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Original Article

Shelter-housed cats show no evidence of faecal shedding of canine parvovirus DNA

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ABSTRACT

Canine parvovirus (CPV) and feline panleukopenia virus (FPV) are deoxyriboncucleic acid (DNA) viruses in the taxon *Carnivore protoparvovirus 1*. Exposure of cats to either CPV or FPV results in productive infection and faecal shedding of virus. Asymptomatic shedding of CPVs by one-third of shelter-housed cats in a UK study suggests that cats may be an important reservoir for parvoviral disease in dogs. The aim of this cross-sectional study was to determine the prevalence of faecal shedding of CPVs in asymptomatic shelter-housed cats in Australia. Faecal samples ($n = 218$) were collected from cats housed in three shelters receiving both cats and dogs, in Queensland and NSW. Molecular testing for *Carnivore protoparvovirus 1* DNA was performed by polymerase chain reaction (PCR) amplification followed by DNA sequencing of the VP2 region to differentiate CPV from FPV.

Carnivore protoparvovirus 1 DNA was detected in only four (1.8%, 95% confidence interval 0.49–4.53%) faecal samples from a single shelter. Sequencing identified all four positive samples as FPV. Faecal shedding of CPV by shelter-cats was not detected in this study.

While the potential for cross-species transmission of CPV between cats and dogs is high, this study found no evidence of a role for cats in maintaining CPV in cat and dog populations through faecal shedding in the regions tested.

Introduction

Canine parvovirus (CPV) and feline panleukopenia virus (FPV) are small, (5 Kb) single-stranded, icosahedral DNA viruses in the taxon *Carnivore protoparvovirus 1*. CPV and FPV share greater than 98% nucleotide identity and both viruses can infect and cause disease in felids (Hoelzer and Parrish, 2010). Viral host range and pathogenicity are determined by binding of the viral VP2 capsid region to cellular transferrin receptors (TfR) (Allison et al., 2013). Frequent cross-species transmission of parvoviruses between wildlife carnivore species suggests that CPV and FPV have evolved independently from common sylvatic ancestors (Allison et al., 2013; Allison et al., 2014).

The clinical syndrome of feline panleukopenia, characterised by enteritis and panleukopenia, has been recognised in cats for over a hundred years (Hoelzer and Parrish, 2010), although it was not until 1965 that FPV was first isolated (Johnson, 1965). When CPV emerged as a new virus in dogs in the mid-to-late 1970s, the virus, termed CPV-2, was unable to infect cats (Parrish, 1999). However, by 1980 a new variant, CPV-2a, had emerged, replacing CPV-2 that had acquired the feline host range through four mutations in VP2 (L87M, I101T, A300G, D305Y) that likely enable capsid binding to the feline TfR. Two additional CPV variants VP2 N426D (CPV-2b) and VP2 D426E (CPV-2c) have since emerged that co-circulate with CPV-2a in varying proportions in different geographic regions (Hoelzer and Parrish, 2010; Decaro and Buonavoglia, 2012).

CPV variants have been isolated from the blood (Ikeda et al., 2000) and faeces of cats worldwide, in both natural (Mochizuki et al., 1993; Mochizuki et al., 1996; Truyen et al., 1996; Gamoh et al., 2003a; Battilani et al., 2006; Battilani et al., 2011) and experimental (Nakamura et al., 2001) infections. While FPV is the most common aetiological agent of feline panleukopenia, CPVs can cause clinical signs in cats that are indistinguishable from those caused by FPV (Stuetzer and Hartmann, 2014). DNA sequencing of

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parvovirus isolates from cats presenting with signs of feline panleukopenia has revealed most to be FPV, with CPVs causing approximately 5% of feline panleukopenia cases (Truyen et al., 1996). Clinical disease caused by CPV variants have been reported in Italy, Germany, USA, Japan and in Portugal (Mochizuki et al., 1996; Truyen et al., 1996; Gamoh et al., 2003b; Decaro et al., 2010; Miranda et al., 2014). In the UK, faecal shedding of CPV was detected in more than a third of faecal samples collected from healthy cats from two mixed canine and feline shelters (Clegg et al., 2012). The ability of CPV variants to infect cats has raised concerns about the role of cats as reservoirs of infection for dogs, and has important implications for biosecurity especially in mixed animal shelters housing both cats and dogs (Clegg et al., 2012).

