Revista de la Facultad de Medicina Veterinaria y de Zootecnia

ISSN-L: 0120-2952

ARTÍCULOS DE INVESTIGACIÓN, REPORTES DE CASO Y REVISIÓN

VOL. 72 N.º 1
ENERO-ABRIL
2025

Facultad de Medicina Veterinaria
y de Zootecnia
Sede Bogotá

UNIVERSIDAD
NACIONAL
DE COLOMBIA

Revista de la Facultad de Medicina Veterinaria y de Zootecnia

UNIVERSIDAD
NACIONAL
DE COLOMBIA

Artículos de Investigación, Reportes de Caso y Revisión

Volumen 72 n.º 1, enero-abril 2025

UNIVERSIDAD NACIONAL DE COLOMBIA

FACULTAD DE MEDICINA VETERINARIA Y DE ZOOTECNIA
Vol. 72 n.º1, enero-abril de 2025
ISSN-enlace (ISSN-L): 0120-2952
ISSN en línea: 2357-3813
DOI: 10.15446/rfmvz (CrossRef)
http://www.revistas.unal.edu.co/index.php/remevez/index
Correo electrónico: rev_fmvzbog@unal.edu.co
Bogotá, D. C., Colombia

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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.
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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.
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https://doi.org/10.15446/rfmvz.v72n1.118231

Climate change: challenge or opportunity for agro-livestock systems?

Climate change, defined as long-term variations in the climate patterns of the planet, is a natural process that has driven the evolution and development of diverse organisms and ecosystems. However, human activities have accelerated this process to unsustainable levels, causing abrupt changes in global temperatures, disruptions in rainfall patterns, and an increased frequency of extreme weather events.

These changes present significant challenges to agro-livestock systems, considering that climate, as a regulating agent, determines the conditions under which organisms can survive within specific ranges of temperature, humidity, water availability, and other essential environmental factors. Disruptions to these parameters not only threaten the survival of organisms but also compromise the sustainability and functionality of agricultural production systems, which must adapt to variable conditions and adopt strategies to endure within a challenging environment.

Among the most evident effects of climate change are heat stress and reduced availability of water and forage, which diminish animal productivity and, consequently, the efficiency of the production chain. In sectors such as cattle farming, this results in lower milk and meat production, directly impacting the economic stability of producers. Meanwhile, intensive production systems, such as poultry and swine farming, face additional challenges related to air quality, management of biological waste, and increased incidence of diseases due to climatic conditions that favor the survival and spread of pathogens and associated vectors.

In the face of these challenges, climate change also offers opportunities for the agro-livestock sector to implement innovative strategies that enhance resilience to external stressors. Concepts such as *climate-smart agriculture* have gained prominence for providing holistic approaches to ensure food security in changing environments. These approaches promote practices like the integration of agroforestry systems and efficient water use, which not only protect natural resources but also strengthen the incomes of producers by offering sustainable economic alternatives.

The adoption of strategies focused on climate adaptation and mitigation positions the agro-livestock sector as a key element in addressing this global issue. By implementing practices aligned with national climate commitments, such as Nationally Determined Contributions (NDCs), the sector plays a strategic role in achieving the Sustainable Development Goals (SDGs). This represents a paradigm shift, recognizing the sector as an essential pillar in the transition towards more resilient, sustainable, and climate-conscious production systems.

In this context, industry associations and producers play a critical role as catalysts for change and facilitators of the implementation of climate-responsible strategies. Prominent sectors of animal production in Colombia, such as poultry farming, have taken the lead by conducting studies that provide key tools for national producers to adapt to and effectively address the ongoing challenges posed by climate change. Through resource mobilization, training programs, and the promotion of sustainability-oriented policies, these stakeholders drive the adoption of resilient practices, transforming climate challenges into strategic opportunities.

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Cambio climático: ¿reto u oportunidad para los sistemas agropecuarios?

El cambio climático, entendido como aquellas variaciones en los patrones climáticos del planeta en el largo plazo, es un proceso natural que ha permitido la evolución y el desarrollo de los diferentes organismos y ecosistemas del planeta; no obstante, la actividad humana ha acelerado este proceso a ritmos insostenibles, lo que ha causado variaciones abruptas en la temperatura global, alteraciones en los patrones de lluvia y mayor frecuencia de fenómenos climáticos extremos.

Estos cambios representan, entonces, un desafío para los diferentes sistemas agropecuarios, dado que el clima como agente regulador ha determinado las condiciones bajo las cuales un ser vivo puede subsistir o no bajo ciertos rangos de temperatura, humedad, disponibilidad hídrica y otros factores ambientales esenciales. La alteración de estos, no solo compromete la supervivencia de los organismos, sino también la sostenibilidad y el funcionamiento de los sistemas de producción agropecuarios, que tienen que acoplarse a condiciones variables y adoptar estrategias que les permitan perdurar en un entorno desafiante.

Entre los efectos más evidentes del cambio climático se encuentran el estrés por calor y la disminución de la disponibilidad de agua y forraje, lo que reduce la productividad de los animales y, por ende, la eficiencia de la cadena productiva. En sectores como la ganadería bovina, esto se traduce en menor producción de leche y carne, lo que impacta directamente la economía de los productores. Por otra parte, los sistemas intensivos de producción, como la avicultura y la porcicultura, enfrentan desafíos adicionales relacionados con la calidad del aire, el manejo de desechos biológicos y el aumento de enfermedades debido a las condiciones climáticas más favorables para la subsistencia y propagación de patógenos y vectores asociados.

Frente a estos retos y desafíos, el cambio climático abre una ventana de oportunidades para que el sector agropecuario implemente estrategias innovadoras que le permitan aumentar su resiliencia ante agentes externos. Conceptos como *agricultura climáticamente inteligente* han cobrado relevancia al permitir enfoques integrales que ayuden a garantizar la seguridad alimentaria en entornos cambiantes, pues promueven prácticas como la incorporación de sistemas agroforestales y el uso eficiente del agua, que no solo protegen los recursos naturales, sino que también fortalecen los ingresos de los productores al ofrecer alternativas económicas sostenibles.

La implementación de estrategias enfocadas en la adaptación y mitigación del cambio climático transforma al sector agropecuario en un actor clave para enfrentar esta problemática global. Al adoptar prácticas alineadas con los compromisos climáticos nacionales, como las Contribuciones Determinadas a Nivel Nacional (NDC, por sus siglas en inglés), el sector asume un papel estratégico en el cumplimiento de los Objetivos de Desarrollo Sostenible (ODS). Lo anterior refleja un cambio de paradigma al reconocer al sector como un pilar esencial en la transición hacia sistemas productivos más resilientes, sostenibles y comprometidos con la acción climática y la sostenibilidad ambiental.

En este proceso, los gremios y los productores desempeñan un papel fundamental como catalizadores del cambio y facilitadores en la implementación de estrategias climáticamente responsables. Sectores destacados de la producción animal en Colombia, como el avícola, han tomado la delantera al desarrollar estudios que brindan herramientas clave para que los productores nacionales se adapten y respondan eficazmente a los desafíos que el cambio climático presenta de manera continua. A través de la movilización de recursos, la organización de programas de capacitación y la promoción de políticas orientadas hacia la sostenibilidad, estos actores impulsan la adopción de prácticas resilientes, haciendo del clima no un desafío sino su mejor aliado.

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https://doi.org/10.15446/rfmvz.v72n1.113703

Factors associated with the presence of antibiotic residues in raw milk from cows in the canton of El Carmen, Manabí, Ecuador

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Recibido: 28/03/2024 Aprobado: 24/01/2025

ABSTRACT

Milk, an essential component of human nutrition, can also serve as a vehicle for external contaminants such as antibiotics. To date, no data are available regarding the presence of antibiotic residues in raw milk from El Carmen (Manabí), the largest dairy basin on the Ecuadorian coast. Therefore, the objective of this study was to determine the presence of these residues and the associated risk factors. A total of 126 raw milk samples were collected from 42 dairy farms on three different occasions between September 2022 and January 2023. Of these, 78.57% (99/126) contained antibiotic residues below the maximum limits established by the Codex Alimentarius, whereas 21.43% (27/126) exceeded these limits. Sulfonamides were the most frequently detected residues (15.57%), followed by β-lactams (4.76%) and tetracyclines (0.79%). Among the risk factors analyzed, the frequency of antibiotic use in animals per year had the greatest influence (Odds Ratio: 0.001), compared with other covariates. The findings indicate a high prevalence of antibiotic residues in raw milk from the sampled region, primarily due to the indiscriminate use of these drugs by dairy farmers. Therefore, the role of regulatory agencies is crucial in preventing the commercialization of milk containing these residues. Additionally, training programs for producers are essential to mitigate this form of chemical contamination.

Keywords: antibiotics, raw milk, risk factors, Manabí.

Factores asociados a la presencia de residuos antibióticos en leche cruda de vacas en el cantón El Carmen, Manabí, Ecuador

RESUMEN

La leche, alimento esencial en la nutrición humana, también puede ser el vehículo de contaminantes externos, como los antibióticos. Al respecto, no se posee datos de la presencia de residuos de estos fármacos en la leche cruda del cantón El Carmen (Manabí), la mayor cuenca lechera de la costa ecuatoriana, por lo que el objetivo de la presente investigación fue determinar su presencia y los factores asociados. Se recolectaron 126 muestras de leche cruda de 42 ganaderías, por 3 ocasiones, entre septiembre 2022 y

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enero 2023, de las cuales el 78,57% (99/126) no presentaba presencia de residuos de antibióticos por encima de los límites permitidos por el Codex Alimentarius, frente a un elevado 21,43% (27/126) de muestras que sí presentaron, donde las sulfamidas mostraron mayor presencia (15, 57%), seguido de betalactámicos (4, 76%) y tetraciclinas (0, 79%). Entre los factores de riesgo, el de mayor influencia es la frecuencia al año con la que se usa los medicamentos en animales (Odd Ratio 0, 001), frente a otras covariables. Se concluye que existe alta presencia de residuos de antibióticos en la leche cruda del cantón muestreado, por uso indiscriminado de los ganaderos, por lo que el accionar de los organismos de control es fundamental a fin de impedir que se expenda el producto con estos residuos, así como se hace fundamental la capacitación a los productores para evitar esta contaminación química.

Palabras clave: antibióticos , leche cruda, factores, Manabí.

INTRODUCTION

Agriculture and livestock farming are among the most important economic sectors in Ecuador, ranking fourth and contributing 9.96% to the national gross domestic product (GDP). Dairy production is primarily carried out by small-scale farmers, who account for 80% of total milk production (Contero Callay *et al.* 2021; Ioneta 2022; Terán 2019). In 2022, Ecuador's daily milk production reached 5.5 million liters, with Manabí ranking as the country's fourth-largest milk-producing province, contributing 11.32% of the total. Manabí also represents the primary dairy basin of the coastal region, with El Carmen Canton being the area with the largest bovine population and the highest milk production (INEC-ESPAC 2023; CFN 2022).

One of the most critical challenges in the dairy industry is that raw milk and its derivatives often reach Ecuadorian households without undergoing quality analysis to ensure their suitability for human consumption or further processing (CIL 2015; Guapi Guamán *et al*, 2017; Terán 2019). In Manabí, bovine diseases such as mastitis and lameness are highly prevalent. Previous studies conducted in Chone Canton have reported the presence of various antibiotic residues in raw milk (Andrade & Saldarriaga 2018).

