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Política editorial

La *Revista de la Facultad de Medicina Veterinaria y de Zootecnia* fue creada en 1929 por el doctor Doménico Geovine, decano de la Escuela Nacional de Medicina Veterinaria, hoy Facultad de Medicina Veterinaria y de Zootecnia. En el medio universitario y en el área pecuaria, es la revista del área de mayor antigüedad. Desde su creación su objetivo ha sido ofrecer un medio escrito de expresión para toda la comunidad académica interna y externa, en el cual exponer sus ideas, resultados de investigación, ensayos etc., en relación con el quehacer científico en el área de las Ciencias Animales y otras afines. Su filosofía ha sido tener un carácter abierto, decididamente transparente y democrático, no solo en la participación de los articulistas sino en los procedimientos internos de gestión. La Revista busca cumplir con sus objetivos de divulgar los trabajos de investigación, documentos críticos y de revisión técnico científica, permitiendo la difusión del conocimiento entre profesionales de las áreas pecuarias; siempre en la búsqueda de información pertinente y actualizada de temas relacionados con el sector y propendiendo a obtener reconocimiento en la comunidad en general, editando una revista que permita la interacción de la academia con el medio.
Periodicidad: Publicación continua (3 números por año).

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Los manuscritos y propuestas de publicación serán evaluados por medio de criterios explícitos, según el tipo de material, por pares académicos externos mediante la modalidad de doble ciego con cuando menos dos evaluadores por manuscrito. La evaluación procurará identificar los aportes a la innovación científica tecnológica o pedagógica de las propuestas, frente al estado vigente de conocimiento en una disciplina; los pares académicos externos deben emitir un concepto de aprobación, modificación o reprobación y en caso de un concepto dividido será el Comité Editorial quien determine la decisión final. Así mismo, el Comité Editorial o el editor en jefe podrán recomendar o negar la publicación del manuscrito, o solicitar la corrección de forma o de fondo del mismo.
Los criterios por aplicar en la evaluación académica de los manuscritos y propuestas son los siguientes:

- Pertinencia de contenido o temática: los textos deberán abordar las cuestiones que resulten relevantes de manera directa o indirecta, para la comprensión de alguna de las disciplinas y profesionales de la salud y la producción animal.
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- Claridad conceptual: correspondencia entre términos científicos o técnicos empleados en la finalidad temática.

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Innovations in veterinary medicine and animal science: the era of molecular biology and genomic data analysis

The generation and analysis of genomic data in veterinary medicine and animal science are of great importance due to their ability to provide a deep understanding of molecular processes, thereby enhancing animal treatment and production. This analysis includes preprocessing, filtering, descriptive analysis, statistical analysis, and gene network construction. It offers a comprehensive view of the molecular landscape at the cellular level.

Interest in genomic data for animals began with the sequencing of model organism genomes and the use of genetic sequence variations (particularly single nucleotide polymorphisms) to explain and select traits of productive or heritable resistance interest. This process started about 20 years ago, but in the past decade there has been a significant increase in interest in the generation and analysis of genomic data, especially transcriptomic data (messenger RNA quantification). These data provide a broader perspective than purely genetic data by including epigenetic and environmental factors that also shape molecular processes.

In Colombia, interest in genomic data has developed almost simultaneously. In 2020, for the first time, students from the Faculty of Veterinary Medicine and Animal Science took the Genomic Statistics course I offer in the Department of Statistics at the Universidad Nacional de Colombia (Bogotá Campus). Since then, several students have participated in the course using their own data generated at the University or through collaborative projects, gaining expertise in data analysis. Recently, there has been a growing focus on using genomic data analysis to find solutions for extracting information from single-cell data using molecular biology techniques that have been developed since around 2010. Currently, most of this data is generated for human studies, but similar data is now also available for animals.

The global and Colombian research communities in veterinary medicine and animal science have recognized the power of genomic data analysis and its potential to enhance understanding of biological processes for: 1. improving animal health by facilitating more precise diagnostics and treatments; 2. selecting animals with desirable traits such as disease resistance, higher meat or milk quality, and greater productive efficiency; 3. understanding responses to environmental stressors; 4. innovating in animal nutrition to design more effective feeding plans; 5. conserving species; and 6. supporting biotechnological development.

Single-cell data analysis can further support research in these areas by providing a deeper understanding of molecular biology and animal immunology. In veterinary medicine, it can also: 1. support early disease detection for more effective intervention; 2. contribute to the development of personalized therapies in the future; and 3. enhance reproductive and developmental studies by allowing investigation into cellular differentiation and development processes in embryos and reproductive tissues.

A significant advantage of having veterinarians and animal scientists at our University who possess the knowledge and skills for genomic data analysis is that it enables us

to remain competitive internationally with the most current data analysis techniques. Additionally, numerous freely available genomic datasets in repositories such as NCBI (**https://www.ncbi.nlm.nih.gov/**) can expand our knowledge and contribute to the development of veterinary medicine and animal science in all of the aforementioned areas. This access allows for the formulation of biological hypotheses before generating our own data, thus making research in Colombia more efficient.

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Innovaciones en veterinaria y zootecnia: la era de la biología molecular y el análisis de datos genómicos

La generación y el análisis de datos genómicos en veterinaria y zootecnia es de gran importancia debido a su capacidad para ofrecer una comprensión profunda de los procesos moleculares y, con ello, mejorar el tratamiento y la producción animal. Su análisis abarca el preprocesamiento, el filtro, la descripción, el análisis estadístico y la construcción de redes génicas. Esto da un panorama completo del paisaje molecular a nivel celular.

El interés por este tipo de datos en animales inició con la secuenciación de los genomas de animales modelo y el uso de las variaciones a nivel de la secuencia genética (especialmente polimorfismos de nucleótido simple) para explicar y seleccionar rasgos de interés productivo o de resistencia heredables. Este proceso comenzó hace unos veinte años, pero, en la última década, el interés en la generación y el análisis de los datos genómicos, sobre todo transcriptómicos (cuantificación del ARN mensajero), ha aumentado de manera considerable. Estos ofrecen una visión más amplia que la puramente genética por incluir aspectos epigenéticos y ambientales que también moldean los procesos moleculares.

En Colombia, el interés ha sido casi simultáneo. En 2020, por primera vez, estudiantes de la Facultad de Medicina Veterinaria y Zootécnica tomaron el curso Estadística Genómica, que ofrezco en el Departamento de Estadística de la Universidad Nacional de Colombia (Sede Bogotá). Desde ese momento, varios estudiantes han participado en el curso con datos propios generados en la Universidad o proyectos colaborativos y se han formado en el análisis de estos datos. Recientemente, el interés del análisis de datos genómicos se ha volcado en encontrar soluciones para extraer información de los datos de célula única con técnicas de biología molecular que se generaron alrededor de 2010. En la actualidad, la mayoría de estos datos se generan para humanos, pero ya existen también en animales.

La comunidad de investigadores en veterinaria y zootecnia a nivel mundial y en Colombia ha comprendido el poder del análisis de los datos genómicos y cómo este puede aumentar la comprensión de los procesos biológicos para 1. la mejora de la salud animal, al facilitar diagnósticos y tratamientos más precisos; 2. seleccionar animales con características deseables como resistencia a enfermedades, mejor calidad de carne o leche, mayor eficiencias productiva, entre otras; 3. comprender respuesta a estreses ambientales; 4. innovar en nutrición animal para el diseño de planes de alimentación más efectivos; 5. conservar especies y 6. apoyar el desarrollo para biotecnología.

El análisis de datos a nivel de célula única puede apoyar la investigación en los mismos sentidos con una comprensión aún más profunda de la biología molecular y la inmunología animal. En medicina veterinaria puede, además, 1. apoyar la detección temprana de enfermedades para una intervención más efectiva, 2. contribuir al desarrollo de terapias personalizadas en el futuro y 3. mejorar la reproducción y el desarrollo al permitir investigar los procesos de diferenciación y desarrollo celular en embriones y tejidos reproductivos.

En nuestra Universidad, la gran ventaja de contar en la actualidad con médicos veterinarios y zootecnistas con los conocimientos y las competencias para el análisis de datos genómicos es que, además de ser competitivos a nivel internacional en las técnicas más actuales del análisis de datos, en los bancos de datos genómicos como el NCBI (https://www.ncbi.nlm.nih.gov/) hay numerosos conjuntos de datos libres para ampliar el conocimiento y aportar al desarrollo de la medicina veterinaria y la zootecnia en todos los aspectos mencionados arriba, lo que permite emitir hipótesis biológicas antes de generar datos propios y hacer más eficiente la investigación en Colombia.

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Prevalence of gastrointestinal parasites in pre-slaughter cattle in the municipality of Quevedo, Ecuador

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ABSTRACT

This study aimed to assess the prevalence of gastrointestinal parasites in pre-slaughter cattle at the Quevedo abattoir, Ecuador. A total of 240 fecal samples were collected between August 2022 and July 2023. Variables such as age, sex, origin of the cattle, and season were analyzed to identify potential risk factors associated with parasite presence. Parasite identification was conducted using the flotation technique, which revealed the presence of *Strongyloides* and *Paramphistomum*. Statistical analysis involved calculating the Odds Ratio (OR) to assess exposure to risk factors, while the Chi-square test evaluated associations between variables and prevalence rates. Infection was significantly associated with male sex and animals originating from the Pucayacu area, attributed to the high humidity characteristic of this region. The overall parasite prevalence exceeded 50%, underscoring the need for implementing control measures in high-risk areas. Data analysis was performed using the statistical software InfoStat.

Keywords: risk factor, *Strongyloides*, *Paramphistomum*.

Prevalencia de parásitos gastrointestinales en bovinos pre-faenado en el municipio de Quevedo, Ecuador

RESUMEN

Este estudio tuvo como objetivo evaluar la prevalencia de parásitos gastrointestinales en bovinos pre-faenados en el camal de Quevedo, Ecuador. Se analizaron 240 muestras fecales recolectadas entre agosto de 2022 y julio de 2023. Se consideraron como variables la edad, el sexo, el lugar de procedencia del bovino y la época del año para identificar posibles factores de riesgo asociados a la presencia de parásitos. La identificación de las noxas se realizó mediante la técnica de flotación, lo que reveló la presencia de *Strongyloides* y *Paramphistomum.* El análisis estadístico incluyó el uso del Odds Ratio para determinar la exposición a factores de riesgo y la prueba de Chi cuadrado evaluó la asociación entre variables, además de la prevalencia. La infección estuvo significativamente asociada con el sexo macho y la procedencia de los animales de la zona de Pucayacu, por la alta humedad que caracteriza a la región. La prevalencia de parásitos fue superior al 50%, lo que resalta la importancia de efectuar medidas de control en estas áreas de mayor riesgo. El procesamiento de los datos se llevó a cabo con el *software* estadístico InfoStat.

Palabras clave: factor de riesgo, S*trongyloides, Paramphistomum*.

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INTRODUCTION

In Latin America, livestock farming plays a pivotal role due to the substantial availability of productive land, serving as a significant source of employment and nutrition across various social strata (Carizi *et al.* 2019). However, the expansion of the livestock sector has heightened the risk of disease, driven by anthropogenic changes that increase interactions among wildlife, humans, and livestock (Rodríguez *et al.* 2016).

The presence of endoparasites in cattle is a common issue, adversely affecting productivity and health, and leading to considerable economic losses (Pineda *et al.* 2018; Abdala *et al.* 2020). Proper identification and management of parasitic loads in cattle are essential to mitigate and prevent the negative impacts of parasitism (Samaniego *et al.* 2022).

Parasitic control in ruminants, especially against nematodes, predominantly relies on the frequent use of anthelmintics. However, indiscriminate and epidemiologically unsound use of these drugs has resulted in widespread parasite resistance (Leal *et al.* 2019). To maximize livestock productivity, understanding the epidemiology of gastrointestinal parasites specific to each region is crucial, as these parasites directly affect the health, production, and reproductive performance of cattle (Nastasi 2015).

The relevance of this research lies in its contribution to the existing scientific knowledge concerning the threat posed by parasites to livestock productivity and animal welfare. Despite the importance of this issue, most existing studies have focused on broader geographical areas or other provinces, limiting the understanding of local epidemiological conditions. Therefore, this study aimed to evaluate the prevalence of gastrointestinal parasites in pre-slaughter cattle at the municipal abattoir of Quevedo, Ecuador, providing specific and relevant data for this region and enhancing the understanding of the local epidemiological situation.

MATERIALS AND METHODS

The study was conducted at the municipal abattoir of Quevedo, located in the San Camilo parish at 1° 20' 30" S latitude and 79° 28' 30" W longitude, with an elevation of 69 m above sea level. The meteorological conditions of the area are characterized by an average temperature of 24 °C, relative humidity of 85%, and an annual rainfall of 2,224 mm. Coproparasitological analyses were carried out in the Microbiology Laboratory of the State Technical University of Quevedo, situated at the Experimental Farm La María, 7.5 km along the Quevedo-El Empalme road, Los Ríos Province, at 1° 3' 18" S latitude and 79° 25' 24" W longitude, with an elevation of 73 m above sea level.

Ethical considerations

The study did not require approval from an ethics committee, as no experiments were performed on the animals. However, the ethical compliance certificate with reference number CERT-ÉTICA-003-DICYT-2024, issued by the Research Directorate of Universidad Técnica Estatal de Quevedo, confirms that this *in situ* research adhered strictly to animal welfare guidelines. Throughout the study, all procedures ensured that cattle were never subjected to cruelty, in full compliance with the Organic Animal Welfare Law (LOBA) in Ecuador, as stipulated in Article 585 of the Ecuadorian Civil Code. The study focused exclusively on observational data

collection, maintaining adherence to established legal and ethical standards for animal welfare in Ecuador.

Sample selection

In 2021, the municipal abattoir of Quevedo had an annual average of 6,430 slaughtered cattle. The sample size was calculated using the formula: $n = N \cdot z^2 \cdot p \cdot q / e^2 (N - 1) + z^2 \cdot p \cdot q$, with a 95% confidence coefficient. A total of 240 cattle were randomly selected for the study (Aguilar, 2005).

Sample collection

Fecal samples were collected directly from the rectum of the animals using palpation sleeves, obtaining approximately 10 g per sample, which were placed in sterile plastic containers. Information on sex, age, origin, and season of the year for each animal was recorded. The samples were then stored in thermal containers and transported to the UTEQ laboratory for processing and evaluation (Caro *et al.* 2011).

Coproparasitological flotation analysis

To perform the flotation technique (Petters *et al.* 2019), a saturated saline solution was prepared by dissolving 250 g of sugar and 200 g of sodium chloride in 500 mL of distilled water. A 4 g fecal sample was weighed and mixed with 57 mL of the solution in a sterilized container. The mixture was gently stirred and strained through a sieve into another sterile container. Using a pipette, the suspension was transferred into a test tube until a convex meniscus formed. A coverslip was placed on top, and the sample was allowed to stand for 5 minutes to enable parasite eggs to float to the surface. The eggs were then examined under a microscope at 10X and 40X magnification, with identification based on morphological characteristics (Pinilla *et al.* 2019).

Prevalence determination

Prevalence was calculated based on sex, age, origin, and season using the formula: Prevalence = (Number of positive animals / Number of animals sampled) × 100 (Guamán et al. 2021).

Odds Ratio determination

Statistical analysis included the calculation of the Odds Ratio (OR) to quantify the association between exposure to risk factors and outcomes such as parasitic infection. This measure identified the most significant factors contributing to parasite transmission, with values greater than 1 indicating an increased likelihood of infection. Variables such as age, sex, origin, and season were analyzed to determine the factor most closely associated with parasite presence in cattle (Solis *et al.* 2022).

Statistical analysis

Statistical analyses were performed using InfoStat software, employing the Chi-square test at a 95% confidence level to evaluate the association between variables and parasite presence. Pearson's Phi coefficient, Cramér's V, and the contingency coefficient were used to assess the strength of relationships between variables when no significant associations were detected. These coefficients provided a measure of the relationship strength, with values close to 1 indicating a strong association and values near 0 suggesting no significant relationship. This multifaceted approach ensured a comprehensive and accurate analysis of the risk factors associated with parasitic infections in pre-slaughter cattle.

RESULTS

Identification of gastrointestinal parasite eggs using the coproparasitological flotation method. The coproparasitological analysis enabled the specific identification of infective larvae in the fecal samples. Out of the 240 cattle examined, 160 tested positive for gastrointestinal parasites, while 80 tested negative (table 1).

Classification of gastrointestinal parasite eggs

Direct microscopic observation of the samples confirmed the presence of parasite eggs in the cattle. It was determined that 150 samples were positive for *Strongyloides* and 47 for *Paramphistomum* (table 2).

Prevalence of *Strongyloides* across variables and risk factor determination

Table 3 shows a higher prevalence of *Strongyloides* infection (76.92%) in cattle aged 5 years, with an OR of 2.07 (95% CI: 0.55-7.74). Although parasitism was observed across all age groups, the infection intensity for cattle aged 5 years had a frequency of 0.07, which did not represent a significant risk factor. The prevalence of *Strongyloides* was significantly higher

TABLE 1. Identification of eggs of gastrointestinal parasites in cattle

Cases	Number of cases	%
Positive	160	66.6
Negative	80	33.3
Total	240	100

Source: own elaboration.

TABLE 2. Classification of eggs of gastrointestinal parasites in cattle

	Results		Total cattle
Strongyloides	Positive	150	240
	Negative	90	
	Total	240	
Paramphistomum	Positive	47	240
	Negative	193	
	Total	240	

Source: own elaboration.