The aim of this study was to determine the prevalence of faecal shedding of CPV in asymptomatic shelter-housed cats to understand whether faecal shedding of CPV by cats poses a significant infection risk in mixed cat-dog shelters in the regions tested.

Materials and methods

Samples

Shelters 1 and 2

Faecal samples ($n = 118$) were collected from healthy cats from two mixed cat-dog rescue shelters in Brisbane, Queensland within 24 h of admission to the shelter. Cats were individually housed except for litters where up to three litter-mates shared an enclosure. On entry to the shelter all cats were administered an attenuated trivalent vaccination containing feline herpesvirus-1, feline calicivirus and feline panleukopenia virus (Companion F3, MSD Animal Health Australia). Sixty-one faecal samples were collected from Shelter 1 on four occasions between 19th October 2015 and 1st February 2016 and 57 faecal samples were collected from Shelter 2 on three occasions between 4th December 2015 and 20th January 2016. The holding capacity of shelter 1 was 180 dogs and 150 cats, and for shelter 2 was 110 dogs and 120 cats. At both shelters the majority of cats were housed one to a cage, but there were a few group housing rooms. Veterinary shelter personnel could move freely between canine and feline areas of the shelter.

Shelter 3

Faecal samples ($n = 100$) were collected from a mixed cat-dog shelter in Sydney, NSW on three occasions at any time point after admission to the shelter (March 2016–February 2017). An outbreak of feline panleukopenia occurred at this shelter in the two months prior to the third sample collection. On admission to the shelter dogs were routinely administered two inactivated canine parvovirus vaccines, 7 days apart (Parvac, Zoetis Australia, Silverwater Australia). Cats were not vaccinated. Healthy cats were housed individually or in groups of up to six cats in enclosures ($n = 14$). One faecal sample was collected from individually caged cats while all faecal samples were collected from multicat enclosures. In some multicat enclosures the number of faecal samples collected exceeded the number of cats because individual cats had defecated more than once since the litter trays had been cleaned. The shelter also housed approximately 100 dogs. All shelter personnel moved freely between canine and feline areas of the shelter.

At all shelters sampled, enclosures were cleaned daily and litter trays were emptied and cleaned at least once daily. At each visit, fresh faecal samples were collected from the litter tray or enclosure floor prior to cleaning. Data including age, sex, breed and date of admission of the cats was obtained at each visit. Faecal samples were stored at −20 °C or −80 °C until processing.

Molecular detection of CPV and FPV from faeces

Shelters 1 and 2

DNA was extracted from faecal samples using the QIAmp DNA Stool Mini Kit (Qiagen) to remove PCR inhibitors as described previously, and stored at −20 °C until PCR testing (Meers et al., 2007). A duplicate set of DNA extractions was performed using boiling and homogenization, as described previously and stored at 4 °C for PCR testing within 7 days of extraction (Clegg et al., 2012).

The presence of parvovirus in faecal samples was detected in duplicate DNA samples extracted by both methods using a conventional PCR to amplify the VP2 gene of *Carnivore protoparvovirus 1*, using primer pair JS1F and JS2R as previously described (Table 1) (Meers et al., 2007). For the kit-extracted DNA samples, each 25 μL reaction contained 0.1 μL of 5U/μL KAPA Taq DNA polymerase (KAPA Biosystems Inc., Wilmington, MA), 2.5 μL KAPA Buffer A (containing 15 mM $MgCl_2$), 0.5 μL of 10 mM dNTP mix, 0.3 μL DMSO (final concentration of 1.5%), 1.25 μL betaine (final concentration 0.25 mM), 1 μL of 0.2 μmol of each primer (10 μM each) and 2 μL DNA template. For the DNA extracted by homogenization and boiling, each 25 μL reaction contained 2.5 μL of 10× reaction buffer (containing 15 mM $MgCl_2$), 0.5 μL of 25 mM $MgCl_2$, 4 μL of 1.25 mM dNTPs, 10 pmol of each primer, 0.5 μL of 5U/μL HotStar Taq DNA polymerase (Qiagen Australia) and 5 μL DNA template. Cycling conditions were initial denaturation at 94 °C for 5 min, followed by 40 cycles of denaturation at 94 °C for 30 s, annealing at 52 °C for 30 s and polymerization at 72 °C for 2 min. A negative control (no DNA) and a positive control were included in each run. Positive controls were faecal DNAs from a dog with parvovirus enteritis infected with CPV-2b and/or from a cat with feline panleukopenia infected with FPV diagnosed using the methods above.