Cow's milk is an essential source of nutrients for consumers due to its high energy, protein, and fat content, which are fundamental for maintaining a healthy, balanced diet. Additionally, dairy farming plays a crucial role in employment generation, particularly for small-scale producers (FAO 2020 & 2023). However, its rich nutritional composition also makes it highly susceptible to contamination by external agents, including pathogenic microorganisms and chemical pollutants, which can lead to the development of diseases that pose health risks to consumers (Brown *et al.* 2020; Sachi *et al.* 2019; Terzi Gulel *et al.* 2020). Among chemical contaminants, antibiotics are of particular concern due to their widespread use in livestock farming for the treatment of bovine diseases. When withdrawal periods are not adequately observed, antibiotic residues can remain in raw milk and its derivatives, leading to significant health risks, such as antimicrobial resistance (Arrieta *et al.* 2019; Özdemir & Tuncer 2020) and hypersensitivity reactions in individuals with allergies (Díaz 2019).

Given the lack of recent data on the presence of antibiotic residues in raw milk from El Carmen Canton, this study aimed to determine the occurrence of these residues and identify the factors associated with their presence in raw milk from this region of Manabí Province, Ecuador.

MATERIALS AND METHODS

Population and sample

This study was a cross-sectional observational study conducted based on convenience sampling. Raw cow's milk samples were collected from various local dairy farms in the canton of El Carmen, Manabí province, specifically in the parishes of San Pedro de Suma, Wilfrido Loor Moreira, El Carmen, La Manga del Cura, La 14, and Santa María. Dairy farms in the region were selected through a census of local raw milk producers who expressed their willingness to participate in the study, with each collected sample representing an observational unit. Sample collection was carried out between September 2022 and January 2023 from 42 dairy farms at three different time points. By the end of the study, a total of 126 samples had been collected. Samples were obtained directly from either the refrigeration tank or storage containers at the farms, ensuring collection occurred immediately after the milking process.

To ensure proper sample collection, personal biosafety measures were observed, and sampling was conducted following the protocol for raw milk sample collection described by Agrocalidad (2020). A sterile ladle specifically adapted for cooling tanks and storage containers was used. The ladle was immersed twice into the container or tank, mixing the contents before drawing the sample from a depth of at least 15 cm below the milk's surface. The collected sample was then transferred into a pre-sterilized 500 mL polyethylene container. A representative sample volume of 30 mL of raw milk was taken, with agitation performed 5 to 6 times prior to sampling. In dairy farms with multiple aluminum or stainless-steel storage containers, the same milk volume was drawn from each container after agitating them 5 to 6 times. These subsamples were then pooled into a single container to ensure sample homogenization.

Sample analysis

After sample collection, the containers were labeled, and the milk was analyzed using a diagnostic test for antibiotic detection. The results were recorded on a control sheet, which included details such as location, sample volume, and time of sampling. Antibiotic detection was performed using the BIOEASY rapid test kit (Shenzhen Bioeasy Biotechnology Co., Ltd., China), a field test designed to detect β-lactams, sulfonamides, and tetracyclines, providing results in approximately nine minutes, following the manufacturer's instructions.

Coagulated milk was not permitted for testing, and tests exposed to inappropriate conditions (e.g., direct sunlight and humidity) were excluded. In cases of positive results, a second confirmatory test was conducted. For result analysis and interpretation, the presence of the upper control line was verified. Tests lacking this control line were deemed invalid and discarded. Upon detecting the control line, the test line signal was compared with the control line sequentially. A negative result was recorded if all lines exhibited uniform coloration. Conversely, if any line remained uncolored or was fainter than the others, the sample was considered positive for that antibiotic family.

Producer surveys

Surveys were conducted among all farm owners and/or individuals responsible for the selected herds to identify factors potentially contributing to the presence of antibiotics in raw milk. The survey included questions regarding the use of antibiotics to treat diseases affecting cattle on farms located in the canton of El Carmen, Manabí. Measurement variables were determined based on instruments designed in a similar study conducted in the Netherlands (Speksnijder *et al.* 2015) and an instrument used to assess antibiotic application in farms located in Bogotá, Colombia (Rodríguez & Acero Plazas 2014). The indicators assessed in these instruments were adapted to meet the research needs of the present study. The survey was validated by three faculty members from the Faculty of Veterinary Medicine at Universidad Central del Ecuador. Additionally, prior to survey administration, informed consent was obtained from the farm owners who participated in the study.

The variables analyzed in this study included the education level of the responsible personnel, age, common diseases affecting the dairy herds, medications used to treat these diseases (specifically antibiotics), dosage, withdrawal period, routes of administration, the individual or professional responsible for diagnosing the disease, and the frequency of medication use.

Statistical analysis

The data obtained from the test results and survey responses were recorded in a Microsoft Excel spreadsheet. Statistical processing was performed using the R Studio software, which was used to organize and classify the data into tables and figures reflecting the study's descriptive results. Frequency and percentage distributions were used to determine the number of cases in which antibiotic residues were detected in raw milk samples collected from the canton of El Carmen, Manabí.

Additionally, risk factor identification was analyzed using a binary logistic regression model to estimate the adjusted odds ratio or by fitting a linear model using weighted least squares with a 95% confidence interval. A bimodal regression was conducted, considering different factors (covariates) that could influence the detection of antibiotic residues in the analyzed raw milk samples (intercept). The covariates in this analysis corresponded to the data obtained from each survey question, while the intercept variable corresponded to the laboratory test results indicating the presence or absence of antibiotic residues. Using the R Studio software, the backward method was applied to eliminate covariates (i.e., risk factors influencing antibiotic residue presence) that had the least impact on the intercept or constant variable.

RESULTS AND DISCUSSION

Results

Presence of antibiotic residues in collected milk

Of the 126 raw milk samples analyzed from the dairy farms, a high proportion (21.43%; 27/126) contained antibiotic residues exceeding the limits established by the Codex Alimentarius, while the remaining 78.57% (99/126) tested negative for residues (table 1). This percentage is considered high, as antibiotic residues should not be present in the final milk supply intended for commercialization due to their implications for public health.

Regarding the types of antibiotics detected, sulfamide residues were found in 15.87% (20/126) of the total samples, β-lactam residues in 4.76% (6/126), and tetracycline residues in 0.79% (1/126) (table 1).

Risk factors

Educational level

The educational level of the farm owners and/or personnel responsible for the sampled farms was predominantly primary education (57.14%), followed by university degree holders (28.57%), and finally, secondary education (14.29%). These data suggest a moderate educational level among the respondents, considering that most have some technical knowledge (table 2).

Age

The age of the study participants (farm owners or workers from the sampled farms) ranged from 32 to 68 years, with a mean age of 44 years. Based on this distribution, participants were categorized into age groups, as detailed in table 3

TABLE 1. Presence of residues and families of antibiotics in raw milk from El Carmen, Manabí

Presence	n	%	Families	n	%
Negative	99	78.57%	Negative	99	78.57%
			Sulfamides	20	15.87%
Positive	27	21.43%	Beta-lactams	6	4.76%
			Tetracyclines	1	0.79%
Total	126	100%	Total	126	100%

Source: own elaboration.

TABLE 2. Educational level of those in charge of the farms

	Frequency	%
Technical	72	57.14%
Professional	36	28.57%
High school	18	14.29%
Total	126	100%

Source: own elaboration.

TABLE 3. Age group of farm managers

	Frequency	%
32 to 50 years	96	76.19%
Above 50 years	30	23.81%
Total	126	100%

Source: own elaboration.

These findings indicate that the personnel working on and managing the farms where samples were collected are relatively young, with 76.19% falling within the 32 to 50-year age range, while 23.81% were in the older age group (above 50 years).

Common diseases in dairy herds

Regarding the most frequently occurring diseases in dairy herds, the results indicated that mastitis was the most prevalent condition (26.19%), followed by infectious respiratory tract diseases with an incidence of 23.81%. Other identified diseases included bacterial infections (19.05%), digestive tract infections, bovine viral diarrhea, and atrophic rhinitis, each with a prevalence of 7.14% (table 4, figure 1).

Antibiotic use in disease treatment

Among the antibiotics used for disease management, tetracyclines were the most frequently administered, detected in 35.71% of the samples. This preference

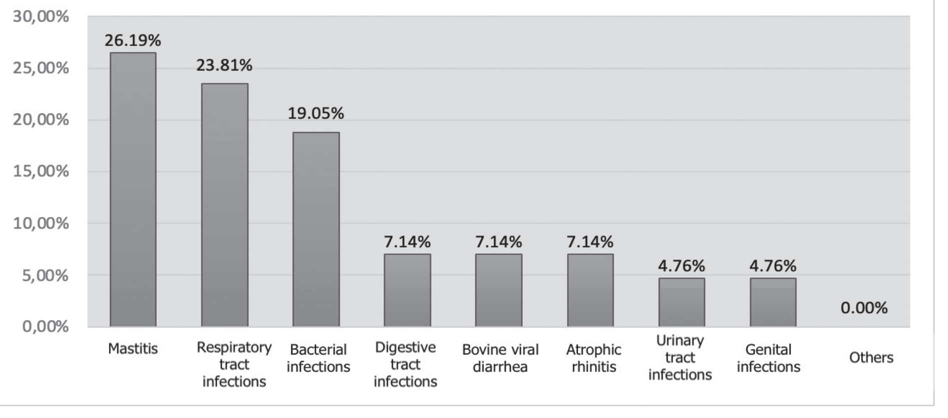

FIGURE 1. Common diseases in dairy herds.

Source: own elaboration.

TABLE 4. Common diseases in dairy herds.

	Frequency	%
Mastitis	33	26.19%
Respiratory tract infections	30	23.81%
Bacterial infections	24	19.05%
Digestive tract infections	9	7.14%
Bovine viral diarrhea	9	7.14%
Atrophic rhinitis	9	7.14%
Urinary tract infections	6	4.76%
Genital infections	6	4.76%
Total	126	100%

Source: own elaboration.

may be attributed to their relatively non-invasive characteristics and broad-spectrum efficacy in addressing multiple conditions affecting dairy herds. β-lactam antibiotics were also commonly used, reported in 35.71% of cases, followed by sulfonamides at 28.57% (table 5).

Dosage and withdrawal period

Respondents indicated that antibiotic administration was generally based on certain herd characteristics, such as weight and age. Specifically, the most commonly reported dosage ranged between 5 and 20 mL per adult cow (64.29% of respondents), while 16.67% reported administering 21–50 mL for more severe conditions, and 19.05% used doses exceeding 50 mL. Notably, some respondents did not adhere to the dosage recommendations provided by the manufacturers, which is a concerning finding.

Additionally, 64.29% of dairy farmers and farm managers reported implementing a withdrawal period of one to five days before selling milk from treated animals, while a smaller proportion observed a waiting period of 5 to 20 days. The antibiotic withdrawal period, as well as the waiting time before milk commercialization, may vary depending on the treated disease and the type of antibiotic used. However, these factors were often overlooked by some producers.

Dosage and withdrawal periods of the medications used

Analysis of the relationship between antibiotic dosage and withdrawal period revealed that 66.67% of dairy farmers adhered to the recommended waiting times before resuming milk production following antibiotic treatment. These respondents indicated that they followed prescribed treatment durations, including waiting at least 15 days after vaccinations or previous treatments before administering additional medication. However, 33.33% of respondents did not comply with these recommendations (table 6).

TABLE 5. Medications used in diseases of dairy herds.

	Frequency	%
Tetracyclines	45	35.71%
Beta-lactams	45	35.71%
Sulfamides	36	28.57%
Total	126	100%

Source: own elaboration.

TABLE 6. Dosage and withdrawal time of medication

	Frequency	%
Within the recommended periods	84	66.67%
Higher than recommended periods	42	33.33%
Total	126	100%

Source: own elaboration.