TABLE 3. Prevalence of *Strongyloides* in relation to age

Age (year)	Total	Positive	Frequency	Prevalence (%)	P	OR	IC
2	77	41	0.27	53.25	0.05	0.56	0.32-0.98
3	99	62	0.41	62.63	0.05	1.01	0.59-1.72
4	51	37	0.25	72.55	0.05	1.78	0.90-3.51
5	13	10	0.07	76.92	0.05	2.07	0.55-7.74
TOTALS	240	150		62.50			

P: statistical significance OR: Odds ratio

IC: Confidence interval

Source: own elaboration.

in male cattle, with an infection rate of 67.5% compared to 52.5% in females. The OR for males was 1.88 (95% CI: 1.08-3.26), indicating that being male nearly doubled the risk of infection with this parasite (table 4).

Association between month and *Strongyloides* presence

Regarding the association between infection prevalence and month, the prevalence varied from 50% to 80% throughout the year. Notably, February had the highest prevalence at 80%. The OR for February was 31.20 (95% CI: 9.66-100.74), indicating that this month represented a significantly higher risk factor compared to the others (table 5).

Prevalence of *Strongyloides* Based on the Place of Origin

Table 6 presents the prevalence of *Strongyloides* according to the place of origin of the cattle. The prevalence in animals

TABLE 4. Prevalence of *Strongyloides* in relation to sex

Sex	Total	Positive	Frequency	Prevalence (%)	P	OR	IC
Male	160	108	0.72	67.50	0.05	1.88	1.08-3.26
Female	80	42	0.28	52.50	0.05	0.53	0.31-0.92
TOTALS	240	150		62.50			

Source: own elaboration.

TABLE 5. Prevalence of *Strongyloides* in relation to time of year

Months	Total	Positive	Frequency	Prevalence (%)	P	OR	IC
August	20	11	0.07	55.00	0.05	0.71	0.28-1.79
September	20	11	0.07	55.00	0.05	0.71	0.28-1.79
October	20	11	0.07	55.00	0.05	0.71	0.28-1.79
November	20	10	0.066	50.00	0.05	0.57	0.23-1.43
December	20	11	0.07	55.00	0.05	0.71	0.28-1.79
January	20	12	0.08	60.00	0.05	0.89	0.35-2.27
February	20	16	0.11	80.00	0.05	31.20	9.66-100.74
March	20	13	0.09	65.00	0.05	1.13	0.43-2.93
April	20	13	0.09	65.00	0.05	1.13	0.43-2.93
May	20	15	0.1	75.00	0.05	1.89	0.66-5.39
June	20	14	0.093	70.00	0.05	1.44	0.53-3.90
July	20	13	0.09	65.00	0.05	1.13	0.43-2.93
TOTALS	240	150		62.50			

Source: own elaboration.

from Santo Domingo and Quevedo was not statistically significant. However, cattle from Pucayacu exhibited a prevalence of 80.73%. The calculated OR for this region was 4.66 (95% CI: 2.59-8.39), indicating that Pucayacu poses a nearly fivefold higher risk of infection with this parasite compared to the other locations assessed.

Prevalence of *Paramphistomum* across variables and risk factor determination

The highest prevalence related to age was observed in 4-year-old cattle (25.49%). However, no age group was identified as a risk factor since the OR were close to 1, and the confidence intervals included the unit, indicating no significant association between age and the presence of the parasite (table 7).

Association between sex and *Paramphistomum*

Data analysis revealed that the prevalence of *Paramphistomum* was higher in females (20%) compared to males (19.38%), with OR of 1.04 and 0.96, respectively, indicating that sex is not a significant factor influencing the presence of this parasite (table 8).

TABLE 6. Prevalence of *Strongyloides* in relation to place of proceeding

Place	Total	Positive	Frequency	Prevalence (%)	P	OR	IC
Pucayacu	109	88	0.59	80.73	0.05	4.66	2.59-8.39
Santo Domingo	126	60	0.4	47.62	0.05	0.24	0.14-0.43
Quevedo	5	2	0.01	40.00	0.05	0.39	0.06-2.39
TOTALS	240	150		62.50			

Source: own elaboration.

TABLE 7. Prevalence of *Paramphistomum* in relation to age

Age (year)	Total	Positive	Frequency	Prevalence (%)	P	OR	IC
2	77	15	0.32	19.48	0.05	0.99	0.50-1.96
3	99	18	0.38	18.18	0.05	0.86	0.45-1.65
4	51	13	0.28	25.49	0.05	1.56	0.75-3.24
5	13	1	0.02	7.69	0.05	0.33	0.04-2.59
TOTALS	240	47		19.58			

Source: own elaboration.

TABLE 8. Prevalence of *Paramphistomum* in relation to sex

Sex	Total	Positive	Frequency	Prevalence (%)	P	OR	IC
Male	160	31	0.66	19.38	0.05	0.96	0.49-1.89
Female	80	16	0.34	20.00	0.05	1.04	0.53-2.04
TOTALS	**240**	**47**		**19.58**			

Source: own elaboration.

Prevalence of *Paramphistomum* over the year

As shown in table 9, the months of October, November, and December exhibited the highest prevalence rates compared to the rest of the year, with OR ranging from 3.92 to 10.16, indicating a significant risk factor during these months.

Prevalence of *Paramphistomum* based on place of origin

Table 10 shows that cattle from Pucayacu exhibited the highest prevalence (29.36%) and an OR of 3.21 (95% CI: 1.63-6.33), indicating that this geographical area posed a threefold higher risk for *Paramphistomum* infection.

TABLE 9. Prevalence of *Paramphistomum* in relation to time of year

Months	Total	Positive	Frequency	Prevalence (%)	P	OR	IC
August	20	0	0.00	0.00	0.05	0.00	0.00
September	20	0	0.00	0.00	0.05	0.00	0.00
October	20	12	0.25	60.00	0.05	7.93	3.02-20.81
November	20	9	0.19	45.00	0.05	3.92	1.52-10.11
December	20	13	0.28	65.00	0.05	10.16	3.78-27.31
January	20	7	0.15	35.00	0.05	2.42	0.91-6.46
February	20	6	0.13	30.00	0.05	1.87	0.68-5.16
March	20	0	0.00	0.00	0.05	0.00	0.00
April	20	0	0.00	0.00	0.05	0.00	0.00
May	20	0	0.00	0.00	0.05	0.00	0.00
June	20	0	0.00	0.00	0.05	0.00	0.00
July	20	0	0.00	0.00	0.05	0.00	0.00
TOTALS	240	47		19.58			

Source: own elaboration.

TABLE 10. Prevalence of *Paramphistomum* in relation to the place of proceeding

Place	Total	Positive	Frequency	Prevalence (%)	P	OR	IC
Pucayacu	109	32	0.68	29.36	0.05	3.21	1.63-6.33
Santo Domingo	126	15	0.32	11.90	0.05	0.35	0.18-0.68
Quevedo	5	0		0.00	0.05	0.00	0.0
TOTALS	240	47		19.58			

Source: own elaboration.

DISCUSSION

The parasites with the highest prevalence identified in this study were *Strongyloides* and *Paramphistomum*. This finding aligns with the reports by Samaniego *et al*. (2022), who investigated the prevalence of gastrointestinal and pulmonary parasites in Ecuador and determined that the infection levels were attributed to parasites such as *Cooperia* spp., *Trichuris* spp., *Ostertagia* spp., *Haemonchus* spp., *Strongyloides*, *Eimeria* spp., *Fasciola*, and *Dictyocaulus*. They noted that livestock could be affected by multiple parasites, which limit their zootechnical performance through direct impacts on health and productivity or indirectly by increasing prevalence due to associated risk factors.

Pinilla *et al.* (2019) indicated a correlation between age and the intensity of *Strongyloides* infection, with higher rates observed in animals younger than one year, decreasing with age due to transmission pathways that favor greater infection rates in calves under poor hygienic conditions. However, in this study, the animals were aged between two and five years, possessing a more developed immune system, and no significant differences in infection rates were observed across age groups. This contrasts with the findings of Benavides and Polanco (2017), who reported that the immune response against gastrointestinal nematodes matures with the age of the livestock, thus indicating that no age group is considered a risk factor. Nonetheless, this should be viewed as a protective factor against infections, which may arise from poor hygiene and sanitary practices in cattle herds.

Regarding the prevalence of *Strongyloides* in relation to sex, male cattle exhibited greater susceptibility to infection. Patiño *et al.* (2017) suggested that this increased predisposition in males' results from physiological and behavioral differences, as males typically have greater body mass, food intake, and metabolic rates, which increases their exposure to infective larvae during grazing. Additionally, *Strongyloides* can survive throughout the year in the environment as infective larvae, enabling them to maintain their prevalence regardless of changing environmental conditions. This is consistent with the findings of Pinilla *et al.* (2019), who did not observe significant seasonal variations in the presence of these intestinal parasites throughout the year. Unlike other parasites, such as *Eimeria* spp., the presence of *Strongyloides* remained constant over the months sampled and was not influenced by the timing of the sampling. Both studies demonstrated that the prevalence of these nematodes in cattle did not exhibit seasonal variation correlated with the months of the year.

Pucayacu, characterized by a humid climate with temperatures averaging 20 °C, represents a significant risk factor for *Strongyloides* infection, particularly when compared to Quevedo and Santo Domingo, which have temperatures of 24 °C and 23 °C, respectively. Munguía *et al.* (2021) suggest that geographic origin is a determining factor in the transmission of these parasites, as certain environmental attributes, such as humidity and favorable conditions for the development of infective larvae, facilitate the infection of new hosts and the continuation of the life cycle. Their findings indicate that more humid environments enhance the survival and availability of infective forms of *Strongyloides*, thereby increasing the risk of infection in cattle from these regions.

The results of the current study demonstrated that the presence of *Paramphistomum* is not significantly associated with the age

of the cattle, suggesting that all age groups are equally exposed to infection. However, Andrade *et al.* (2020) reported a higher incidence of positive cases in cattle under four years of age, positing that developing cattle are more susceptible to infection and subsequently acquire a degree of immunity that protects them from reinfection. Nonetheless, even adult cattle may harbor small quantities of infective larvae, which aligns with the current study's findings regarding prevalence.

Despite this correlation, the odds ratio analysis revealed that age does not act as a risk factor; rather, it may be considered a protective factor. Therefore, control and prevention strategies should be uniformly implemented across all age groups of cattle.

The low intensity of *Paramphistomum* infection in relation to sex and age is attributed to the fact that this infection is more closely related to seasonal environmental conditions than to individual factors such as age or sex. The findings suggest that variations in prevalence are more influenced by specific environmental factors related to the time of year than by the individual characteristics of the cattle. Consequently, control strategies should focus on mitigating the environmental conditions that favor the spread of the parasite, rather than concentrating on variables such as age or sex (Arroyo *et al.* 2022).

Regarding geographic origin, it was observed that cattle from certain regions exhibit greater exposure to infectious agents due to climatic and management factors (Casado *et al.* 2020). These variations in ecological and environmental conditions among tropical regions influence the presence and proliferation of intermediate hosts of the parasite. Fluctuations in climate, such as humidity and seasonal temperatures, along with region-specific management practices, create environments that may promote the development and survival of parasites, thus increasing cattle exposure to infections (Arroyo *et al.* 2022).

The prevalence percentages of *Paramphistomum* significantly increased during the rainy season. In months with abundant rainfall, higher infection levels were detected compared to the dry season. According to Baquero *et al.* (2022), environmental conditions during the winter months, including temperature, humidity, and precipitation, promote the presence of intermediate hosts of *Paramphistomum*, thereby enhancing its biological cycle and facilitating transmission.

CONCLUSIONS

Parasitic load was identified using the flotation technique, which successfully detected intestinal parasite eggs in 66.6% of the examined cattle. Infections caused by *Strongyloides* and *Paramphistomum* were identified. The infection with *Strongyloides* exhibited a significant association with both the sex of the animals and their geographic origin, indicating that male cattle and those from certain regions represent risk factors. In contrast, the infection by *Paramphistomum* showed a slight association with age, being more prevalent in cattle under four years old, while geographic origin and the time of year were considered significant risk factors for this infection.

CONFLICT OF INTEREST

The authors of this article declare that there are no conflicts of interest related to the conduct of the study and the preparation of the manuscript.

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DECLARATION OF ARTIFICIAL INTELLIGENCE USE

We declare that no artificial intelligence has been used in the preparation of this article.

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Risk factors associated with leptospirosis in dual-purpose cattle based on the One Health approach in the department of Huila, Colombia

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ABSTRACT

Leptospirosis significantly impacts beef and dairy cattle production, particularly in tropical regions, although it remains a disease of global relevance. This zoonotic disease primarily causes abortions during the final third of gestation. The present study aimed to analyze the risk factors associated with leptospirosis in the southern Andean region of Colombia. To this end, 360 cattle were sampled across 24 municipalities within the department of Huila. A seroprevalence of 49.2% was determined using enzyme-linked immunosorbent assays (ELISA). These results were subsequently correlated with various variables obtained through epidemiological surveys.

The findings revealed that the primary risk factors for leptospirosis were linked to inadequate livestock management practices. These included improper methods of food storage, failure to implement quarantine protocols for infected animals, the introduction of new fattening cattle into the herd without proper precautions, infrequent weighing of animals, poor storage management of veterinary medications, lack of segregation of sick animals, insufficient estrus detection practices, inadequate hand hygiene when interacting with the herd, and the use of unclean instruments during routine procedures. Conversely, the presence of well-defined internal pathways and the separation of poultry production from cattle operations were identified as protective factors. Understanding these risk factors is critical from a One Health perspective, as it facilitates the implementation of preventive measures aimed at safeguarding both animal and human health.

Keywords: *Leptospira* spp., abortions, ELISA, livestock, human health.

Factores de riesgo asociados con la leptospirosis en bovinos doble propósito basado en el enfoque Una Salud en el departamento del Huila, Colombia

RESUMEN

La leptospirosis afecta la producción de ganado vacuno de carne y leche principalmente en regiones tropicales, aunque es una enfermedad de importancia mundial. Esta enfermedad ocasiona abortos en el último tercio de la gestación. El propósito del presente estudio es desarrollar planes de prevención de la enfermedad que se ajusten a la realidad

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del entorno, considerando el enfoque Una Salud. En este contexto, se seleccionaron 360 bovinos de 24 municipios del departamento del Huila. Se determinó una seroprevalencia de 49,2% para leptospirosis mediante ensayos inmunoabsorbentes ligados a enzimas (ELISA). Los resultados se correlacionaron con una serie de variables obtenidas a través de encuestas epidemiológicas. Los principales factores de riesgo están asociados a las malas prácticas de manejo del ganado, que incluyen los métodos de almacenamiento de alimentos, la aplicación de protocolos de cuarentena para animales infectados, la introducción de nuevos animales de engorde al hato, la frecuencia de pesaje en báscula, el manejo adecuado del almacenamiento de medicamentos, la segregación de animales enfermos, la detección de estros, el lavado de manos al interactuar con el hato y el uso de instrumentos limpios utilizados en los procedimientos habituales. Además, se encontraron factores protectores como tener vías internas bien definidas y mezclar la producción con aves. Comprender estos factores de riesgo es de gran utilidad desde una perspectiva de Una Salud, ya que permite la implementación de medidas para proteger tanto la salud animal como la humana.

Palabras clave: *Leptospira* spp., aborto, ELISA, ganado, salud humana.

INTRODUCTION

Zoonotic diseases are infections that vertebrate animals can transmit to humans. Under natural conditions, the infectious agents responsible for these diseases include bacteria, viruses, parasites, fungi, rickettsia, among others (Tomori & Oluwayelu 2023). Among these, the bacteria causing leptospirosis have garnered significant interest due to the disease's notable impact on public health and its contribution to both reproductive and non-reproductive losses in cattle production (O'Doherty *et al.* 2014). While the disease predominantly affects beef and dairy cattle production in tropical regions, it remains a concern of global significance (Bharti *et al.* 2003). Mammals, including humans, are the most frequent hosts in endemic areas. The challenge of clinical diagnosis is further compounded by the similarity of leptospirosis symptoms to those of other diseases such as dengue, parvovirus, and hepatitis (Faine 1994; Romero *et al.* 2010). It is crucial to recognize that animal species maintain a relationship with specific *Leptospira* serovars. Advances in isolation methods from environmental samples have enabled the identification of 68 *Leptospira* species to date (Arent *et al.* 2022).

Leptospirosis in beef cattle significantly compromises livestock production due to its severe reproductive consequences, including stillbirths and abortions (Sohm *et al.* 2023). This disease remains a critical subject of study and evaluation to guide future strategies for its control, prevention, and potential eradication. In 1980, leptospirosis accounted for approximately 40.8% of abortions in beef cattle in Venezuela, while in Ireland, this figure reached 49.7% (Ellis *et al.* 1982). Despite its impact, leptospirosis has not been classified as a notifiable disease by the Colombian Agricultural Institute (ICA, by its initials in Spanish), as it is not mandated by the World Organization for Animal Health (WOAH) (Instituto Colombiano Agropecuario 2015). Consequently, there is limited official information on the economic burden of leptospirosis on Colombia's national dairy and beef production.

Several studies conducted in Colombia identified a total of 2,426 serovars from 1,586 positive cases, with *L. hardjo* being the most frequently detected, reflecting its status as a reservoir in ruminants, pigs, and equines (Ensuncho–Hoyos *et al.* 2017; Olivera *et al.* 2018; Pulido *et al.* 2017; Estupiñán *et al.* 2018; Vargas *et al.* 2018; Baena *et al.* 2018; Guzmán–Barragán *et al.* 2022). Among bovines, the most common reservoirs identified were *L. hardjo*, *L. tarassovi*, *L. pomona*, and *L. grippotyphosa* (Balamurugan 2016).

Humans can acquire *Leptospira* spp. either directly or indirectly through the skin or via the ocular, oropharyngeal, and nasopharyngeal mucosa (Suárez Conejero *et al.* 2015). The primary reservoirs of this disease are rodents and dogs, which excrete the bacteria in their urine, thereby contaminating both indoor and outdoor environments (Abgueguen 2014). In beef cattle, transmission typically occurs indirectly through contact with contaminated water or soil, as well as through uterine secretions, semen, and urine (Munoz–Zanzi *et al.* 2020). Consequently, the infection enters the body via mucous membranes or damaged skin (Ellis 2015).

