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# High prevalence and risk factors of feline leukemia virus infection in Chilean urban cats (*Felis catus*).

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ABSTRACT

Feline Leukemia Virus is a retrovirus causing fatal disease in domestic cats. While FeLV has been controlled in many countries, it remains a major concern in Latin American countries. This study conducted an epidemiological survey of FeLV in 182 Chilean domestic cats using PCR to detect provirus infection. The results were analysed using Multivariate Logistic Regression to examine risk factors associated with FeLV detection. The FeLV prevalence was 54.95 %, and statistically significant associations ($p < 0.05$) were found for two protective factors and one risk factor. Cats from Concepcion city (95 %CI 0.08–0.56 %) and cats sampled in 2022 (95 %CI 0.1–0.06 %) had lower odds ratios for provirus positivity, whereas non-vaccinated cats (95 %CI 2.3–15.8 %) had an increased odds ratio. No other factors were statistically significant. The high FeLV prevalence is similar to other Latin American countries and the geographical differences highlighted in this study likely correspond to the socioeconomic status of the owners. This study highlights the need for improved FeLV control measures such as promoting FeLV vaccination, implementing health screening prior to adoption of new cats, and educating owners about FeLV to control its circulation.

## 1. Introduction

Feline Leukemia Virus (FeLV) is a gamma-retrovirus with a worldwide distribution among domestic cat populations. The main clinical signs are related to neoplasia, aplastic anaemia, bone marrow suppression, lymphoma, leukemia and ultimately death (Little et al., 2020). The virus is excreted in bodily fluids and can be horizontally transmitted during close contact (grooming, food and water dishes, blood contact, milk, during fights) (Tandon et al., 2005). It also can be transmitted trans-placentally, but less frequently (Hardy et al., 1976). The wide distribution and the high mortality rate mean that FeLV is one of the most common and important infectious threats to domestic and non-domestic felids (Hartmann, 2012).

Cats are routinely tested for FeLV. This is carried out using point-of-care tests to detect free viral p27 antigen in blood, determining antigenemia in viraemic cats (Levy et al., 2006). The test has the advantage that is easy and quick to use in daily clinical practice (Little et al., 2020). Although this test has shown high sensitivity and specificity, under certain circumstances these can produce false-negative results, therefore PCR is suggested to confirm results (Westman et al., 2019a, 2019b, Beall et al., 2021).

There are three main courses of infection outcomes produced by FeLV, led by host immune and viral factors, and these outcomes can cause discrepancies between the tests (Hofmann-Lehmann and Hartmann, 2020). The most severe outcome is progressive infection. The cat does not develop an efficient and early immune response, being persistently infected leading to positive results for DNA (provirus) and antigen tests (p27) (Torres et al., 2005). In a regressive infection the cat's immune response is efficient enough to control the viral replication. In consequence, the antigen test will be negative 1 to 6 weeks later. However, the provirus is integrated into the host genome and it will remain positive to PCR and the infection can be reactivated under stress situations (Tandon et al., 2005; Hartmann and Hofmann-Lehmann, 2020). The most benign infection course is abortive infection. A competent immune system eliminates the virus before proviral insertion, in consequence, there are no antibodies detectable, nor DNA or RNA in peripheral blood. The cat will be negative to all type of test (Hartmann and Hofmann-Lehmann, 2020).

FeLV prevalence has a wide range of levels, based on factors such as: country, cultural issues, veterinary care availability, characteristics of the cat population, and geographic area. In general, developed countries have lower prevalence rates than developing countries (Gleich et al.,

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2009; Burling et al., 2017; Studer et al., 2019; Ortega et al., 2020). During the last decades, developed countries have reported significant decreases in rates of FeLV infection. This has been mainly attributed to efforts in cat vaccination, continuous testing in vet clinics, and improved measures to reduce virus circulation in cat shelters such as: testing prior to animal adoption, and elimination or isolation of progressively infected cats (Little et al., 2009; Hofmann-Lehmann and Hartmann, 2020; Westman et al., 2019b).

For individual cats the main risk factors for FeLV have been associated with outdoor access, male sex and younger cats (Hardy et al., 1976; Little et al., 2009; Gleich et al., 2009). However, there are more factors involved in FeLV prevalence including: populations of stray cats, multicat household, shelters without hygiene measures, irresponsible breeders (Garigliany et al., 2016; Studer et al., 2019; Westman et al., 2019b; Muz et al., 2021; Rungsuriyawiboon et al., 2022).