Route of drug administration

Regarding the administration route of antibiotics, 52.38% (66/126) of respondents reported using the intramuscular route, while 23.81% (30/126) primarily used intravenous administration. Additionally, 14.29% (18/126) applied intramammary administration, and 9.52% (12/126) used subcutaneous injection. The method of antibiotic administration largely depends on the drug type and manufacturer guidelines. However, adherence to these guidelines is critical, as proper administration ensures treatment efficacy and minimizes the risk of antimicrobial resistance.

Disease diagnosis

Concerning disease diagnosis, 64.29% of respondents reported that a veterinarian diagnosed illnesses in their herds, whereas in 35.71% of cases, diagnoses were made by the farm owner or milking personnel. The lack of professional veterinary involvement may lead to inappropriate medication use, including exceeding recommended dosages, prolonging treatment durations, or administering incorrect drugs for specific conditions.

To further investigate antibiotic usage frequency, respondents were asked to estimate the number of times they administered antibiotics to their dairy herds within a year. The results indicated an average of 22 antibiotic applications annually. Moreover, 76.19% of respondents reported administering antibiotics more than ten times per year. This frequency suggests that certain seasons exhibit higher disease incidence, prompting increased antibiotic use to mitigate potential outbreaks, prevent mass infections, and reduce livestock mortality.

Statistical analysis of risk factors

Statistical analysis determined that the frequency of antibiotic use was the only risk factor associated with the presence of antibiotic residues in raw milk from dairy herds.

Initial model including all variables

Initially, all identified variables from the dataset were entered into the R software for logistic regression analysis. This allowed for the identification of residual standard deviation to assess the relationship between two dissimilar variables and predict the probability of an increase or decrease in antibiotic residues in the raw milk samples (table 7):

Table 7 presents a summary of the estimation performed in R, using the appropriate function based on the obtained fit to calculate the residual variance. Upon analyzing table 8, it was observed that none of the variables were statistically significant in predicting the presence or absence of antibiotic residues in raw milk from the 126 samples, indicating a model error. Subsequently, the backward elimination method was applied using R software to gradually remove variables that do not contribute to predicting the presence or absence of antibiotics in raw milk.

TABLE 7. Residual deviation

Min.	1st Qu.	Median	3Q	Max.
-1.461e-06	-1.461e-06	-1.461e-06	-1.461e-06	1.461e-06

Source: own elaboration.

TABLE 8. Variables considered from the database.

	Estimation Std.	Error z	Value
(Intercept)	2.757e+01	1.037e+06	0
Educational level: high school	-1.334e-08	4.833e+05	0
Educational level: technical	-5.177e-11	1.900e+05	0
Years old	3.465e-12	161e+04	0
Diseases: bovine viral diarrhea	-3.747e-10	6.157e+05	0
Diseases: bovine viral diarrhea, mastitis	-6.272e-07	1.119e+06	0
Diseases: bacterial infections	-1.670e-09	5.223e+05	0
Diseases: urinary tract infections	-1.553e-09	6.881e+05	0
Diseases: genital infections	-1.249e-09	5.596e+05	0
Diseases: infections of the digestive tract	-1.188e-09	7.302e+05	0
Diseases: infectious digestive tract, mastitis	-6.379e-07	1.205e+06	0
Diseases: infectious diseases of the respiratory tract	-1.370e-09	5.818e+05	0
Diseases: infectious diseases of the respiratory tract, mastitis	8.919e-06	1.261e+06	0
Diseases: mastitis	-1.742e-09	4.569e+05	0
Diseases: atrophic rhinitis	-1.450e-09	6.999e+05	0
Antibiotics: penicillins	-2.807e-09	9.873e+05	0
Antibiotics: sulfamiridoxines	-3.273e-09	6.973e+05	0
Antibiotics: sulfamiridoxines , Beta-lactams	-8.741e-06	9.850e+05	0
Antibiotics: tetracyclines	-2.507e-09	6.720e+05	NA
Dose: higher	-1.284e-10	5.408e+05	0
Administration: intramuscular	4.423e-10	7.165e+05	0
Administration: intramuscular , intramammary	NA	NA	NA
Administration: intravenous	4.103e-11	2.539e+05	0
Administration: subcutaneous	NA	NA	NA
Diagnostician: owner	1.420e-07	9.599e+05	0
Diagnoser: veterinarian	1.419e-07	9.139e+05	0
Frequency	-1.098e-11	8.041e+03	0
Antibiotic type: none	-5.513e+01	3.246e+05	0
Antibiotic type: sulfonamides	7.204e-07	3.098e+05	0
Antibiotic type: tetracyclines	-8.260e-06	7.890e+05	0

(Dispersion parameter for binomial family taken to be 1)
Null deviance: 1.3093e+02 on 125 degrees of freedom
Residual deviance: 2.6892e-10 on 98 degrees of freedom
AIC: 56
Number of Fisher Scoring iterations: 26

Source: own elaboration.

Selecting the best model using the backward method

The backward elimination method was applied using Akaike's Information Criterion (AIC), which enables the identification of variables that should be retained in the model until the optimal model is obtained. Based on AIC, after assessing each variable individually, it was determined that the best model included the *Coefficient, Intercept, and Frequency*. In other words, the covariate *frequency* was the only significant predictor of the presence or absence of antibiotic residues in raw milk.

Odds ratio analysis

The odds estimate the risk of the presence or absence of antibiotics in milk based on the cofactor of interest, such as an increase in the frequency of antibiotic use in dairy animals from the sampled herds. The OR analysis yielded a coefficient of 0.01103499, indicating a negligible effect size (0.011), which suggests an insignificant probability of detecting antibiotic residues in raw milk. This finding aligns with the overall negative results observed in most of the analyzed samples (table 9).

Model significance

The significance of the model was evaluated to determine whether the presence of antibiotic residues in raw milk was statistically meaningful, with the detailed results provided in table 10. The analysis revealed that the significance level for the variable frequency was 16, which falls within the 0.001 threshold, indicating that *frequency* is a statistically significant predictor of antibiotic presence in the milk samples collected from El Carmen, Manabí.

Moreover, the analysis showed that when antibiotic usage frequency reached an approximate or higher average of 90 doses per year, laboratory results were positive for sulfonamides and β-lactams (figure 2). This suggests that increased antibiotic administration is associated with a higher likelihood of detecting residues in raw milk.

TABLE 9. ODDS Ratio to express the possibility of an event

Variable	Coefficient	Odds_Ratio
1 Constant	-4.506684	0.01103499
2 Frequency	0.048392	0.01103499

Source: own elaboration.

TABLE 10. Model meaning

	Df	Residual desviation	Df	Resid. Dev	Pr (>Chi)
Null			125	130.934	
Frequency	1	70.88	124	60.054	<2.2e-16***
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1					

Source: own elaboration.

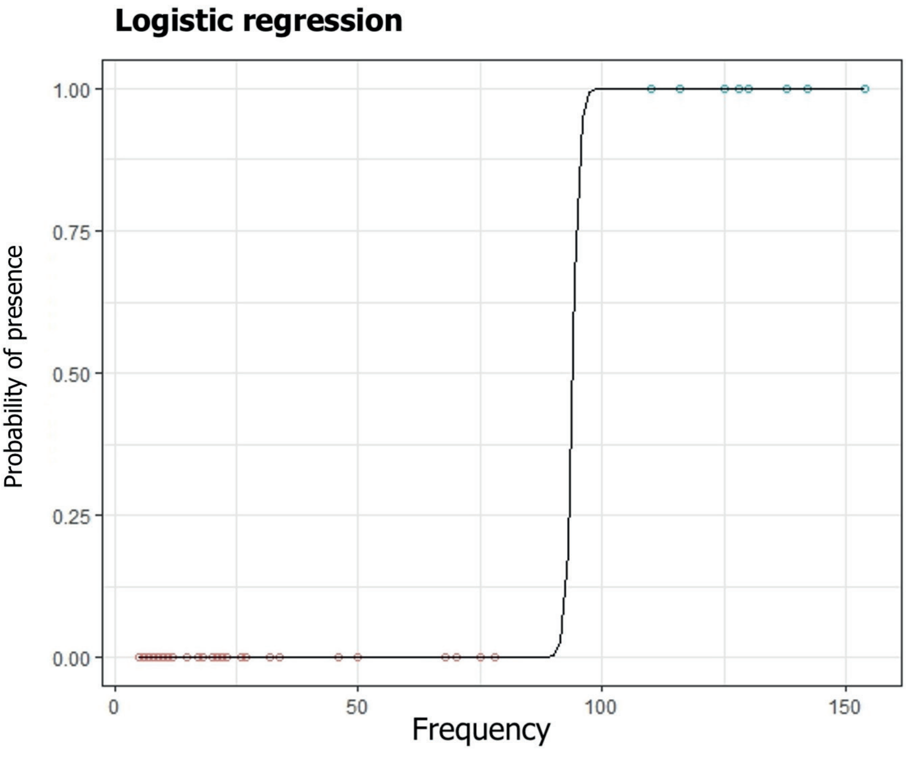

FIGURE 2. Logistic regression.

Source: own elaboration.

Discussion

Following the descriptive and statistical interpretation of the results obtained for raw milk from the canton El Carmen, Manabí, it was identified that 78.57% (99 out of 126 samples) tested negative for antibiotic residues. This finding aligns with reports from Colombia (Madera n.d.), where a study conducted on 40 samples from various farms found that 67.5% also exhibited non detectable antibiotic residues, meeting the national regulatory standards for commercialization and consumption. However, it is important to note that studies on raw milk in Ecuador have shown considerable heterogeneity, with results varying across locations and regions (Puga–Torres *et al.* 2022). For instance, a study conducted in collection centers and small farms in the cantons of Cayambe and Pedro Moncayo (Pichincha, Ecuador) found no antibiotic residues in any of the samples analyzed (Salguero *et al.* 2023).

One notable finding of this study was the prevalent use of sulfonamides (15.87%, 20 out of 126 samples) to treat diseases affecting dairy herds. This result is comparable with another study that reported a 19% prevalence (8 out of 100 samples) for sulfonamide use in dairy herds (Vásquez & Olivera 2012). However, our results do not align with those of Rey et al. (2020), who found that penicillins were more frequently detected than sulfonamides in raw milk samples previously exposed to antibiotics (Rey *et al.* 2020).

Another key aspect to discuss is the prevalence of diseases in dairy herds, which directly influences antibiotic use. This study identified mastitis (26.19%) and infectious respiratory tract diseases (23.81%) as the most frequent health conditions (33 and 30 out of 126 samples, respectively). These findings are consistent with research conducted on dairy farms, where climatic and viral factors often trigger major disease outbreaks in dairy herds. If not addressed promptly, these conditions can lead to mass infections and high mortality rates, significantly impacting farmers' economic stability and threatening food security (Chiesa *et al.* 2021). Antibiotics are commonly used not only for disease treatment but also for preventive purposes and growth promotion. However, there is substantial scientific evidence indicating that food-producing animals (dairy, meat, and egg producers) serve as reservoirs of antimicrobial-resistant bacteria (Gutiérrez *et al.* 2020).

Regarding associated factors, the variable *frequency of antibiotic use* emerged as the most influential determinant. This finding is consistent with the study conducted by Vásquez and Olivera (2012), which reported that in over 50% of cases, annual antibiotic treatments exceeded 100 applications.

Similarly, our findings align with research conducted in Peru, which also identified mastitis as the most prevalent disease in dairy herds. This study further emphasized that antibiotic treatment for mastitis can lead to detectable residues in milk, as a portion of the administered antibiotics is excreted in the milk during the milking process. Additionally, cows with lower milk production tend to eliminate antibiotics over a longer period, prolonging the presence of residues in milk. The study also highlighted that failure to adhere to prescribed dosages and application times is a key factor contributing to antibiotic residues in raw milk and the development of bacterial resistance (Choque–Quispe *et al.* 2020).

