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PEQUEÑAS ESPECIES**

Tema: **Prevalence of some viral infections in feral cats from San Cristobal Island,
Galapagos, Ecuador**

*Protocolo del trabajo de investigación, previo la obtención del título de
Magister en Medicina Veterinaria, Mención Clínica y Cirugía de Pequeñas
Especies.*

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“ **Prevalence of some viral infections in feral cats from San Cristobal Island, Galapagos, Ecuador**” es el tema del proyecto de investigación de mi AUTORÍA, previo a la obtención del Título de Cuarto Nivel o Posgrado correspondiente a Magister en Medicina Veterinaria, Mención Clínica y Cirugía de Pequeñas Especies, por lo que, asumo su originalidad y el uso de fuentes de terceros registradas según las normas APA vigentes.

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DEDICATORIA

A mi hijo Joaquín, quien ha sido mi fuente de inspiración para lograr todos los proyectos de mi vida.

Priscilla Espin Vargas

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Content

Presentation	i
Certificación de los Asesores	ii
Certificación de Autoria	iii
Dedicatoria	iv
Agradecimiento	v
INTRODUCTION	1
ABSTRACT	3
Chapter I	4
MATERIALS AND METHODS	4
1.1 Study area	4
1.2 Feral cat colony selection	4
1.3 Capture	4
1.4 Identification, sampling and release	5
1.5 Analysis and serological test	5
1.6 Calculation of prevalence	5
1.7 Statistical analysis of the results	5
Chapter II	6
RESULTS AND DISCUSSION	6
2.1 Most common viral infections	6
2.2 Prevalence according to gender and sampling area	8
2.3 Prevalence according to age	10
5. CONCLUSIONS	12
6. ETHICS	12
7. REFERENCES	12

INTRODUCTION

Cats have been associated with the development of zoonotic conditions in humans, including fungal infections, as well as conditions caused by bacteria, dermatophytes, helminths, fleas, mites, mycobacteria, ticks, unicellular parasites, and viruses, including cowpox and leishmaniasis, which are diseases recently reported as transmissible by felines (Peterson and Barnes, 2020; Vioti et al., 2021).

Yamaguchi et al. (1996) state that, as companion animals, cats have the possibility of infecting humans with *Toxocara cati* and *Toxascaris leonina* and, more seriously, with *Toxoplasma gondii*, which causes toxoplasmosis and also affects cattle; rota-viruses, including feline rotavirus, have been isolated from numerous mammals and cause acute diarrhea in humans, cattle, sheep, and pigs; while feline panleukopenia virus causes panleukopenia, which is highly infectious not only to Felidae but also to carnivores such as Mustelidae, Procyonidae, and Viverridae.

Toxoplasmosis is one of the most widespread zoonotic diseases, with a significantly higher burden in low-income countries, and cats are a key reservoir host (Torgerson and Mastroiacovo, 2013). Cats can become infected after ingesting *T. gondii* bradyzoites that are found in tissue cysts of infected rodents or birds and harbor the sexual cycle of the parasite and subsequently shed millions of *T. gondii* oocysts in their feces, contaminating the soil or water bodies, which sporulate in the environment and become infectious for intermediate hosts and people (de Wit et al., 2019).

Dokuzeylül et al. (2016) state that feline leukemia virus (FeLV) and feline immunodeficiency virus (FIV) are the main non-traumatic causes of death in adult cats and are associated with immunosuppression that causes feline acquired immunodeficiency syndrome, a major secondary infection; while feline herpesvirus 1 (FHV) and feline calicivirus (FCV) cause feline upper respiratory tract viral disease ("cat flu"), a major veterinary problem.

Knowledge about the presence of infectious diseases in wildlife is crucial to understand the potential consequences that these can have on the conservation of wild species and assess the threat they pose to human health (Valenzuela-Sánchez and Medina-Vogel, 2014). Anthropogenic factors, such as the introduction of exotic species, climate change, habitat fragmentation and loss, and human encroachment on natural areas, can increase the risk of disease transmission from wild reservoirs to domestic animals and humans (Daszak et al., 2001).

