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Absence of Domestic Cat Hepadnavirus DNA in Clinical Samples from a Large Cat Population in the Porto Alegre Metropolitan Area, Southern Brazil

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ABSTRACT

***Background*:** Infectious liver diseases are frequent in domestic cats. However, the etiological diagnosis remains challenging due to the nonspecific nature of clinical and laboratory findings. Domestic cat hepadnavirus (DCHV) (*Orthohepadnavirus felisdomestici*) is a circular DNA virus belonging to the genus *Orthohepadnavirus* within the family *Hepadnaviridae*, recently identified in domestic and wild felines. Similar to human hepatitis B virus (HBV), DCHV has been associated with chronic hepatitis and hepatocellular carcinoma in cats. The DCHV was firstly reported in Australia in 2018 and subsequently across multiple countries and continents countries, including Brazil. DCHV has been primarily detected in serum, whole blood, and liver tissue samples. However, viral DNA has also been identified in kidney, intestine, brain, heart, and lymph node tissues, suggesting the possibility of systemic. DCHV is currently classified in genotypes A and B, where genotype A is the most frequent reported worldwide, and genotype B was only reported in Japan and Brazil. The present study aimed to detect DCHV in serum and liver in a large domestic cat population from Porto Alegre and its metropolitan area, contributing to a better understanding of this virus molecular epidemiology.

***Materials, Methods & Results*:** In total, 528 individual clinical samples were analyzed, obtained from domestic cats of different age groups and clinical histories. Of these, 451 were serum samples collected in veterinary diagnostic laboratories, and 77 were liver tissue samples from cats necropsied at the Department of Veterinary Pathology of the Federal University of Rio Grande do Sul (UFRGS). Detection of DCHV was performed using two different PCR assays, both aiming to amplify the polymerase (P) gene, including internal controls in all reactions. The second DCHV PCR was designed to amplify both genotypes A and B. None of the samples analyzed yielded a positive result for DCHV genome. This finding suggests the absence or a low frequency of DCHV in the domestic cat population from Porto Alegre metropolitan area, South Brazil.

***Discussion*:** DCHV has been identified in cats from different regions worldwide, with prevalence varying among domestic cat populations. In the present study, a comprehensive investigation of DCHV occurrence was carried out in a large domestic cats sampling from Porto Alegre and its metropolitan area using two clinical specimens (serum and liver) as well as two different PCR assays. DCHV DNA was not detected in any of the animals evaluated, suggesting the absence or low frequency of this virus in the here evaluated domestic cat population. These findings are consistent with previous studies reporting low DCHV detection rates in some geographic regions, including Brazil. In contrast, higher frequencies have been described in locations such as Hong Kong, Thailand, and Malaysia, indicating that regional, population-related, specific epidemiological factors may influence viral transmission dynamics. Expanded surveillance and further investigations are essential to determine whether the currently low detection frequency reflects truly limited viral circulation or arises from sampling and methodological constraints. Considering the hepatotropic potential of DCHV, the results of this study highlight the importance of continuous monitoring of this agent in feline populations. Expanded investigations across diverse epidemiological contexts will be essential to determine whether the absence of DCHV here observed reflects truly limited viral circulation or arises from methodological constraints.

Keywords: *Orthohepadnavirus felisdomestici*, *Hepadnaviridae*, molecular diagnosis, hepatotropic viruses, PCR.

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INTRODUCTION

The *Hepadnaviridae* family comprises small (3.0-3.4 kb), enveloped viruses with a partially double-stranded DNA genome [8,11]. This family is divided into 5 genera, with *Orthohepadnavirus* being of relevance, as it includes hepatitis B virus (HBV) and domestic cat hepadnavirus (DCHV). The latter was discovered in Australia in 2018 [1] and subsequently classified as a new hepadnavirus species, *Orthohepadnavirus felisdomestici* [11,19]. Following its initial description, DCHV has been identified in domestic cats from multiple regions worldwide [6,12,13].

It has been suggested that DCHV probably has similar transmission pathways as HBV in humans, highlighting direct contact with blood and body fluids, sexual transmission, and vertical (maternal-fetal) [3,9,21]. In addition, coinfection with feline immunodeficiency virus (FIV) and feline leukemia virus (FeLV) may act as aggravating factors in DCHV infection, analogous to what is observed in HBV coinfection with human immunodeficiency virus (HIV) [1,2].

Despite the increasing DCHV detection in different countries, a limited number of studies have been performed in Brazil to date [13,22]. Moreover, the heterogeneity of reported prevalence rates highlights the importance of region-specific investigations based on representative sampling. In this context, the objective of the present study was to analyze the presence of DCHV in domestic cats from Porto Alegre and its metropolitan region, contributing to a better understanding of the circulation of this virus in feline populations in southern Brazil.

