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Bovine herpesvirus 4 DNA is not detected in free-ranging domestic cats from California, Colorado or Florida

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Abstract

Objectives—Several studies have reported that domestic cats can be naturally infected with bovine herpesvirus 4 (BHV4). Cats experimentally inoculated with BHV4 developed clinical signs involving the urinary tract, leading to the hypothesis that natural infection with BHV4 may be associated with feline lower urinary tract diseases. However, the question of whether BHV4 infection is common in cats remains equivocal. In this study, we sought to determine whether BHV4 is a common natural infection of domestic cats in the USA.

Methods—We used a sensitive nested PCR protocol specific to the BHV4 thymidine kinase gene to screen free-ranging domestic cat blood DNA samples (n = 101) collected from California, Colorado and Florida.

Results—Cats within this cohort were positive for seven other common pathogens of domestic cats, demonstrating the relatively high exposure of this population to endemic feline infections. In contrast, all domestic cat blood samples were negative for BHV4, while BHV4-containing tissue culture extracts were strongly positive.

Conclusions and relevance—BHV4 has been detected in tissues of latently infected cattle, though viral DNA is typically also detected in peripheral blood cells throughout infection. Our results suggest that persistent presence of BHV4 DNA in the blood of domestic cats is either rare or non-existent. We thus conclude that BHV4 is unlikely to be a major pathogen of cats.

Introduction

Bovine herpesvirus 4 (BHV4) is a ubiquitous γ -herpesvirus of cattle that is typically asymptomatic,^{1–3} but in some cases has been associated with urogenital, ocular and respiratory diseases.^{4–7} Infected cattle usually shed the virus through respiratory and genital secretions. New infections are typically acquired via the same routes, though the virus has been shown to multiply after intravenous, intradermal, intramammary and intratesticular

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inoculations.^{8,9} The virus persists for the life of the infected host and can typically be detected by PCR or recovery of infectious virus in circulating leukocytes and multiple anatomic sites.^{7,8} BHV4 has been isolated in various species of large and small ruminants,⁸ and has also been shown to be experimentally transmissible to rabbits.¹⁰

Several reports from the 1970s and 1980s provided serologic and experimental evidence that domestic cats (*Felis catus*) could become infected with a strain of BHV4 identified as feline cell-associated herpesvirus.^{11–14} This agent was also posited as a possible etiologic agent of feline lower urinary tract disease.^{15,16} Attempts to replicate these initial intriguing findings, however, have been largely unsuccessful.^{17–21} In a more recent study, evidence of BHV4 exposure and active infection was detected using an indirect fluorescent assay test and PCR in free-ranging cats in Michigan. Out of 104 cats tested, 61 (58.7%) were seropositive and 28 (26.9%) were viremic by PCR.²²

We recently utilized degenerate nested pan- γ -herpesvirus (GHV) PCR primers to identify DNA sequences of three novel GHVs present in domestic cats, bobcats and pumas.²³ Subsequent virus-specific real-time quantitative PCR assays found that 16% of free-ranging domestic cats were infected with a virus that we named *Felis catus* γ -herpesvirus 1 (FcaGHV1). FcaGHV1 clusters phylogenetically with GHVs of the *Percavirus* genus and is divergent from BHV4, a member of the *Rhadinovirus* genus.²³ When we screened domestic cat DNA samples (n = 60) using the pan-GHV PCR, we were able to confirm that all positive samples contained novel FcaGHV1; curiously, none detected sequences that were similar to BHV4. These degenerate primers were capable of amplifying BHV4 but likely have relatively poor sensitivity due to their degeneracy (data not shown). This result suggested to us that BHV4 infection may be relatively uncommon in cats. To investigate this hypothesis, we sought to test domestic cat DNA samples for BHV4 using a high-sensitivity assay. We adopted a previously published nested PCR assay that targeted the BHV4 thymidine kinase (TK) gene.⁷ These primers have been used in several studies for sensitive and specific detection of BHV4,^{24–26} and have been validated against 31 strains of BHV4 by Wellenberg et al.²⁵ We tested 101 samples from free-ranging cats in three US states that had previously been tested for a suite of seven feline pathogens, including FcaGHV1. All cats were negative for BHV4 DNA, suggesting that BHV4 infection is likely uncommon in US cats, at least relative to other more common feline agents such as FcaGHV1.