Furthermore, it is crucial to ensure that treatment durations do not exceed recommended limits, as non-compliance with withdrawal periods increases the risk of antibiotic residues in raw milk. In the present study, it was observed that cases testing positive for antibiotic residues involved treatments that exceeded the recommended dosage and withdrawal times. Therefore, strict adherence to prescribed dosages and the minimization of antibiotic application are essential in managing diseases in dairy herds (Choque– Quispe *et al.* 2020; Reyna & Arteaga 2022).

CONCLUSIONS

This study determined the high prevalence of antibiotic residues in raw milk from the sampled canton, primarily due to the indiscriminate use of veterinary antibiotics by dairy farmers. A total of 21.43% of the analyzed samples contained antibiotic residues, with sulfonamides (15.87%) being the most frequently detected, followed by beta-lactams (4.76%). Statistical analysis performed using R software, based on Akaike's Information Criterion (AIC), identified *frequency of antibiotic use* as the most influential factor in the presence of antibiotic residues, compared to other covariables. However, the odds ratio (0.011) indicated that an increase in antibiotic usage frequency could raise the probability of detecting antibiotic residues in raw milk.

It is crucial to provide training to dairy farmers in this key milk-producing region of Ecuador regarding the risks associated with the indiscriminate use of antibiotics in livestock, particularly concerning public health implications when withdrawal periods are not respected. Additionally, governmental intervention is necessary to prevent contaminated milk from reaching consumers.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ACKNOWLEDGMENTS

The authors extend their gratitude to the dairy farmers of El Carmen, Manabí, Ecuador, for their generous cooperation.

FUNDING DECLARATION

This research was self-funded by the authors.

ETHICAL COMMITTEE APPROVAL

Ethical committee approval was not required for this study, as samples were collected directly from milk containers following the milking process performed by farm personnel. At no point did the researchers collect samples directly from the animals or have direct contact with them. Additionally, the survey conducted did not include personal or confidential questions directed at farm owners or animal caretakers.

DECLARATION ON THE USE OF ARTIFICIAL INTELLIGENCE

No artificial intelligence was used in this study.

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Forma de citación del artículo:

Vera, M.D., & Puga-Torres, B. (2025). Factors associated with the presence of antibiotic residues in raw milk from cows in the canton of El Carmen, Manabí, Ecuador. Rev Med Vet Zoot. 72(1): e113703. https://doi.org/10.15446/rfmvz.v72n1.113703

https://doi.org/10.15446/rfmvz.v72n1.117797

Current epidemiological situation of bovine viral diarrhea and infectious bovine rhinotracheitis in Colombia

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Recibido: 29/11/2024 Aprobado: 11/02/2025

ABSTRACT

Viral diseases such as bovine viral diarrhea (BVD) and infectious bovine rhinotracheitis (IBR) impact livestock production, causing significant economic losses in dairy and beef cattle, due to reproductive and respiratory issues. This research aims to identify risk factors contributing to the occurrence of IBR and BVD in the department of Huila, and to determine biosecurity measures for effective management in livestock production. The sampling was made on 360 cattle across 24 municipalities with blood samples tested for BVD and IBR antibodies using ELISA. Of the 360 samples analyzed, 52.7% tested positive for BVD, 55.6% for IBR, and 31.1% for both. Key risk factors identified included estrus detection, contact with neighboring animals, and limited access restrictions for BVD. In contrast, IBR risk factors involved animal purchases, contact with neighboring herds, and infrastructure like footbaths. Protective factors including regular pregnancy diagnostics, tool disinfection, and animal isolation protocols. This descriptive research highlights the importance of managing IBR and BVD in livestock, identifying a lack of awareness and farming practices especially in prevention, sanitation, and biosecurity as critical issues impacting production.

Keywords: ELISA, risk factors, protective factors, biosecurity.

Situación epidemiológica actual de diarrea viral bovina y rinotraqueítis infecciosa bovina en Colombia

RESUMEN

Las enfermedades virales como la diarrea viral bovina (DVB) y la rinotraqueítis infecciosa bovina (RIB) afectan la producción ganadera, causando pérdidas económicas significativas tanto en el ganado lechero como en el de carne debido a problemas reproductivos y respiratorios. Esta investigación tiene como objetivo identificar los factores de riesgo que contribuyen a la aparición de RIB y DVB en el departamento del Huila, y determinar medidas de bioseguridad para un manejo efectivo en la producción ganadera. El muestreo se realizó en 360 bovinos en 24 municipios con muestras de sangre analizadas

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para anticuerpos de DVB y RIB mediante ELISA. De las 360 muestras analizadas, el 52,7% dio positivo para DVB, el 55,6% para RIB y el 31,1% para ambos. Los factores de riesgo clave identificados incluyeron la detección de celo, el contacto con animales vecinos y las restricciones de acceso limitadas para DVB, mientras que los factores de riesgo de RIB involucraron la compra de animales, el contacto con rebaños vecinos e infraestructura como baños de pies. Los factores de protección se identificaron como diagnósticos regulares de preñez, desinfección de herramientas y protocolos de aislamiento animal. Esta investigación descriptiva resalta la importancia del manejo de IBR y BVD en el ganado, identificando la falta de concientización y prácticas agrícolas especialmente en prevención, saneamiento y bioseguridad como problemas críticos que impactan la producción.

Palabras clave: ELISA, factores de riesgo, factores de protección, bioseguridad.

INTRODUCTION

Viral diseases have affected livestock production for centuries, and more zoonotic diseases have been detected in recent years to have a negative impact on their performance. Bovine viral diarrhea (BVD) and infectious bovine rhinotracheitis (IBR) have been reported to cause several economic losses worldwide, both in dairy and beef livestock (Rondón 2006). These diseases can impact the herds depending on the immunity status, management, and nutritional status, also they can cause miscarriage, embryo and embryo death, weight loss, and negative impact on dairy production.

First, BVD is caused by pestivirus with a single-stranded, positive-sense RNA genome from the family Flaviviridae. This virus has two biotypes (cytopathic and non-cytopathic) based on its behavior in cell culture and two genotypes (I and II) based on its genetic sequence. The severity of clinical conditions may vary according to the infecting strain, this could cause severe mucosal damage and lethal effects on the fetus (La Polla *et al.* 2022).

On the other hand, IBR is caused by bovine herpesvirus type 1 (BHV-1), a member of the subfamily Alphaherpesvirinae. It affects individuals of all ages and causes several economic impacts on production due to respiratory and reproductive issues. Transmission occurs directly through contact between infected animals and their aerosols. The incubation period for this disease is 3 to 7 days, and the virus has been detected at least 2 weeks after infection in nasal and ocular secretions. Indirect transmission may occur via fomites, including contaminated equipment, tools, and materials used by veterinarians and other individuals involved in the production process. Additionally, venereal transmission can occur during mating when there is contact with the genital secretions of infected cattle or during artificial insemination with contaminated semen (Nettleton & Russell 2017).

This investigation aims to establish the seroprevalence of IBR and BVD in dual-purpose production systems in the department of Huila and to identify the factors that may contribute to the increased prevalence of these diseases, as well as to identify the possible factors contributing to the occurrence of IBR and BVD, which significantly impact cattle producers' income and their operations in the department of Huila, Colombia.

MATERIALS AND METHODS

This study was conducted as a cross-sectional collaboration between University Corporation of the Huila Region (Corhuila), Colombian Agricultural Research Corporation (Agrosavia), Committee of Huila Cattlemen (CGH), and the Governorate of Huila. Additionally, all the activities carried out in this study were approved through the ethics, bioethics and scientific integrity committee of Colombian Agricultural Research Corporation (Agrosavia), under Act N.° 2 of 2021.

A total of 360 cattle over 24 months old were randomly selected from the 150 farms associated with livestock organizations. The sampling was conducted in 24 municipalities of Huila, distributed across four regions as follows: the central region (Altamira, Garzón, Gigante, Pital, Tarqui, and Suaza), the northern region (Aipe, Algeciras, Baraya, Campoalegre, Colombia, Palermo, Rivera, Tello, Villavieja, and Yaguará.), the western region (La Plata, Nátaga, Paicol, and Tesalia), and the southern region (Acevedo, Pitalito, San Agustín, and Timaná).

Blood samples were collected via jugular vein puncture. The samples were centrifuged at 3.070 Relative Centrifugal Force (RCF) for 5 minutes to separate the sera and preserved at -20 °C to avoid degradation.

Epidemiological surveys were used to collect information on sanitary, socioeconomic, and animal management conditions for each farm, including a monthly follow-up over the course of one year. Additionally, a clinical examination was performed on every animal included in the study.

Bovine viral diarrhea detection

The ELISA analysis was performed using the commercial kit Ingezim BVD DAS (INGENASA, Madrid, Spain). This assay is based on Double Antibody Sandwich ELISA technique using monoclonal antibodies (MAb), which are specific for p80/p125 BVDV protein.

The samples and kit components were thawed at room temperature. Then, 100 µl of the samples were dispensed into each well in duplicate, as established in our laboratory's standardized protocol under ISO 17025 accreditation, to ensure the validity of the results. This approach was not explicitly required by the manufacturer but was implemented based on our internal validation process.

The plate was incubated at room temperature for 18 hours. After incubation, the washing was performed as follows: the plate was inverted to discard the contents while preventing cross-contamination between wells, 300 µl of washing solution were dispensed, the plate was shaken for 30 seconds, the contents were discarded, and the process was repeated six times according to the kit insert. After the washing process, 100 µl of conjugate 1 were added to each well and incubated for 1 hour at 37 °C.

The plate was washed 6 times according to the manufacturer's recommendations. Conjugate 2 was added by 100 µl to each well and then incubated at room temperature for 30 minutes. Another washing was performed. Finally, 100 µl of stop solution were added to each well. The plate was read in the Microplate reader BMR-100 (BOECO Germany) at 450 nm within 5 minutes after the addiction of stop solution.

Infectious bovine rhinotracheitis detection

The detection was performed using the commercial kit Ingezim IBR 2.0 (INGENASA, Madrid, Spain). This kit uses the indirect ELISA method to detect specific antibodies against bovine herpesvirus 1 (BoHV1).

The samples were added by duplicate by 100 µl to each well including the kit controls. Then the plate was incubated at 37 °C for 45 minutes. After the incubation, the plate was washed 4 times following the manufacturer's instructions. Additionally, 100 µl of conjugate were added to each well and incubated for 45 minutes at 37 °C. The plate was washed 5 more times according to the instructions. The substrate solution was added by 100 µl and incubated by 15 minutes at room temperature. Finally, 100 µl of stop solution was added to the plate. The plate was read in the Microplate reader BMR-100 (BOECO Germany) at 450 nm within 5 minutes after the addition of stop solution.

Statistical analysis

The study aimed to investigate the relationship between socioeconomic and demographic factors and the presence of the viruses bovine viral diarrhea and infectious bovine rhinotracheitis.

Binary logistic regression models were developed, using ELISA test results as the dependent variable (0: negative, 1: positive for one of the viruses). The prevalence was compared using the Chi-Square test across the different variable levels, including age groups and regions. Determinant factors, including vaccination status, farm size, and animal density, were compared, and odds ratios (OR) were calculated to examine disparities. The existence of these factors was verified through a combination of survey responses and on-site validation during a one-year productive follow-up. Vaccination status was confirmed both through the survey and by cross-referencing vaccination records during the follow-up period. To avoid interference from vaccine-induced antibodies, sample collection timing was carefully planned in relation to vaccine administration, minimizing the risk of false positives. Statistical significance was determined at a 95% confidence level ($P < 0.05$), enhancing the validity of the selected environmental factors.