MATERIALS AND METHODS

Sampling population and ethical statements

A total of 451 serum samples from live cats were obtained from veterinary clinics and diagnostic laboratories in Porto Alegre and its metropolitan region. In addition, 77 liver samples from necropsied cats were included in the study, originating from the Veterinary Pathology Sector of the Federal University of Rio Grande do Sul (UFRGS). The demographic and clinical profile of the sampled cats were assessed reviewing the prontuaries of the animals and collecting age, sex, breed and FIV/FelV status[1]. All samples were stored at -80°C for subsequent analyses.

DNA isolation and PCR

DNA isolation was performed using the DNeasy Blood & Tissue Kit[2], initially processing 100 µL of serum samples and 180 µL of homogenized tissue samples, in accordance with the manufacturer's instructions. Two PCR assays were employed for the detection of DCHV, both aiming to amplify the polymerase (P) gene. The 1st assay targeted the amplification of a 256-base pair (bp) fragment using the primers FeHep.2116F (3′-GCACCTGGATTCGCACAC-5′) and FeHep.ModR (3′-TCCTTGAKRGAGTAAAGCC-5′) [15]. The 2nd assays aimed to amplify a 488-bp using the primers HBV Fel F (3′-AGACTSGYGGTGGACTTCTC-5′) and HBV Fel R (3′-ATAAGCMAACACCATACAATG-5′), which were specifically designed for this study. This second assay were designed aiming to amplify both DCHV genotypes A and B. The primers were constructed using Geneious software version 9.0.5 using complete DCHV complete sequences representative of genotypes A and B available in GenBank dataset.

For both PCR protocols, PCR cycling conditions consisted of an initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 40 s, with a final extension at 72°C for 7 min. Amplification products were visualized by electrophoresis on 1.5% agarose gels stained with ethidium bromide and examined under ultraviolet light.

A synthetic DNA positive control[3] was constructed from the genomic region corresponding to that amplified by the PCR protocols employed and was used as a positive control in both DCHV PCR reactions [22]. Moreover, the detection limit of both PCR assays was evaluated analyzing the positive control in 10-fold dilutions.

To assess the integrity of the extracted DNA, an additional PCR assay targeting a fragment of the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene was performed according previously described [14]. Moreover, the quality and quantity of the DNA were assessed through spectrophotometry and fluorometry performed with NanoDrop™[3].

RESULTS

General epidemiological data

The present study comprised the analysis of 528 samples from domestic cats from Porto Alegre and

its metropolitan region, with each sample corresponding to a different animal. Age information was available for 323 of 528 cats (61.2%), ranging from 2 months to 20 years, with a mean age of 6 years. Of the total number of cats evaluated, 262/528 (49.6%) were male and 266/528 (50.4%) were female. Regarding breed distribution, mixed-breed cats were 518/528 (98.1%), followed by Persian cats (4/528; 0.8%), Siamese cats (3/528; 0.6%), and American Shorthair, Bengal, and British Shorthair cats, each representing 1/528 (0.2%).

Information on FIV and FeLV status was available for 381 of 528 cats (72.2%). Diagnosis was performed using a commercial immunochromatographic test. Among these animals, 15/381 (3.9%) tested positive for both FIV and FeLV, 22/381 (5.8%) were positive for FIV only, 131/381 (34.4%) were positive for FeLV only, and 213/381 (55.9%) tested negative for both viruses. No information was available regarding the animal's lifestyle (indoor or outdoor). Detailed information on age, sex, FIV and FeLV status, and sample type (tissue or serum) is provided in Table 1 and Table 2.

PCR

In the present study, sera and liver of 528 cats were evaluated using 2 DCHV distinct PCR protocols. Both assays were evaluated for their detection limit, and both presented a detection limit of 10 DNA copies per reaction.

Among all 528 animals, none tested positive for DCHV using either of the PCR assays employed. Amplification of the GAPDH gene was successful in all samples, confirming DNA integrity into the samples.

Table 1. Information about the liver tissue samples analyzed in the present study. FIV and FeLV status are presented as positive / negative / not determined (n). Animals classified as "FIV and FeLV" in the original dataset are included as positive for both viruses.

Age group	n	Breed	FIV status	FeLV status
< 6m	1	Mixed breed (n=1)	0 / 1 / 0	1 / 0 / 0
6m-1y	5	Mixed breed (n=5)	0 / 5 / 0	0 / 5 / 0
1-3y	13	Mixed breed (n=12); British Short Hair (n=1)	2 / 11 / 0	4 / 9 / 0
4-7y	27	Mixed breed (n=27)	4 / 20 / 3	12 / 12 / 3
8-10y	12	Mixed breed (n=12)	0 / 10 / 2	2 / 8 / 2
11-14y	7	Mixed breed (n=6); Persian (n=1)	0 / 7 / 0	3 / 4 / 0
15y	3	Mixed breed (n=3)	0 / 2 / 1	0 / 2 / 1
>15y	5	Mixed breed (n=5)	1 / 3 / 1	1 / 3 / 1
ND	4	Mixed breed (n=4)	0 / 3 / 1	3 / 0 / 1

FIV and FeLV status are presented as positive / negative / not determined (n). Animals classified as "FIV and FeLV" in the original dataset are included as positive for both viruses. [m: months; y: years; M: male; F: female; ND: not determined].