Although parasites of domestic cats have been extensively studied, little is known about their prevalence in free-roaming cat populations. Studying and evaluating the health of free-ranging populations is important because they are likely to harbor and transmit disease more easily than domestic cats, as they are subject to the stress of wildlife and have more opportunities to interact with other cats and feeding on wild rodents (Luria et al., 2004)

Islands are home to a large proportion of the world's biodiversity and an even greater proportion of the threatened biodiversity (Mittermeier et al., 2004). Through a combination of environmental uniqueness and

isolation, islands have become hotspots of endemism: approximately 15% of the world's vertebrate species and 20% of the world's vascular plants are island endemics (Millennium Ecosystem Assessment, 2005). The high densities of human population, the acceleration of the processes of in-vasion of existing spaces and the creation of new ones, have turned them into critical points of extinction and threat of species. Nearly half of all recorded animal extinc-tions have been species that were endemic to islands (Duncan et al., 2013; Tershy et al. 2015). In this sense,

In the Galapagos Islands, domestic animals that have become feral, such as goats and pigs, severely affect the endemic plant and animal populations of the islands (Stone et al., 1994). Such affectations involve habitat deterioration (Coblentz, 1990), parasitism (Schofield, 1989), competition and predation (Case and Bolger, 1991).

McManus et al. (2014) indicate that the options for the management of feral cats generally fall into four categories: temporary accommodation in short-term traditional animal shelters, awaiting adoption or euthanasia; lifetime housing in long-term sanc-tuaries; foster home care programs and community cat trap, neuter and return pro-grams.

Under the context described above, the objective of this research was to evaluate the prevalence of some viral infections in feral cats from San Cristobal Island, Gala-pagos, Ecuador, as support in the decision-making process in population management.

ABSTRACT

With the purpose of evaluating the prevalence of some viral infections in feral cats from San Cristobal Island, Galapagos, Ecuador, a sampling of 91 animals was carried out in 14 locations in the urban area, and 4 in the rural area. The capture of cats was made with Tomahawk-type traps with baits. The diseases considered for the study were: Feline Leukemia (FeLV), Feline Immunodeficiency (HIV), Feline Calicivirus (FCV), Feline Herpesvirus (FHV) and Feline Panleukopenia (FPLV). Cats older than 4 months were evaluated from blood drawn from the jugular vein. For the detection of FeLV antigens and antibodies against HIV, Anigen Rapid FIV Ab/FeLV Ag tests were used as an immunoassay by blood serum chromatography. The prevalence of each disease was compared between genders, sampling areas, and localities through Chi-square method with 95% confidence. Regression equations were adjusted according to the age of the most common diseases. FCV and FPLV were the most prevalent viral infections, with 83.52% and 64.84%, respectively. 57% of the feral cats had concomitant infections with both viral agents. The presence of HIV or FHV was not detected. There were no statistical differences in relation to gender and capture areas; the trend was towards higher prevalence in males and in urban areas. A prevalence of 45% for FPLV and 80% FCV were obtained in cats under one year of age. The highest prevalence of FPLV occurred in cats aged 4-5 years and for FCV there was a linear increase with age. FCV and FPLV were the most prevalent viral infections, with 83.52% and 64.84%, respectively. 57% of the feral cats had concomitant infections with both viral agents. The presence of HIV or FHV was not detected. There were no statistical differences in relation to gender and capture areas; the trend showed a higher prevalence in males and in urban areas. Prevalences of 45% for FPLV and 80% FCV were obtained in cats under one year of age. The highest prevalence of FPLV occurred in cats aged 4-5 years and for FCV there was a linear increase with age.

Keywords: Panleukopenia; feline viral leukemia; Feline viral immunodeficiency; Feline calicivirus; feline herpes virus.

Chapter I

MATERIALS AND METHODS

1.1 Study Area

The study was carried out in the province of Galapagos, in San Cristobal town, San Cristobal Island. Eighteen points located in the urban area (14 locations) and the rural area (4 locations) of the island were sampled according to Figure 1.