Materials and methods

Animal procedures were approved by appropriate animal care committees prior to initiation of sample collection. Feline blood samples were collected from 101 free-ranging domestic cats in California (n = 33), Colorado (n = 37) and Florida (n = 31) during trap–neuter–release campaigns conducted by animal shelters.²⁷ Total DNA was extracted from blood using a DNeasy blood and tissue kit (Qiagen). Cats had previously been tested for seven common feline pathogens as part of a larger disease survey²⁸ using PCR, Western blot (WB) and enzyme-linked immunosorbent assays (ELISA) performed and interpreted following previously described standardized protocols.^{23,27} These pathogens included *Bartonella* species (*Bartonella henselae*, *Bartonella clarridgeiae* and *Bartonella vinsonii* subspecies

berkhoffii), feline calicivirus, feline herpesvirus-1, feline parvovirus, feline immunodeficiency virus (FIV), FcaGHV1 and the feline hemoplasmas (Table 1).^{23,29–33}

First-round PCR reactions for BHV4 were prepared in a total volume of 50 µl using GoTaq Master Mix (Promega) with primers BHV4-p1 and BHV4-p2 at 400 nM using 50–500 ng DNA.⁷ Second-round nested PCR reactions were prepared in a total volume of 50 µl using GoTaq Master Mix with primers BHV4-p3 and BHV4-p4 at 400 nM using 2 µl first-round PCR product.⁷ The final product length was 260 bp.⁷ BHV4 viral DNA was extracted from cell culture supernatant containing BHV4 using the QIAamp DNA blood kit (Qiagen). Log dilutions of this BHV4-positive control DNA were tested using the thymidine kinase PCR and were sensitive for BHV4 detection to a 1:10,000 dilution. Water and blood cell DNA prepared from seven specific pathogen-free colony cats served as negative controls. Both rounds of PCR were completed using the following cycling conditions: one step at 94°C for 2 mins, followed by 35 cycles of 94°C for 30 s, 60°C for 30 s and 72°C for 30 s. Amplification was concluded with a final step at 72°C for 5 mins. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) PCR was utilized to ensure the presence of DNA in 99/101 free-ranging cat samples using previously described protocols.³⁴ Ten microliters of the PCR products were analyzed by electrophoresis in a 1.5% agarose gel using GelRed (Biotium) DNA intercalating dye and visualizing bands under ultraviolet light.

Results

We consistently found a strong BHV4-specific band in positive control samples. GAPDH PCR confirmed amplifiable DNA was present in all 99 samples tested for which adequate DNA samples were available (data not shown). DNA from the seven specific pathogen-free cats was negative for BHV4 by PCR. We did not detect PCR amplicons for BHV4 in any of the 101 cats tested from California, Colorado and Florida (Figure 1). While BHV4 could not be detected in these cats, previous tests revealed that a variety of other bacterial and viral agents could be detected using PCR, WB and ELISA (Table 1). Calicivirus (6.5–18.2%), FcaGHV1 (2.7–33.3%) and at least one of the hemoplasmas (8.1–60.6%) were detected by PCR in all three locations, with varying prevalence. PCR was also used to detect *Bartonella* species in Californian and Floridian cats, and FHV-1 in Californian cats. Evidence of FIV seroconversion in 21.2% of Californian cats and feline parvovirus in 6.5% of Floridian cats was documented. These data provide evidence that these feral cats were exposed to a variety of pathogens found within the environment and among the population (Table 1).

