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BRIEF REPORT

Viral diagnostic criteria for *Feline immunodeficiency virus* and *Feline leukemia virus* infections in domestic cats from Buenos Aires, Argentina

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KEYWORDS
Feline immunodeficiency virus;
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Abstract A cross-sectional study was carried out on cats attending the Small Animal Hospital at the Faculty of Veterinary Sciences of the University of Buenos Aires to assess the prevalence and associated risk factors of *Feline immunodeficiency virus* (FIV) and *Feline leukemia virus* (FeLV) in the city of Buenos Aires, Argentina. Blood samples from 255 cats with symptoms compatible with FIV or FeLV infection, collected between 2009 and 2013 were analyzed by serology (immunochromatography, IA) and by hemi-nested PCR (n-PCR). The IA and n-PCR assays showed similar percentages of positivity for FIV while the n-PCR test was more sensitive for FeLV. Differences between the diagnostic tests and their choice according to the age of the animal are discussed. The clinical histories of ninety of the 255 cats showed blood profiles similar to others previously reported and revealed a higher risk of infection in male adult cats with outdoor access.

PALABRAS CLAVE
Virus de la inmunodeficiencia felina;
Virus de la leucemia felina;
Inmunocromatografía;
PCR

Criterios diagnósticos para la infección por el virus de la inmunodeficiencia felina y el virus de la leucemia felina en gatos domésticos de Buenos Aires, Argentina

Resumen Para determinar la prevalencia en la ciudad de Buenos Aires del virus de la inmunodeficiencia felina (FIV) y del virus de la leucemia felina (FeLV), y analizar los factores de riesgo que pudieran estar asociados a ellos, se realizó un estudio transversal en gatos atendidos en el Hospital de Pequeños Animales de la Facultad de Ciencias Veterinarias de la Universidad de Buenos Aires. Se analizaron por serología (inmunocromatografía [IA]) y por hemi-*nested* PCR (n-PCR) 255 muestras de sangre de gatos con síntomas compatibles con infección por FIV o FeLV.

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La IA y la n-PCR revelaron porcentajes similares de animales positivos para FIV, mientras que para FeLV el diagnóstico por n-PCR resultó más sensible. Se discuten las diferencias halladas entre los métodos diagnósticos y su elección según la edad del animal. Las historias clínicas de 90 de los 255 gatos mostraron perfiles sanguíneos similares a otros ya reportados y revelaron el mayor riesgo de infección con ambos virus en machos adultos con acceso al exterior.

Feline immunodeficiency virus (FIV) and *Feline leukemia virus* (FeLV) are retroviruses that can infect both domestic and wild cats[3,7]. FIV, which is a lentivirus, produces a progressive deterioration of the immune system of the animal leading to prominent secondary infections, in a very similar fashion to the *Human immunodeficiency virus* type 1. FeLV, which is a *gammaretrovirus*, is associated with proliferative, degenerative and oncogenic diseases in erythroid, myeloid and lymphoid cell lineages. Diagnosis of these infections can be performed by different methods. Detection of antibodies against structural proteins is the choice for FIV diagnosis using immunochromatography-based kits. The same kits are designed to detect FeLV antigens[8]. Alternatively, polymerase chain reaction (PCR) tests targeting conserved sequences such as those present within the *pol* region can also be used[2,6].

Reports of the prevalence of these agents around the world are numerous. However, in Latin America information is scarce. There have been few reports on the FIV situation in Argentina, where specific antigens and antibodies have been detected in cats since 1994[13]. In order to investigate the prevalence of these infections in domestic cats in the city of Buenos Aires we tested a total of 255 cats with clinical symptoms compatible with FIV or FeLV infection in the period 2009–2013. These animals were treated in the Small Animal Hospital at the Faculty of Veterinary Sciences (University of Buenos Aires) and they resided in the city of Buenos Aires and their surrounding areas. Testing, which is ordered on a regular basis by clinicians in suspected cases, was performed with a commercial immunochromatographic assay (IA) and by a nested PCR (n-PCR). These tests were performed at the Virology Department of the same faculty. Only samples from adult cats (>2 years old) were included in this study. Cats under two years old were not tested due to the possible interference of maternal antibodies. Clinical and laboratory data were also examined and compared between infected and uninfected cats as well as with data from other countries.

Blood samples (2 ml) were collected in tubes containing 1% EDTA and processed during the following 12 h. They were divided into two aliquots; one was centrifuged at 2000 rpm for 15 min and the collected serum was tested for antibodies against FIV (gp40) and antigens for FeLV (p27 group specific) with a commercial immunochromatographic assay (Speed DUO FeLV-FIV, BIO VETO Test®, BVT Virbac). The other aliquot was centrifuged with 1 ml of Histopaque®-1077 (Sigma-Aldrich), at 400 × *g* for 15 min. The opaque interface containing the mononuclear cell fraction was carefully aspirated. Finally, proviral DNA extraction was performed with High Pure Viral Nucleic Acid Kit® (Roche).

A n-PCR was performed for FeLV following a previously described procedure that amplifies a 166 base-pair (bp) fragment from the *ul3* gene[9]. For FIV, a hemi-nested PCR protocol that amplifies a 338 bp fragment from p24 of the *gag* gene was adapted from previous studies[5].

Both PCR were carried out in a total volume of 50 μl, containing 0.2–1 μg of genomic DNA, 1.5 mM $MgCl_2$, 1 mM each of the four deoxynucleotide triphosphates, 50 pmol of each primer for FIV (for both rounds) and 3.5 pmol and 15 pmol for FeLV (first and second round, respectively), 1 U of GoTaq polymerase and 5 μl of 10X GoTaq buffer (Promega).

Negative and positive controls (DNA from infected cats) were included in each assay. Cycling conditions for both rounds of the FIV hemi-nested PCR were: one cycle at 94 °C for 2 min, 35 cycles at 94 °C for 30 s, 52 °C for 30 s and elongation at 72 °C for 30 s, and finally, a 10-min elongation stage at 72 °C. For FeLV a previously described n-PCR method proposed by Hofmann-Lehmann et al.[9] was followed. Primers for both amplifications are detailed in Table 1. Complete clinical histories were only available for 90 cats. The group analyzed was composed by female and male cats and was divided into two categories: juvenile cats (two to five years old) and elderly cats (more than five years old). To determine the association between positivity and the clinical variables an χ^2 test and a two-tailed Fisher corrected test were performed using the Epi Info™ 7 (7.1.5) software. The odds ratio (OR) was calculated using the same program. The level of significance was $p >$ or $=0.05$.

Results indicated that of the total of 255 samples, the overall prevalence by IA was 21.45% (55/255) for FIV and 7.69% (14/255) for FeLV, while 20.34% (52/255) for FIV and 11.82% (30/255) for FeLV were observed by n-PCR (Fig. 1A). Only 3 samples of the 255 (0.9%) were positive for both viruses by n-PCR. Age distribution of the infected animals showed that cats between eight and eleven years old had the highest prevalence (Fig. 1B).

The clinical histories provided information about the clinical signs, laboratory diagnosis and anamnesis data at the moment of the veterinary consultation. From this information, it was found that 78.6% of FIV or FeLV positive cats were male while only 21.4% were female. Sixty two percent were castrated while 70% had access to the outside environment or lived with other cats. In relation to the clinical signs, 42.9% had oral lesions (ulcers or gingivitis), 41% experienced weight loss and 38.1% had skin infections. Blood cell counts and protein values data showed that 36% of the positive animals had an alteration in the ratio of total proteins versus albumin and 66% showed anemia or polymorphonuclear cell abnormalities (Table 2).

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