Figure 1. Distribution of feral cat capture sites on San Cristobal Island, Galapagos province, Ecuador.

1.2 Feral Cat Colony Selection

For the calculation of the sample size, the study carried out by Grijalva et al. (2019), which estimates 1.09 cats per hectare on San Cristobal Island was taken as the main reference. The capital of the island has around 738,103 ha, and the rural area has 8,300 ha, then the estimated population of stray cats is around 9,850 cats (Government Council of the Galapagos Special Regime, 2016). Based on this data, the sample size was set at 91 cats using the "Sample Size Calculation" tool of the EpiTools online pro-gram (Sargento, 2018), starting from an estimated prevalence of 50%, a confidence level of 95% and a margin error of 10%.

1.3 Capture

Tomahawk-type traps with baits were set in the areas of greatest cat sightings in the urban and rural areas of the island. Trapping was carried out in rotation between the 4 sampling points in the rural area and 14 in the urban area. The traps were placed throughout the day and checked every hour for three hours. The age of the animals was estimated by their external exploration, size of the animal and by the conformation and structure of the teeth. All animals older than 4 months were included in the study and all individuals younger than this age were discarded to prevent mater-nal antibodies from influencing the results.

1.4 Identification, Sampling and Release

The following viral diseases were considered in the study: Feline Leukemia (FeLV), Feline Immunodeficiency (FIV), Feline Calicivirus (FCV), Feline Herpesvirus (FHV) and Feline Panleukopenia (FPLV). All animals were thoroughly checked for clinical signs, alterations in vital signs, or any type of indication of disease, for that purpose an exploration sheet was designed to register all the variables noted. The animals suitable for the study were taken to the ABG facilities where they were weighed and subsequently anesthetized using the combination of xylazine – ketamine (Ket-A-Xyl®) with a dose of 10 mg*kg⁻¹ intramuscularly (Simon & Steagall, 2020). Subsequently, 3 ml of blood was extracted from the jugular vein with 3 ml syringes and 21 x 1 needles. The animals part of the study was identified with a tattoo on the left ear and were returned to the site where they were capture once recovered from anesthesia.

1.5 Analysis and Serological Test

Anigen Rapid FIV Ab/FeLV Ag tests were used as an immunoassay by chromatography in feline serum, plasma, or whole blood for the detection of vLF antigens and antibodies against vIFV.

The tests were performed with the serum of the samples obtained, and they were frozen at a temperature of -80°C (Electrolux International Freezer, model MUFF21X7HW1, USA) until processed. Subsequently, these were removed from the freezer and placed at room temperature for 15 min; at the same time, the kits that were refrigerated were placed at room temperature for 15 to 30 min for greater effectiveness, continuing the analysis of the sample with the protocol indicated by the manufacturer.

1.6 Calculation of prevalence

Prevalence estimates were calculated for each disease and reported as the percentage of cats with a positive test result according to Equation 1 (Tique et al., 2009).

$$\text{Prevalencia} = \frac{\text{Number of cats testing positive} * 100}{\text{Total population of study}} \quad (\text{Equation 1})$$

1.7 Statistical analysis of the results

The results obtained from the analyzes carried out on the 91 patients were organized in dynamic tables to perform a descriptive statistical analysis. Prevalence comparisons between the sexes of the animals, between rural and urban areas, and between different localities were made through the Chi-square test at a confidence level of 95%. In the case of variations in the prevalence of diseases according to the age of the animals, regression analyzes were performed and the respective explanatory equations were determined. All analyzes were performed with the statistical software SPSS®, version 24 (IBM, 2017).

Chapter II

RESULTS AND DISCUSSION

2.1 Most common viral infections

The most common viral infections on San Cristobal Island were feline calicivirus with a prevalence of 83.52% and feline panleukopenia with a prevalence of 64.84%; while the feline viral leukemia virus was only detected in one animal, showing a prevalence of 1.10%. It is important to note that the presence of animals infected with feline immunodeficiency virus or feline herpesvirus was not detected (Table 1).