The study utilized R version 4.3.3 to conduct descriptive analyses, which facilitated the identification of logistic regression models and environmental variables associated with the infections. This approach allowed for identifying significant determinants, indicating their association with either the presence or absence of viruses.

RESULTS AND DISCUSSION

Of the 360 samples analyzed, 190 (52.7%) showed antibodies for BVD. Meanwhile, 200 of the 360 samples (55.6%) tested positive for IBR. Additionally, 112 of the 360 samples (31.1%) tested positive for both diseases. In the same vein, 278 out of 360 animals (77.2%) tested positive for either of the two diseases. The age group of 66-85 months showed the highest number of seropositive animals, with 82.3% testing positive for at least one of the two diseases. This was followed by the age groups ≥106 months and 46-65 months, with 80% and 78.3%, respectively. On the other hand, the age of eight individuals could not be determined and, therefore, they could not be included in the age groups. Similarly, the distribution of positive cases for each disease was comparable(table 1).

Regarding the geographical distribution of the disease, the region with more animals analyzed was the northern region: 49.1% (177/360); followed by the southern region: 18.0% (65/360); then, the western region: 16.6% (60/360), and the central region: 16.1% (58/360). Similarly, the highest seroprevalence for either of the two diseases was in the central region with 86.2%, followed by the northern region (81.9%), western region (70%), and southern region (63.1%). However, for bovine viral diarrhea, the highest seropositivity was observed in the western region at 60%, followed by the northern region (54.8%), southern region (47.7%), and central region (44.8%) (table 2).

Regarding BVD, the risk factors found using binary logistic regression analysis were the tools cleaning before the procedures, estrus detection, contact with neighboring animals, and restriction on animal access (table 3). In contrast, for IBR the risk factors identified were purchasing animals for production, contact with neighboring animals, and the presence of infrastructure like footbaths and housing facilities. However, activities such as regular pregnancy diagnostics, disinfecting work tools, consistently analyzing the reproductive potential of males, implementing protocols for cow synchronization, and isolating sick animals in the production process are considered protective factors

TABLE 1. Distribution of positive cases for BVD, IBR, and BVD or IBR diseases by age groups

Variable		n	BVD			IBR			BVD or IBR		
			+	%	I.C. 95%	+	%	I.C. 95%	+	%	I.C. 95%
Age	0 to 45	59	27	45.8	(0.33-0.58)	25	42.4	(0.30-0.55)	40	67.8	(0.56-0.80)
	46 to 65	115	61	53.0	(0.44-0.62)	63	54.8	(0.46-0.64)	90	78.3	(0.71-0.86)
	66 to 85	124	74	59.7	(0.51-0.68)	77	62.1	(0.53-0.71)	102	82.3	(0.75-0.89)
	86 to 104	24	11	45.8	(0.26-0.66)	10	41.7	(0.22-0.62)	17	70.8	(0.52-0.90)
	106 or more	30	13	43.3	(0.26-0.61)	20	66.7	(0.49-0.84)	24	80.0	(0.65-0.94)
			χ^2:5.10; gl:5; p:0.404			χ^2: 9.86; gl: 5; p: 0.079			χ^2: 6.51; gl: 5; p: 0.259		

Source: own elaboration.

TABLE 2. Cattle distribution by geographic region in the department of Huila and the corresponding positive cases for BVD, IBR, and BVD or IBR diseases

Variable		n	BVD			IBR			BVD or IBR		
			+	%	I.C. 95%	+	%	I.C. 95%	+	%	I.C. 95%
Region	Southern	65	31	47.7	(0.36-0.60)	21	32.3	(0.21-0.43)	41	63.1	(0.51-0.75)
	Central	58	26	44.8	(0.32-0.57)	42	72.4	(0.61-0.84)	50	86.2	(0.77-0.95)
	Northern	177	97	54.8	(0.47-0.62)	106	59.9	(0.53-0.67)	145	81.9	(0.76-0.88)
	Western	60	36	60.0	(0.48-0.72)	31	51.7	(0.39-0.64)	42	70.0	(0.58-0.82)
			χ^2:3.69; gl:3; p:0.297			χ^2: 22.6; gl: 3; p: 0.001			χ^2: 14.01; gl: 3; p: 0.003		

Source: own elaboration.

TABLE 3. Logistic regression of independent variables for risk factors with the seroprevalence of bovine viral diarrhea

Variable		N	β	gl	P	OR	IC OR 95%	
							ICI	ICS
Region	Central	58	-	-	-	-	-	-
	Northern	177	0.30	1	0.188	1.49	0.82	2.71
	West	60	0.37	1	0.100	1.85	0.89	3.83
	Southern	65	0.36	1	0.750	1.12	0.55	2.28
Self-medication	No	56	-	-	-	-	-	-
	Yes	303	-0.66	1	0.030*	0.52	0.52	0.28
Estrus detection	No	155			-	-		
	Yes	204	0.44	1	0.041*	1.55	1.02	2.36
Synchronization procedures	No	231			-	-		
	Yes	128	0.27	1	0.216	1.32	0.85	2.03
Pregnancy diagnosis	No	64	-	-	-	-	-	-
	Periodically	132	-0.40	1	0.190	0.67	0.37	1.22
	Sporadically	164	0.16	1	0.597	1.17	0.65	2.09
Purchase of fattening animals	No	286	-	-	-	-	-	-
	Yes	74	0.06	1	0.805	1.07	0.64	1.78
Contact with neighboring animals	No	148	-	-	-	-	-	-
	Yes	210	0.52	1	0.017*	1.68	1.10	2.56
Work tool disinfection	No	28	-	-	-	-	-	-
	Yes	329	-0.05	1	0.895	0.95	0.44	2.06
Male evaluation	No	147	-	-	-	-	-	-
	Yes	59	-0.21	1	0.494	0.81	0.44	1.48
Housing facilities	No	56	-	-	-	-	-	-
	Yes	304	-0.21	1	0.477	0.81	0.46	1.44
Isolation of sick animals	No	64	-	-	-	-	-	-
	Yes	295	-0.25	1	0.362	0.776	0.45	1.33
Introduction of external animals	No	331	-	-	-	-	-	-
	Yes	29	-0.35	1	0.337	0.707	0.33	1.52
Restrict access to the herd	No	126	-	-	-	-	-	-
	Yes	234	0.72	1	0.001*	2.046	1.32	3.18
Implement quarantine	No	146	-	-	-	-	-	-
	Yes	211	0.22	1	0.502	0.865	0.57	1.32

Source: own elaboration.

(table 4). Finally, the risk factors for the occurrence of either of the two diseases are: keeping animals in the northern and western regions, implementing reproductive protocols for cow synchronization, and restricting the access of other animals. Meanwhile, the protective factors are pregnancy diagnosis, selling breeding animals, introducing external animals, and implementing quarantine (table 5).

The seroprevalence observed in this study for IBR was 55.6% (200/360), while for BVD it was 52.7% (190/300). Previous studies in Colombia have reported seroprevalence rates for BVD ranging between 30% and 35% (Ochoa *et al.* 2012; Rivera *et al.* 2018) and between 50.0% and 58.0% (Motta Giraldo *et al.* 2013; Rivera *et al.* 2018).

On the other hand, regarding geographic areas, it is evident that for the presentation

TABLE 4. Logistic regression of independent variables for risk factors with the seroprevalence of IBR

Variable		n	β	gl	P	OR	IC OR 95%	
							ICI	ICS
Region	Central	58	-	-	-	-	-	-
	Northern	177	-0.56	1	0.089	0.57	0.30	1.09
	West	60	-0.90	1	0.022*	0.41	0.19	0.88
	Southern	65	-1.70	1	0.000*	0.18	0.08	0.39
Self-medication	No	56	-	-	-	-	-	-
	Yes	303	0.09	1	0.762	1.09	0.62	1.94
Estrus detection	No	155	-	-	-	-	-	-
	Yes	204	0.04	1	0.844	1.04	0.69	1.59
Synchronization procedures	No	231	-	-	-	-	-	-
	Yes	128	0.55	1	0.015*	1.74	1.11	2.71
Pregnancy diagnosis	No	64	-	-	-	-	-	-
	Periodically	132	-0.82	1	0.009*	0.44	0.24	0.81
	Sporadically	164	-0.11	1	0.729	0.90	0.49	1.64
Purchase of fattening animals	No	286	-	-	-	-	-	-
	Yes	74	0.79	1	0.005*	2.20	1.27	3.82
Contact with neighboring animals	No	148	-	-	-	-	-	-
	Yes	210	0.65	1	0.003*	1.91	1.25	2.93
Work tool disinfection	No	28	-	-	-	-	-	-
	Yes	329	-0.95	1	0.036*	0.39	0.16	0.94
Male evaluation	No	147	-	-	-	-	-	-
	Yes	59	-0.74	1	0.019*	0.48	0.26	0.89
Housing facilities	No	56	-	-	-	-	-	-
	Yes	304	0.96	1	0.001*	2.61	1.44	4.73
Isolation of sick animals	No	64	-	-	-	-	-	-
	Yes	295	-0.69	1	0.019*	0.50	0.28	0.89
Introduction of external animals	No	331	-	-	-	-	-	-
	Yes	29	-0.32	1	0.412	0.73	0.34	1.56
Restrict access to the herd	No	126	-	-	-	-	-	-
	Yes	234	0.39	1	0.076	1.48	0.96	2.29
Implement quarantine	No	146	-	-	-	-	-	-
	Yes	211	-0.31	1	0.152	0.73	0.48	1.12

Source: own elaboration

TABLE 5. Logistic regression of independent variables for risk factors with the seroprevalence of both BVD and IBR

Variable		n	β	gl	P	OR	IC OR 95%	
							ICI	ICS
Region	Central	58	-	-	-	-	-	-
	Northern	177	-0.32	1	0.452	0.72	0.31	1.68
	West	60	-0.98	1	0.038*	0.37	0.15	0.94
	Southern	65	-1.30	1	0.005*	0.27	0.11	0.67
Self-medication	No	56	-	-	-	-	-	-
	Yes	303	-0.22	1	0.536	0.80	0.39	1.63
Estrus detection	No	155	-	-	-	-	-	-
	Yes	204	0.23	1	0.362	1.26	0.77	2.06
Synchronization procedures	No	231	-	-	-	-	-	-
	Yes	128	0.77	1	0.008*	2.15	1.22	3.79
Pregnancy diagnosis	No	64	-	-	-	-	-	-
	Periodically	132	-1.03	1	0.008*	0.36	0.17	0.77
	Sporadically	164	-0.06	1	0.877	0.94	0.43	2.07
Purchase of fattening animals	No	286	-	-	-	-	-	-
	Yes	74	0.64	1	0.072	1.89	0.94	3.79
Contact with neighboring animals	No	148	-	-	-	-	-	-
	Yes	210	0.65	1	0.11	1.91	1.16	3.15
Work tool disinfection	No	28	-	-	-	-	-	-
	Yes	329	-0.34	1	0.505	0.71	0.26	1.93
Male evaluation	No	147	-	-	-	-	-	-
	Yes	59	-0.70	1	0.44	0.49	0.25	0.98
Housing facilities	No	56	-	-	-	-	-	-
	Yes	304	0.15	1	0.66	1.16	0.60	2.24
Isolation of sick animals	No	64	-	-	-	-	-	-
	Yes	295	-0.70	1	0.69	0.50	0.23	1.06
Introduction of external animals	No	331	-	-	-	-	-	-
	Yes	29	-0.81	1	0.47*	0.45	0.20	0.99
Restrict access to the herd	No	126	-	-	-	-	-	-
	Yes	234	0.75	1	0.003*	2.13	1.29	3.51
Implement quarantine	No	146	-	-	-	-	-	-
	Yes	211	-0.63	1	0.020*	0.53	0.31	0.91

Source: own elaboration.

of BVD alone, there is no statistically significant difference, while for IBR, the western and southern regions behave as protective factors (OR: 0.41 and 0.18, respectively) compared to the central regions. Similarly, for the presentation of either of the two diseases, the western and southern regions were protective factors (OR: 0.37 and 0.27, respectively) in comparison with the southern region. These results highlight how the specific climatic characteristics of different regions in Huila may influence the prevalence of diseases such as IBR and BVD. In the central region, where a temperate climate predominates, the conditions may favor the survival and transmission of certain pathogens due to lower humidity, which could allow viruses to remain viable for longer in the environment. In contrast, the temperate climate of the west and the cold climate of the south appear to offer some degree of protection, possibly due to lower animal density and different management practices associated with these climates (Murcia–Mono *et al.* 2025).