Leptospirosis progresses through distinct phases. The disease begins with an acute or septicemic phase, lasting approximately one week. This is followed by the immune phase, during which antibodies are produced, and *Leptospira* spp. is excreted in the urine for a brief period. The quantity of bacteria excreted during this phase is minimal, and the bacteria are typically non-viable, classifying the host as accidental (Monte *et al.* 2015). In the chronic stage, the pathogen primarily localizes in the kidneys and the genital tract of carrier animals (Faine 1994).

Leptospirosis has been extensively studied across various species worldwide; however, research focusing on beef cattle remains insufficient (Goarant *et al.* 2019). As noted by Sohm *et al.* (2023), it is critical for public health to increase awareness and attention to leptospirosis due to its significant economic impact. Collaborative efforts between health professionals and veterinarians can assist farmers in preventing the spread of the disease, particularly in regions not yet classified as endemic. The growing practice of open grazing in Europe has further contributed to the resurgence of leptospirosis, underscoring the importance of studies on beef cattle for improving epidemiological understanding of the disease (Sohm *et al.* 2023).

Risk factors such as geographic location, inadequate management practices, animal purchasing, livestock movement, and biosecurity measures significantly influence the presence of leptospirosis (O'Doherty *et al.* 2014). Accordingly, the present study aims to analyze the risk factors associated with leptospirosis in the southern Andean region of Colombia.

MATERIALS AND METHODS

This study was conducted with the approval of the Ethics, Bioethics, and Scientific Integrity Committee of the Colombian Agricultural Research Corporation (Agrosavia, by its acronym in Spanish), as per Act N.° 2 of 2021. All biosecurity measures established on the farms were strictly followed, and sampling procedures adhered to a standardized protocol.

A cross-sectional investigation was undertaken in collaboration with the University Corporation of the Huila Region (Corhuila, by its acronym in Spanish), the Colombian Agricultural Research Corporation (Agrosavia, by its acronym in Spanish), the Committee of

Livestock Farmers of Huila (CGH, by its initials in Spanish), and the Governorate of Huila. Cattle were randomly selected from livestock farms located in the department of Huila. Out of the 37 municipalities in the department, 24 were randomly chosen and grouped into four geographic zones: the northern zone (Aipe, Algeciras, Baraya, Campoalegre, Colombia, Palermo, Rivera, Tello, Villavieja, and Yaguará); the central zone (Altamira, El Pital, Garzón, Gigante, Suaza, and Tarqui); the western zone (La Plata, Nátaga, Paicol, and Tesalia); and the southern zone (Acevedo, Pitalito, San Agustín, and Timaná).

The criteria for selecting the farms included membership in a livestock association, availability of suitable land for livestock use, maintenance of production records, and possession of sufficient economic resources for disease treatment. A total of 150 farms meeting these inclusion criteria were selected, and 360 cows were randomly chosen for the study. Data collected for each animal included a problem-oriented clinical record, an epidemiological survey, and a socioeconomic survey. Clinical examinations of all cattle across the herds were performed by veterinary medicine and zootechnics professionals. Clinical parameters evaluated included heart rate, respiratory rate, temperature, body condition, and weight. Additionally, the morphophysiological integrity of various systems (cardiovascular, respiratory, digestive, reproductive, and integumentary) and body parts was assessed. Blood samples were collected via jugular vein puncture from each bovine, and the presence of bovine antibodies was determined using the commercial Cow Leptospira spp. Antibody ELISA kit (Abbexa LTD, Cambridge, UK). The ELISA kit has a reported sensitivity of 94% and specificity of 99%, as per the manufacturer.

To identify risk factors associated with leptospirosis, both epidemiological and socioeconomic surveys were administered. These surveys comprised 230 potential risk factors, including biosecurity, sanitation, transportation, traceability, medication use, animal health, and feeding practices (epidemiological survey).

ELISA protocol

Blood samples were collected in 10 mL red-cap tubes and centrifuged at 3,070 Relative Centrifugal Force (RCF) for 5 minutes to separate the serum. To ensure preservation, the samples were stored at -20 °C to prevent protein degradation and denaturation.

Prior to processing, ELISA kit components and serum samples were thawed to room temperature. Serum samples were diluted with Sample Diluent Buffer at a 1:5 ratio, while the Wash Buffer was prepared by diluting it with distilled water at a 1:30 ratio, following the manufacturer's instructions.

A total of 50 µL of positive, negative, and blank controls (Sample Diluent Buffer) were aliquoted in duplicate, alongside 50 µL of each diluted sample, into the respective wells of the ELISA plate. The plate was incubated for 30 minutes at 37 °C in a dry oven (ARI Medical Technologies). After incubation, the plate was washed five times using 1X Wash Buffer. Next, the Detection Reagent was added, and the plate was incubated again at 37 °C for 30 minutes, followed by additional washes as specified by the manufacturer.

To develop the reaction, TMB Substrate A and TMB Substrate B were added to each well, and the plate was incubated at 37 °C for 15 minutes. Subsequently, 50 µL of Stop Solution was added to inactivate the enzymatic reaction. The optical density

(OD) of the samples was measured within 3 minutes after adding the Stop Solution, using a BOECO BMR-100 Microplate Reader equipped with a 450 nm filter.

STATISTICAL ANALYSIS

A Pearson correlation coefficient was calculated to evaluate the relationship between variables, focusing on the association between socioeconomic and demographic factors and the presence of the pathogen *Leptospira* spp. Binary logistic regression models were developed, where the dependent variable represented the ELISA test results (0: negative; 1: positive for leptospirosis). Risk factors were analyzed, and odds ratio (OR) were calculated for each, with a 95% confidence level (P-value < 0.05) to ensure the validity of the identified risk factors.

Descriptive analysis was performed using the statistical software R (version 4.3.3). This approach facilitated the determination of logistic regression models, and the identification of risk factors associated with the occurrence of the disease. Risk factors with significant associations, whether with the presence or absence of leptospirosis, were identified and highlighted.

RESULTS AND DISCUSSION

Out of the 360 samples analyzed, 177 (49.2%) tested positive for leptospirosis. The seroprevalence across different age groups was as follows: 12-45 months, 39% (23/59); 46-65 months, 49.6% (57/115); 66-85 months, 54% (67/124); 86-105 months, 50% (12/24); and ≥106 months, 40% (12/30) (table 1).

The highest prevalence by region was observed in farms located in the northern zone, with 52.5% (93/177), followed by the western zone at 48.3% (29/60), the central zone at 46.6% (27/58), and the southern zone at 43.1% (28/65) (table 2).

Out of the 360 cows analyzed, 85 (23.6%) had contact with canines, and 42 of these (49.4%) tested positive for leptospirosis. Similarly, 101 cattle (29.1%) had contact with equines, with 52 (51.5%) testing seropositive. Additionally, 28 cattle (7.8%) had contact with ovines, among which 13 (46.4%) tested positive for leptospirosis (table 3).

TABLE 1. Seroprevalence of leptospirosis according to different age groups

Age groups (months)	Frequence	Seroprevalence	95% Confidence interval	
			Lower limit	Upper limit
12-45	23/59	39.0	26.5	51.4
46-65	57/115	49.6	40.4	58.7
66-85	67/124	54.0	45.3	62.8
86-105	12/24	50.0	30.0	70.0
≥106	12/30	40.0	22.5	57.5
not received	6/8	75.0	45.0	105.0
χ^2: 6,781; **gl:** 5; **p:** 0.237.				

Source: own elaboration.

TABLE 2. Seroprevalence of leptospirosis according to the different regions of the department of Huila.

Region	Frequence	Seroprevalence	95% Confidence interval	
			Lower limit	Upper limit
Northern	93/177	52.5	45.2	59.8
Western	29/60	48.3	35.9	61.0
Central	27/58	46.6	33.9	59.6
Southern	28/65	43.1	32.7	54.8
χ^2: 1,947; **gl:** 3; **p:** 0,584.				

Source: own elaboration.

TABLE 3. Contact with other domestic species associated with the seroprevalence of leptospirosis in cattle from the department of Huila

Contact with other species		Frequence	Seroprevalence	χ^2	p	OR ICI	IC OR 95% ICS	
Canines	Yes	42/85	49.4	0.002	0.959	1.01	0.62	1.65
	No	135/275	49.1					
Equines	Yes	52/101	51.5	0.302	0.583	1.14	0.72	1.80
	No	125/259	48.3					
Sheep	Yes	13/28	46.4	0.091	0.763	0.89	0.41	1.92
	No	164/332	49.4					
Birds	Yes	10/42	23.8	12.82	0.001	0.28	0.13	0.59
	No	167/318	52.5					

Source: own elaboration.

Additional clinical and morphophysiological integrity parameters were assessed in the cows, including heart rate, respiratory rate, temperature, body condition, and weight. The integrity of various systems (cardiovascular, respiratory, digestive, reproductive, and integumentary) was also evaluated, along with other body parts. However, no statistically significant differences were observed.

Of the 230 variables analyzed through independent logistic regression models, only 12 were identified as statistically significant, either as protective or risk factors (table 4). A multiple logistic regression model incorporating these significant variables was subsequently developed, resulting in 4 variables that demonstrated statistical significance ($p \leq 0.05$) (table 5).

TABLE 4. Independent variable logistic regression of risk factors with the seroprevalence of leptospirosis

Variables		β	gl	p	OR	IC OR 95%	
						ICI	ICS
Purchase cattle for fattening	No		-	-	-	-	-
	Yes	0.665	1	0.013*	1.94	1.15	3.28
Have defined internal pathways	No			-	-	-	-
	Yes	-1.33	1	0.011*	0.26	0.095	0.73
Storage of concentrate	No			-	-	-	-
	Barrel	0.574	1	0.037*	1.78	1.03	3.05
	Pallet	0.351	1	0.264	1.42	0.77	2.63
	Pallet/ Barrel	2.170	1	0.049*	8.76	1.01	75.77
	Floor	0.27	1	0.942	1.03	0.50	2.11
Weigh on scale	No			-	-	-	-
	Periodically	0.984	1	0.008*	2.67	1.29	5.53
	Sporadically	0.427	1	0.126	1.53	0.89	2.65
Rodent control program	No			-	-	-	-
	Yes	0.523	1	0.016*	1.69	1.10	2.58
Medications stored and classified	No			-	-	-	-
	Yes	0.674	1	0.008*	1.96	1.19	3.22
Quarantine	No			-	-	-	-
	Yes	0.493	1	0.023*	1.64	1.07	2.50
Isolation	No			-	-	-	-
	Yes	0.748	1	0.009*	2.11	1.20	3.72
Heat detection in cows	No			-	-	-	-
	Yes	0.569	1	0.008*	1.77	1.16	2.69
Handwashing and disinfection	No			-	-	-	-
	Yes	0.554	1	0.014*	1.74	1.12	2.70
Prior cleaning of objects	No			-	-	-	-
	Yes	1.188	1	0.000*	3.28	1.71	6.27

Note: * = variable with significance p ≤0.05;–= reference variable

Source: own elaboration.

TABLE 5. Multiple logistic regression analysis of risk factors with the seroprevalence of leptospirosis.

Independent variables	Coefficients β	gl	p	OR	IC OR 95%	
					ICI	ICS
Intercept	0.56	1	0.31	1.76		
Mix cattle production with birds	-1.28	1	0.00	0.28	0.13	0.59
Have defined internal pathways	-1.39	1	0.01	0.25	0.09	0.7
Isolation	0.59	1	0.05	1.8	1	3.3
Heat detection in cows	0.66	1	0.04	1.9	1.2	3.0
Omnibus test p: 0.000			**Nagelkerke R^2:** 0.124			

Source: own elaboration.

The One Health approach emphasizes the interconnectedness of human health, animal health, and ecosystem integrity, advocating for collaborative, multidisciplinary efforts to achieve balance within this complex system (Roberts 2019). Furthermore, many emerging zoonotic diseases originate from wildlife due to ecosystem degradation (Taddei 2021). Leptospirosis, a zoonotic disease, affects humans as well as wild and domesticated animals (Vera *et al.* 2019). However, the epidemiological role of wild animals in disease transmission remains a subject of debate (Pal *et al.* 2021).

Studying the ecosystem dynamics of bovine leptospirosis, including its epidemiological, demographic, and social determinants within specific populations, will enhance public health strategies aimed at reducing human transmission. Additionally, identifying risk factors associated with bovine disease will support the development of prevention and control measures to mitigate its adverse effects on human health.

The prevalence of leptospirosis in the tested individuals was 49.2% (177/360), with *Leptospira* seropositivity analyzed using the ELISA assay. According to the literature, no studies on leptospirosis in cattle have been conducted at the regional level. However, at the national level, research is scarce, and the seroprevalence observed in this study is considerably higher than the average reported. At the international level, several studies have investigated leptospirosis in livestock due to its significant impact. For instance, a study in southern Andaman reported a seroprevalence of 42.15% (180/247), which is similar to the findings of the present study; however, the study used the MAT technique and identified serovars such as *L. hardjo*, *L. australis*, *L. pomona*, and *L. canicola* (Sunder *et al.* 2017). Similarly, in Japan, a study identified 44 (12.8%) dairy cows as seropositive using the TMA technique, with *L. sejroe* being the most prevalent serovar. Additionally, PCR testing of urine culture samples revealed a prevalence of 5.12% (Koizumi & Yasutomi, 2012). In another study conducted in a Nigerian abattoir, a 3.5% seroprevalence was found, with a preference for the *L. hardjo* serovar (Ngbede *et al.* 2012). Furthermore, in Monte Negro, Brazil, the prevalence was 52.8% (1,114/2,109) (Aguiar *et al.* 2006).

At the national level, in the department of Caquetá, the Colombian Cattle Ranching Association (Fedegan, by its acronym in Spanish) analyzed data from several ICA

diseases and determined a prevalence of 46% (Fedegan 2010). In Mexico, a study using the ELISA technique found a seroprevalence of 8.58% (17/198) (Andrade *et al.* 2001). Similarly, in Egypt, a 2022 study using the ELISA technique found a seroprevalence of 39.33% (236/600) (Ibrahim *et al.* 2022).

The TMA technique is traditionally considered the gold standard for diagnosing leptospirosis; however, its use is limited to certain laboratories, which complicates its widespread application (Hartskeerl & Smythe 2014). On the other hand, PCR testing detects the presence of the antigen, and protocols have demonstrated its sensitivity (100%) and specificity (99%), making it a reliable, fast, and accurate method (Hernández–Rodríguez *et al.* 2011).

The technique employed in the present study was ELISA, which does not detect the antigen but instead identifies reactive antibodies to *Leptospira* spp. Therefore, the results do not necessarily indicate that the cattle were diseased at the time of sampling. However, the ELISA technique provides valuable information regarding the effectiveness of preventive and biosecurity measures implemented across different cattle herds. A positive result suggests that these measures were breached at some point, leading to infection.

Culturally, the implementation of rodent control programs is often perceived as necessary when there is a high level of infestation. Rodents, the primary reservoir for *Leptospira* spp., are commonly found in production environments. As such, rodent control programs are typically enacted in response to their presence. These animals act as significant vectors of contamination due to their behavior, such as urinating and deliberately walking in various areas. Consequently, the presence of rodents is closely associated with one of the most important risk factors for disease transmission. According to Garoussi *et al.* (2006), because rodents have direct contact with cattle feed, cattle are particularly susceptible to infection (Garoussi *et al.* 2006).

This research also identifies the storage of bovine concentrate in bins as a risk factor, due to the ease with which rodents can access the feed. The inadequate and insecure storage methods allow rodents to infiltrate the concentrate, facilitating the spread of the pathogen. This finding is consistent with Fávero *et al.* (2017), who reported a positive correlation between rodent access to cattle concentrate and the occurrence of leptospirosis (Fávero *et al.* 2017).

Moreover, medicines are at risk of contamination by *Leptospira* spp. through the urine left behind by rodents as they move around the storage areas. As a result, farmers may believe they are storing medicines appropriately without recognizing the influence of rodents on disease transmission (Mwachui *et al.* 2015). In the present study, this variable is also associated with leptospirosis as a risk factor (OR = 1.96), with statistical significance (p = 0.008).

In the present investigation, the mixing of poultry with cattle was identified as a protective factor (OR = 0.283), as rodent populations may be specifically concentrated near the poultry houses within the herd (Miño *et al.* 2007). Consequently, the separation of livestock and poultry may reduce the risk of leptospirosis contamination in cattle. However, according to Castillo (2014), the mixing of poultry could also pose a risk factor or potential source of infection, as birds may act as vectors by ingesting infected rodents (Castillo Hernández 2014). Additionally,

strains of *Leptospira* spp. that infect cattle have been identified in chickens (Bracken 1955). Despite this, Castillo suggests that birds may help control the disease by preying on rodents, which are the primary reservoir for *Leptospira* spp.

The introduction of external or replacement animals into the herd is considered a risk factor (OR = 1.94), as cattle themselves can act as reservoirs for the disease. This finding aligns with the work of Williams and Windens (2014), who identified the introduction of external cattle as a predisposing factor for the presence of *Leptospira* spp. in herds. Therefore, the concept of a *closed farm*, which enforces strict biosecurity measures to prevent the introduction of external diseases, is essential (Van Schaik *et al.* 2002). In this context, it is also critical to adopt good husbandry practices (GHP), including quarantine protocols and comprehensive diagnostic screening for all diseases prior to the introduction of new cattle.

On the other hand, internal roads were identified as a protective factor. This is due to the organization of cattle herds and their mobility, which reduces contact between cattle and potential reservoirs such as stagnant water (e.g., puddles, floods). Several studies have shown that contaminated water and rainfall are the primary sources of infection in both animals and humans (Barbagelata *et al.* 2013; Romero–Vivas *et al.* 2013). Additionally, water helps maintain the viability of the antigen over extended periods by preventing desiccation (Andre–Fontaine *et al.* 2015).