In a wider perspective, human social factors heavily affect FeLV prevalence. European and North-American countries have more awareness about the disease, and there is a greater emphasis on animal welfare compared with other zones of the world (De Boo and Knight, 2005). However, this also influences decisions that could increase the FeLV risk, for instance, leaving cats unneutered and protecting stray cat colonies (Spada et al., 2012; Studer et al., 2019). On the other hand, from an economic perspective, meta-analysis of FeLV prevalences, at the country level, has demonstrated a relationship between national gross domestic product (GDP) per country, through purchasing power parity (PPP), and FeLV prevalence (Ludwick and Clymer, 2019; Studer et al., 2019). This has been explained by resources being available to finance shelters, measures to control feral cats, programs for sterilization, and veterinary care by owners being affordable and available (Ludwick and Clymer, 2019).

Multiple reports have described FeLV prevalence using different diagnostic methods. Some studies have performed comparisons between antigen and PCR methods. These studies have demonstrated that prevalence can vary considerable according to the method used. This is mainly explained by broader detection by PCR and hence higher prevalence with this method (Szilasi et al., 2020; Muz et al., 2021). In consequence, provirus detection is likely more adequate for examining virus exposure (Little et al., 2020; Hartmann and Hofmann-Lehmann, 2020).

The prevalences described for detection of provirus (PCR based) in South-America have demonstrated high variability: In Brazil between 3 % to 47.2 % (Coelho et al., 2008; Lacerda et al., 2017); 11.82 % in Argentina (Galdo Novo et al., 2016); 56 % in Montevideo, Uruguay (Acevedo et al., 2020); 35 % in areas of Guayaquil, Ecuador (Santana et al., 2022); and between 23 % to 59.4 % in Colombia (Tique et al., 2009; Ortega et al., 2020).

Although there is information about FeLV prevalence in other countries there are only a few studies of FeLV prevalence in Chile. Chile extends over 4000 km long, and 350 km wide (in the widest zone). Seventy-eight-point 9 % of people live in the central area and 87.9 % live in urban areas. Regarding the cat population, there are estimated to be around 4 million owned cats, with approximately 86 % of them living in urban zones (INE, 2018). In domestic cat populations, FeLV prevalences between 3 and 33 % were previously described (Bilbao, 2008; Mora et al., 2015; Tabilo, 2018; Sacristán et al., 2021). However, two of these studies were conducted on a small group of animals, around 50 cats (Bilbao, 2008; Tabilo, 2018). Meanwhile, the other two studies were carried out in rural areas, to determine transmission of FeLV between domestic and non-domestic felids (Mora et al., 2015; Sacristán et al., 2021). The aim of this study is to provide evidence about the prevalence of FeLV in Chilean domestic cats in Bio-Bio and Ñuble regions from Chile, and to evaluate risk factors commonly associated with the infection.

## 2. Methods

### 2.1. Sample collection for the prevalence study and molecular analysis

A total of 182 blood samples from domestic cats were collected in EDTA, and kept at −20 °C. The samples were collected over 2 years (2021−2022) from 16 veterinary clinics from 10 communes, located between the Bio-Bio and Nuble Region (Chile) (Fig. 1 and Table 1). All procedures and handling were performed by veterinarians during clinical diagnostics under the owner's consent.

Samples were processed in Haiken Laboratory in Concepcion (Chile). Nucleic acid extractions were performed using a commercial kit (Geneaid®) following manufacturer's instructions. PCR diagnosis specific for exogenous FeLV was performed to detect provirus using 3′LTR primers, Forward: 5′-CTACCCCAAAATTTAGCCAGCTACT-3′ and Reverse 5′ AAGACCCCCGAACTAGGTCTTC-3′), previously described by Cattori et al. (2006).

### 2.2. Data collection for regression analysis

Overarching ethical approval for this study was granted by the University of Nottingham School of Veterinary Medicine and Science Committee for Animal Research and Ethics (CARE) (Number 3682 220,923). Basic data were available for all cats (sex, age, sampling date and commune/location). Additionally, risk factors were retrospectively collected from each veterinary clinic and any personal data was anonymised.

The survey was sent and all owner or cat identifying data was anonymised in compliance with General Data Protection Regulation (GDPR). The survey was completed by each cat's veterinary surgeon and included the following variables: breed, origin (rescued as stray cat or rehomed/purchased/born in-house), test reason (sick or prophylaxis), lifestyle (indoor-outdoor), reproductive status (neutered or intact), multi-household (single cat or multiple cats living in the same house), and FeLV vaccination status. Response were received for 182 cats. The dataset was managed using Excel Microsoft 365 (Version 2308).