In the epidemiological survey, 13 sanitary and socioeconomic conditions were identified as being related to the seroprevalence observed in the diseases ($P < 0.05$). Self-medication was identified as a protective factor for BVD in this study (OR: 0.52), based on field observations about metaphylactic measures implemented to safeguard the health of their animals. A survey conducted with 23 veterinarians in North America revealed that 95.65% of respondents recommend metaphylaxis (Terrell *et al.* 2011). Additionally, Schlochtermeier (2018) mentions that metaphylactic treatment in large groups of animals reduces both morbidity and mortality and is also effective in administering parenteral products to young cattle at high risk of developing bovine respiratory syndrome (BRS). At the national level, the use of popular antimicrobials such as tulathromycin, enrofloxacin, oxytetracycline, and sulfamethazine, among others, is well known and has demonstrated favorable efficacy.

Generally, the spread of antigens is due to poor farming practices (González–Stagnaro 2011), such as personnel spreading the viruses between infected and non-infected animals. In this study, estrus detection was considered a risk factor (OR: 1.55). The implementation of Good Agricultural Practices (GAP) is crucial as antigens can persist in areas and objects, facilitating their spread. Therefore, we infer that rather than the estrus detection itself, the lack of implementation of the aseptic practices followed during the detection process could increase the prevalence. Studies show the importance of sanitary management in promoting health, improving production, and preventing animal diseases (Benavides & Rosenfeld 2009). Walz *et al.* (2010) emphasized the need to eliminate contact between livestock and neighboring fences, properly disinfect equipment, and restrict the entry of outsiders into production areas. Furthermore, there are various methods for detecting estrus in cows, ranging from visual observation and record-keeping to the use of pressure-sensitive devices, chalk or paint at the base of the tail, and estrus detection patches (Guáqueta 2009).

On the other hand, synchronization protocols were identified as a risk factor for the occurrence of IBR alone (OR: 1.74) and for the concurrent occurrence of both BVD and IBR (OR: 2.15). Romero–Salas *et al.* (2013) concluded that, on dual-purpose cattle farms, natural breeding contributes to the transmission of IBR; therefore, the adoption of artificial insemination could help reduce its prevalence. Villar (2015) mentions that artificial insemination or mixed natural breeding are risk factors (OR: 7.89) for bovine viral diarrhea compared to farms that only use natural breeding. However, Abad–Zavaleta *et al.* (2016) stated that farms that do not perform artificial insemination or embryo transfers have a higher risk of IBR and BVD, results that differ from Villar (2015).

One of the advantages of synchronization protocols is to avoid direct contact of fluids between animals, and thus the disease, making it essential to maintain good livestock practices, such as the asepsis of reproductive tools (González–Stagnaro 2011). However, despite the divergences in the research findings, the need for rigorous biosecurity measures and good livestock practices is evident.

Synchronization protocols seem to be a valuable tool to reduce disease transmission, but they must be complemented with strict hygiene and rigorous health control of breeding animals to maximize their effectiveness. Additionally, continuous training for producers in risk identification and management is crucial to reduce the spread of these diseases. The implementation of specific protocols adapted to local conditions and the constant evaluation of their effectiveness are essential for efficient health management in the dual-purpose livestock farming in Huila.

Various pathogens, such as the IBR virus, can affect pregnant cows, causing embryonic death, mummification, abortions, infertility, and the birth of stillborn or weak calves that die shortly after birth (Magaña–Urbina *et al.* 2005). Early disease detection is crucial for identifying and isolating infected animals to prevent further transmission. However, common diagnostic methods, such as rectal palpation, can pose a risk of disease spread if not performed under proper biosecurity measures.

This study focuses solely on early detection methods, excluding reproductive management practices. Our findings highlight the need for producer training in proper waste disposal and biosecure animal handling during diagnostic procedures. While early diagnosis is essential for disease control, implementing best practices is equally important to minimize transmission risks.

There are common methods for pregnancy diagnosis that allow for early detection of the disease and proper isolation to prevent further infections. In this study, pregnancy diagnosis is considered a protective factor (OR: 0.44) for the occurrence of IBR.

The purchase of fattening animals was identified as a risk factor in this research (OR: 2.20), due to the possible acquisition of persistently infected animals and the limited control in diagnostics for the detection of IBR. Delgado *et al.* (2022) noted that the main factor in the spread of the disease is the lack of control when acquiring an animal. In 2022, a study on respiratory diseases in cattle in Villavicencio determined that the purchase of fattening animals is a risk factor (OR: 1.44), associated with the transportation of animals (Ballesteros González & Briñez Castiblanco 2022). Taylor *et al.* (2010) indicated that purchased animals, typically transported to their place of origin, may be exposed to different management variables, including stress, which could act as a "necessary but not sufficient" association for the occurrence of respiratory diseases in cattle. Therefore, it is recommended to follow a strict health protocol when integrating new animals into herds to preserve the health of the animals already present on the farm (Narváez Morales & Sangucho Lema 2021).

There are preventive measures to control IBR when the first symptoms of the disease manifest. In this research, the separation of sick animals is considered a protective factor (OR: 0.50). This suggests that the isolation practices implemented by farmers are effective in reducing the presence of the disease. When clinical signs suggesting infections in the herd are observed, it is recommended to take preventive isolation measures (Obando & Rodríguez 2005).

Similarly, Alvarez *et al.* (2007) mentions that if a lower prevalence is observed, concentrated in adult animals, it is possible to perform individual serology on all animals and proceed to eliminate the seropositive animals after vaccinating them every six months, which allows the eradication of the disease in production.

Currently, good livestock practices provide producers with the opportunity to establish proper sanitary and commercial conditions for the export of their products. These practices not only strengthen export capacity but also implement various prevention and control programs aimed at protecting the herds from any eventuality that could put them at risk. This research has identified that the disinfection of equipment is a significant protective factor (OR: 0.39), attributable to the good practices promoted by farmers regarding effective disinfection protocols on farms to prevent the spread of diseases (Benavides & Rosenfeld 2009).

Semen evaluation is crucial in bovine reproduction, allowing the identification of bulls with subfertility or infertility. These evaluations should be performed periodically, including three fundamental steps: physical evaluation, libido assessment, and semen collection and analysis (Lozano & Arias 2008). In the present study, productions that perform exams to determine the male's potential are considered protective factors for the occurrence of IBR (OR: 0.48). When analyzing semen quality and detecting any abnormalities, the bull can be treated or removed from the production system. Additionally, viruses such as the one causing IBR can affect semen quality, as this antigen may be present in subclinical infected bulls.

The IBR virus (BHV-1) can affect reproductive tissues, compromising semen quality and bull fertility. This virus may cause inflammation of testicles and epididymitis, interfering with spermatogenesis and sperm motility, which significantly reduces semen quality. Therefore, semen evaluation can serve as an indicator of IBR infection (Bierema *et al.* 2009; O'Toole *et al.* 2011).

On the other hand, even when BVD may affect reproductive health, it has not been reported a direct affectation to the semen quality being these effects less evident. The common impacts of these diseases are reflected in abortions, fetal malformations, and more vulnerability to secondary infections, but not in the bull reproductive health (Miller *et al.* 2008; Robson *et al.* 2015).

The statistical limitations, such as sample size, may have affected the ability to accurately diagnose semen quality and identify an association with BVD. Additionally, the variability in exposure and the different clinical manifestations of the disease may have hindered the identification of a correlation in this study.

Animal health guarantees the well-being and genetic expression of herds, and its control should be based on the proper structuring and monitoring of a health program. Maintaining animal health is essential to ensure the stability of production in agricultural enterprises, controlling the emergence of infectious and contagious disease outbreaks (Sánchez–Villalobos 2014). The infrastructure of farms plays a crucial role in managing epidemiological outbreaks; adequate facilities allow for the timely isolation of infected animals, preventing the spread of diseases. However, in this research, housing facilities were identified as a significant risk factor (OR: 2.61) for the occurrence of IBR. We infer that this is due to the limited available space in productions for isolation, which could facilitate faster spread. This study included facility observations conducted by professionals who periodically visited the farms to ensure the reliability of the data. During these follow-up visits, the professionals assessed the isolation facilities. However, specific questions regarding isolation facilities and procedures were not included in the survey. Given the impact of isolation practices on disease transmission, it is important for future investigations to include specific questions to better evaluate this determinant.

Various studies have shown results contrary to our findings. For example, González *et al.* (2009) reported a seroprevalence of 58.9% in productions with closed system infrastructures and 81.5% in productions with open systems, demonstrating a significant association between infection and open field systems. Likewise, Ferrara *et al.* (2024) mentioned that house accommodation is a protective factor (OR=0.16) compared to keeping animals in a partial grazing system. Although the results obtained in this study indicate a situation contrary to the literature review, we conclude that having housing facilities on the farm does not necessarily serve as a protective factor, but rather the combination of its size and the biosecurity measures implemented is what truly matters.

Infectious agents enter the herd through individuals with chronic infections, so any biosecurity plan must begin within the farms. Thus, it is necessary to maintain the fences in pastures in optimal conditions to control the entry and exit of animals, thereby minimizing contact with neighboring herds (Hoet & Boscán 2005). Therefore, preventing the entry of foreign cattle into the production system was a protective factor in this study (OR: 0.47). However, this result was completely contradictory for those herds where the entry of both vehicles and visitors is restricted (OR: 2.13).

Furthermore, quarantine was also considered a protective factor (OR: 0.53) for the occurrence of either disease, as they are transmitted through direct contact between sick and susceptible animals (Hoet & Boscán 2005).

CONCLUSIONS

Bovine viral diarrhea (BVD) and infectious bovine rhinotracheitis (IBR) are not officially controlled diseases in Colombia, yet they are critically associated with the bovine reproductive complex. Given their significant contribution to reproductive pathologies and their consequential impact on livestock production systems, their management remains a priority in veterinary health programs. This study evaluated various socioeconomic, demographic, and sanitary determinants, identifying 13 factors significantly associated with the epidemiology of these diseases. Specifically, IBR was correlated with six protective factors and four risk factors, whereas BVD was associated with one protective factor and three risk factors. The primary challenge identified was the limited awareness and implementation of Good Agricultural Practices (GAP). This includes deficiencies in primary prevention strategies, such as immunization protocols, environmental sanitation measures, and adherence to biosecurity protocols, which are essential to mitigating the prevalence and impact of these diseases.

The surveys were complemented by direct measures carried out during follow-up visits. In this way, the data obtained through the surveys were validated with the results of field measurements, allowing for greater robustness in the conclusions.

However, we recognize that this approach may still have limitations, such as the possible variability in respondents' perceptions and in facility conditions over time. Therefore, it is suggested that future studies follow a similar approach but incorporate advanced technologies or additional tools to make more accurate and objective measurements of these factors.