Table 2. Information about the serum samples analyzed in the present study. FIV and FeLV status are presented as positive / negative / not determined (n). Animals classified as "FIV and FeLV" in the original dataset are included as positive for both viruses.

Age group	n	Breed	FIV status	FeLV status
<6m	19	Mixed breed (n=19)	1 / 18 / 0	7 / 12 / 0
6m–1y	40	Mixed breed (n=39); Siamese (n=1)	0 / 35 / 5	11 / 24 / 5
1–3y	48	Mixed breed (n=47); Persian (n=1)	5 / 39 / 4	15 / 29 / 4
4–7y	65	Mixed breed (n=63); American Short Hair (n=1); Persian (n=1)	11 / 48 / 6	17 / 42 / 6
8–10y	34	Mixed breed (n=31); Siamese (n=2); Persian (n=1)	7 / 25 / 2	8 / 24 / 2
11–14y	31	Mixed breed (n=29); Bengal (n=1); Persian (n=1)	6 / 17 / 8	2 / 21 / 8
15y	5	Mixed breed (n=5)	0 / 4 / 1	1 / 3 / 1
>15y	9	Mixed breed (n=9)	0 / 7 / 2	2 / 5 / 2
ND	200	Mixed breed (n=200)	0 / 89 / 111	57 / 32 / 111

FIV and FeLV status are presented as positive / negative / not determined (n). Animals classified as "FIV and FeLV" in the original dataset are included as positive for both viruses. [m: months; y: years; M: male; F: female; ND: not determined].

DISCUSSION

DCHV was initially detected in Australia [1]. After, it has since been identified in several other countries, with prevalence varying across regions [5,6,10,13,15]. Aiming to detect this pathogenic virus in our region, clinical samples (serum and liver tissues) from a total of 528 domestic cats were analyzed with 2 different PCR assays. Interestingly, there were not any positive results, suggesting the absence or a very low frequency of DCHV in the here evaluated geographic region. Previous studies have already reported a low frequency of DCHV in some regions [5,13,22]. Importantly, 1 of the PCR assays was designed and validated to be used in this study in order to detected both DCHV genotypes A and B, since only genotype B was reported in Brazil to date [13,22]. However, no positive samples were obtained in the present study. Taken together, findings reported here and previous reports in closer cities [22] suggest a very low DCHV prevalence in South Brazil. These results suggest that DCHV circulation may be heterogeneous across different regions and that, in certain epidemiological contexts, the virus may exhibit low prevalence or limited detectability.

In contrast, higher prevalences of DCHV have been reported in specific regions, including Hong Kong [2,3], Thailand [17], and Malaysia [2], suggesting that regional, population-related, or epidemiological factors may favor more intense viral circulation in these locations. This pattern highlights a marked geographic heterogeneity in the distribution of DCHV, reinforcing the complexity of its transmission dynamics across different epidemiological settings [2,4,10,13,15,16].

This scenario underscores the importance of continuous monitoring of potentially hepatotropic viruses in feline populations, particularly considering the genetic and biological similarities between DCHV and HBV [21]. Expanded surveillance and further investigations are essential to determine whether the currently low detection frequency reflects truly limited viral circulation or arises from sampling and methodological constraints. As additional studies are conducted and diverse populations are examined, a more accurate assessment of the global distribution and epidemiological impact of DCHV will become possible.

Considering the hepatotropic potential of DCHV and its genetic and biological similarities to HBV, the data presented underscore the importance of continuous and systematic monitoring of this agent in domestic cats. The expansion of studies employing diverse study designs, populations, and diagnostic strategies will be essential to determine whether the low frequency observed reflects truly limited viral circulation or results from methodological constraints. In this context, the present work contributes to the understanding of DCHV epidemiology in southern Brazil and highlights the need for ongoing surveillance of emerging viruses with potential impact on feline health.

CONCLUSION

The present study investigated the presence of DCHV in a large sampling of cats from Porto Alegre and its metropolitan region, using sensitive molecular approaches and distinct PCR protocols. Despite the analysis of 528 samples, including serum and liver tissues, no evidence of DCHV infection was detected in any of the animals evaluated, suggesting a low frequency of viral circulation in this population. These findings are consistent with previous studies that also reported low DCHV detection rates in certain regions, reinforcing the hypothesis that DCHV circulation is geographically heterogeneous. In contrast to areas where higher prevalences have been described, the results obtained here indicate that regional, epidemiological, and possibly methodological factors may influence DCHV detection in feline populations.

MANUFACTURERS

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Ethical approval. The project was registered and approved with the Ethics Committee on Animal Use (CEUA) of the Universidade Federal do Rio Grande do Sul (UFRGS) under the protocol number no. 46127.

Declaration of interest. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of paper.

Artificial intelligence statement. The authors declare that no artificial intelligence (AI) tools were used to generate, analyze, or interpret the data or to produce the scientific content of this manuscript. The authors are solely responsible for all aspects of the work.

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