### 2.3. Descriptive analysis

Descriptive analyses including prevalence were performed in STATA/SE 18 (StataCorp, 2023). The Graph was created in GraphPad Prism software (GraphPad, San Diego, CA) and geographical visualization was done in Rstudio v4.1.3 (2023.09.1 + 494) (Rstudio Team, 2020) using the "ggmap" (Kahle and Wickham, 2013) and "rnaturalearth" package (Massicotte and South, 2023).

### 2.4. Regression analysis

Protective and risk factors associated with positive PCR results for FeLV were evaluated by univariate analysis. The age variable was categorized for contingency analysis. This was grouped in four categories: Kittens (between 1 month to <6 months), young adult (>7 months to 2 years), adult (>2 years to 6 years) and old (over 6 years old). Univariate frequencies were compared in GraphPad with Fisher's exact test, and the Baptista-Pike method to calculate odds ratio (OR) with 95 % CI (confidence interval) was used. Afterwards, a multivariate logistic regression analysis was performed with variables with *p*-values <0.2.

Variables with the highest *p*-value were manually excluded and included to find the best model. Once the variables were selected, and if these were biologically relevant, these were evaluated for significance. The multivariate model suitability, was assessed using Hosmer-Lemeshow goodness-of-fit test (Petrie and Watson, 2006) and the limit of statistical significance was defined as $p < 0.05$. Multivariate statistical analyses were conducted in Rstudio v4.1.3 using the packages "ResourceSelection" (Lele et al., 2023), "broom" (Robinson, 2014) and "caret"(Kuhn, 2018).

