoV-II and TGEV (Lorusso et al., 2008).

In this study, an epidemiological survey for CPV and CCoV in several Western European countries was conducted and the viruses were characterised at the molecular level in order to assess the relative prevalence of the various CPV and CCoV types.

Materials and methods
Sample collection and preparation

An epidemiological survey for CPV and CCoV was conducted in Spain, Italy, France, Germany, the United Kingdom, Belgium and the Netherlands during the period January 2008–April 2009. Specimens were collected by practitioners from...
dogs with acute gastroenteritis housed in rescue and rehoming shelters and commercial breeding colonies, or presented to veterinary clinics located in different areas of the same country (Table 1). Each veterinarian participating to the survey was provided with kit boxes, shipping materials and a guidance booklet, including submission forms for collection of full anamnesis (age, breed, sex, clinical signs and vaccination status). The inclusion criteria were (1) occurrence of severe gastroenteritis especially in rescue shelters or large breeding kennels, and (2) shipping of frozen samples following the cool chain by using the provided dispensable packaging materials.

Specimens were homogenised (10% w/v) in Dulbecco’s modified Eagle’s medium (DMEM) and subsequently clarified by centrifuging at 2500 g for 10 min. Viral DNA was extracted from the supernatants of faecal homogenates by boiling for 10 min and chilling on ice. This extraction method does not appear to alter viral DNA (Desario et al., 2005). To reduce residual inhibitors of DNA polymerase activity to ineffective concentrations, the DNA extract was diluted 1:10 in distilled water (Decaro et al., 2006b). The clarified faecal suspension (140 μL) was also used for RNA extraction using the QIAamp Viral RNA Mini Kit (Qiagen), following the manufacturer’s protocol and the RNA templates were stored at −70 °C until used.

Table 1
Summary of the sampling sites and their geographical location.