Table 1. Prevalence of viral diseases in feral cats from San Cristobal Island, Galapagos, Ecuador.

	feline leukemia	Immunodeficiency	panleukopenia	herpes virus	Calicivirus	Feline leukemia + Panleukopenia + Calicivirus	panleukopenia + Calicivirus
Total cases	1	0	59	0	76	1	52
Prevalence	1.10	0.00	64.84	0.00	83.52	1.10	57.14

According to these results, Dall'Ara et al. (2019) reported seropositive results for FPV, FHV-1, and FCV of 45.7%, 37.1%, and 85.4%, respectively, in cats studied in the city of Milan, Italy. The results confirm previous findings indicating a greater distribution and worldwide seroprevalence of FCV in cats compared to FPV and FHV-1. This is probably due to the ability of FCV to mutate and escape host immune response, easy transmission with oronasal and conjunctival secretions, carrier status of infected cats, social behavior in the populations studied, along with its high resistance in the environment (Afonso et al., 2016; Afonso et al., 2016 Afonso et al., 2017).

Feline viral leukemia (FeLV) is a disease of great importance due to the economic and emotional impact to the owners of domestic cats. The saliva is the main means of transmission, although the causal agent can also be found in blood and other bodily secretions, for which intimate contact with mucous membranes, mutual licking behavior, breastfeeding, sharing dishes and fighting, favor the transmission of the virus (Little et al., 2020).

Ramirez et al. (2016) indicate that FeLV is responsible for causing several pathologies by causing immunosuppression, which predisposes the cat to opportunistic infections, in addition to generating neoplasms and myeloproliferative disorders, which in several cases lead to aggressive behaviors; these characteristics cause highly variable clinical pictures compared to any other agent, which makes its diagnosis difficult, being responsible for several deaths related to the disease. Little et al. (2020) reported that in North America, the prevalence of FeLV varies between 2.3 and 7.5%, in Australia it reaches 2%, while in Europe it is higher, from 3.6 to 15.6%.

Feline panleukopenia virus, or feline parvovirus, is a highly contagious pathogen that can cause severe disease in cats, especially young animals, with a mortality rate of 50–90% (Barrs, 2019). Jacobson et al.

(2019) indicate that FPV transmission occurs primarily through the fecal-oral route, but large amounts of virus are shed in the saliva, urine, feces, and vomit of infected cats; clinical signs may be nonspecific, particularly early in the disease; early acute signs include lethargy, pyrexia, anorexia, and vomiting; while the peracute symptoms includes septic shock and sudden death.

Tuzio (2021) states that the domestic cat is the primary host, but FPV can infect all members of the Felidae worldwide, including tigers, panthers, and leopards; it also infects species in the families Mustelidae, Procyonidae, and Viveridae, which include the raccoon, ringtailed cat, and mink. The virus is associated with disease in the fox and is known to replicate in ferrets without causing disease; in dogs, it replicates in the thymus, spleen, and bone marrow; the virus does not infect humans, although it has been isolated from a monkey (Sykes, 2014).

Barrs (2019) indicates that the most common presentation of the disease is characterized by an acute course of several days with high fever, lethargy, anorexia, vomiting, diarrhea and severe dehydration; in cases of peracute disease, sudden death from septic shock without premonitory signs can occur, especially in kittens younger than 2 months. On the other hand, Url et al. (2003) note that, depending on the stage of pregnancy at which infection occurs, infected queens may miscarry (early pregnancy) or give birth to kittens with eye and central nervous system defects (late pregnancy), including cerebellar hypoplasia, hydrocephalus, hydranencephaly, retinal dysplasia and optic nerve hypoplasia.