Shelter 3

DNA was extracted from faeces by homogenization and boiling as described previously, (Clegg et al., 2012). The resulting supernatants were diluted in molecular-grade water (1:10, 1:20, and 1:50) and stored at 4 °C for PCR testing within 7 days of extraction. To test for PCR inhibition, DNA extracts (1:10 and 1:20 dilutions) were spiked with 1 μL of feline genomic DNA (138 ng/μL) extracted from the lymph node of a healthy cat. A conventional PCR using primer set GAPDH-F and GAPDH-R to amplify the feline housekeeping GAPDH gene was performed (Table 1) (McLuckie et al., 2016). Each 50 μL reaction contained 5 μL of 10 X PCR buffer (containing 10 mMol dNTP and 75 mM MgCl2), 1 μL (10 pmol) of each primer, 0.2 μL (0.2 U) of Taq QIAGEN DNA Polymerase (QIAGEN Germantown, MD), 1 μL of feline genomic DNA template and 5 μL faecal DNA. Cycling conditions were initial denaturation at 94 °C for 3 min, followed by 35 cycles of denaturation at 94 °C for 45 s, annealing at 55 °C for 30 s and polymerization at 72 °C for 30 s, with final extension 72 °C for 7 min. If PCR inhibition was detected additional dilutions of the sample were tested to identify the dilution at which PCR inhibition was abolished.

The presence of parvovirus in each faecal sample was detected using a conventional PCR to amplify the VP2 gene using primer pair 555-F and 555-R (Decaro et al., 2008) (Table 1). PCR screening of all faecal samples was performed using both 1:10 and 1:20 dilutions, as well as 1:50 dilutions for samples in which PCR inhibition was detected. Each 50 μL reaction contained 10 μL PCR buffer (5 mM dNTPs, 15 mM $MgCl_2$), 0.5 μL (0.5U) MyTaq Hot Start polymerase (Bioline, Meriden Life Science, Alexandria, Australia), 1 μL (10 pmol) forward and reverse primer, 7 μL DNA and 30.5 μL water. Cycling conditions were initial denaturation at 94 °C for 1 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s and polymerization at 72 °C for 30 s, with final extension 72 °C for 1 min. PCR was performed using sample dilutions that had no evidence of PCR inhibition. A blank DNA control (PBS used during the extraction process) and a positive control were included in each run. The positive control was faecal DNA from a dog with parvovirus enteritis extracted and sequenced using the methods above, and identified as CPV-2a.

The sensitivity of this conventional PCR was determined using DNA extracted from a feline faecal sample confirmed to contain FPV by sequencing. The FPV copy

Table 1
Primers used in this study for PCR amplification of feline DNA (GAPDH) and of canine and feline parvovirus DNA, targeting the VP2 region (VP2).

Primer name	Target	Sequence	Size (nt[a])	Reference
JS1F	VP2	5′-AGCTACAGGATCTGGGAACG-3′	1975	Meers et al. (2007)
JS2F		5′-CCACCCACACCATAACAACA-3′		
GAPDH-F	GAPDH	5′-AAGGCTGAGAACGGGAAAC-3′	80	McLuckie et al. (2016)
GAPDH-R		5′-CATTTGATGTTGGCGGGATC-3′		
555-F	VP2	5′-CAGGAAGATATCCAGAAGGAA-3′	583	Decaro et al. (2008)
555-R		5′-GGTGCTAGTTGATATGTAATAAACA-3′		
2655F	VP2	5′-CCAGATCATCCATCAACATCA-3′	837	Decaro et al. (2008
3511R		5′-TGAACATCATCTGGATCTGTACC-3′		
3381F	VP2	5′-CCATGGAAACCAACCATACC-3′	717	Decaro et al., 2008
4116R		5′-AGTTAATTCCTGTTTTACCTCCAA-3′		

[a] Nucleotide.