Weighing animals is a routine practice on farms, and it is common for farmers not to clean the scale thoroughly between weighing different animals. In this study, this lack of cleaning is considered a risk factor for the transmission of leptospirosis, as animals carrying the antigen can contaminate the scale, facilitating the spread of the disease. Furthermore, when animals are weighed, they are typically arranged sequentially before reaching the scale. This practice increases the likelihood of disease transmission among the animals. According to Loureiro and Lilenbaum (2020), *Leptospira* spp. can form biofilms to enhance their survival, and high concentrations of these bacteria may be present in the vaginal discharge of cows, even in asymptomatic individuals (Loureiro & Lilenbaum 2020). In a similar vein, rectal palpation and/or ultrasonography are commonly used to verify estrus. However, if these procedures are not performed properly and proper sanitation measures are not followed, particularly in the herd at the time of the procedure, poor management practices could contribute to disease spread (Barrett *et al.* 2018).

The implementation of quarantine and/or the separation of sick animals was identified as a risk factor (OR=1.64; OR=2.11, respectively) in the present investigation. This is generally associated with poor management practices and a lack of awareness of the disease by the farmers. Tinoco Rangel (2024) reports that a farm located in the department of Cesar has a sanitary plan that includes preventive measures (quarantine guidelines) as well as control measures, which include instructions for the separation of sick animals, particularly for officially controlled diseases such as brucellosis and tuberculosis (Tinoco Rangel 2024). On the other hand, quarantine measures are intended to reduce the spread of diseases between farms (Becker *et al.* 2020). Santos *et al.* (2019) analyzed risk factors associated with leptospirosis in pigs and found that quarantine had no significant effect

(P=0.117) (Santos *et al.* 2019). However, in farms where quarantine was implemented, the disease prevalence was 44.3% (35/79), compared to 54.8% (102/186) in those that did not practice quarantine. In contrast, Valença *et al.* (2013) found that quarantine was a protective factor (OR=0.37) with statistical significance (P=0.004), with prevalence rates of 13.9% and 30.4%, respectively, for farms that did and did not perform quarantine (Valença *et al.* 2013). However, effective quarantine requires obtaining negative results from detection tests, which incurs higher costs and labor, especially when considering the number of animals entering the farm (Van den Brink *et al.* 2023). Similarly, the separation of animals is commonly used as a preventive measure to manage diseases, but it primarily helps improve the signs and symptoms without eliminating the possibility that the animal remains a carrier (Moreira *et al.* 2019).

Finally, to maintain a healthy sanitary environment within the facilities, daily processes are implemented, including the cleaning of objects to be used with the herd, the farm itself, and the respective washing and disinfection of hands. However, statistical analysis reveals these variables as risk factors (OR=3.28; OR=1.74, respectively) with statistical significance. This suggests that the processes associated with these variables are either being performed incorrectly or not at all. For instance, inadequate management of excreta contributes to the spread of diseases, as it serves as a reservoir for *Leptospira* spp. and other pathogens (Contreras–Gómez *et al.* 2017). Additionally, utensils and equipment commonly used in bovine management (e.g., transducers, palpation gloves, intravaginal devices) may not be properly cleaned. Similarly, proper milking practices require hand washing before milking to reduce the risk of bacterial infection (Cerón–Muñoz *et al.* 2015). These findings highlight the need for training in good husbandry practices, with a particular emphasis on adequate hygiene standards for farmers. Although certain variables are expected to prevent disease, they may, in fact, contribute to its occurrence.

CONCLUSIONS

It is crucial to promote and train personnel responsible for herd management to enhance livestock practices, including animal welfare, food quality and safety management, environmental sustainability, and health and disease control, in order to prevent the emergence of diseases such as leptospirosis, which can lead to significant economic losses. The study concluded that the seroprevalence of leptospirosis in the department of Huila is high. However, the primary goal was to identify the key risk factors influencing the dissemination of the pathogen. The presence of cattle in areas frequented by rodents was associated with other risk factors and is considered a major trigger for the disease's onset. Additionally, the design and management of internal pathways are critical for maintaining herd health, as they acted as a protective factor by reducing the likelihood of puddles, which, along with rodents, serve as a reservoir for the disease.

Finally, the factors evaluated in this study are not the only potential triggers; further research is needed to investigate and identify additional risk factors for the occurrence of leptospirosis. Given the lack of alternative solutions for infected individuals and the zoonotic risk posed to public health, prevention remains the most

effective mitigation strategy. In this way, progress can be made toward achieving the goals of the One Health approach, as cattle production is directly linked to human consumption and, therefore, plays a vital role in public health.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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DECLARATION OF USE OF ARTIFICIAL INTELLIGENCE

We declare that no artificial intelligence was used in the preparation of this work.

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Addition of cyclodextrins saturated with cholesterol and its effect on the cooling of goat semen

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ABSTRACT

Caprine spermatozoa exhibit a low cholesterol-to-phospholipid ratio in their plasma membrane, which affects its structure and functionality, as well as sperm survival after the cryopreservation process. The presence and incorporation of cholesterol into the plasma membrane enhances membrane stability, thereby increasing sperm survival. The objective of this study was to evaluate the effect of adding cholesterol-loaded methyl-β-cyclodextrin (CLC) on the viability and membrane integrity of chilled semen. Four Anglo Nubian x Boer crossbred males were used, with 5 ejaculates collected per male, resulting in a total of 20 ejaculates. Each ejaculate was divided into four groups: a control without CLC and three treatment groups (1, 2, and 3 mg of CLC per 120 million spermatozoa). The samples were refrigerated at 5 °C for 24 and 48 hours for evaluation. No statistical differences were observed between the treatment means for semen viability after 24 and 48 hours. However, in treatment 3, a statistically significant decrease in membrane integrity was observed after 24 and 48 hours. It is concluded that, although a biological improvement was observed, it was not statistically significant.

Keywords: reproduction, caprine, spermatozoa, cryopreservation.

Adición de ciclodextrinas saturadas con colesterol y su efecto en la refrigeración de semen caprino

RESUMEN

Los espermatozoides caprinos presentan una baja relación colesterol/fosfolípidos en la membrana plasmática, lo que afecta su estructura y funcionalidad, como también la supervivencia del espermatozoide tras el proceso de criopreservación. La presencia de colesterol y su incorporación a la membrana plasmática aumenta su estabilidad, lo cual incrementa la supervivencia de los espermatozoides. El objetivo de este trabajo fue evaluar el efecto de la adición de metil-β-ciclodextrina cargada de colesterol (CLC) sobre la viabilidad e integridad de membrana del semen refrigerado. Se emplearon 4 machos cruza Anglo Nubian x Boer y se obtuvieron 5 eyaculados por macho, con un total de 20 eyaculados. Cada eyaculado fue dividido en cuatro: control sin CLC y tres tratamientos (1, 2 y 3 mg de CLC/120 millones de espermatozoides). Las muestras fueron refrigeradas a 5 °C durante 24 y 48 horas para su evaluación. No hubo diferencias

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estadísticas entre las medias de los tratamientos para la viabilidad del semen refrigerado luego de 24 y 48 horas. Sin embargo, en el tratamiento 3 se observó una diferencia estadística en la integridad de membrana que disminuyó luego de las 24 y 48 horas. Se concluye que, a pesar de encontrar una mejora desde el punto de vista biológico, no tiene significancia estadística.

Palabras clave: reproducción, caprinos, espermatozoide, crioconservación.

INTRODUCTION

Artificial insemination (AI) is one of the most widely used assisted reproduction techniques worldwide. Due to its numerous benefits, AI has been implemented in breeding and conservation programs for both livestock species of zootechnical interest and wild species. The success of AI outcomes depends on semen quality, as well as processing and preservation methods (Viñán Díaz *et al*. 2019). Semen can be used either fresh or cryopreserved. When inseminating with fresh semen, it must be used shortly after collection, as sperm motility and viability rapidly decline under these conditions due to the accumulation of lactic acid in the ejaculate. Refrigerated semen, however, can be preserved for approximately 48 hours, providing greater flexibility for its use. Regarding storage temperature, numerous studies have been conducted, indicating that semen can be stored at temperatures ranging from 2 to 15 °C. This is based on the rationale that reducing sperm motility and metabolism in a reversible manner extends its fertilizing capacity (Castro Bedriñana *et al.* 2017; Puente *et al.* 2022).

The results described for the viability and fertility of ovine and caprine spermatozoa refrigerated for 5-8 hours are satisfactory, but longer periods (beyond 12-24 hours) show a reduction in fertility (Leboeuf *et al.* 2000; Paulenz *et al.* 2002). Protection of cells against low temperatures is enhanced by the addition of compounds such as egg yolk or skim milk, as they increase resistance to permeability changes and prevent sperm from accumulating calcium due to alterations in membrane exchange systems (Mocé *et al.* 2020). The presence of cholesterol is essential, and its incorporation into plasma membranes prior to refrigeration increases the cholesterol-to-phospholipid ratio, enhancing membrane stability and consequently sperm survival (Ccalta Hancco *et al.* 2022). Improvements in motility, viability, and membrane integrity have been observed when equine, bovine, ovine, and porcine spermatozoa are treated with cholesterol-saturated cyclodextrins prior to cryopreservation (Combes *et al.* 2000; Ferré *et al.* 2018; Moore *et al*. 2005). However, Zahn *et al.* (2002) and Purdy *et al.* (2004) did not observe improvements in motility.

Studies conducted in various animal species have reported improvements in semen quality parameters when spermatozoa were treated with cholesterol-loaded cyclodextrins (CLC), including both methyl-β-cyclodextrin and 2-hydroxypropyl-β-cyclodextrin. These improvements have been observed in donkeys (Álvarez *et al.* 2006), sheep (Castillo Cevallos *et al.* 2019; Mocé *et al.* 2010), and swine (Zeng and Terada 2001; Galantino–Homer *et al.* 2006; Souza *et al.* 2021).

The objective of this study was to evaluate the effect of adding cholesterol-loaded methyl-β-cyclodextrin (CLC) to refrigerated semen and its impact on sperm viability and membrane integrity variables.

MATERIALS AND METHODS

Animals

Four sexually mature Anglo Nubian x Boer (Nubor) crossbred male goats were used for this study. The animals were fed a diet consisting of alfalfa pellets, corn, pasture hay, and had *ad-libitum* access to water. The study was conducted at the experimental facility of the Faculty of Agricultural Sciences at the National University of Lomas de Zamora, located in the Santa Catalina field station in Llavallol, Buenos Aires, Argentina.

Semen collection

Semen was collected using an artificial vagina and a female in estrus. The artificial vagina was maintained at a temperature of 42-45 °C. A total of 5 ejaculates were obtained from each male, amounting to 20 ejaculates in total. Each ejaculate was evaluated macroscopically for volume and color. A 10 µl drop was then placed on a glass slide over a heated stage to assess mass motility using an optical microscope (10X). The semen was subsequently diluted at a 1:10 ratio.

Preparation of cyclodextrin

The preparation of cholesterol-loaded cyclodextrin (CLC) followed the technique described by Purdy and Graham (2004). A total of 200 mg of cholesterol (Sigma) was dissolved in 1 ml of chloroform. Separately, 1 g of methyl-β-cyclodextrin (Sigma) was dissolved in 2 ml of methanol in a glass tube.

A 0.45 ml aliquot of the cholesterol solution was added to the cyclodextrin solution, and the mixture was stirred until homogenized into a clear solution, which was then poured into a glass Petri dish. The dish was placed in a fume hood at room temperature for 24 hours, allowing the solution to crystallize. The resulting crystals were removed from the dish, stored, and kept in a glass container with an airtight lid at room temperature.

Treatments

The ejaculates were centrifuged at 1,500 rpm for 5 minutes at room temperature. The supernatant was then removed, and the sperm pellet was resuspended in Tris-citric acid-fructose (TCF) diluent, restoring it to the initial volume. Afterward, 20% egg yolk was added. From each sample, one control (Semen + TCF) and three treatments were prepared: 1- (Semen + TCF + 1 mg of CLC per 120 million spermatozoa), 2- (Semen + TCF + 2 mg of CLC per 120 million spermatozoa), and 3- (Semen + TCF + 3 mg of CLC per 120 million spermatozoa).

The semen was evaluated immediately after collection and after storage at 5 °C for 24 and 48 hours. Viability was assessed using the 5% Eosin-Nigrosin staining technique. A 10 µl aliquot of diluted semen was mixed with 10 µl of the stain solution, homogenized, and a smear was prepared. The smear was air-dried at room temperature before being examined under an optical microscope (100x). Membrane integrity was assessed using the hypoosmotic swelling (HOS) test (Van der Ven *et al.* 1986), which involved mixing 10 µl of diluted semen with 490 µl of a hypoosmotic solution containing fructose and sodium citrate. The mixture was incubated at 36 °C in a water bath for 30 minutes. A 10 µl aliquot of the solution was then placed on a glass slide and observed under an optical microscope (40x).

Ethics for animal experimentation

All ethical requirements of the institution where the study was conducted were followed, as outlined by the Comité Institucional para el Cuidado y Uso de Animales de Experimentación (Cicuae, for its acronym in Spanish) under Resolution CAA/123 Expte. A/22839/2017.

Statistical analysis

Statistical analysis was performed using Infostat software (Di Rienzo *et al.* 2020). An analysis of variance (Anova) for a completely randomized design (CRD) was conducted for a fixed effects model. The assumption of normality was tested using the Shapiro–Wilk test, and the assumption of homogeneity was tested using Levene's test. Subsequently, mean comparisons were made using the Duncan's Multiple Range Test (DGC), with a significance level set at 5%.

RESULTS

After refrigeration for 24 hours at 5 °C, there was no significant variation among the three treatments in terms of the percentage of live spermatozoa compared to the control, which exhibited the highest percentage (76.69%). After 48 hours of refrigeration, no significant differences were observed among the three treatments compared to the control group. However, the treatment with 1 mg of CLC showed a higher percentage of live spermatozoa (73.69%) (table 1).

Regarding membrane integrity data, at the 24-hour assessment, a significant difference was observed between the control and treatment 3, which had the lowest percentage (35.23%). Treatments 1 and 2 with CLC did not show significant differences from the control group, which had the highest percentage (63.69%) (table 1).

TABLE 1. Percentage of live sperm and integrity of the plasma membrane, after refrigeration at 5 °C for 24 and 48 h with 0 (control), 1, 2, and 3 mg of CLC

Treatment	Time (hours)	Percentage of live sperm	Percentage of sperm with plasma membrane integrity
Extender with 0 mg CLC	24	76.69 ±12.09 [a]	63.69 ± 7.43 [a]
	48	70.31 ±10.42 [a]	58.54 ± 6.46 [a]
Extender with 1 mg CLC	24	73.23 ± 9.58 [a]	61.69 ± 5.96 [a]
	48	73.69 ±10.98 [a]	58.62 ± 6.98 [a]
Extender with 2 mg CLC	24	72.69 ±11.56 [a]	60.08 ± 7.02 [a]
	48	70.00 ±11.78 [a]	56.31 ± 7.42 [a]
Extender with 3 mg CLC	24	67.85 ±11.64 [a]	35.23 ± 7.62 [b]
	48	64.38 ± 12.06 [a]	51.85 ± 7.55 [b]

Values represent the mean ± SD (n = 20). Treatments with different letters are significantly different from each other ($p > 0.05$).

Source: own elaboration.

Data observed after 48 hours of refrigeration indicate that treatment 3 with CLC had a lower percentage (51.85%), showing significant differences compared to the other treatments. There were no significant differences between treatments 1 and 2 with CLC and the control group; however, treatment with 1 mg of CLC (58.62%) exhibited the highest percentage of membrane integrity (table 1).

DISCUSSION

During cryopreservation, spermatozoa undergo intracellular and extracellular changes that lead to a reorganization of membrane lipids and proteins, as well as osmotic changes, which can damage sperm membranes and result in cell death (Purdy & Graham 2004).

The understanding of the relationship between phospholipids and cholesterol in sperm membranes and their resistance to thermal shock is not recent. Darin–Bennet and White (1977) and Parks and Lynch (1992) established a correlation between the ratio of polyunsaturated to saturated fatty acids in sperm phospholipids and the role of cholesterol in cold tolerance. These observations have spurred research into the amount of cholesterol in spermatozoa and its correlation with sensitivity to thermal decline. The ratio of phospholipids to cholesterol in membranes varies among species. Darin–Bennet and White (1977) and Purdy and Graham (2004) observed differences in the cholesterol-to-phospholipid ratio between species, noting that species with a lower ratio, such as boars (0.26), stallions (0.36), rams (0.38), bulls (0.45), or bucks (0.59), were more susceptible to cryopreservation compared to species with a higher ratio, close to 1, such as rabbits (0.88) or humans (0.99).

The addition of cholesterol improves the survival rate during cryopreservation. Its incorporation into the sperm membrane is facilitated by cyclodextrins, with β-cyclodextrin showing the highest affinity for cholesterol, as demonstrated by Ccalta Hancco *et al.* (2022) in alpacas, using concentrations of 0, 1.5, and 3 mg of cholesterol.

Castillo Cevallos *et al.* (2019) refrigerated and froze ram semen with 1 and 2 mg of cholesterol-loaded cyclodextrin (CLC), finding no differences between treatments after refrigeration. However, differences were observed after freezing, with better results obtained using 2 mg of CLC. These results are consistent with those of Moraes *et al.* (2010) using bull semen, in contrast to the findings of Purdy *et al.* (2010), who did not observe significant differences.