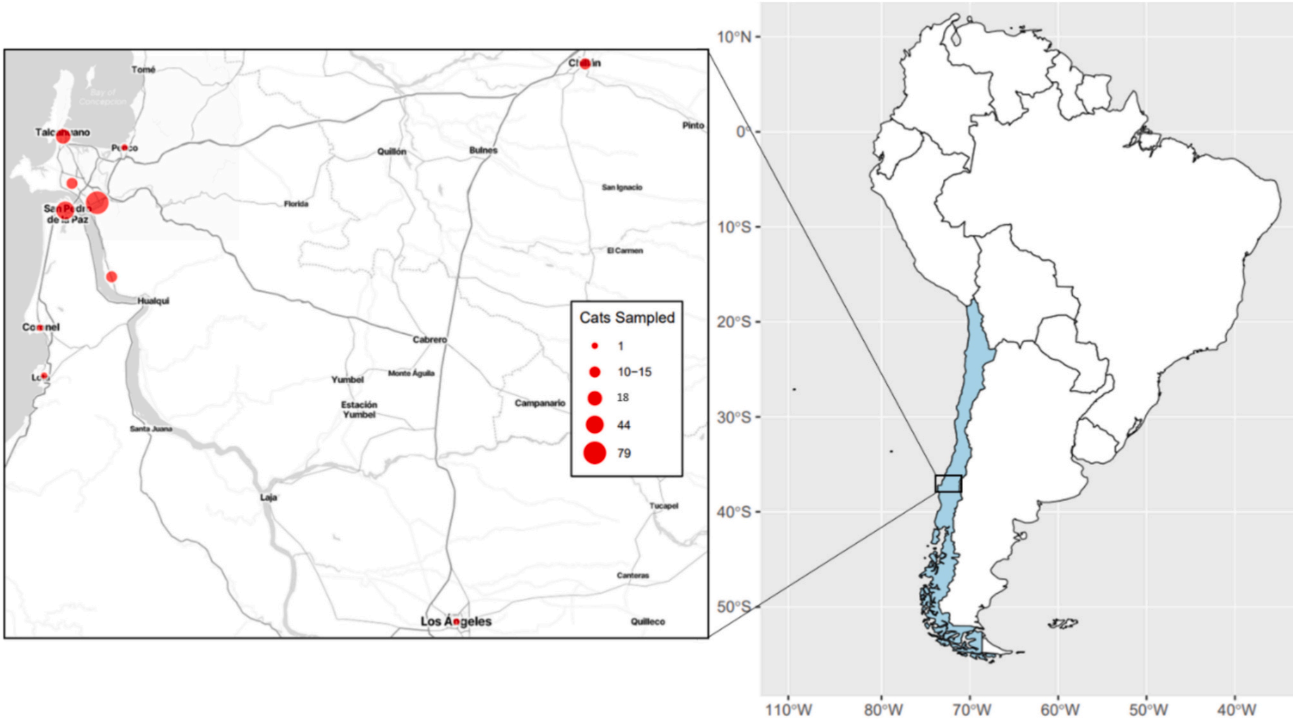


**Fig. 1.** Right panel show map of South America and Chile geographical location (in light blue). The small square shows the area where FeLV provirus presence was assessed. The left panel show the map of sampling sites in Bio Bio and Nuble regions. Red circles sizes are proportional to the number of cats sampled and each circle indicate the sampling area. The sampling sites were, as follow: San Pedro ($n$ = 44), Concepcion ($n$ = 79), Chiguayante ($n$ = 13), Talcahuano ($n$ = 18), Chillan ($n$ = 11), Hualpen ($n$ = 12), Lota (n = 1), Los Angeles (n = 1), Penco (n = 1), and Coronel (n = 1). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

## 3. Results

### 3.1. Description of the population

A total of 182 cats were assessed using PCR, 100 of them were positive for provirus (54.95 %; 95 %CI: 47–62 %). Samples were collected during 2021 (26.37 %) and 2022 (73.63 %) from veterinary facilities from 10 communes, between the Bio-Bio and Nuble regions (Fig. 1). The residence sites for the cats were as follows: San Pedro (24.18 %, $n$ = 44), Concepcion (43.4 %, $n$ = 79), Chiguayante (7.1 %, $n$ = 13), Talcahuano (9.8 %, $n$ = 18), Chillan (6 %, $n$ = 11), Hualpen (6.5 %, $n$ = 12), and other locations (2.7 %, $n$ = 5), which includes one case per location: Lota, Los Angeles, Penco, and Coronel. (Fig. 1).

The age distribution per year is shown in Fig. 2. This category was grouped in 4 modalities for the logistic regression analysis: Kittens (11.54 %) and old cats (10.44 %) were the least represented, while Young Adult (29.67 %) and Adult (48.35 %) were the most represented (Table 1).

Only slight differences were observed between clinically healthy cats (50.55 %), and cats suspected of infection (49.95 %). The cat's origin variable showed that most of cats had been born as stray cats, and they were rescued and resettled in a home (93.9 %). Only a small number were non-stray cats, rehomed from breeders or other houses (6.04 %). There was also a group for which it was not possible to determine where they had been born (11.54 %). Regarding to the cat's sex, female (48.35 %) cats were slightly less represented than male cats (51.65 %). Almost all samples were mixed breed cats (93.96 %), contrasting with a few pure breed cats (6.04 %). The pure breed cats were Abyssinian, Maine coon, Persian, and Siamese. Reproductive status indicates that the majority of cats were neutered (86.81 %), with only a few intact cats (11.54 %) and some data missed (1.65 %). The data about household composition was available for 161 cats, from them, near to half were multiple household cats (57.14 %) and a third were single cats (31.32 %). A similar situation in the access variable was observed. Most of the cats were indoor (69.23 %), and close to a quarter were outdoor (27.47 %), there were 6 (3.3 %) cats for whom it was not possible retrieve this data. The vaccination rate was 20.33 % and not vaccinated were 76.37 % (Table 1).

Cats were tested for FeLV for two main reasons: rehoming or initial health checks for healthy animals or a suspicion of FeLV related disease. The age distribution categorized by the reason for testing is shown in Fig. 3, as follows: 18 (85.7 %) healthy and 3 (14.3 %) suspect kittens; 26 (48.1 %) healthy and 28 (51.9.3 %) suspect young; 34 (38.6 %) healthy and 54 (61.4 %) suspect adults; and 14 (73.7 %) healthy and 5 (26.3 %) suspect old cats were sampled. Old cats were primarily healthy animals being tested for rehoming and likely represent animals with regressive infections.

### 3.2. Risk factors and regression analysis

Each variable was evaluated through univariate analysis and those with $p$-value <0.20 were selected (Table 1). For the multivariate analysis vaccination status, year sampled, and area were considered. All of them showed at least one statistically significant modality (Table 1). Compared to San Pedro, all areas were considered as protective factor, but only Concepcion was statistically significant (OR = 0.22; 95 % CI 0.08 to 0.56). Cats sampled during 2022 were less likely to be infected (OR = 0.25; 95 % CI 0.1–0.6) and vaccination was a protective factor compared with not vaccinated cats (OR = 5.73; 95 % CI 2.3–15.8). The Hosmer-Lemeshow goodness of fit test showed that the model fit the data acceptably ($x^2$ = 4.96, df = 3, $p$-value = 0.1747).