| Country     | Site no. | Town                        | State/region/province | Number of samples |
|-------------|----------|-----------------------------|-----------------------|-------------------|
| Spain       | 1        | Lugo                        | Galicia               | 4                 |
|             | 2        | La Cartuja Baja             | Aragón                | 4                 |
|             | 3        | Sevilla                     | Andalucia             | 6                 |
|             | 4        | Dos Hermanas                | Andalucia             | 4                 |
|             | 5        | Barcelona                   | Catalonia             | 5                 |
|             | 6        | Mataró                      | Catalonia             | 3                 |
|             | 7        | Mataró                      | Catalonia             | 5                 |
|             | 8        | Madrid                      | Madrid                | 2                 |
|             | 9        | Madrid                      | Madrid                | 1                 |
|             | 10       | Mejorada del Campo          | Madrid                | 8                 |
|             | 11       | Bilbao                      | Basque country        | 5                 |
| Total       |          |                              |                       | 47                |
| Italy       | 1        | Turin                       | Piedmont              | 2                 |
|             | 2        | Turin                       | Piedmont              | 4                 |
|             | 3        | Genoa                       | Liguria               | 1                 |
|             | 4        | Voghera                     | Lombardy              | 2                 |
|             | 5        | Codogno                     | Lombardy              | 2                 |
|             | 6        | Madignano                   | Lombardy              | 1                 |
|             | 7        | Cordenons                   | Friuli Venezia-Giulia | 2                 |
|             | 8        | Casirate d’Adda             | Lombardy              | 5                 |
|             | 9        | Este                        | Veneto                | 2                 |
|             | 10       | Lastra a Signa              | Tuscany               | 4                 |
|             | 11       | Jesi                        | Marche                | 1                 |
|             | 12       | Macerata                    | Marche                | 2                 |
|             | 13       | Roma                        | Lazio                 | 1                 |
|             | 14       | Follonica                   | Lazio                 | 4                 |
|             | 15       | Palma Campania              | Campania              | 2                 |
|             | 16       | Pomigliano d’Arco           | Campania              | 2                 |
|             | 17       | Bari                        | Apulia                | 2                 |
| Total       |          |                              |                       | 39                |
| France      | 1        | Bayeux                      | Basse-Normandie       | 4                 |
|             | 2        | Pleyben                     | Bretagne              | 3                 |
|             | 3        | Saint-Méen-le-Grand        | Bretagne              | 1                 |
|             | 4        | La Mothe-Saint-Héray        | Poitou-Charentes      | 1                 |
|             | 5        | Le Blanc                    | Centre                | 1                 |
|             | 6        | Saint-Jean-de-Monts         | Pays de la Loire      | 1                 |
|             | 7        | Flers-en-Escuebiers         | Nord-Pas-de-Calais    | 1                 |
|             | 8        | Aix-en-Provence             | Provence-Alpes-Côte d’Azur | 5           |
|             | 9        | Ba-Mauco                    | Aquitaine             | 6                 |
|             | 10       | Saint-Médard-en-Jalles      | Aquitaine             | 2                 |
|             | 11       | Le Bourget-du-Lac           | Rhône-Alpes           | 1                 |
| Total       |          |                              |                       | 26                |
| Germany     | 1        | Berlin                      | Berlin                | 1                 |
|             | 2        | Berlin                      | Berlin                | 2                 |
|             | 3        | Berlin                      | Berlin                | 4                 |
|             | 4        | Königs Wusterhausen         | Land Brandenburg      | 9                 |
|             | 5        | Erfurt                      | Freistaat Thüringen   | 1                 |
|             | 6        | Reuveler                    | Nordrhein-Westfalen   | 1                 |
|             | 7        | Hannover                    | Land Niedersachsen    | 2                 |
|             | 8        | Erfurt                      | Freistaat Thüringen   | 21                |
| Total       |          |                              |                       | 21                |
| The United Kingdom | 1  | Merstow Green               | West Midlands         | 1                 |
|             | 2        | London                      | Greater London        | 2                 |
|             | 3        | London                      | Greater London        | 5                 |
| Total       |          |                              |                       | 8                 |
| Belgium     | 1        | Aalst                       | Flanders              | 1                 |
|             | 2        | Varsenare                   | Flanders              | 4                 |
|             | 3        | Koersel                     | Flanders              | 5                 |
| Total       |          |                              |                       | 10                |
| The Netherlands | 1  | Zeist                       | Utrecht               | 1                 |
|             | 2        | Ermelo                      | Gelderland            | 4                 |
| Total       |          |                              |                       | 5                 |
Detection of CPV DNA was obtained by real-time PCR using a conventional TaqMan probe targeting the VP2 gene (Decaro et al., 2005a). Real-time PCR was carried out in a 25 μl reaction volume containing 12.5 μl of iCycler supermix (Bio-Rad), 600 nM of primers CPV-Forward (AAACAGGAAATATATATATATATA) and CPV-Reverse (AAATTTGACCATTTGGATAAACT), 200 nM of probe CPV-Pb (FAM-TGGTCCTTTA-ACTCCTGAAATTATATGACCT-GG-200), and 200 nM of probe CPV-c (FAM-AGGATATCCGACGACGACCCAATGATC). Reverse transcription (RT-PCR ThermoScript One-Step System (Invitrogen) and the following oligonucleotide primer pair CPVa/b-Forward (AGGAAGATATCCAGAAGGAGATTGGA) and CPVa/b-Reverse (CAATTGATGCTTGTGCACTATAACA) (type 2a/2b assays) or CPV-c/Forward (GAAGATATCCGACGACGACCCAATGATC) and CPV-c/Reverse (ATGCAGTAAGCAGACCTATGATTTGATC) (type 2c assay). All samples were tested in parallel using real-time RT-PCR ThermoScript One-Step System (Invitrogen) and the following oligonucleotide primer pair CPVa/b-Forward (AGGAAGATATCCAGAAGGAGATTGGA) and CPVa/b-Reverse (CAATTGATGCTTGTGCACTATAACA) (type 2a/2b assays) or CPV-c/Forward (GAAGATATCCGACGACGACCCAATGATC) and CPV-c/Reverse (ATGCAGTAAGCAGACCTATGATTTGATC) (type 2c assay). For real-time RT-PCR, the MGB groove binder (MGB) real-time PCR assays specific for types 2a/2b and 2b/2c, based on single nucleotide polymorphisms in the VP2 gene of the variant pairs (Decaro et al., 2006b). The reactions were carried out in a total volume of 25 μl containing 10 μl of template or standard DNA, 12.5 μl of iCycler supermix (Bio-Rad), 900 nM of primers CPVb-Forward (AGGAAGATATCCAGAAGGAGATTGGA) and CPVb-Reverse (CAATTGATGCTTGTGCACTATAACA) (type 2a/2b assays) and CPV-c/Forward (GAAGATATCCGACGACGACCCAATGATC) and CPV-c/Reverse (ATGCAGTAAGCAGACCTATGATTTGATC) (type 2c assay). A total of 156 faecal samples meeting the inclusion criteria were collected in several countries, namely Spain (n = 47), Italy (n = 39), France (n = 26), Germany (n = 21), the UK (n = 8), Belgium (n = 10), and the Netherlands (n = 5) (Table 1). The age of the sampled dogs ranged from 4 weeks to 12 years (mean ± standard deviation (SD): 1.11 ± 2.46 years); 133 were pups aged under 3 months and 23 were dogs ≥ 1 year of age. Seventy-seven dogs had either not been vaccinated or had not completed the full vaccination protocol. There were minimal differences in the vaccination protocols among the different countries. All vaccine formulations contained modified live CPV (either type 2 or 2b) strains and (when administered) killed CCoV strains. All the samples analysed were epidemiologically unrelated to each other, being representative of different disease outbreaks.