Ferré *et al.* (2018) demonstrated that the process of sexing semen in cattle could exacerbate the detrimental effects of thermal decline on membranes. These authors conducted an experiment involving the stabilization of plasma membranes in sexed spermatozoa by adding cholesterol-loaded cyclodextrin (CLC) at concentrations of 1.5, 3, and 6 mg, followed by freezing. They observed that spermatozoa treated with 3 mg of CLC exhibited higher motility and vigor.

In goats, according to the results of Salmón *et al.* (2016), to test whether treatment with cholesterol-saturated cyclodextrins protects motility and membrane integrity, a study was conducted where fresh semen was treated with 1, 3, and 6 mg of CLC prior to freezing. After thermal decline, the proportions of motile and viable

spermatozoa with intact acrosomes were twice as high as in the control group. This beneficial effect of CLC on cold resistance was evident at all three concentrations of CLC evaluated, with no differences observed between these treatments.

According to our results, with the concentration of 1 mg of CLC, an increase in the evaluated semen quality parameters was observed after refrigeration. Conversely, at a concentration of 3 mg, the evaluated parameters decreased compared to the control group.

The differing results observed in various studies may be attributed to the species of animal or to different individuals within a species who may respond differently to cyclodextrins. Factors contributing to this variability in results include the use of different diluents and cryopreservation protocols. For example, Ccalta Hancco *et al.* (2022) used concentrations of 0, 1.5, and 3 mg of CLC in alpacas, with a tris-based diluent, egg yolk, and dimethylformamide, and performed a freezing curve over 2 hours and 45 minutes. In contrast, Castillo Cevallos *et al.* (2019) used concentrations of 0, 1, and 2 mg of CLC in ram semen, with a freezing procedure lasting 20 hours.

CONCLUSION

It is concluded that refrigeration at 5 °C for 24 hours of goat semen with the addition of CLC at concentrations of 1, 2, and 3 mg does not show a significant difference in viability compared to the control group, with the control group exhibiting the highest results. Regarding plasma membrane integrity, the 3 mg concentration significantly yielded lower results.

After 48 hours, no significant differences in viability were observed among the concentrations of 1, 2, and 3 mg compared to the control group. However, for plasma membrane integrity, the 3 mg concentration again showed significantly lower results.

Although no statistical differences were found among the treatments, refrigeration of semen at 5 °C for 24 or 48 hours provides greater flexibility for use in artificial insemination programs.

CONFLICT OF INTEREST

The authors declare no commercial or personal conflicts of interest related to this research.

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DECLARATION OF USE OF ARTIFICIAL INTELLIGENCE

The authors declare that no artificial intelligence tools were used during the development of the research or the preparation of the article resulting from this research.

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Endodontic treatment of a dental fracture with pulp exposure of the right upper fourth premolar in a canine: case report

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ABSTRACT

Dental fractures represent a specific set of injuries affecting the tooth and/or its supporting structures. In veterinary dentistry, crown fractures with pulp chamber exposure are among the most common endodontic injuries. While tooth extraction is one possible treatment for these cases, root canal therapy offers a more specialized and less invasive alternative, particularly suitable for horizontal supragingival tooth fractures. This report presents the case of a 5-year-old male Cavalier King Charles Spaniel, neutered, evaluated and treated by the Amico Dental veterinary team in Brazil. During the clinical examination, mild gingivitis and a dental fracture involving enamel, dentin, and pulp of tooth 108 were observed. Radiographic examination revealed signs of periapical bone lysis, suggesting a localized infection. This case emphasizes the significance of dental fractures and presents endodontic treatment through root canal obturation as an alternative to tooth extraction in cases of pulp exposure.

Keywords: pulp exposure, root canal, treatment**,** dental fracture.

Tratamiento endodóntico en fractura dentaria con exposición pulpar de cuarto premolar superior derecho en un canino: reporte de caso

RESUMEN

Las fracturas dentales son un conjunto de lesiones específicas que afectan al diente y/o a sus estructuras de soporte. En la odontología veterinaria, la fractura de la corona dental con exposición de la cámara pulpar es una de las lesiones endodónticas más comunes. Aunque la extracción dental es uno de los tratamientos que se puede utilizar para estos casos, el tratamiento del canal radicular es un procedimiento más especializado y menos invasivo, por lo cual es una alternativa viable para fracturas supragingivales horizontales del diente. Este trabajo reporta el caso de un canino macho de raza Cavalier King Charles

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Spaniel, castrado y de 5 años, evaluado y tratado por el equipo veterinario Amico Dental en Brasil. Durante el examen clínico, se observó gingivitis leve y una fractura dental que afectaba el esmalte, la dentina y la pulpa en el diente 108. El examen radiográfico mostró indicios de lisis ósea periapical, lo que sugirió una posible infección localizada. Este caso subraya la importancia de las fracturas dentales y presenta el tratamiento endodóntico mediante la restauración radicular con la técnica de obturación del canal pulpar como una opción frente a la extracción dental en casos de exposición pulpar.
Palabras clave: canal radicular, exposición pulpar, fractura dental, tratamiento.

INTRODUCTION

Dental fractures in canine species, commonly referred to as traumatic dentoalveolar injuries (TDI), are defined, according to Soukup *et al.* (2015), as a specific set of injuries affecting the tooth (either the crown or root) and/or its supporting structures, such as the periodontal ligament and alveolar bone, due to direct traumatic force. The classification system for dental fractures used in many veterinary studies is based on a human classification system that identifies 14 types of dentoalveolar injuries and categorizes dental fractures according to the tissues affected (Soukup *et al.* 2015). Within this framework, crown fractures with pulp chamber exposure or enamel-dentin-pulp fractures are the most common endodontic injuries in veterinary dentistry (Jucan *et al.* 2023).

Tooth extraction is recommended in cases of vertical or subgingival root fractures, while root canal treatment and restoration are indicated for horizontal supragingival fractures (Bladowski *et al.* 2013). It is worth noting that this classification applies when the affected part is above the gumline, potentially involving both the tooth crown and supporting structures. Although dental extraction is an option for teeth affected by endodontic disease, a more specialized and less invasive procedure, such as endodontic therapy, offers an alternative for preserving the structure and functionality of the tooth (Carvalho *et al.* 2024).

Endodontic treatment encompasses procedures designed to remove damaged or infected dental pulp and seal the tooth's canals. This approach helps prevent infections and maintains the health of the tooth and alveolar bone. In applying this technique to animals, well-defined procedural principles have been developed, primarily by adapting endodontic methods from human dentistry to the specific anatomy of canine and feline teeth (Girard *et al.* 2006).

Over the years, studies have been conducted on different types of dog teeth to assess the success rate of therapy based on the number of roots treated (Adrian *et al.* 2022; Strom *et al.* 2018). Some of these studies indicate that, due to the more complex morphology of multirooted teeth, outcomes tend to be less favorable. However, in dogs specifically, various studies have shown that root canal therapy is consistently successful (Adrian *et al.* 2022; Kuntsi–Vaattovaara *et al.* 2002). When strict criteria were applied, the success rate ranged from 69% to 71%. In contrast, when *flexible criteria* (a combination of success and absence of evidence of failure) were used, higher success rates were observed, reaching between 95% and 96% (Jucan *et al.* 2023).

This case report aims to highlight the significance of pulp disease and presents an alternative to tooth extraction in cases of dental fractures with pulp canal exposure, detailing the technique and feasibility of endodontic obturation.

CASE DESCRIPTION

Anamnesis

A 5-year-old neutered male Cavalier King Charles Spaniel weighing 10.4 kg was presented at an authorized veterinary clinic in the region. After an initial evaluation, the dog was referred to the Amico Dentale team in São Paulo, Brazil. According to the owner, the dog had sustained a dental fracture over a year prior without receiving treatment. The owner reported no symptoms of dysphagia or behavioral changes, suggesting that the fracture might have resulted from the dog's habit of biting the door.

Clinical examination findings

A general physical examination indicated that all physiological parameters were within normal limits. However, the examination revealed mild gingivitis and a supragingival horizontal dental fracture with pulp exposure in tooth 108 (figure 1), identified according to the modified Triadan system (Floyd 1991).

Diagnostic aids

For preoperative assessments, a complete blood count, chest radiograph, Doppler echocardiogram, and electrocardiogram were requested, all of which showed no relevant clinical abnormalities. Subsequently, the team performed a comprehensive radiographic study of the oral cavity using intraoral projections of the entire dental arch. The primary objective was to detect any periodontal bone defects in both the affected tooth and the apparently healthy teeth. Two radiographic techniques were employed: the parallel technique

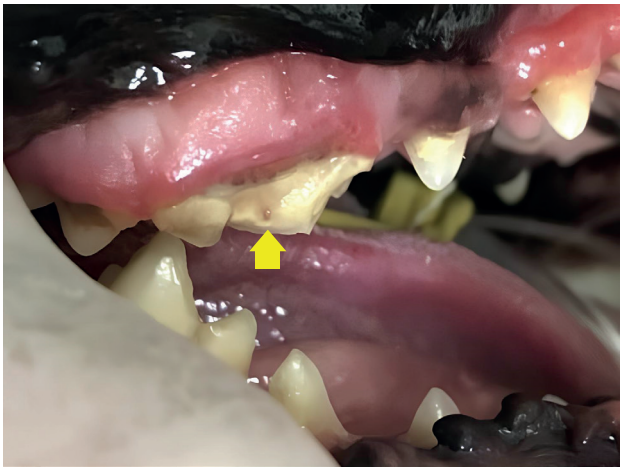

FIGURE 1. Right maxillary fourth premolar (108) with crown–root fracture, showing pulp exposure (yellow arrow).

Source: own elaboration.

(P technique), where the film is positioned parallel to the teeth and the X-ray beam is directed perpendicular to both the tooth planes and the film, forming a 90° angle; and the bisecting angle technique (B technique), where the radiographic film is placed perpendicular to the tooth (90° to the tooth plane). The X-ray beam is then directed towards the film at a 45° angle, bisecting the angle between the film and the tooth (Carvalho *et al.* 2019).

These two comprehensive radiographic techniques allow visualization of the teeth and surrounding structures in both humans (Yen & Yeung 2023) and in dogs and cats (Hennet & Girard 2005; Nepomuceno *et al.* 2013; Lobprise & Dodd 2019). In tooth 108, a chip fracture of the dental crown with pulp exposure was observed, classified as a complicated enamel-dentin fracture with pulp involvement according to Soukup *et al.* (2015). Periapical bone lysis was also noted (figure 2). No other abnormalities were identified in the remaining teeth.

Based on radiographic findings and a detailed oral examination, a diagnosis of dental fracture with pulp exposure in the right maxillary fourth premolar (modified Triadan 108) was made, and endodontic obturation of the three roots was chosen as the optimal treatment option. This approach aimed to eliminate or significantly reduce the bacterial population in the non-vital pulp and to maintain or restore the health of the adjacent periodontal tissues.

Clinical procedure

On the day of the procedure, the patient underwent an 8-hour food fasting and a 2-hour liquid restriction. The dog was alert, with no signs of pain or discomfort,

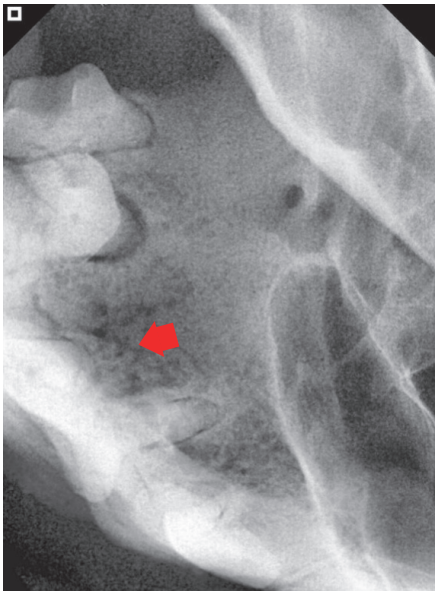

FIGURE 2. Right maxillary fourth premolar dental radiograph, using the bisecting angle technique. Periapical lysis of the alveolar bone is evident (red arrow).

Source: own elaboration.

showed cranial symmetry, and had normal submandibular lymph nodes upon palpation. During the specific oral examination (without sedation), the gingival mucosa appeared normocolored and hydrated. However, the patient exhibited grade II dental calculus across the dental arch, as classified by Lobprise and Dodd (2019), along with mild gingivitis in the premolars and molars, moderate halitosis, and pulp exposure in tooth 108.

The patient was taken to the operating room, where preanesthetic medication was administered with acepromazine at 0.02 mg/kg and methadone at 0.15 mg/kg, both intramuscularly. Next, the middle third of the right forearm was shaved, and the area was antiseptically prepared with gauze soaked in 70% alcohol. A 22-gauge catheter was inserted and secured in the cephalic vein for venous access. For anesthetic induction, propofol at 2 mg/kg combined with ketamine at 1 mg/kg was administered, followed by maintenance with inhaled isoflurane at 1.5%.

Surgical technique

The treatment followed the global dental guidelines of the World Small Animal Veterinary Association, as described by Niemiec *et al.* (2020). First, dental scaling (both mechanical and manual) was performed on all dental arches using an ultrasonic scaler. Next, coronal polishing was completed using a dental brush and prophylactic paste on a low-speed handpiece. The entire oral cavity was then rinsed with pressurized water using a three-way air-water syringe. Finally, topical antisepsis was applied with 0.12% chlorhexidine, and a local anesthetic block was performed using 2% lidocaine. To block the maxillary nerve, a 24-gauge needle was inserted into the pterygopalatine fossa, and 0.3 ml of 2% lidocaine was injected at a dose of 5 mg/kg.

The upper fourth premolar has three dental roots: mesial, palatal, and distal. To facilitate the procedure given the tooth's anatomy, endodontic access was made parallel to the long axis of the distal root of tooth 108. A 0.12% chlorhexidine spray was used to reduce contamination, and diamond-tipped burs attached to a high-speed motor were used for access, following the recommendations of Hennet and Girard (2005).

In line with the methods outlined in the literature (Lobprise & Dodd 2019; Girard *et al.* 2006; Hennet & Girard 2005), the pulp chamber was first aseptically cleaned with a 1% sodium hypochlorite solution due to its effectiveness in dissolving organic matter; this solution can be supplemented with coadjuvant solutions like Endo-PTC (León *et al.* 2003). For root canal exploration, a Hedstroem® endodontic file (H) number 15 was inserted through the access site to measure the distance between the entry point and the apex of the root (working length determination). An additional radiographic projection was taken using the bisecting angle technique (figure 4). The apparent tooth length, which measured 21 mm, was determined using a millimeter-calibrated endodontic ruler.

Before obturation, the canal was instrumented to enlarge the cervical portion, facilitating improved irrigation and disinfection of the area. The WaveOne Gold® reciprocating file system (Dentsply-Maillefer, Ballaigues, Switzerland) was used as described by Freitas *et al.* (2018) to remove debris, shape, and smooth the dentinal walls of the root canal through alternating clockwise and counterclockwise partial rotations. Initially, the WaveOne Gold Glider® file (#15.02) was used for

preliminary canal enlargement, followed by the WaveOne Gold Small® file (#20.07) for further shaping and dentin debris removal, and concluding with the WaveOne Gold Primary® file (#25.07) (figure 3). After each instrument change, the canal was irrigated with 1% sodium hypochlorite and dried with an absorbent paper point.

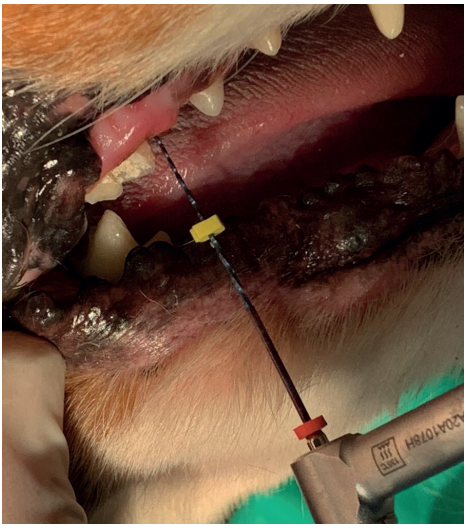

FIGURE 3. Distal root canal instrumentation of tooth 108 using WaveOne Gold Primary® reciprocating file system (25.07) coupled to a high-speed air turbine.

Source: own elaboration.

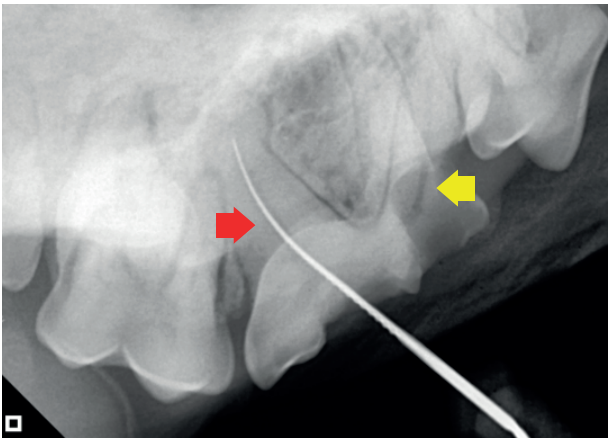

FIGURE 4. Tooth 108 dental radiograph, using the bisecting angle technique. The image shows the Hedstroem #15 endodontic file in the distal root canal (red arrow); the widening of the mesial root canal is also seen (yellow arrow).

Source: own elaboration.

A polymer-based filling cone (number 25 gutta-percha) was then inserted into the root canal, and an additional radiographic examination was performed to confirm that the cone had reached the actual working length. After verifying the filling of the root canal with gutta-percha, the sealing cement was placed in the canal alongside the gutta-percha cone. Upon confirming proper obturation, the canal was filled with a zinc oxide-eugenol-based restorative cement, and the gutta-percha was trimmed with a curette, applying pressure to the apical region. A vertical condenser was then used to compact the gutta-percha within the root canal.