## 4. Discussion

The aim of this study was to determine the FeLV prevalence in a South-Central area of Chile. Although FeLV transmission has been

**Table 1**
Sample characteristics and estimated odds ratios (OR) and *p*-values obtained in univariate analysis and Multivariate logistic Regression of the factors associated to provirus detection ($n = 182$).

| Variable | Modalities | Cat Sampled / FeLV+ | Univariate analysis | | Multivariate Logistic Regression | |
|---|---|---|---|---|---|---|
| | | | *p*-value | OR (95 % CI) | *p*-value | OR (95 % CI) |
| Test Reason | Healthy | 92 (50.55) / 47 (51.09) | Ref | – | – | – |
| | Suspect | 90 (49.95) /53 (58.89) | 0.30 | 1.37 (0.77–2.47) | – | – |
| Origin | House | 11 (6.04) / 5 (45.45) | Ref | – | – | – |
| | Rescued | 150 (82.42) / 84 (56) | 0.54 | 1.52 (0.42–4.63) | – | – |
| | n/a | 21 (11.54) / 11 (54.38) | >0.99 | 1.32 (0.32–6.03) | – | – |
| Sex | Female | 88 (48.35) / 45(51.14) | Ref | – | – | – |
| | Male | 94 (51.65) / 55(58.51) | 0.37 | 1.34 (0.75–2.42) | – | – |
| Reproductive Status | Neutered | 158 (86.81) / 88 (55.70) | Ref | – | – | – |
| | Intact | 21 (11.54) / 9 (42.86) | 0.35 | 0.59 (0.25–1.43) | – | – |
| | n/a | 3 (1.65) / 3 (100) | | | | |
| Age | Kitten | 21 (11.54) / 9 (42.86) | Ref | – | – | – |
| | Young Adult | 54 (29.67) / 29 (53.70) | 0.44 | 1.54 (0.53–4.23) | – | – |
| | Adult | 88 (48.35) / 50 (56.82) | 0.33 | 1.75 (0.67–4.34) | – | – |
| | Old | 19 (10.44) / 12 (63.16) | 0.22 | 2.28 (0.68–7.73) | – | – |
| Breed | Pure | 11 (6.04) / 5 (45.45) | Ref | – | – | – |
| | Mixed | 171 (93.96) / 95 (55.56) | 0.54 | 1.41 (0.55–3.87) | – | – |
| Household environment | Single | 57 (31.32) / 27 (47.37) | Ref | – | – | – |
| | Multi | 104 (57.14) / 57 (54.81) | 0.41 | 1.34 (0.70–2.62) | – | – |
| | n/a | 21 (11.54) / 16 (76.19) | 0.038 | 3.55 (1.12–9.64) | – | – |
| Access | Indoor | 126 (69.23) / 69 (54.76) | Ref | – | – | – |
| | Outdoor | 50 (27.47) / 29 (58) | 0.73 | 1.14 (0.58–2.18) | – | – |
| | n/a | 6 (3.3) / 2 (33.33) | 0.41 | 0.41 (0.07–1.83) | – | – |
| FeLV Vaccination | Yes | 37 (20.33) / 9 (24.32) | Ref | – | – | – |
| | No | 139 (76.37) / 88 (63.31) | <0.000 | 5.36 (2.24–12.75) | <0.00* | 5.73 (2.3–15.8) |
| | n/a | 6 (3.3) / 3 (50) | 0.32 | 3.11 (0.61–14.78) | 0.09 | 5.11 (0.70–37.2) |
| Year Sampled | 2021 | 48 (26.37)/ 37 (77.08) | Ref | – | – | – |
| | 2022 | 134 (73.63)/ 63 (47.01) | <0.000 | 3.791 (1.82–8.34) | 0.002* | 0.25 (0.10–0.60) |
| Area | Concepcion | 79 (43.41) / 31 (39.24) | <0.000 | 0.166 (0.07–0.39) | 0.001* | 0.22 (0.08–0.56) |
| | San Pedro | 44 (24.18) / 35 (79.55) | Ref | – | – | – |
| | Chiguayante | 13 (7.14) / 8 (61.54) | 0.14 | 2.47 (0.81–7.37) | 0.71 | 0.75 (0.16–3.64) |
| | Talcahuano | 18 (9.89) / 11 (61.11) | 0.11 | 2.43 (0.89–6.31) | 0.20 | 0.43 (0.12–1.59) |
| | Hualpen | 12 (6.59) / 7 (58.33) | 0.22 | 2.16 (0.65–6.68) | 0.41 | 0.54 (0.12–2.45) |
| | Chillan | 11 (6.04) / 7 (63.64) | 0.19 | 2.71 (0.74–8.72) | 0.24 | 0.40 (0.09–1.95) |
| | Others | 5 (2.75) / 1 (20) | 0.64 | 0.38 (0.03–2.56) | 0.14 | 0.15 (0.00–1.49) |

n/a: Not Available; Ref: Reference; Numbers between brackets indicate percentage; CI: Confidence interval.