For its part, FCV is host-specific for animals of the Felidae family, and no zoonotic potential or changes in host range have been observed (Spiri et al., 2019). Feline calicivirus is a highly contagious pathogen with a wide distribution in the feline population, and can even be found in asymptomatic carriers; cats can be infected with FCV directly from cat to cat, or indirectly via fomites, as infection occurs via the nasal, oral, or conjunctival routes (Berger et al., 2015).

Spiri (2022) points out that FCV-induced disease has various manifestations and can be categorized into ulcerative disease, gingivostomatitis, lameness syndrome, and virulent-systemic disease. Cats are commonly affected by an ulcerative disease of the upper respiratory tract, with clinical signs such as oral ulcerations, gingivitis-stomatitis, lethargy, oral ulcers, gingivostomatitis, hypersalivation and fever; at the field level, coinfections with feline herpes virus (FHV), Chlamydia felis, and Mycoplasma felis are common.

FCV infection is widespread in the general cat population, Kratzer et al. (2020) state that the prevalence is directly proportional to the number of cats in a home, reaching values of up to 32%; while the prevalence within colonies and individual refuges is variable, with values ranging from 50 to 90% (Hofmann-Lehmann et al., 2022). High FCV prevalence within a colony is associated with high FCV strain diversity, related to a small number of persistently infected cats and reinfection of other members of the cat population with a variant of the same FCV strain or with a different strain (Coyne et al., 2007). In relation to the detection of concomitant infections, of the total of 91 animals, it was possible to

determine the confluence of the infectious agents of feline leukemia, panleukopenia and calicivirus in one of the patients evaluated; while 52 of them had concomitant infections of the infectious agents of panleukopenia and calicivirus, for a prevalence of 57.14% (Table 1).

Conde (2016) reported a prevalence of 5.2% of stray cats with the presence of more than one infectious agent and 6.8% with lesions compatible with herpesvirus/calicivirus infection and other agents in the city of Madrid, Spain.

2.2 Prevalence according to gender and sampling area

The statistical comparisons, carried out through the Chi-square test, did not detect differences in the prevalence of feline panleukopenia, feline calicivirus and feline viral leukemia in relation to the sex of the animals evaluated; however, in all cases the tendency to obtain higher values was found in male cats, with prevalences of 39.6, 50.5 and 1.1%, respectively (Table 2).

Similarly, no statistical differences were found when comparing the prevalence of diseases in the two animal capture areas, showing a tendency to obtain higher values in urban areas, where a prevalence of 45.1% was obtained for panleukopenia, 60.4% for calicivirus and 1.1% for feline viral leukemia (Table 2).

Table 2. Prevalence of viral diseases in feral cats according to sex and sampling area in San Cristobal Island, Galapagos, Ecuador.

	Sex			Chi squared	Zone			Chi squared
	Female	Male	Total		Rural	Urban	Total	
Panleukopenia	25.3%	39.6%	64.8%	0.466	19.8%	45.1%	64.8%	0.942
Calicivirus	33.0%	50.5%	83.5%	0.320	23.1%	60.4%	83.5%	0.144
Feline leukemia	0.0%	1.1%	1.1%	0.395	0.0%	1.1%	1.1%	0.503

These results partially agree with those of Dall'Ara et al. (2019) who reported that both females and males showed higher seroprevalences of FCV compared to FPV and FHV-1, and, the percentage of seropositive males was lower than that of females for the three pathogens.

In contrast, Zheng et al. (2021) found that the percentage of positive FCV was higher for males than for females, even though the difference was not statistically significant, which they associated with the aggressive nature of male cats; leading to an increased risk due to bite wounds. Similar results were reported by Kruse et al. (2010).

Regarding to the prevalence of viral diseases in feral cats according to the sampling location on San Cristobal Island, Chi-square comparisons did not detect statistical differences between locations for

panleukopenia and calicivirus diseases. In the case of the urban area, the highest prevalence of panleukopenia was 6.6% and occurred in the locations of the Municipal Market, Fragatas, Peñas Altas, and Playa de Oro; while, for the rural area, the highest prevalence was for the location of El Progreso, followed by La Soledad and La Amancay, with percentages of 9.9, 5.5 and 4.4%, respectively (Table 3).