Seventy-six faecal samples (48.7%, 95% confidence interval [CI] 40.9–58.5%) tested positive for CPV, including 13/47 Spanish (27.7%, CI 14.9–40.5%), 21/39 Italian (53.8%, CI 38.2–69.4%), 16/26 French (61.5%, CI 42.8–80.2%), 15/21 German (71.4%, CI 52.1–90.7%), 7/8 British (87.5%), 4/10 Belgian (40.0%), and 0/5 Dutch (0.0%) specimens. Distribution of the CPV types varied based on the country of origin of the samples (Fig. 1a). The variant CPV-2c was predominant in Spain (9/13 CPV-positive samples) and widespread in France (7/16) and Germany (7/15), where types 2b and 2a were detected at higher frequency. In Italy, 5/21 positive samples were found to contain CPV-2c strains, with type 2a being prevalent. All the CPV strains detected in the UK (n = 7) were characterised as type 2b, whereas all the Belgian CPV strains (n = 4) were type 2a.

By TaqMan real-time RT-PCR, CCoV was detected in 60/156 tested samples (38.5%, CI 30.9–46.1%) with higher detection rates in Italy (20/39, 51.3%, CI 35.6–67.0%), Belgium (8/10, 80.0%), and the Netherlands (3/5, 60.0%) (Fig. 1b). By using genotype-specific assays, CCoV-I was detected less frequently (24/60 samples) than CCoV-II (56/60 samples). Simultaneous infections by both CCoV genotypes were identified in 20 specimens. A single sample tested positive for CCoV in the UK and was characterised as CCoV-II. CPV and CCoV were found in mixed infections in 28/156 samples (17.9%, CI 11.9–23.9%), including 4 Spanish (8.5%), 12 Italian (30.8%), 1 French (3.8%), 6 German (28.6%), 1 British (12.5%) and 4 Belgian (40.0%) specimens.

### Results

Seventy-six faecal samples (48.7%, 95% confidence interval [CI] 40.9–58.5%) tested positive for CPV, including 13/47 Spanish (27.7%, CI 14.9–40.5%), 21/39 Italian (53.8%, CI 38.2–69.4%), 16/26 French (61.5%, CI 42.8–80.2%), 15/21 German (71.4%, CI 52.1–90.7%), 7/8 British (87.5%), 4/10 Belgian (40.0%), and 0/5 Dutch (0.0%) specimens. Distribution of the CPV types varied based on the country of origin of the samples (Fig. 1a). The variant CPV-2c was predominant in Spain (9/13 CPV-positive samples) and widespread in France (7/16) and Germany (7/15), where types 2b and 2a were detected at higher frequency. In Italy, 5/21 positive samples were found to contain CPV-2c strains, with type 2a being prevalent. All the CPV strains detected in the UK (n = 7) were characterised as type 2b, whereas all the Belgian CPV strains (n = 4) were type 2a.

By TaqMan real-time RT-PCR, CCoV was detected in 60/156 tested samples (38.5%, CI 30.9–46.1%) with higher detection rates in Italy (20/39, 51.3%, CI 35.6–67.0%), Belgium (8/10, 80.0%), and the Netherlands (3/5, 60.0%) (Fig. 1b). By using genotype-specific assays, CCoV-I was detected less frequently (24/60 samples) than CCoV-II (56/60 samples). Simultaneous infections by both CCoV genotypes were identified in 20 specimens. A single sample tested positive for CCoV in the UK and was characterised as CCoV-II. CPV and CCoV were found in mixed infections in 28/156 samples (17.9%, CI 11.9–23.9%), including 4 Spanish (8.5%), 12 Italian (30.8%), 1 French (3.8%), 6 German (28.6%), 1 British (12.5%) and 4 Belgian (40.0%) specimens.

### Discussion

CPV and CCoV are the most common canine enteric pathogens worldwide and, in recent years, both viruses have given rise to new genotypes or variants. A new CPV variant, type 2c, emerged around 2000 (Buonavoglia et al., 2001) and is now circulating worldwide along with the former CPV types 2a and 2b (Greene and Decaro, in press). Likewise, CCoV-I was only detected in 2003 (Pratelli et al., 2003).