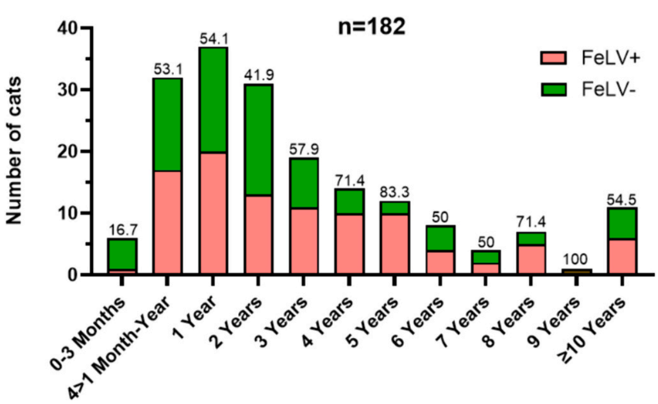


**Fig. 2.** Age distribution per year of cats sampled. In light red are the Feline Leukemia Virus (FeLV) positive and in green are the negative cats. Numbers above bar indicate FeLV positive percentages. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

successfully controlled in several countries, this is still a large problem for domestic cat health in Latin America. Comprehensive studies in North America have shown a prevalence rate of 3.1 % in healthy cats (Levy et al., 2006). In Europe the overall prevalence is low, 3.9 % in sick cats and 1.6 % in healthy cats (Studer et al., 2019). European countries with higher prevalence are Portugal, Hungary and Italy with prevalences between 5.7 and 11.8 % (Studer et al., 2019; Szilasi et al., 2019). In Southeast Asia, low prevalences also have been described (~4 %), except for Thailand with 18.5 % (Capozza et al., 2021). However, FeLV still has higher prevalence in some areas of the world such as, Turkey, 69.7 % of proviral DNA positivity in stray cats (Muz et al., 2021), 76 % in Mexico (Ramírez et al., 2016), 56 % in Uruguay (Acevedo et al., 2020), 59.4 % in Colombia (Ortega et al., 2020). In Brazil, there are studies describing of 3 % and 4.5 % prevalence in some areas of Brazil (Poffo et al., 2017; Lacerda et al., 2017), while there are higher rates such as 47.2 % (Coelho et al., 2008), 22.3 % in Santa Catarina (Cristo et al., 2019) or 31 % in Rio Grande do Sul (da Costa et al., 2017).

This report has demonstrated a much higher prevalence rate (54.95 %) in Chile, than previous reports. Two previous studies in one Chillan city (one of the cities evaluated here) have been conducted. These evaluated a limited number of cats ($n = 50$), and both reports sampled cats from the same university's veterinary clinic, a decade apart. In the first study a 3 % of prevalence using end-point PCR was described (Bilbao, 2008), in the second study a 20 % prevalence was reported using real-time PCR (Tabilo, 2018). In Valdivia, a smaller and southern city in Chile, one study described 14.9 % seroprevalence in 308 cats sampled (Arauna, 2015). However, this cannot be compared directly with our results, as they used an antigen-based test, and higher prevalences are expected when provirus PCR diagnostics are used (Muz et al., 2021; Szilasi et al., 2020; Capozza et al., 2021; Giselbrecht et al., 2023). This study identified two risk factors in a univariate analysis: sick cats and no veterinary checks, but no factors in the multivariate analysis were statistically significant (Arauna, 2015).