The same technique was applied to access the pulp chamber, perform antisepsis, measure working length and fill the root canal with gutta-percha and dental cement in the mesial and palatal roots of tooth 108. An additional radiographic projection was taken using the bisecting angle technique (figure 5). To complete the procedure, a light-cured composite resin was applied using ultraviolet light (figure 6), and the oral cavity was thoroughly cleaned to remove any residual material, concluding the filling and restoration treatment (figure 7). A bolus of metronidazole at 15 mg/kg IV was administered, and the animal was monitored until recovery from anesthesia.

Following the procedure, the patient was admitted for monitoring and discharged the same day, with a follow-up appointment scheduled for 15 days later. The patient was prescribed dipyrone at 25 mg/kg every 8 hours for 5 days and tramadol hydrochloride at 4 mg/kg every 8 hours for 5 days due to the synergistic effect of these two medications. Additionally, a 0.12% chlorhexidine mouthwash spray was recommended twice daily for 7 days.

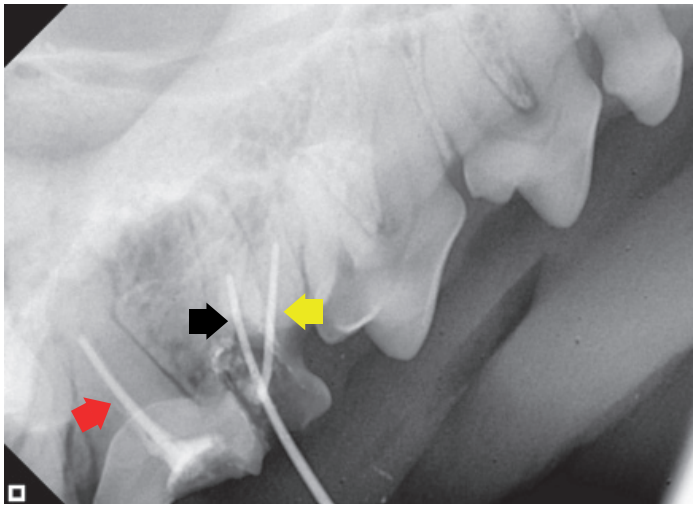

FIGURE 5. Tooth 108 dental radiograph, using the Clark technique, showing the completed filling of the mesial (yellow arrow) and distal roots (red arrow), as well as the filling of the palatal root canal with 21mm gutta-percha (black arrow).

Source: own elaboration.

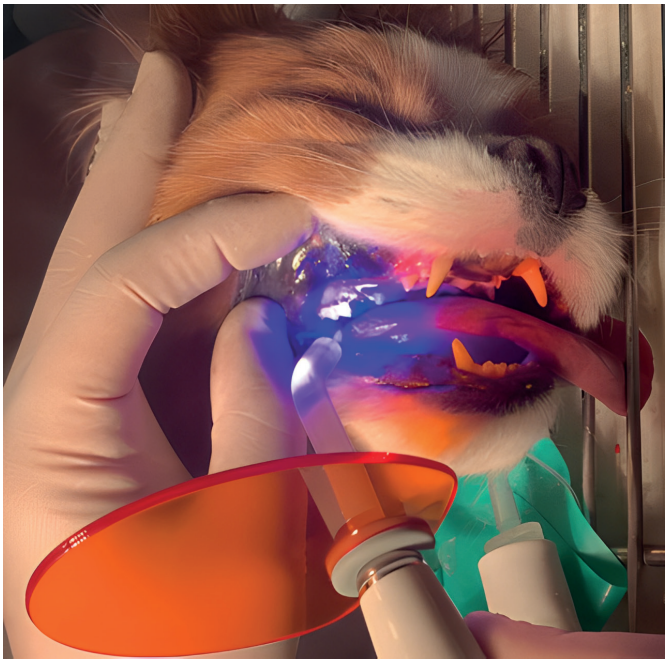

FIGURE 6. Photopolymerization of composite resin (restoration) with ultraviolet light.

Source: own elaboration.

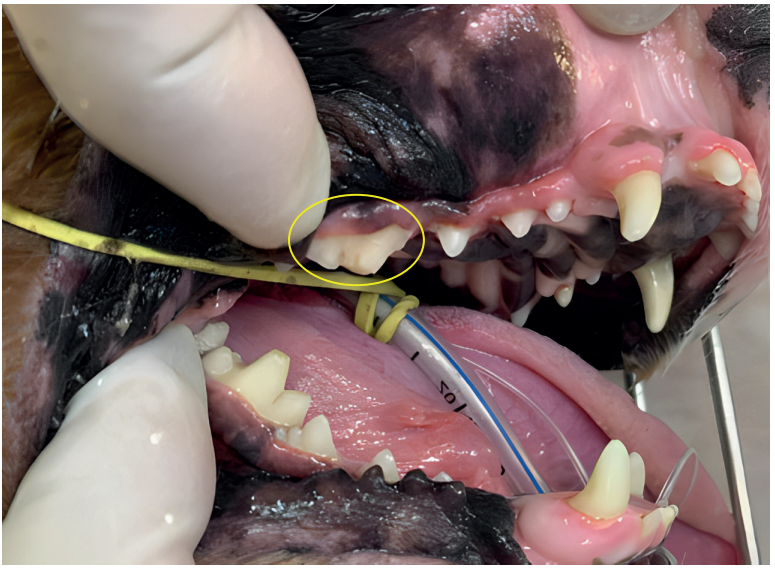

FIGURE 7. Tooth 108 post-restoration (yellow circle).

Source: own elaboration.

DISCUSSION

Dental fractures with pulp exposure provide a direct route for bacteria to invade the tooth's internal tissues, resulting in pain, inflammation, contamination, and infection, leading to endodontic disease (Campbell *et al.* 2016). Endodontic disease can progress to more severe pulp necrosis, potentially causing systemic complications in some cases (Jucan *et al.* 2023; Lobprise & Dodd 2019). In this case, the patient exhibited no signs of systemic infection; however, mild gingivitis was observed around the fractured tooth, and radiographs indicated localized bone lysis in the alveolar bone of the affected tooth, consistent with a localized infection.

According to Souza *et al.* (2018), one of the most common causes of endodontic injury in veterinary dentistry is the habit of chewing hard objects, as seen in this case. Unfortunately, animals with endodontic disease often suffer for extended periods before diagnosis and definitive treatment. Niemiec (2009) notes that most endodontic cases remain undiagnosed due to the absence of external signs other than a fractured or discolored tooth, as dogs and cats rarely display overt signs of oral pain or disease progression.

The diagnosis of dental fractures is crucial and begins with a thorough patient history, physical examination, and intraoral radiographs. It is essential to document all teeth radiographically for an accurate diagnosis of oral lesions; as this procedure requires general anesthesia, it should be properly integrated into dental treatments (Carvalho *et al.* 2019). Radiographic examination played a key role in this case, as it not only revealed typical endodontic disease findings such as an enlarged pulp chamber, periapical lysis, and crown fracture (Campbell *et al.* 2016), but also effectively guided the precise execution at each stage of the protocol, as described by Hennet and Girard (2005). This ensured correct instrumentation within the tooth during the operative phase and facilitated proper canal obturation, which was instrumental in achieving the high quality of the final procedural outcome.

The use of reciprocating instrumentation has become an advanced and effective solution for root canal preparation in endodontics. This method is notable for the use of a single disposable instrument, leading to reduced working time, decreased instrument requirements, and increased procedural safety (Freitas *et al.* 2018). Although these instruments are designed for single use, it is common for alternative files to be used to prepare multiple canals within the same tooth. This practice is especially prevalent in molars with three or four root canals, which present challenges due to the complexity of their anatomy (Kirchhoff *et al.* 2018).

The versatility of reciprocating instrumentation allows dental professionals to address difficult cases more effectively. However, it is crucial to follow recommended usage guidelines to ensure treatment quality and patient health. In summary, reciprocating instrumentation not only optimizes the endodontic process but also promotes a safer and more efficient approach in clinical practice.

The success of endodontic treatment largely depends on the thorough cleaning and obturation of the root canals (Tandir *et al.* 2024). However, the surgeon's skills, knowledge, and experience are indispensable. This treatment is performed in veterinary dentistry in an attempt to maintain periodontal and endodontic health in strategically important teeth

affected by pulp necrosis. The goal is to remove the infected or necrotic pulp while shaping, cleaning, and disinfecting the root canal, followed by obturation and restoration (Jucan *et al.* 2023).

The authors suggest that endodontic therapy should be initially considered for class IV dental fractures, a technique that has been extensively studied in recent years (Mareschi *et al.* 2020; Adrian *et al.* 2022; Lee *et al.* 2022). On the other hand, although Hennet and Girard (2005) recommend the use of prophylactic antimicrobial therapy prior to endodontic surgery, it was only administered during the intraoperative period in this case to reduce post-procedural bacteremia. According to Siqueira (2002), there is evidence that the infection site is not affected by systemic antibiotics, as they fail to reach and eliminate microorganisms in the root canals due to the lack of blood circulation in the exposed pulps. For this reason, antibiotic therapy was not used, and instead, the use of 0.12% chlorhexidine was prescribed postoperatively. In accordance with the study by Michelotto *et al.* (2008), 0.12% chlorhexidine was effective against both aerobic and strict anaerobic bacteria in the oral cavity.

It is worth noting that nonsteroidal anti-inflammatory drugs (NSAID) are recommended for both pain and inflammation in endodontic treatments (Hennet & Girard 2005). However, in this case, they were not prescribed. It would be interesting to use them in the postoperative phase to help maintain the viability of the tooth and its supporting and protective structures (gingiva, alveolar bone, and periodontal ligaments).

A significant limitation that persists across all areas of veterinary practice is that pet owners often fail to return for follow-up visits, which hinders the ability to assess medium- and long-term progress, as was the case in this study. Authors such as Jucan *et al.* (2023) recommend conducting radiographic follow-ups at least once a year for patients receiving endodontic treatment.

CONCLUSIONS

Radiographic studies are essential and fundamental in the diagnosis and treatment of dental diseases. However, intensive training is required for veterinarians to acquire and interpret radiographic projections clearly and efficiently to reach an accurate diagnosis. The root canal obturation technique described in this report followed the steps and recommendations outlined in the literature, resulting in a successful outcome. Therefore, it represents an excellent alternative to dental extraction. However, regular dental check-ups and follow-up care every six months are crucial to ensure favorable long-term outcomes.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

FUNDING SOURCES

The surgical procedure was funded by the owner.

USE OF ARTIFICIAL INTELLIGENCE

The authors declare that no artificial intelligence was used in this study.

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Marsupialization of lacrimal granuloma on the third eyelid in a canine. Case report

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ABSTRACT

The aim of this study was to describe the formation of a granuloma as a surgical complication following repositioning of the third eyelid lacrimal gland and the success of its surgical treatment using marsupialization in a dog. A three-month-old male French Bulldog was presented at the Uningá Veterinary Clinic with a primary complaint of a nodule in the lower medial region of the right eye. This medical complication developed after a previous surgical procedure by another veterinarian to reposition the third eyelid lacrimal gland. Suspecting a granuloma, cyst, or neoplastic growth, the animal underwent a marsupialization procedure, during which a fragment of the nodule wall was collected for histopathological analysis, which confirmed the diagnosis of a granuloma. Nine months of follow-up post-surgery showed complete recovery without complications. This case concluded that histopathological analysis is essential for accurate differential diagnosis of a lacrimal granuloma. Furthermore, careful burial of the third eyelid gland is recommended as an important measure to prevent granuloma formation. Finally, the study demonstrates that marsupialization can yield excellent results without compromising lacrimal function.

Keywords: canine, marsupialization, ophthalmology, surgery.

Marsupialización de granuloma lacrimal en el tercer parpado en un canino. Reporte de caso

RESUMEN

El objetivo de este estudio fue describir la formación de un granuloma como intercurrencia quirúrgica del reposicionamiento de la glándula lagrimal del tercer párpado y el éxito de su tratamiento quirúrgico por la técnica de marsupialización en un perro. Un Bulldog francés, macho, de tres meses de edad, fue atendido en la Clínica Veterinaria Uningá con la principal queja de la presencia de un nódulo en la región medial inferior del ojo derecho. Esta complicación médica se generó después de que otro profesional

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veterinario había reposicionado la glándula lagrimal del tercer párpado. Ante la sospecha de granuloma, quiste o neoformación tumoral, el animal fue sometido a un procedimiento quirúrgico de marsupialización, donde se recuperó un fragmento de la pared del nódulo para un análisis histopatológico que confirmó el diagnostico de granuloma. Se realizó seguimiento del caso nueve meses después del procedimiento quirúrgico y el resultado obtenido mostró una recuperación total del paciente sin ningún tipo de complicaciones. Se concluyó en este caso que la realización de un análisis histopatológico es fundamental para un diagnóstico diferencial acertado del granuloma lagrimal. Adicionalmente, se resalta que es muy importante la realización de una técnica cuidadosa de entierro de la glándula del tercer párpado con el fin de prevenir la formación de granulomas. Finalmente, se demuestra que la técnica de marsupialización puede aportar excelentes resultados sin comprometer la función lagrimal.

Palabras clave: canino, cirugía, marsupialización, oftalmología.

INTRODUCTION

The third eyelid, or nictitating membrane, is an ocular structure that functions to protect the cornea. Located within the nasal portion of the inferior conjunctival sac between the cornea and the lower eyelid, this structure is mobile (Peruccio 2018). In dogs, the third eyelid contains a cartilaginous component referred to as the "T" of hyaline cartilage, which provides rigidity to the membrane. At the base of the third eyelid lies the nictitating membrane gland, responsible for producing approximately 30% of the aqueous portion of the tear film (Wouk *et al.* 2009).

Gland prolapse is one of the most frequently reported conditions affecting the third eyelid in dogs (Barbé *et al.* 2016). Breeds most affected include Bulldogs, followed by Lhasa Apsos and, lastly, Shih Tzus (Queiroz *et al.* 2015). The etiology of third eyelid gland prolapse remains unclear, though one cause may involve laxity in the connective tissue that anchors the gland to the periorbital tissues (Barbé *et al.* 2016).

Surgical repositioning of the gland is considered the most effective treatment. Various techniques have been described for gland burial, which either anchor the gland to the periorbital periosteum and the base of the cartilaginous "T" or involve creating a conjunctival pocket to reposition and stabilize the gland in its anatomical location (Swanson & Hermann 2005).

The etiology of lacrimal cyst formation is primarily attributed to iatrogenic damage resulting from gland repositioning procedures; however, its occurrence is rare and may affect any area containing tear-producing glandular tissue (Lima *et al.* 2020). Given that one potential cause of lacrimal granuloma development in this case could be associated with prior surgical repositioning of the third eyelid gland, the objective of this study was to alert veterinarians to the possibility of granuloma formation as a surgical complication of lacrimal gland repositioning. Additionally, this study aimed to demonstrate a definitive diagnosis through histopathological analysis and, finally, to report the successful outcome achieved with surgical treatment using the marsupialization technique.

CASE REPORT PRESENTATION

Patient overview and reason for consultation

A three-month-old male French Bulldog, weighing four kilograms, was presented at the Uningá Veterinary Clinic by his owner with the primary complaint of a nodule in the lower region of the right eye. This nodule appeared following a procedure performed by a previous veterinarian to reposition the third eyelid gland.

Anamnesis and physical examination

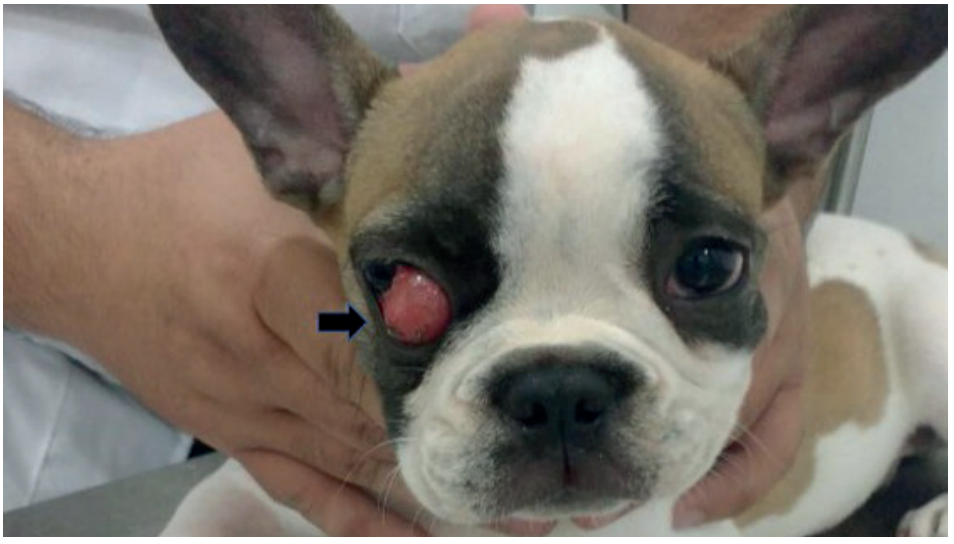
The owner reported that, after the appearance of the nodule, the animal had been taken back to the original veterinarian, who performed a puncture and complete drainage of the nodule, during which a transparent fluid was identified, according to the owner. While the drainage initially resolved the nodule, it recurred a few days later.

On general physical examination, all parameters were within the normal range for the species. Ophthalmic examination revealed a unilateral, pinkish, translucent nodule with a soft, painless consistency located in the lower medial region of the eye near the third eyelid (figure 1).

Initial clinical suspicions and surgical approach

Based on the information provided by the owner and the clinical findings, initial suspicions included lacrimal granuloma, conjunctival cyst, and less likely, neoplastic growth of the third eyelid. The owner was informed of the potential risk of dry keratoconjunctivitis resulting from excision of the third eyelid. Therefore, marsupialization of the palpebral conjunctiva of the third eyelid was chosen, along with an incisional biopsy for histopathological analysis to achieve a definitive diagnosis while preserving the natural

FIGURE 1. Photographic image of a three-month-old French Bulldog showing a unilateral pink nodule indicative of a lacrimal granuloma (arrow).