Except for a large European survey on the distribution of CPV types in 2006–2007 (Decaro et al., 2007b), only studies describing the molecular epidemiology at a national or regional level have been published in Europe (Decaro et al., 2005b; Benetka et al., 2006; Davies, 2008; Vieira et al., 2008). Based on those studies, CPV-2c appeared to be widespread in Italy (Decaro et al., 2005b), whereas it was virtually absent in the UK and Belgium, where CPV types 2b and 2a were predominant, respectively (Decaro et al., 2007b; Davies, 2008). In the present study, CPV-2c was shown to be still common in Italy and Germany, albeit less frequent than CPV-2a, suggesting a temporal fluctuation (Decaro et al., 2007b). In contrast, the new variant CPV-2c was found to be predominant in Spain and frequent in France.

Although cross-protection between CPV-2 and its variants has been demonstrated (Spibey et al., 2008), some concerns have been raised that antigenic differences may have decreased the effectiveness of the old vaccines that are based on the original type CPV-2, a virus no longer circulating in the field (Pratelli et al., 2001a; Cavalli et al., 2008; Decaro et al., 2008). Based on these concerns, vaccines containing CPV-2b strains have been developed and licensed.

Limited data have been gathered on CCoV molecular epidemiology. Both CCoV genotypes have been found to be widespread in Italy and Austria with CCoV-I being more common (Decaro et al., 2005b; Benetka et al., 2006). In contrast, in the current survey, whereas CCoV-I was detected in most European countries, it appeared to be far less common than CCoV-II. In the UK, CCoV-I was not detected, although only a limited number of samples were available. Based on studies from the early 1990s, it may be hypothesised that the actual prevalence of CCoV in the UK is much higher (Tennant et al., 1991, 1993). Simultaneous infections by CCoV...
types I and II were detected, but at a lower frequency than in previous investigations, where up to 75% of mixed infections were documented (Decaro et al., 2005b).

An interesting finding was the presence of mixed CPV/CCoV infections in 28/156 samples. Except for the hypervirulent CCoV strains (Decaro and Buonavoglia, 2008), CCoV is usually regarded as a mild canine pathogen, since it can frequently be detected in dogs with no overt clinical signs (Schulz et al., 2008). However, CCoV may also cause severe diarrhoea and exacerbate diseases caused by other canine viruses, such as CPV (Pratelli et al., 1999; Decaro et al., 2006c) and canine adenovirus (Pratelli et al., 2001b; Decaro et al., 2007a). Although recording the complete anamnesis of diarrhoeic dogs was beyond the scope of the present study, it would have been of interest to assess whether mixed infections affected the severity and outcome of the disease. Inactivated vaccines are available in Europe and can be used to confer some protection against CCoV, thereby reducing the effects of mixed infections (Decaro and Buonavoglia, 2008). Since inactivated vaccines can protect dogs from CCoV-induced disease but not from infection, modified live virus vaccines administered oronasally have been proposed as a reliable alternative (Pratelli et al., 2004).

Our sampling procedure was planned to cover different areas of each country, but for some countries (UK, Belgium and The Netherlands), samples from only a few areas could be obtained, thus making our results not fully representative. Nevertheless, the presence of particular CPV variants and/or CCoV genotypes is reported here for the first time in some countries and this is relevant in terms of the potential efficacy of current vaccines. Since there were no remarkable differences in the vaccination protocols among the various countries, the differences in epidemiological findings are most likely related to different trading flows of the dogs imported from foreign countries rather than to vaccination protocols (Decaro et al., 2007b). For instance, it is known that there is a prosperous trade of purebred pups between Eastern and Western European countries, and this may lead to the introduction and spread in a naïve country of novel virus strains (Decaro et al., 2007a; Martella et al., 2007). However, it cannot be ruled out that some differences in the distribution of virus variants/genotypes are due to a sampling bias, at least for the countries where the number of samples was low.

Conclusions

Although the study lacks valid statistical support, it provides a snapshot of the epidemiological situation of CPV and CCoV infections in some Western European countries. This first CPV/CCoV combined epidemiological survey in Europe documents the presence and relative distribution of new viral genotypes (CCoV-I) and variants (CPV-2c) in several European countries.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisation that could inappropriately influence or bias the content of the paper.