Additionally, other two studies in Chile have been conducted to evaluate risk transmission to native non-domestic felids. These sampled domestic cats from rural communities, adjacent to habitats of wild felids. They described a 33 % prevalence in domestic cats from Chiloe

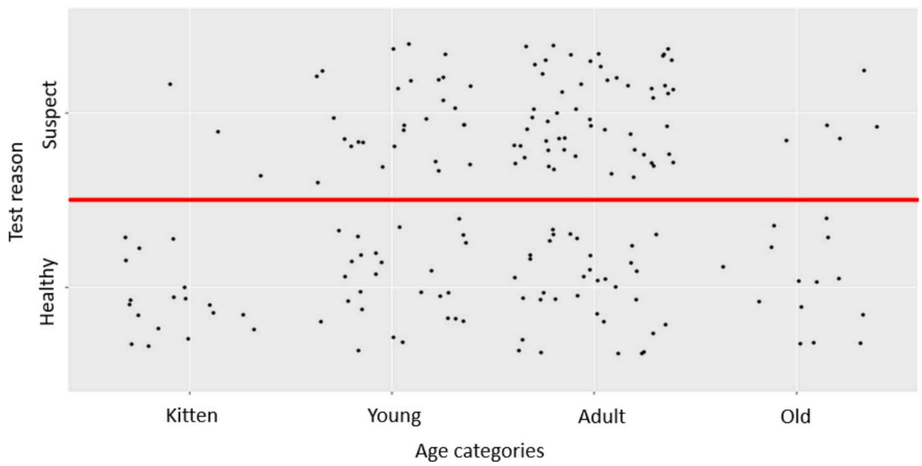


**Fig. 3.** Graph of age distribution compared with test reason. Age categories are sorted by younger from the left to the older in the right. Black dots indicate number of cats sampled and red line divide between healthy from infection suspect cats. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Island (Mora et al., 2015), and a 20.6 % prevalence in a Chilean central area (Sacristán et al., 2021). However, 86 % of Chilean cats live in urban areas and only 14 % live in rural areas (Subdere, 2022). We evaluated one of the most crowded cities in Chile (Concepcion) and transmission dynamics in urban areas with higher cat density areas may change the transmission risk (Spada et al., 2012; Capozza et al., 2021).

Although the recruitment to our study was biased towards cats that veterinarians had a suspicion of FeLV or pre-vaccination test (hence presented for testing), the cat population sampled was consistent with the general population characteristics in the national pet survey (Subdere, 2022). Multivariate analysis showed a statistical association between FeLV PCR positivity with vaccination rate, residence area, and year sampled. Notably in 2021, the Chilean government established movement restrictions for people during the COVID-19 pandemic and this likely influenced a reduction in preventive exams in healthy cats. The geographic location was another statistically significant factor in FeLV infection status. Cats living in Concepcion were less likely to be FeLV infected than cats in the other communes. This commune is wealthier and better pet care has been described than in other communes (Subdere, 2022). FeLV status of the cats is likely associated with better owner socio-economic conditions and better veterinary care, linked to a higher ratio of vaccination and tested cats (Studer et al., 2019; Ludwick and Clymer, 2019).

Vaccination as a protective factor was expected, according to our results, unvaccinated cats are approximately 5 times more likely to be infected by FeLV. These results confirm the efficacy of the current FeLV vaccines protecting against progressive infection (Little et al., 2020; Diesel et al., 2024). The vaccination rate in our results is still low compared with wealthier European countries (48 %–81.5 %) where FeLV circulation has been controlled (Studer et al., 2019). However, it can be considered better than Italy with 17.8 %, Portugal with 14.2 % (Studer et al., 2019) or Thailand with 19.96 % (Rungsuriyawiboon et al., 2022). In South America, the FeLV vaccination rate is rarely described, for instance, in Santa Catarina (Brazil) 40.15 % of cats have some type of vaccine and only 8.39 % of cats were vaccinated against FeLV, in Buenos Aires (Argentina), according to estimations only 12 % of cats have at least one vaccine, and only 1 % are vaccinated against FeLV (Gómez, 2016). In Peru only 7.8 % of cats have received some type of vaccine (Gil et al., 2022).

Although other variables such as origin, reproductive status or breed are commonly mentioned as risk factors in other studies, this was not the case in our multivariate model. We have only a small number of animals in these modalities, and it is possible that with increased numbers these factors might be statistically significant. It is also possible that concept of a fully indoor cat was not understood by the owners. Some indoor cats can occasionally have access to outside and have contact with other cats, but the owners may have declared these as indoor cats (with access to the exterior). We did not identify statistical significance between FeLV infection with multi-cat household status or age (Levy et al., 2006; Garigliany et al., 2016; Burling et al., 2017).

An unexpected result is the similar infection rate observed between older and younger cats. Infections in younger cats typically reduce survival time between 2 and 5 years post-infection in progressively infected animals. Consequently, a lower prevalence is expected in older cats due to early mortality (Spada et al., 2018), although a recent study in progressively infected cats has suggested that older cats could have longer survival times compared to younger cats (Westman et al., 2024).