Source: own elaboration.

lacrimal function of the third eyelid gland. Complete blood count, serum alanine aminotransferase (ALT), and creatinine levels were within normal limits. The patient was subsequently prepared for surgery. Anesthetic induction was achieved with Propofol (4 mg/kg) and Ketamine (2 mg/kg) administered intravenously, with anesthetic maintenance via inhaled isoflurane.

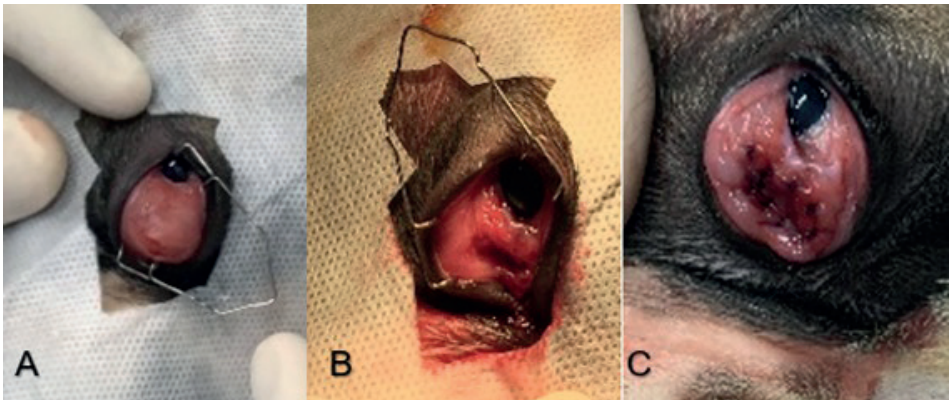
The surgical site was thoroughly aseptic-ated with a 10% povidone-iodine solution diluted in 0.9% NaCl (1:100). Sterile drapes were positioned, and a blepharostat was used to keep the eyelids open.

The procedure involved a full-thickness elliptical incision, approximately 1.0 cm in horizontal orientation, through the granuloma wall (palpebral conjunctiva of the third eyelid). This incision allowed for drainage of the contents, which appeared clear, suggesting a lacrimal origin. Following drainage, a simple interrupted suture was placed between the inner wall of the granuloma and the external surface of the palpebral conjunctiva of the nictitating membrane, thus creating a marsupialization. A 5-0 synthetic absorbable suture (polyglycaprone) was used (figure 2). The elliptical fragment excised from the cyst wall was submitted for histopathological analysis.

Clinical progression and follow-up plan

Post-surgery, the following medications were prescribed: Meloxicam (0.1 mg/kg orally every 24 hours for 3 days), Dipyrone (25 mg/kg orally every 12 hours for 5 days), Tobramycin (1 drop in the right eye every 6 hours for 5 days), and Diclofenac (1 drop in the right eye every 12 hours for 5 days). The use of an Elizabethan collar was recommended. After providing these instructions to the owner, a follow-up appointment was scheduled for 12 days post-surgery. Histopathological examination results indicated a focus of moderate fibroblast hyperplasia, disorganized and interspersed with a small number of lymphocytes and plasma cells. No bacterial, fungal, or parasitic agents were detected

FIGURE 2. Photographic images of a three-month-old French Bulldog during surgery. A. Pre-surgical setup with sterile drapes and blepharostat retractor; B. Full-thickness elliptical incision through the granuloma wall; C. Interrupted single sutures placed between the granuloma wall and the incision edges of the palpebral conjunctiva of the third eyelid.

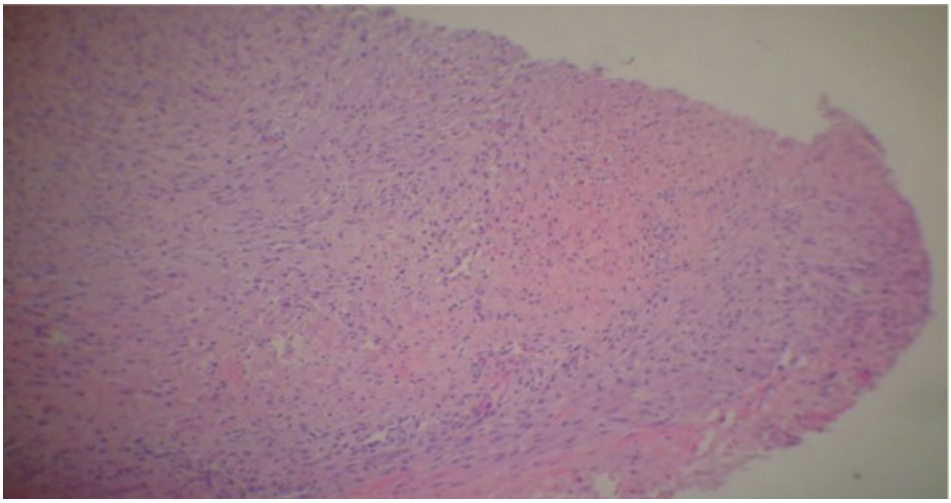


Source: own elaboration.

in the sample analyzed, supporting a granuloma diagnosis (figure 3).

Approximately two months post-surgery, the animal returned for evaluation, showing significant improvement with no visible abnormalities (figure 4). Consequently, the patient was discharged from the hospital.

FIGURE 3. Photographic image of the histopathological examination of a lacrimal granuloma on the eyelid at 40x magnification.



Source: own elaboration.

FIGURE 4. Photographic images of a five-month-old French Bulldog showing full recovery two months post-surgery.

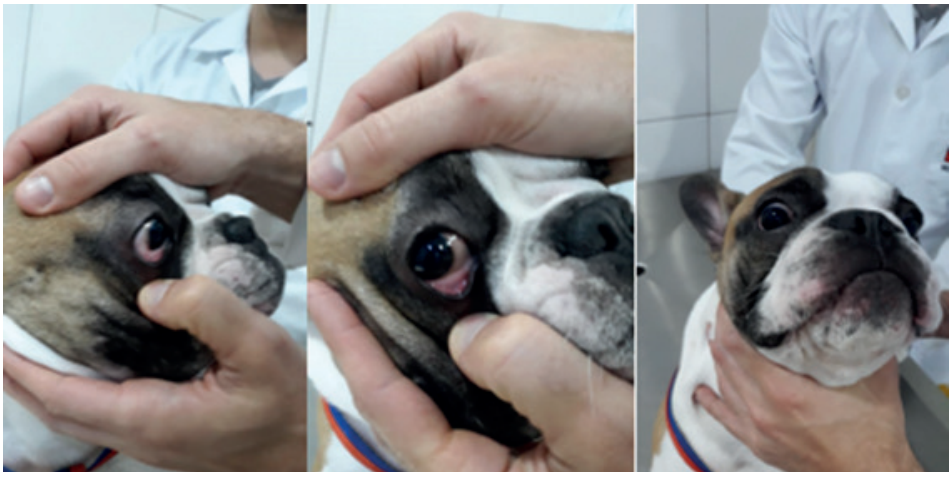
Source: own elaboration.

In subsequent evaluations conducted five and twelve months after the marsupialization of the granuloma, the patient demonstrated complete recovery without any complications (figures 5 and 6).

DISCUSSION

A cyst is defined as a cavity lined by epithelium and filled with liquid or semi-solid material. In contrast, a granuloma can be round or oval, characterized by a

FIGURE 5. Photographic images of an eight-month-old French Bulldog, showing no changes observed during the ophthalmologic examination five months after the surgical procedure.



Source: own elaboration.

FIGURE 6. Photographic images of a fifteen-month-old French Bulldog, showing no changes observed during the ophthalmologic examination twelve months after the surgical procedure.

Source: own elaboration.

middle area composed of granulomatous inflammatory cells surrounded by a peripheral zone of fibroblasts, which may contribute to the formation of a fibrous capsule (Ackermann 2018). While the distinction between a cyst and a granuloma is straightforward in pathology, it poses challenges during clinical evaluation, even when imaging studies are conducted (Pinheiro *et al.* 2007). Therefore, histopathological evaluation was essential for differentiating these conditions, including the exclusion of a neoplastic process (Barbé *et al.* 2016).

The differential diagnoses for lacrimal granuloma include neoplastic processes, cysts, subconjunctival fat protrusion, and myiasis (Delgado 2013; Lamagna *et al.* 2012). The challenge in clinically distinguishing between granulomas and cysts leads to the misclassification of many cases described in the literature as lacrimal cysts, as not all studies employed histopathology as a diagnostic tool (Barbé *et al.* 2016). Therefore, we suspect that granulomas may be more common than previously reported in the literature. Consequently, in the absence of documented cases of lacrimal granuloma, we will reference lesions described in the literature as lacrimal cysts to facilitate the discussion of this case.

The occurrence of primary cysts in the lacrimal gland and duct is uncommon. Lacrimal cysts may arise from a variety of causes, including congenital factors such as developmental defects in the lacrimal ducts, as well as acquired causes such as trauma or the presence of a foreign body in the ducts (Giuliano 2021). In this case, the tear granuloma developed after the third eyelid gland repositioning technique, with a suspicion of iatrogenic closure of the tear drainage ducts or obstruction due to cicatricial stenosis.

To prevent the formation of a tear granuloma, the surgeon must exercise caution to avoid obstructing the tear drainage system. In the case of the repositioning technique of Morgan, which is the most commonly used method by veterinarians in Brazil (Lopes 2019), we recommend that incisions made in the bulbar conjunctiva of the third eyelid at the margins of the prolapsed gland do not meet at their ends, as such union may critically obstruct tear drainage.

The protrusion of the gland is one of the most common changes observed in the third eyelid of dogs, particularly in Bulldogs, followed by Lhasa Apsos and Shi Tzus (Queiroz *et al.* 2015). This is consistent with the current case, in which the patient is a young French Bulldog that exhibited protrusion of the third eyelid gland prior to the development of the lacrimal granuloma.

The ocular clinical signs associated with the lacrimal granuloma in this patient align with the clinical manifestations described in the literature for lacrimal cysts. These signs include the presence of floating, elevated masses with rounded or ovoid shapes, characterized by a pinkish, translucent wall and a soft, painless texture upon palpation (Dawson *et al.* 2015).

Drainage of cysts and granulomas is a viable option; however, as demonstrated in the current case, recurrence is common. Therefore, the definitive technique to prevent recurrence is marsupialization, which involves creating a communication channel between the cyst and the external environment (Lima *et al.* 2020).

The third eyelid gland is responsible for producing 30-57% of the tear film, making its resection inadvisable (Holzlsauer *et al.* 2021). Furthermore, electron microscopy studies have shown that removal of this

gland leads to exfoliation of surface cells and a reduction in the thickness of the corneal cell layers (Saito *et al.* 2004).

Considering the critical function of the third eyelid gland, the choice of the marsupialization technique in this case is well justified, as it preserves the function of the gland and the integrity of the cornea.

Marsupialization is a well-documented technique used in the treatment of various conditions, including prostatic cysts (Smith 2008), arachnoid cysts (Zang *et al.* 2017), and sublingual mucoceles (ranulas) (Radlinsky & Fossum 2021). However, its application in the treatment of lacrimal cysts and granulomas is rarely reported. In this case, marsupialization proved to be a safe and effective method, yielding significant short-term and long-term results (Barbé *et al.* 2016; Lima *et al.* 2020).

The use of marsupialization for lacrimal granuloma treatment has led to satisfactory improvement in the patient, with no recurrences noted up to 360 days postoperatively. Additionally, this technique has maintained adequate corneal hydration, a benefit not typically observed following the excision of the third eyelid gland, which can result in dry keratoconjunctivitis (Barbé *et al.* 2016).

CONCLUSION

This case highlights the essential role of histopathological analysis in diagnosing lacrimal granuloma. Additionally, it underscores the precautions veterinarians must take when performing the repositioning technique of the third eyelid gland to prevent the formation of granulomas. Finally, it demonstrates that the marsupialization technique can yield excellent outcomes while preserving lacrimal function.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ETHICAL APPROVAL STATEMENT

As this publication is a case report, approval from an ethics committee was not required. The guardian of the patient provided consent for both the surgical procedure and the scientific dissemination of the case report.

ARTIFICIAL INTELLIGENCE STATEMENT

It is hereby stated that no artificial intelligence technologies were employed in the preparation of this case report.

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Case report: *Hepatozoon* sp. in a canine in Bogotá

Cañón–Cocunubo, E. [1]*

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ABSTRACT

This case report describes a diagnosis of hepatozoonosis in Bogotá in a one-year-old mixed-breed female dog, successfully treated with imidocarb dipropionate. The patient initially presented with persistent cough, organomegaly, poor body condition, and mucosal pallor. A complete blood count revealed normocytic normochromic anemia, thrombocytopenia, and hyperglobulinemia, suggesting hemoparasitism as the primary differential diagnosis. *Hepatozoon* sp. was confirmed by Polymerase Chain Reaction (PCR), leading to the initiation of specific treatment with imidocarb dipropionate, supplemented with atropine and doxycycline to target potential coinfections. Clinical and hematological follow-up showed significant improvement, with resolution of anemia and thrombocytopenia. This case highlights the importance of including *Hepatozoon canis* in the differential diagnoses in areas above 2,600 meters above sea level, particularly in dogs from shelters, due to the risk of underdiagnosis in non-endemic areas and the potential for vertical transmission. The occurrence of hepatozoonosis in Bogotá suggests an emerging risk for the region, emphasizing the need to disseminate this case along with the diagnostic and therapeutic approaches used. In this context, the availability of molecular tools, such as PCR, is crucial for accurate diagnosis and effective management of this infection in newly affected areas.

Keywords: hemoparasites, imidocarb, Colombia, emerging risk.

Reporte de caso: *Hepatozoon* sp. en un canino en Bogotá

RESUMEN

Este reporte de caso describe un diagnóstico de hepatozoonosis en Bogotá en una hembra canina mestiza de un año tratada exitosamente con dipropionato de imidocarb. La paciente presentó inicialmente tos persistente, organomegalia, condición corporal baja y palidez de las mucosas. Tras realizar un hemograma, se detectó anemia normocítica normocrómica, trombocitopenia e hiperglobulinemia, lo que sugirió hemoparasitismo como primer diagnóstico diferencial. La confirmación de *Hepatozoon* sp. mediante PCR permitió iniciar un tratamiento específico con dipropionato de imidocarb, complementado con atropina y doxiciclina dirigida contra posibles coinfecciones. El seguimiento clínico y hematológico mostró una notable mejoría, con resolución de la anemia y la trombocitopenia. Este caso resalta la importancia de incluir *Hepatozoon canis* en los diagnósticos diferenciales en zonas de altitud superior a los 2.600 m s. n. m., particularmente en caninos provenientes de refugios, debido al riesgo de subdiagnóstico en áreas no endémicas y la posibilidad

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de transmisión vertical. La aparición de hepatozoonosis en Bogotá sugiere un riesgo emergente para la región, lo que subraya la necesidad de divulgar este caso junto con los métodos diagnósticos y terapéuticos empleados. En este contexto, la disponibilidad de herramientas moleculares, como la PCR, es crucial para un diagnóstico preciso y un manejo adecuado de esta infección en zonas de reciente incidencia.

Palabras clave: hemoparásitos, imidocarb, Colombia, riesgo emergente.

INTRODUCTION

Canine hepatozoonosis is a parasitic disease caused by protozoa of the genus *Hepatozoon*, belonging to the phylum Apicomplexa, class Conoidasida, and family Hepatozoidae. These parasites have complex biological cycles that involve both vertebrate and invertebrate hosts, with hematophagous ticks acting as vectors. In dogs, the two species of greatest clinical significance are *Hepatozoon canis* and *Hepatozoon americanum*. While *H. canis* typically causes mild to moderate pathogenicity, *H. americanum* is associated with severe clinical manifestations, such as myositis and myocarditis, which can significantly compromise the health of infected dogs (Mastrantonio *et al.* 2023). The primary route of transmission of these protozoa in dogs is through the ingestion of infected ticks (Ewing & Panciera 2003).

Regarding its epidemiology, *Hepatozoon canis* utilizes the tick *Rhipicephalus sanguineus*, commonly known as the brown dog tick, as its definitive host (Freire 2019). Although there are reports identifying *Hepatozoon americanum* oocysts in *Amblyomma maculatum* ticks, these are not considered significant in the transmission of the parasite (Arcila *et al.* 2005). In Brazil, A*mblyomma cajennense* has been determined to lack importance in the transmission of *Hepatozoon canis*, suggesting that this tick does not play a relevant role in the epidemiology of canine hepatozoonosis in the region. Nevertheless, the potential existence of different subspecies of *Amblyomma ovale* with varying capacities to act as pathogen vectors has been proposed, highlighting the need for further studies to clarify the impact of these subspecies on the transmission of *Hepatozoon canis* and other infectious agents (Demoner *et al.* 2013). Additionally, while Rubini *et al.* (2009) suggested that *Amblyomma ovale* might be implicated in the transmission of *Hepatozoon canis,* a significant impact of this species on the epidemiology of the disease has yet to be confirmed. Therefore, further studies are required to more accurately assess its role as a vector and its relevance in the transmission cycle of this parasite.

The primary route of transmission for *Hepatozoon canis* is through the ingestion of infected ticks. The parasite's life cycle begins when the tick ingests gamonts while feeding on the blood of an infected dog, leading to the development of oocysts within the vector. Once the dog ingests the tick carrying the oocysts, these pass through the gastrointestinal wall (Beugnet *et al.* 2018), releasing sporozoites. The sporozoites invade mononuclear phagocytic cells and endothelial cells of various organs, such as the bone marrow, spleen, muscles, liver, and lungs. In these tissues, cysts containing different forms of meronts are formed, which perpetuate the infection within the host (Ettinger *et al.* 2017).