Acknowledgements

This study was funded by Fort Dodge Animal Health and by Grants of University of Bari, Italy (contribution to PRIN07, Project ‘Infezione da parvovirus nei carnivori: aspetti molecolari, patogenetici ed immunologici’; Ateneo 2009, Project ‘Epidemiologia molecolare del parvovirus del cane nei carnivori domestici e selvatici’), and of Italian Ministry of Health (Ricerca finalizzata 2007, Project ‘Mammalian coronaviruses: molecular epidemiology,
vaccine development and implications for animal and human health). We are grateful to Carlo Armenise, Donato Narcisi, and Arturo Gentile for their continuous technical assistance and to Maria-rosaria Marinaro for her critical revision of the manuscript.

References

Benetka, V., Kolodziejek, J., Walk, K., Rennhofer, M., Möstl, K., 2006. M gene analysis of atypical strains of feline and canine coronavirus circulating in an Austrian animal shelter. Veterinary Record 159, 170–174.

Buonavoglia, C., Martella, V., Pratelli, A., Tempesta, M., Cavalli, A., Buonavoglia, D., Bozzo, G., Elia, G., Decaro, N., Carmichael, L.E., 2001. Evidence for evolution of canine parvovirus type-2 in Italy. Journal of General Virology 82, 1555–1560.

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

Cavalli, A., Martella, V., Decaro, N., Camero, M., Bellacicco, A.L., De Palo, P., Decaro, N., Elia, G., Buonavoglia, C., 2008. Evaluation of the antigenic relationships among canine parvovirus type 2 variants. Clinical and Vaccine Immunology 15, 534–539.

Davies, M., 2008. Canine parvovirus strains identified from clinically ill dogs in the United Kingdom. Veterinary Record 163, 543–544.

Decaro, N., Fratelli, A., Campolo, M., Elia, G., Martella, V., Tempesta, M., Buonavoglia, C., 2004. Quantitation of canine coronavirus RNA in the faeces of dogs by TaqMan RT-PCR. Journal of Virological Methods 119, 145–150.

Decaro, N., Elia, G., Martella, V., Desario, C., Campolo, M., Di Trani, L., Tarisitano, E., Tempesta, M., Buonavoglia, C., 2005a. A real-time PCR assay for rapid detection and quantitation of canine parvovirus type 2 DNA in the feces of dogs. Veterinary Microbiology 105, 19–28.

Decaro, N., Martella, V., Ricci, D., Elia, G., Desario, C., Campolo, M., Cavaliere, N., Di Trani, L., Tempesta, M., Buonavoglia, C., 2005b. Genotype-specific fluorogenic RT-PCR assays for the detection and quantitation of canine coronavirus type 1 and type II RNA in faecal samples of dogs. Journal of Virological Methods 130, 72–78.

Decaro, N., Elia, G., Desario, C., Roperto, S., Martella, V., Campolo, M., Lorussso, A., Decaro, A., Buonavoglia, C., 2006a. A minor groove binder probe real-time PCR assay for discrimination between type 2-based vaccines and field strains of canine parvovirus. Journal of Virological Methods 136, 65–70.

Decaro, N., Elia, G., Martella, V., Campolo, M., Desario, C., Camero, M., Giacalone, M.L., Lorussso, A., Lucente, M.S., Narcisi, D., Scala, P., Buonavoglia, C., 2006b. Characterisation of the canine parvovirus type 2 variants using minor groove binder probe technology. Journal of Virological Methods 133, 92–99.

Decaro, N., Martella, V., Desario, C., Bellacicco, A.L., Camero, M., Manso, L., D’Alloja, D., Buonavoglia, C., 2006c. First detection of canine parvovirus type 2c in pups with haemorrhagic enteritis in Spain. Journal of Veterinary Medicine. B, Infectious Diseases and Veterinary Public Health 53, 468–472.

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

Decaro, N., Campolo, M., Elia, G., Buonavoglia, D., Giacalone, M.L., Lorussso, A., Mari, V., Buonavoglia, C., 2007a. Infectious canine hepatitis: an ‘old’ disease reemerging in Italy. Research in Veterinary Science 83, 269–273.

Decaro, N., Desario, C., Addie, D.D., Martella, V., Vieira, M.J., Elia, G., Zicola, A., Davis, C., Thompson, G., Thiry, E., Truyen, U., Buonavoglia, C., 2007b. Molecular epidemiology of canine parvovirus, Europe. Emerging Infectious Diseases 13, 1222–1224.

Decaro, N., Buonavoglia, C., 2008. An update on canine coronaviruses: viral evolution and pathobiology. Veterinary Microbiology 132, 221–234.

Decaro, N., Desario, C., Elia, G., Martella, V., Mari, V., Lavazza, A., Nardi, M., Buonavoglia, C., 2008. Evidence for immunisation failure in vaccinated adult dogs infected with canine parvovirus type 2c. New Microbiologia 31, 123–130.