It is also important to consider that high viral circulation may change the infection dynamics. The infection outcome is determined by host immune status (including previous immunity) and viral factors (as viral strain and loads), which can influence the proportion of infection types (abortive, regressive, progressive) across different populations and countries studied (Englert et al., 2012; Little et al., 2020; Giselbrecht et al., 2023; Diesel et al., 2024). In that respect, our other work on this population (currently in review) has demonstrated distinct geographical clustering of FeLV-A isolates from Chile, though we cannot assess pathogenicity based on sequence data alone (Castillo-Aliaga et al., 2023).

Although, we cannot determine whether our cats were recently infected or they are undergoing a regressive infection, older cats were primarily healthy (66 % were tested as part of preventative medicine examinations), with adult or young cats more likely to be tested for suspicion of disease (only 38.6 % of adult and 48.8 % of young cats were healthy at testing) We speculate that as we are measuring provirus FeLV exposure (whether progressive or regressive) and due to the high viral circulation, the cats are continuously exposed by outdoor access or cohabitation during all life stages, and older cats are likely to have an increased cumulative lifetime risk of exposure (Levy et al., 2006; Little et al., 2020; Ortega et al., 2020). In a prospective study it would be ideal to also assess seroprevalence to gain a better idea of progressive vs regressive infection. However, cost constraints in Chilean primary veterinary practice mean that the in-house lateral flow diagnostic tests commonly used in developed countries are not widely available, hence no data on seroprevalence was accessible for this retrospective study.

The Chilean cat population has some good pet health indicators, for example the neutering rate of cats in this study was 86.81 %, with 92.8 % described in Chile (Salgado-Caxito et al., 2021), this is high when compared with other high FeLV prevalence areas such as: 32–48 % cats neutered in Southeast Asia and Taiwan (Capozza et al., 2021; Rungsuriyawiboon et al., 2022), 63.5 % in Peru (Gil et al., 2022), and 54.38 % in Brazil (Biezus et al., 2019). The Chilean rate of neutered cats is comparable to that in low FeLV prevalence countries where up to 85 % of cats are neutered (Cats Protection, 2023). This may explain why the neutering status or gender were not risk factors for FeLV exposure in this study.

The problem in Chile, similarly to other countries in the region, is that cat owners frequently obtain stray cats without previous examination to incorporate into their house, therefore the other cats in the house are at risk of contracting diseases when new additions are not quarantined, or health checked. To our knowledge, in Chile there are no cat shelters with management and guidelines to control diseases such as there are working in developed countries. Although some shelters run by small animal welfare associations or a small number of city councils exist in Chile (Subdere, 2021). All shelters in Chile lack resources and it is unlikely they have measures to control diseases, having poor sanitary conditions (Subdere, 2021). In countries where FeLV has been controlled, breeders and shelters play a crucial role in controlling feline retroviruses. In these facilities routine pre-adoption screenings are performed and new cats are kept separated, FeLV immunizations are given as core vaccines, and isolation and euthanasia for progressively infected cats in poor condition is applied (Möstl et al., 2015, Westman, Malik, and Norris 2019, Stone et al., 2020, Little et al., 2020). Chilean cat owners declare that the main route to obtaining a cat is through neighbours or social media, indeed 87.1 % of cats owners in Chile reported that their cats never had offspring (Salgado-Caxito et al., 2021).

In the last decade, government programs for animal welfare and professional efforts have significantly improved the awareness of pet health. However, the prevention of FeLV in Chile are strongly veterinary practitioner and pet owner resources dependent, resulting in a limited number of animals being tested in veterinary hospitals. Further analysis, such as antigen testing or viral quantification, may help to elucidate the proportion of each infection type.

Future research should include strain typing, serology and PCR assessment, infection type classification, and prospective analysis with higher number of animals, to evaluate survival time in countries like Chile with higher FeLV prevalence rates This knowledge would help to implement strategies for controlling roaming cat populations and improving domestic cat welfare.

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## CRediT authorship contribution statement

**Cristobal Castillo-Aliaga:** Writing – original draft, Visualization, Software, Methodology, Formal analysis, Data curation, Conceptualization. **Susana Castro-Seriche:** Methodology, Investigation, Data curation. **Alonso Jerez-Morales:** Resources, Methodology, Funding acquisition, Data curation. **Rachael Tarlinton:** Writing – review & editing, Supervision, Software, Methodology, Formal analysis, Conceptualization.

## Declaration of competing interest

The authors declare that there are no conflicts of interest.

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