Table 3. Prevalence of viral diseases in feral cats according to the sampling location in San Cristobal Island, Galapagos, Ecuador.

Location	Urban zone		Location	Rural zone	
	Panleukopenia	Calicivirus		Panleukopenia	Calicivirus
Airport	1.1	4.4	La Amancay	4.4	6.6
Central neighborhood	2.2	3.3	La Soledad	5.5	3.3
Cactus	0.0	1.1	El Progreso	9.9	12.1
Landfill	5.5	5.5	El Mango	0.0	1.1
Estacion	2.2	3.3			
Fragatas	6.6	5.5			
Manzanillo	0.0	0.0			
Market	6.6	6.6			
Palmeras	1.1	1.1			
Peñas Altas	6.6	8.8			
Peñas Bajas	0.0	1.1			
Perimetral	1.1	1.1			
Playa de Oro	6.6	11.0			
Police Station	5.5	7.7			
Total	45.1	60.4	Total	19.8	23.1
Chi squared	0.328ns	0.123ns			

On the other hand, the prevalence of calicivirus in the urban area reached values of 11.0% in Playa de Oro, 8.8% in Peñas Altas, and 7.7% in the Police station; while, in the rural area, the locations with the highest prevalence were El Progreso and La Amancay, with 12.1% and 6.6%, respectively (Table 3). It should be noted that, for both diseases, the highest prevalences corresponded to Playa de Oro and Peñas Altas in the urban area, and to El Progreso and La Amancay in the rural area.

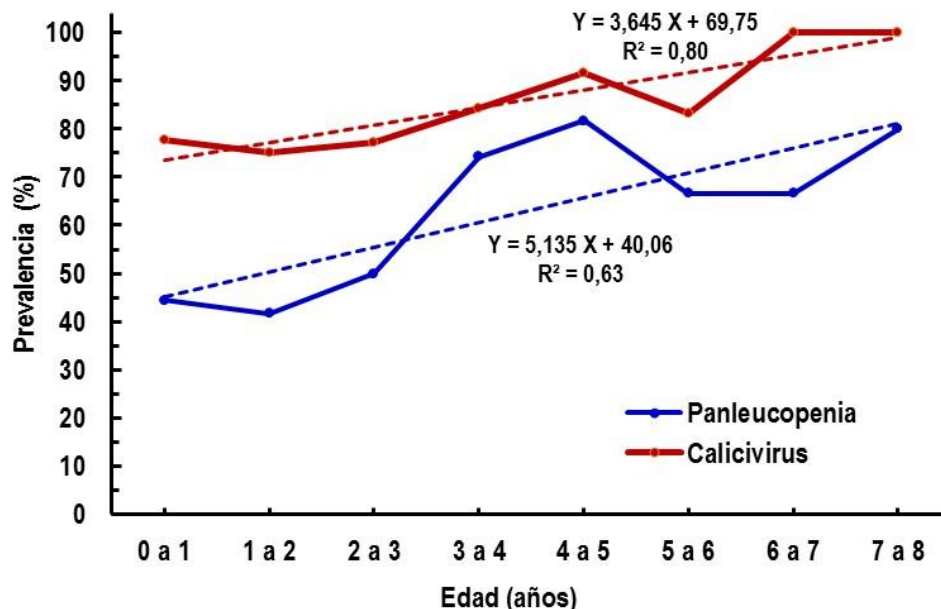
Zheng et al. (2021) indicate that geographic location, sample population, and sample size probably affect feline disease prevalence results, and found lower prevalence than the results of the present investigation, with positive FCV rates of 43.0 % in Hangzhou, China, higher than that detected in 16 Chinese cities (14.2%), but lower the prevalence reported in Beijing (46.3%) and Switzerland (45.0%), presumably associated with carrier status of the virus, as infected cats generally shed FCV more or less continuously. Regarding the prevalence of PVL, Blanco et al. (2009) reported similar results to those obtained in this study, for domestic cats in the metropolitan area of Costa Rica with values of 93%, which agrees with the prevalence reported for captive wild cats in Costa Rica; but differences were found between the number of seropositive animals within the different age categories, but not within the variables of indoor or outdoor cats, households with one or more cats, and vaccinated or unvaccinated cats, suggesting a natural exposure of cats to FPV relatively stable and resistant at early ages in Costa Rica, which could be due to

the high presence of the agent in the feline population.