Finally, it is important to carry out agricultural extension through training for livestock producers on topics related to good livestock practices, sustainable livestock, animal health, animal welfare, One Health, among others, which is essential for production to reach levels of productivity and competitiveness in the region. This should be developed through professionals with experience and methodology according to the population to be targeted.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

FINANCING

The funding for this research came from the Department of Huila through funds from the General Royalties System (SGR, for its abbreviation in Spanish) and the allies of the project named "Sanitary and genomic analysis in dairy cattle with emphasis on breeding for the improvement of productive and competitive characteristics in the department of Huila" BPIN 2021000100300.

ACKNOWLEDGMENT

The authors express their gratitude to the Governorate of Huila, Department of Huila, for funding and overseeing the BPIN 2021000100300 project. They also extend their gratitude to the project allies: Committee of Huila Cattlemen (CGH) for collaborating with the livestock associations in the region. Additionally, they acknowledge the support of the Colombian Agricultural Research Corporation (Agrosavia) through the project "ID1002144: Assessment and multi-animal high genetic value Huila," as well as the execution of the project by the University Corporation of the Huila Region (Corhuila). The authors also appreciate the monitoring of the project by the National Planning Department (DNP, for its abbreviation in Spanish), and the supervision provided by the Ministry of Science, Technology, and Innovation.

DECLARATION OF USE OF ARTIFICIAL INTELLIGENCE

We declare that we did not use artificial intelligence.

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Forma de citación del artículo:

Murcia–Mono, C.A., Falla–Tapias, S., Álvarez–Cubillos, K.Y., Burgos–Paz, W.O. (2025). Current epidemiological situation of bovine viral diarrhea and infectious bovine rhinotracheitis in Colombia. Rev Med Vet Zoot. 72(1): e117797. https://doi.org/10.15446/rfmvz.v72n1.117797

https://doi.org/10.15446/rfmvz.v72n1.116207

Supplemented grazing in Jersey-Holstein cows on two farms in the middle tropics of Valle del Cauca, Colombia

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Recibido: 12/08/2024 Aprobado: 28/11/2024

ABSTRACT

In Colombia, dairy production systems in the mid-altitude tropics rely on grazing and minimal concentrate supplementation. This approach is primarily driven by cost considerations and the need to avoid forage substitution with concentrates. Using rotational grazing on star grass pastures (*Cynodon plectostachyus*) and supplementation with commercial concentrates, animal performance was monitored based on milk yield (liters-cow.day^{-1}) and daily dry matter intake (DMI.day^{-1}) in Jersey-Holstein (Jer-Hol) cows.

The study involved 15 cows per farm, with an average parity of 4 ± 2, at Finca Canadá (FC) (1,500 MASL) and Finca Chiquique (FCH) (980 MASL) in the Valle del Cauca region. Over nine weeks, the grazing behavior of the cows was observed every eight days for 24 hours. On average, across the two environments, cows spent 35.10% of the day grazing (CG), 31.10% ruminating (CR), 22.60% neither grazing nor ruminating (CNGNR), 1.75% drinking (CD), and 9.45% in milking (CM). Bite rate (BR) and chews per bolus (ChB) averaged 23 and 48 per minute, respectively, across both environments. The relative forage value (RFV) was low, at 85 and 82 for FC and FCH, respectively. Milk yield per cow was estimated at 18.00 and 14.80 kg cow.day^{-1}, with DMI at 15.50 and 13.70 kg cow.day^{-1}. These values reflect a biological efficiency of 1.16 and 1.08 liters of milk per kilogram of DMI for FC and FCH, respectively.

Keywords: rotational grazing, animal behavior, biological efficiency, milk production.

Pastoreo suplementado en vacas Jersey-Holstein en dos fincas del trópico medio del Valle del Cauca, Colombia

RESUMEN

En Colombia, los sistemas de producción lechera, en el trópico medio, dependen del pastoreo y suplementación con concentrado, en cantidad mínima, por costo y para no hacer sustitución de forraje por concentrado. Con base en pastoreo rotacional en pastizales de estrella *Cynodon plectostachyus* y suplemento de concentrado comercial, se monitoreó el comportamiento animal con base en la relación producción de leche (litros-vaca.día^{-1}) y la ingesta de materia seca por día (IMS.d^{-1}) en vacas Jersey-Holstein (Jer-Hol). Se utilizó vacas de 4 ± 2 partos, 15 vacas.finca^{-1}, en Finca Canadá (FC) (1.500 m s. n. m.) y Finca Chiquique (FCH) (980 m s. n. m.), en el Valle del Cauca respectivamente.

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Se observó durante 24 horas cada 8 días por 9 semanas el comportamiento en pastoreo y se determinó que, de las 24 horas del día en promedio, para los dos ambientes, las vacas pastaron (VP) 35,10%, rumiaron (VR) 31,10%, no pastorearon ni rumiaron (VNPNR) 22,60%, vacas bebiendo (VB) 1,75% y en ordeño (VO) 9,45% del día respectivamente. La toma de bocado (TB) y rumia por bolo (RB) en promedio para los dos ambientes fue 23 y 48 por minuto, respectivamente. El valor relativo del forraje (VRF) fue bajo, en 85 y 82 para FC y FCH, respectivamente. El desempeño lácteo por vaca se estimó en 18,00 y 14,80 kg vaca.día^{-1} y CMS de 15,50 y 13,70 kg. vaca.día^{-1}, ello supone una eficiencia biológica de 1,16 y 1,08 litros de leche por kilo en CMS para FC y FCH, respectivamente.
Palabras clave: pastoreo rotacional, comportamiento animal, eficiencia biológica, producción lechera.

INTRODUCTION

Dairy production systems in the mid-altitude tropics of Colombia (1,000 to 2,000 MASL) (Echezuría 2019) are characterized by low-quality pastures, which requires strategic supplementation with commercial concentrates. However, this supplementation is managed to avoid critical levels of forage substitution by concentrates (Morales & Ortiz 2018). Grazing behavior reflects voluntary intake in cows and serves as a primary indicator of biological performance, as it is closely associated with animal health and welfare (Werner *et al.* 2019; O'Leary *et al.* 2020; Horn & Isselstein 2022).

Voluntary intake regulation is influenced by several factors, including the social status of a cow within the herd, grazing expertise, hierarchical rank, breed, size, and the intrinsic value of the available fresh forage (AFF) under prevalent tropical environmental pressures (Tarazona *et al.* 2012; Salah 2022; Iqbal *et al.* 2023). For grazing animals, accurately estimating forage dry matter intake (kg cow^{-1} day^{-1}) is the most critical tool for milk production management (Rombach *et al.* 2019; Pinheiro & Gregorini 2022).

Climatic conditions and soil fertility significantly affect forage quality, creating either positive or negative synergies that alter the quantity and quality of AFF. These changes influence key parameters such as neutral detergent fiber (NDF), which affects intake, and acid detergent fiber (ADF), which governs digestibility, energy intake, and water and energy requirements (Lee *et al.* 2021; Iqbal *et al.* 2022; Iqbal *et al.* 2023).

Given these dynamics, field-based data collection was essential to understand the grazing behavior of F1 Jersey-Holstein (Jer-Hol) cows under tropical conditions. Based on this need, the objective of the study was to observe the grazing behavior of supplemented Jer-Hol cows and assess their biological and productive performance at two altitudes within the mid-altitude tropics of Valle del Cauca.

MATERIALS AND METHODS

Location

The fieldwork was conducted at Finca Canadá (FC) and Finca Chiquique (FCH), situated at two altitudes in Valle del Cauca, Colombia (table 1).

Biological material used

Fifteen Jersey-Holstein (Jer-Hol) cows grazed on *Cynodon plectostachyus* (star grass) pastures and were supplemented

TABLE 1. Location and environmental characteristics of the farms where Jer-Hol cows grazed

Descriptor	CF	CHF
Municipality	Restrepo	Yotoco
Geographic coordinates	N 03° 50, 723 W 076° 27, 899	N 03° 82, 984 W 076° 39, 108
Average annual temperature (ºC)	24	25
Relative humidity (%)	75	76
Annual precipitation (mm)	1,256	1,000
Altitude (MASL)	1,500	980
Average distance from the pastures to the milking parlor (m)	270-560	290-460
Average walking time to and from the milking parlor (min)	19.9	12.9

Where: CF= Canada Farm. CHF= Chiquique Farm.

Source: own elaboration.

with commercial concentrate. Starting from a baseline of 10 liters of milk per cow, one kilogram of concentrate was allocated for every additional 5 liters of milk produced. The cows were fitted with numbered collars corresponding to their parity (4 ± 2), monitored during the first 100 days of lactation, and observed grazing for 24 hours every eight days over a nine-week period (Suárez et al. 2014; Balochi et al. 2002; Iqbal et al. 2023).

Ethics committee approval

The research was approved by the Ethics Committee for Research at the Faculty of Agricultural Sciences, Palmira Campus, under certificate CEI-FCA-07/2024 issued on July 2, 2024.

Variables evaluated

The time allocation of the cows for various activities was measured on each farm, including grazing (CG), drinking water (CD), ruminating (CR), inactive behaviors such as resting or sleeping (non-grazing and non-ruminating, CNGNR), and milking (CM), which included walking and waiting in the milking parlor. Additionally, bite rate (BR), chews per bolus (ChB), and weekly milk production per cow (MP) were recorded (adapted from Suárez et al. 2014; Balocchi et al. 2002). Dry matter intake (DMI) was estimated using three mathematical models developed in the doctoral dissertation of Morales Vallecilla (2016).

Double Sampling Model (2M)

This model estimated the available fresh forage (AFF) before cow entry (24-hour grazing period) and the remaining forage (RF) after grazing. Samples were collected using a 0.25 m^2 quadrat frame, following the protocols described by Morales (2016):

$$DMI\ 2M = (AFF - RF) * \%MS$$

Mertens Model (1985)

(Mer): Dry matter intake (DMI) was linked to neutral detergent fiber (NDF) consumption, which ranged from 1.1% to 1.2% of the animal's live weight (LW). Mertens proposed a formula to precisely estimate DMI based on NDF concentration in the diet and the animal's live weight:

DMI Mer = (1,2% * LW) / (%NFD)

NRC 2001 Model (NRC)

This model predicted DMI based on fat-corrected milk (FCM), the animal's metabolic weight, and weeks in lactation (WIL):

The term adjusts for the decline in DMI during the early postpartum weeks.

FCM o LCG= 0,4 * (kg milk/day) + (15 * kg milk fat/day) where 0.4 and 15 are constants.

Microsoft Excel 2016 was used for data processing through univariate statistical analysis of field observations. For forage intake calculations, mathematical models from the literature were applied (Mertens, 1985; NRC, 2001).

RESULTS AND DISCUSSION

Biological characteristics of the pasture

During the experimental period, the pastures at FC and FCH exhibited high levels of neutral detergent fiber (NDF) and acid detergent fiber (ADF), along with moderate protein content (table 2). These values correspond to a fourth-category forage quality based on forage classification criteria (García et al., 2005; Ortiz and Valdés, 2019). The classification ranges from the highest quality level, characterized by protein >19%, ADF <31%, NDF <40%, and a relative forage value (RFV) >150, to the lowest (fifth category), defined by protein <8%, NDF >65%, ADF >45%, and RFV <75.

Forages represent a limiting factor in animal production as they contribute the majority of the NDF, which restricts intake, and the ADF, which determines the rate

TABLE 2. Composition and biological characteristics of the forage consumed by Jer-Hol cows

DESCRIPTOR	CF	CHF
ADF %	38.40	42.20
Lignin %	6.50	6.20
NDF %	61.60	66.00
EE %	1.58	1.05
CP %	14.40	15.61
DMD %	56.00	58.00
DMI %	1.90	1.80
RFV	85.00	82.00

Where: CF= Canada Farm. CHF=Chiquique Farm. ADF= Acid detergent fiber; NDF= Neutral detergent fiber; EE= Ethereal extract; CP= crude protein; DMD= dry matter digestibility; DMI= Dry matter intake live weight %; RFV= Relative feed Value.