According to the study by Schäfer *et al.* (2022), vertical transmission of *Hepatozoon canis* in dogs has also been identified. Additionally, Beneth (2011) reported

that this hemoparasite can be transmitted through the consumption of infected prey. In most cases, *Hepatozoon canis* infection is asymptomatic, and it is common to suspect the infection when a positive patient is identified without evident clinical signs. However, clinical signs can range from mild to severe, depending on the level of parasitemia, the host's immune status, and the presence of coinfections. Dogs with low parasitemia are usually asymptomatic, while those with high parasitemia may exhibit symptoms such as lethargy, fever, and emaciation (Tuna *et al.* 2021). In contrast, *Hepatozoon americanum* infection is associated with more severe clinical signs, including painful myositis, fever, progressive muscle atrophy, weakness, and lameness, which in some cases can lead to paralysis (Eiras *et al.* 2007).

In various regions of the world, coinfections of *Hepatozoon canis* with other pathogens have been described, including *Babesia* sp. (Ciuca *et al.* 2021), *Anaplasma platys* (Anderson *et al.* 2013), and *Ehrlichia canis* (Sukara *et al.* 2023), as well as with distemper, parvovirus, *Anaplasma phagocytophilum, Toxoplasma gondii*, and *Leishmania infantum* (Baneth 2011). It is essential to carefully interpret clinical signs, differentiating those caused by *Hepatozoon canis* from symptoms attributable to other concomitant pathogens. This distinction is particularly important in dogs from shelters or regions with poor sanitary and ectoparasite control, to ensure proper diagnosis and treatment of all present conditions (Greene 2012).

Regarding diagnosis, a study conducted in Argentina by Vezzani (2017) found that 56.9% of dogs infected with *Hepatozoon canis* presented with anemia, 36.3% showed leukocytosis, and 7.5% had leukopenia. Among parasitemic dogs, 74.1% exhibited an inflammatory leukogram, 46.4% had eosinophilia, and 17.8% had monocytosis. Hyperglobulinemia (Beugnet *et al.* 2018) and anemia are associated with the chronicity of the infection (Paiz *et al.* 2016). Thrombocytopenia is uncommon, except in cases of coinfection with other hemoparasites (Ettinger *et al.* 2017). According to Tuna *et al.* (2021), 84.38% of patients coinfected with *Ehrlichia canis* presented with thrombocytopenia, while 56.25% showed anemia, being these the most common hematological abnormalities.

The definitive diagnosis of hepatozoonosis is made through blood smear analysis, where gamonts can be visualized in the cytoplasm of neutrophils and occasionally within monocytes (Mastrantonio *et al.* 2023). However, both blood smears and buffy coat preparations have low sensitivity in animals with subclinical infections due to variable parasitic loads, with a positivity rate not exceeding 5% in infected patients (Modrý *et al.* 2017; Arcila *et al.* 2005). Therefore, PCR is considered the best diagnostic option, as it has been shown to be 2.5 times more sensitive (Karagenc *et al.* 2006).

The most commonly used treatment protocol for *Hepatozoon canis* includes the administration of two doses of imidocarb dipropionate at concentrations of 5 to 6 mg/kg, with a 14-day interval between doses (Plumb 2018). This treatment is frequently supplemented with doxycycline, administered orally at a dose of 10 mg/kg per day for 21 days, to address potential coinfections with rickettsiae (Greene 2012). With less success, toltrazuril therapy has also been used at concentrations of 10-14 mg/kg orally, every 24 hours for 7-10 consecutive days (Beugnet *et al.* 2018). Treatment follow-up should include hematological assessments 14 or 28 days

after initiation (Mastrantonio *et al.* 2023). However, prevention remains key, minimizing tick exposure and administering acaricidal products in cases of high-risk exposure (Beugnet *et al.* 2018).

The first report of this hemoparasite in Colombia was in 2005, following the observation of inclusion bodies in neutrophils and monocytes in blood smears (Arcila *et al.* 2005). Isolated cases in Colombia have been of the same class as those from Venezuela, Spain, and Taiwan. The identification of *Hepatozoon canis* in Colombia was accomplished using PCR, and it was first diagnosed in a study conducted with dogs from shelters in Bogotá, Bucaramanga, and Villavicencio, recording a prevalence of 31.8%. However, in that study, no positive animals were found in Bogotá (Vargas–Hernández *et al.* 2011), so the prevalence of hepatozoonosis in the city remains unknown.

Since the initial discovery of *Hepatozoon canis* in Colombia, several studies have reported its prevalence in various regions of the country. For example, in Magdalena, a prevalence of 7.1% was recorded in analyzed canine patients (Thomas *et al.* 2020). In the Valle de Aburrá, it was identified as the primary canine tick-borne pathogen, with a higher prevalence than expected, suggesting that the infection might be underreported or underdiagnosed in the region (Cabrera–Jaramillo *et al.* 2022). In Cúcuta, an asymptomatic case was reported (Cala *et al.* 2018), and a study of dogs with clinical signs suspected of hemoparasitic infection revealed a positivity rate of 8.6% (Chinchilla *et al.* 2020). To date, no cases of *Hepatozoon americanum* have been reported in the country.

In a study conducted in the department of Magdalena, the detection of *Hepatozoon canis* by PCR was 7.1% in the canine patients examined (Thomas *et al.* 2020), a relatively low percentage. On the other hand, in the Valle de Aburrá, *H. canis* was observed to be the primary canine tick-borne pathogen, with a higher prevalence than expected, suggesting that it may be underreported or underdiagnosed (Cabrera–Jaramillo *et al.* 2022). Additionally, two PCR-confirmed studies have been reported in the city of Cúcuta, one of which identified a case with no clinical signs (Cala *et al.* 2018). In the same city, another PCR detection study found a positivity rate of 8.6% in dogs with clinical signs suspected of hemoparasitic infection (Chinchilla *et al.* 2020).

In this sense, the objective of this case report is to inform about the presence of *Hepatozoon canis* in the city of Bogotá and to describe the currently available diagnostic methods and treatment options for its management.

CASE DESCRIPTION

A one-year-old spayed female mixed-breed dog was presented for a general consultation the day after her adoption from a shelter. On the same day, rapid tests using immunochromatography for distemper and canine parvovirus were performed, with negative results. Additionally, the patient received flea and tick treatment (Afoxolaner), deworming (Milbemycin oxime), and a polyvalent vaccination (distemper, parvovirus, coronavirus, parainfluenza, hepatitis, adenovirus, and leptospira), as well as a rabies vaccine. Prior to adoption, the patient had been treated for a possible diagnosis of "kennel cough" (canine respiratory infectious complex) for one week, receiving antibiotics and nebulizations, although the specific medications used were not detailed.

Since her adoption, the patient exhibited a fearful demeanor, intolerance to exercise, nocturnal coughing episodes, and moderate pruritus in the cervical region, abdomen, and distal areas of all four limbs. On physical examination, a moderate non-productive cough reflex was noted, along with pale mucous membranes, abdominal organomegaly in the left mesogastric region (suspected splenomegaly), dyskeratosis on the right abdomen, and an X-shaped scar potentially related to a previous ovariohysterectomy. Additionally, hypotrichosis with crusting was observed in the ventral cervical region and on the hind limbs, along with poor skin and coat quality and a low body condition score (3/9) with a weight of 5 kg.

The differential diagnosis included canine respiratory infectious complex, hemoparasitism (ehrlichiosis, babesiosis, anaplasmosis), malnutrition, dermatophytosis, and mite dermatitis. As part of the initial diagnostic plan, blood samples were collected from the cephalic vein for a complete blood count, Alanine Transaminase (ALT), and creatinine. Blood chemistry analyses were processed using a semi-automatic Rayto RT-1904 CV, while the complete blood count with differentiated proteins was processed using an automated Vetplus 3500 hematology analyzer, with manual confirmation of the count and morphology. The results revealed normocytic normochromic anemia, erythrocyte agglutination, thrombocytopenia, and hyperproteinemia due to hyperglobulinemia (table 1).

The initial treatment consisted of administering doxycycline (10 mg/kg orally, every 12 hours for 21 days), prednisolone (0.5 mg/kg orally, every 12 hours for 8 days, then every 24 hours for an additional 8 days), and Mirrapel® oil (1.5 ml orally every 24 hours until further notice). It was recommended to supplement the diet with commercially available gastrointestinal care food and, after the resolution of coughing, to start weekly medicated baths with 3% chlorhexidine shampoo.

Subsequently, a real-time multiplex PCR with probes for the detection of hemoparasites (canine distemper, *Anaplasma* spp., *Hepatozoon* spp., *Ehrlichia* spp., and *Babesia* spp.) was performed, which tested positive for *Hepatozoon* spp. Based on this result, the treatment was supplemented with imidocarb dipropionate (5 mg/kg via intramuscular injection), preceded by subcutaneous administration of atropine (0.02 mg/kg), with the procedure repeated 15 days later.

Seven and fifteen days after the first injection, a follow-up complete blood count was performed. The results, detailed in table 1, showed improvement with resolution of anemia and thrombocytopenia. At the first follow-up, myelocytes and hyperproteinemia due to hyperglobulinemia persisted; however, these anomalies were resolved by the second follow-up. Clinically, the patient demonstrated complete resolution of the clinical signs and gained 600 grams over a two-week period. By the time of the second injection, her weight had increased to 6 kg, with a body condition score of 4/9 and no abnormalities noted during physical examination. At the final follow-up, conducted seven months after the initial treatment, the patient remained without abnormalities and achieved an ideal body condition with a weight of 7 kg.

RESULTS AND DISCUSSION

The initial approach to the patient was based on the high-risk history, clinical signs,

TABLE 1. Complete blood count tests performed on the patient (abnormal values are highlighted with an asterisk)

	Before diagnosis		First checkup		Second checkup		
Parameter	**Absolute value**	**Relative value**	**Absolute value**	**Relative value**	**Absolutevalue**	**Relative value**	**Reference ranges**
Erythrocytes	4.8*		7.4		6.8		5.5-8.5 x 10 ^ 12/L
Hemoglobin	10.1*		15.6		16.2		12.0-188 g/dL
Hematocrite	31.8*		46.8		48.7		37.0-55.0 %
MCV	66.3		63.3		71.8		60.0-77.0 fL
MCH	21.0		21.1		23.9		19.5-24.5 pg
MCHC	31.8*		33.3		33.3		32.0-36 g/dL
RDW	12.9		13.5		13.8		11.0-16.0 %
Platelets	100*		243		206		200-500 x 10 ^ 9/L
MPV	8.8		8.7		8.4		7.0-12.0 fL
Total protein	8.4*		8.5 *		6.8		6.0-7.5 g/dL
Albumin	2,6		2.76		2.5		2.4-3.9 g/dL
Globulins	5.8*		5.7 *		4.3		2.5-4.5 g/dL
Leukocytes	15.0		7.4		6.8		6.0-17.0 x 10 ^ 9/L
Neutrophils	10050	67	3848	52 *	3445	53 *	3,000-11, 500 ul 60-77 %
Band neutrophils	0	0	0	0	0	0	< 300 ul 0 %
Lymphocytes	4350	29	2146	29	2665	41*	1,000-4, 800 ul 15-35 %
Monocytes	0	0*	1036	14 *	260	4	< 1,200 ul 2-7 %
Eosinophils	600	4	222	3	130	2	100-1,000 ul 2-6 %
Basophils	0	0	0	0	0	0	< 100 ul 0-1 %
Metamyelocytes	0	0	148 *	2 *	0	0	0 ul 0 %

Source: own elaboration.

and previous treatment. The initial basic examinations allowed for the quantification of blood cell lines, and following manual confirmation of cell counts and evaluation of the blood smear, it was possible to infer a low parasitemia due to the absence of hemoparasite gamonts in the blood smear. However, the positive confirmation for *Hepatozoon canis* by PCR indicated that the infection was present but not in a sufficient quantity to be visualized in a single blood smear.

Additional tests included serum measurement of ALT to assess active liver damage and creatinine to detect severe renal damage or severe dehydration. Both values were normal, allowing for appropriate treatment to be directed and differential diagnoses to be established, including hemoparasitism and canine respiratory infectious complex (including distemper). In the case of hemoparasitism, the main suspected infectious agents were ehrlichiosis, anaplasmosis, and babesiosis, as these are the most common in clinical practice.

Dermatological findings were likely attributed to nutritional deficiencies rather than to hepatozoonosis, with notable improvement observed following supplementation with fatty acids, multivitamins, and high-quality food. Regarding diagnosis, the increasing availability of PCR profiles and tests in laboratories, especially for hemoparasites, enabled the identification of this lesser-known disease in Bogotá, which was not initially considered in the differential diagnoses. Without the PCR technique, a definitive diagnosis would not have been possible, as no intracellular inclusions of gamonts were observed in neutrophils or monocytes in routine blood smears. However, in patients with high parasitemia, direct observation of the smear remains a rapid and cost-effective diagnostic tool. It is essential that in non-endemic areas, such as Bogotá, or in cold climates where it has not been reported, blood smear examination be included in each processed complete blood count as part of routine paraclinical evaluation.

Among the molecular tests available in Bogotá, various PCR variants are employed, including multiplex and simplex, end-point, isothermal, real-time, and real-time with probe PCR. The latter was utilized in this case due to its superior sensitivity and specificity (Chinchilla *et al.* 2020). It is important to note that PCR results are qualitative (positive or negative), which are useful for diagnosis but not applicable for quantitative monitoring of parasitemia.

The initial treatment was prescribed based on the clinical history and paraclinical test results, utilizing doxycycline, a broad-spectrum antibiotic (Plumb 2018), at a dosage that covered both the bacteria associated with canine respiratory infectious complex and the most common hemoparasite, *Ehrlichia canis* (Papich 2021). However, this antibiotic is ineffective against *Hepatozoon canis*. Prednisolone was administered as an anti-inflammatory to treat the affected respiratory tract and to manage the potential diagnosis of *Ehrlichia canis* infection. Although the literature advises against the use of corticosteroids in patients with hepatozoonosis (Ettinger *et al.* 2017), by the time the diagnosis was confirmed via PCR, the treatment with prednisolone had already concluded without observed adverse effects. Additionally, nutritional support with high-quality food and multivitamins was provided, which facilitated the patient's overall recovery.

Although the PCR technique identifies only the genus *Hepatozoon*, the absence of severe clinical signs and the possibility of co-infections suggested that the infection was most likely *Hepatozoon canis* (Eiras *et al.* 2007). For this reason, the most appropriate treatment for this condition was chosen, involving the administration of imidocarb dipropionate via the parenteral route. Prior to each injection, premedication with atropine was administered to mitigate potential side effects associated with imidocarb dipropionate (Papich 2021). No adverse reactions were observed during the treatment.

Following the diagnosis of hepatozoonosis, the therapy with doxycycline, which had been previously initiated, was continued to address the canine respiratory infectious complex, given the high likelihood of co-infection in the patient. The presence of coughing and thrombocytopenia is not a common sign of *Hepatozoon* infection, suggesting the possibility of co-infection with another undetected hemoparasite, as the literature well documents the concomitance of multiple tick-borne pathogens (Tuna *et al.* 2021), particularly in patients affected by *Rhipicephalus sanguineus* (Greene 2012). Although no ticks were found during the initial clinical examination, recent treatment with afoxolaner may have eliminated any visible evidence of infestation. Nevertheless, prior contact with ticks cannot be ruled out and remains a key risk factor for the transmission of hemoparasites, as documented in studies identifying the tick species involved in such cases (Arcila *et al.* 2005).

Since no gamont forms of the hemoparasite were detected in the blood smears, a complete recovery cannot be confirmed, as there was no initial positive smear to serve as a reference. However, the success of the treatment can be evaluated based on the clinical improvement of the patient and the resolution of anemia and thrombocytopenia observed in the follow-up complete blood count (Mastrantonio *et al.* 2023). Although quantitative real-time PCR was not performed for follow-up, clinical and paraclinical improvement suggests that this technique could be a useful tool for monitoring treatment effectiveness if needed.

Preventing hepatozoonosis in companion animals presents various challenges due to its easy transmission through ectoparasites. To mitigate the risk of infection, it is essential to reduce exposure to endemic areas with poor vector control and to apply acaricides regularly to both animals and their environments, especially in locations with high animal concentration such as shelters, schools, and kennels (Beugnet *et al.* 2018). Veterinarians play a crucial role, not only in the early detection of the disease but also in implementing tailored preventive strategies adapted to the risk profile of each patient or group. Effective prevention extends beyond the use of antiparasitic drugs, requiring constant vigilance and owner education to ensure long-term animal welfare.

CONCLUSION

Hepatozoonosis is not commonly considered a differential diagnosis among tick-borne diseases in Bogotá, leading to an underdiagnosis of this condition, particularly in animals from shelters with uncertain origins. It is possible that both dogs and cats, as well as the ticks infesting them, may have been transported from endemic areas, and climate change may facilitate the survival of these ectoparasites in previously cooler climates, increasing the risk of infection in urban areas such as Bogotá.

Given that the goal of therapy is not the complete eradication of the parasite but rather the resolution of clinical signs, the treatment was successful according to the literature. Two of the reported medications for treating *Hepatozoon* sp. infection and its possible coinfections were used, suggesting, given the good clinical response, that the infection was likely *Hepatozoon canis.* However, it is important to develop tests that can identify whether the infection is caused by *H. canis* or *H. americanum*, as both species exhibit differences in clinical presentation and treatment, and there is a possibility that both may emerge in the country.

Finally, it is crucial to conduct studies that assess the significance of hepatozoonosis as an emerging disease in Bogotá. The increasing mobility of dogs between cities and the potential for vertical transmission highlight the importance of further investigating the epidemiology of this disease in the country.

CONFLICT OF INTEREST

The author declares no conflict of interest.

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USE OF ARTIFICIAL INTELLIGENCE

No artificial intelligence was used during the diagnostic and treatment process, nor in the preparation of this manuscript.

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