Dolz et al. (2018) point out that viral diseases of domestic cats have been investigated mainly in urban regions of the world, and particularly in the case of Costa Rica; therefore, evaluations in rural regions where contact between domestic and wild felines is more likely are of great importance, and reported that the prevalence of FeLV and FIV was very low compared to reports by Blanco et al. (2009) for the metropolitan area, which they associated with a relatively low density of cats in the San Luis region.

2.3 Prevalence according to age

Figure 2 shows the prevalence of panleukopenia and calicivirus according to the age of feral cats on San Cristobal Island, in which it is highlighted that a large proportion of the animals are infected at an early age, since the prevalence in cats under one year reached values of approximately 45% for panleukopenia and 80% for calicivirus. The highest prevalence values of panleukopenia were obtained in cats aged 4 to 5 years; while, in the case of calicivirus, the trend shows a linear increase with age of the individual was obtained, which approaches 100% from 6 years old. Pearson's linear regression equation $Y = 5.135 X + 40.06$ yielded a significant fit of 63% for panleukopenia; while, in the case of calicivirus, the adjusted equation was: $Y = 3.645 X + 69.75$, with a regression coefficient of 80%.



Figure

Prevalence of panleukopenia and calicivirus according to age in feral cats from San Cristobal Island, Galapagos, Ecuador.

2.

In agreement with these results, Dall'Ara et al. (2019) found that kittens under one year of age and adult

cats were less likely to be seropositive for FPV compared to cats of intermediate age; while in the case of FCV, older cats showed a higher seroprevalence of FCV compared to other age categories, suggesting that age could influence the probability of encountering these viruses and, therefore, it is more likely that older animals become seropositive with higher antibody titers (Tizard, 2017).

Rehme et al. (2022) identified age as a risk factor for feline panleukopenia, reporting that cats younger than 2 years were 72 times more likely to develop signs of feline panleukopenia than cats older than 2 years, because antibodies derived of the mother fall below the protective titles at an early age.

In contrast, Jenkins et al. (2020) found that the prevalence of protective antibody titers against FPV was 94% in the cats studied, but was not associated with ages older than 6 months or older than 1 year; while Zheng et al. (2021) also found no relation-ship between FCV infection and age.

5. CONCLUSIONS

The most common viral infections on San Cristobal Island were feline calicivirus and feline panleukopenia with a prevalence of 83.52% and 64.84%, respectively; 57% of the feral cats had concomitant infections with both viral agents. Feline immunodeficiency virus and feline herpesvirus were not detected.

No statistical differences were detected regarding the prevalence of viral diseases in relation to sex and the area of capture of the animals, obtaining the trend to higher prevalence in males in the urban area.

Cats under one year of age obtained a prevalence of 45% for panleukopenia and 80% for calicivirus. The highest prevalence of panleukopenia was obtained in cats aged 4 to 5 years; while for calicivirus the linear trend of increase with age was obtained. Linear regression equations yielded adjustments of 63% for panleukopenia and 80% for calicivirus.

6. ETHICS

The study was approved by the jury and direction of the master's degree program in Veterinary Medicine, Clinical Mention and Surgery of Small Species of the Catholic University of Cuenca, verifying that the sampling was carried out in accordance with animal welfare and bioethical protocols. The field work in Galapagos was carried out in coordination with the Galapagos Biosafety Agency ABG.

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- **Informed consent statement:** No apply.
- **Data Availability Statement:** The dataset supporting the conclusions of this study is included in this article.
- **Conflicts of interest:** The authors declare no real or potential conflicts of interest.

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