Discussion

We did not detect BHV4 in peripheral blood cells using a highly sensitive PCR assay, capable of detecting one viral DNA copy per reaction.⁷ It is possible that BHV4 could be present in an alternate latent reservoir tissue in cats. In cattle, spleen and nervous tissue have been suggested as possible reservoirs.^{35,36} However, BHV4 DNA is typically detected at high levels in leukocytes of cattle,^{7,8,37} and a previous study characterizing BHV4 prevalence in cats detected viral DNA in peripheral blood cells.²² This suggests that, if infected, cats would be expected to have viral DNA in peripheral cells in the majority of cases. Furthermore, the free-ranging cats examined in this study were exposed to many

common feline pathogens (Table 1), suggesting that if BHV4 was a common infection of cats, we would expect to detect it in this population. Thus, our results suggest that BHV4 may not be as highly prevalent in the cat population as previously thought, at least compared with the recently identified felid GHV FcaGHV1, which is found in the peripheral blood of 10–16% of cats in Australia, the USA and Singapore.^{23,34}

Our findings contrast sharply with the findings from free-ranging cats in central Michigan, where investigators detected BHV4 DNA using a hemi-nested PCR targeted to the glycoprotein B gene in 28/104 cats tested (26.9%).²² Several possible explanations may clarify the discrepancy between BHV4 evaluations in domestic cats. First, our PCR protocol may not afford adequate sensitivity to detect a feline BHV4 strain with significant genetic diversity from bovine isolates. This is unlikely as the PCR protocol used here has been validated for 31 American and European strains,²⁵ and a pan-GHV PCR protocol also did not detect BHV4 in domestic cats.²³ Second, it is possible that there is high geographic variation in BHV4 incidence, as feral cats in different parts of the country may have variable exposure to cattle infected with BHV4. Additional testing could thus reveal positive samples from other regions or in cats with evidence of urinary tract disease. Serology assays may be additionally informative in providing evidence of natural contact of BHV4 in cats. Lastly, it is also possible that previously reported results represented false positives. While Kruger et al utilized hemi-nested primers,²² which were evaluated for sensitivity and specificity, exact values were not reported. Given the difficulty in consistently isolating BHV4 from cats, some investigators postulated that isolation of BHV4 from cat cell cultures in the original experiments was the result of contaminated media or fetal bovine serum,³ as filtering of fetal bovine serum may not have been common practice in 1971. Evaluation of these possibilities is complicated by the fact that, to our knowledge, no DNA sequences of feline BHV4 have ever been published.

Conclusions

Our results offer evidence that BHV4 is not detectable in a cohort of geographically diverse free-ranging cats in California, Colorado and Florida, and provide evidence for the hypothesis that BHV4 is not a widespread viral agent in cats. While additional studies could assist in confirming this observation, given the number of common feline pathogens detected in this set of samples, we conclude that BHV4 is not a common naturally occurring infection in domestic cats.

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Figure 1.

PCR products obtained after amplification of domestic shelter cat samples (lanes 3–6 and 9–12). Negative controls (lanes 2 and 8) and positive controls (lanes 7 and 13) were also included. PCR markers are 50, 150, 300 (demarcated by the arrow), 500, 750 and 1000 bp long (169 × 69 mm)

Table 1

Cat blood samples were used in additional disease pathogen testing for evidence of infection with seven common bacterial and viral diseases of free-ranging cats. Evidence of infection with *Bartonella* species, feline calicivirus, feline herpesvirus-1 (FHV-1), feline immunodeficiency virus (FIV), feline γ -herpesvirus-1 (FcaGHV1), feline parvoviruses (FPV) or the feline hemoplasmas was identified to be present in at least one geographic location. Detection of pathogens was accomplished using PCR, quantitative PCR (qPCR), Western blot (WB) or enzyme-linked immunosorbent assay (ELISA)

		California (n = 33)	Colorado (n = 37)	Florida (n = 31)
<i>Bartonella</i> species	PCR	9.1%	–	41.9%
<i>Hemoplasma</i> species		60.6%	8.1%	19.4%
Calicivirus		18.2%	10.8%	6.5%
FHV-1		12.1%	–	–
FcaGHV1	qPCR	33.3%	2.7%	12.9%
FIV	WB	21.2%	–	–
FPV	ELISA	–	–	6.5%