Source: own elaboration.

of ruminal degradation of forage material (Ortiz & Valdés 2019; Lee *et al.* 2021).

Time spent on daily activities by cows grazing

At FC, the cows spent 34.40% of their daily time (496 min/day) grazing to collect forage directly, while at FCH, they spent 19 minutes more than at FC (table 3). The average grazing time observed across both locations was 35.10% of the daily time (505 min/day). This value falls within the range reported in other studies, such as Balocchi *et al.* (2002) and Rombach *et al.* (2019), who documented grazing times between 32.20% and 38.30% of the day (464 to 552 min/day) under similar conditions.

Furthermore, using Global Positioning System (GPS) collars, it is estimated that adult cows may graze for 33% to 42% of the day (Augustine & Derner 2013). Grazing time is influenced by several factors, including the level of fasting prior to grazing. The production status of each cow affects its foraging dynamics and voracity (Werner *et al.* 2019).

Cows typically enter the pasture energetically after milking and sustain their consumption cadence for two to four hours, after which intake progressively declines. This observation aligns with findings by Sheahan *et al.* (2013), who reported that 94% of cows grazed for one hour following the morning milking. After this period, grazing gradually decreased to 35% over the subsequent four hours.

The grazing level and the time spent grazing are closely related to the availability and quality of the fresh forage dry matter (AFF) offered, which was higher at FC (0.541 kg AFF/m²) compared to FCH (0.384 kg/m²). This relationship indicates that grazing time increases as forage availability decreases, as limited forage availability constrains the cow's ability to efficiently graze the pasture (García *et al.* 2005; Lee et al. 2021). Ultimately, grazing time is mechanically influenced by pasture quality,

TABLE 3. Average time distribution and confidence intervals for activities performed by Jer-Hol cows on grazing land

Activity	CG	CR	CNGNR	CD	CM
			CANADA FARM		
Minutes/Day	496	474	306	24	140
CV %	4.50	2.87	4.36	8.38	7.14
CI	444-547	443-506	275-336	20-29	117-163
			CHIQUIQUE FARM		
Minutes/Day	515	422	344	26	133
CV %	2.77	6.99	8.30	16.50	6.61
CI	482-548	354-490	278-409	16-36	113-154

Where CG= Cows grazing, CR= Cows ruminating, CNGNR= Non-grazing cows do not ruminate and include periods that the animal uses to vegetate and sleep; CD= cows drinking; CM=cows being milked, including the walk to the milking parlor and waiting; CV=Coefficient of variation; CI= Confidence interval.

Source: own elaboration.

as more mature forage exhibits higher levels of neutral detergent fiber (NDF) (table 2), which correlates with reduced intake. This was observed at FCH (table 5), whereas at FC, higher AFF intake was achieved. In our study, early rumen fill at FC was influenced by both NDF content and forage substitution levels, alongside forage availability. As a result, cows spent less time grazing at FC compared to FCH (Zhao & Jurdak, 2016; Burns & Sollenberger, 2002; Reategui *et al.* 2019).

At FC, the cows consumed 4.50 kg of concentrate per cow per day, which was 26.67% more dry matter (DM) than FCH, where cows consumed 2.70 kg of concentrate per cow per day along with 0.60 kg of silage per cow per day (table 5). This likely contributed to a reduction in grazing time, as forage was substituted with concentrate and silage. The substitution effect reduced grazing time by up to 12 minutes per day for each kilogram of dry matter supplement consumed (Bargo *et al.* 2003; Russo *et al.* 2021).

Ruminating

At FC, cows spent 32.9% of their daily time ruminating (474 min/day), which was 52 minutes more than at FCH (table 3). This can be logically explained: cows with a full rumen, as observed at FC, must allocate more time to the mechanical digestion of the bolus (rumination). Daily rumination is influenced by the quality and quantity of feed consumed, which was clearly of lower quality at FCH (Sjaastad *et al.*, 2010; Russo *et al.*, 2021).

The extended rumination time at FC was likely due to reduced mechanical resistance to forage breakdown compared to FCH. The literature indicates that the resistance to particle size reduction during chewing is directly proportional to the NDF content of the feed; lower NDF content corresponds to lower mechanical resistance (table 2) (Romney & Gill 2000; Zhao & Jurdak 2016).

Non-grazing and non-ruminating activities

At FC, cows spent 21.3% of their time resting or sleeping without engaging in grazing or rumination activities (306 min/day), which was 38 minutes less than at FCH (table 3). This reduction at FC resulted from the increased time allocated to rumination, thereby decreasing the time spent on non-grazing, non-ruminating activities (CNGNR). According to Pulido et al. (2001), cows with higher milk production tend to spend more time either ruminating or grazing, significantly reducing the time spent standing or lying idle.

Climatic factors directly influence livestock activities by affecting animal physiology (temperature, relative humidity, internal and external parasites) and indirectly by altering the growth and chemical composition of grass and forages (Pérez *et al.* 2008). These factors positively influenced the animals at FC, providing better comfort and nutritional conditions compared to FCH, which allowed for a more balanced allocation of time across different activities.

Overall, the daily time of the cows at both FC and FCH was primarily spent on grazing (CG), ruminating (CR), and CNGNR activities, collectively accounting for 88.8% of the total time. Within this proportion, 22.6% (325 min/day) was dedicated to CNGNR. This finding is consistent with Balocchi *et al.* (2002), who reported that these three activities represented 87.7% of daily time, with 24.2% dedicated to CNGNR.

Drinking activity

The time spent drinking was similar at FC (1.7%) and FCH (1.8%) of the daily time budget (24 and 26 min/day, respectively). However, these values contrast with the 0.5% (7 min/day) reported by Balocchi et al. (2002). This discrepancy is likely due to the tendency of Bos taurus cattle to consume more water in hot climates (Patiño et al. 2008; Bernabucci *et al.* 2010). The average annual temperatures at FC and FCH were 24 °C and 25 °C, respectively, compared to the 12 °C reported in Balocchi et al. study. As ambient temperature increases, water consumption also rises (Molina et al. 2016).

Milking activity

Milking time was similar at both FC and FCH, accounting for 9.7% and 9.2% of the daily time budget, respectively (140 and 133 min/day). The average distance from pastures to the milking parlor ranged between 270-560 m at FC and 290-460 m at FCH, with both farms using the same mechanical milking system. The values observed in this study align with the literature, where Balocchi et al. (2002) and Pulido et al. (2001) reported milking times of approximately 10% of daily time under similar conditions. In another study, Clavijo (2018) found that milking took 120 min/day without

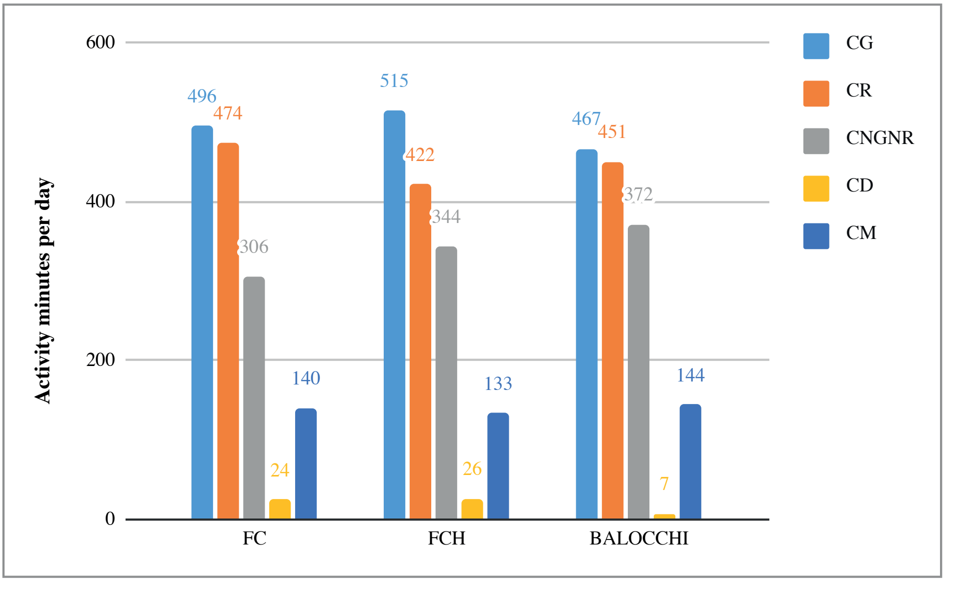

FIGURE 1. Comparison between the daily time dedicated to each activity for cows FC (Canada Farm), FCH (Chiquique farm) and Balocchi.

Where: CG: Cows grazing, CR: Cows ruminating, CNGNR: Non-grazing cows do not ruminate and include periods that the animal uses to vegetate and sleep, CD: Cows drinking and CM=Cows being milked, including the walk to the milking parlor and waiting. Balocchi *et al.* 2002.

Source: own elaboration.

accounting for the time spent walking to the milking parlor.

Figure 1 summarizes the daily time spent on each activity at FC and FCH and compares it with the study by Balocchi *et al.* (2002). A similar pattern is observed, except for drinking activity (CD).

Bite rate (BR, bites min^{-1}):

The bite rate pattern of Jer-Hol cows at FC and FCH showed the highest activity immediately after milking, averaging 24 ± 2 and 21 ± 2 bites min^{-1}, respectively (table 4). These values are lower than the minimum reported in the literature, where BR ranges from 30 to 50 bites min^{-1}. Such rates appear to be common in both cattle and sheep, with total daily bites for adult cattle ranging between 12,000 and 36,000 (Vallentine 2001). BR values in dairy cows under grazing conditions in cold climates range from 45.7 to 55.3 bites min^{-1} (Pulido et al. 2001). Similarly, positive effects on BR have been observed in both heifers and adult cows, with rates of 59 to 60 bites min^{-1} reported in subtropical climates (Abrahamse *et al.* 2008; Kondo 2011; Jiménez & Améndola 2022).

The lower BR observed in this study is likely influenced by forage quality and availability (table 2). At FCH, the star grass matured more rapidly than at FC. At FC, the relative forage value was likely higher due to lower neutral detergent fiber (NDF) content, which influences intake, and lower acid detergent fiber (ADF) content, which affects ruminal degradation. These conditions were likely more favorable at FC, supported by a more temperate climate, as similarly reported for Jersey cows grazing on alfalfa in the Mexican highlands (Lemus *et al.* 2020).

Forage characteristics and consumption dynamics

Forages with low cell wall content, specifically low neutral detergent fiber (NDF) and acid detergent fiber (ADF), are more palatable and digestible, particularly when derived from forage plants grown in favorable climatic conditions (García *et al.* 2005; Tarazona *et al.* 2012; Hernández *et al.* 2024).

The type of pasture (e.g., green forage) used for animal feeding in the subtropical climates of Argentina positively influences

TABLE 4. Average distribution of bite and chewing rate per regurgitated bolus in grazing Jer-Hol cows

DESCRIPTOR	CF	CHF
Average Bite Rate (BR)	24	21
Coefficient of variation %	3.79	5.07
Confidence Interval	22-26	19-23
Average Chews per Bolus (ChB)	49	47
Coefficient of variation %	1.51	2.29
Confidence Interval	48-51	44-49

Where: CF=Canada Farm. CHF=Chiquique Farm; BR= Bite Rate expressed in bites per minute; ChB= Chews per Bolus expressed in chews per minute.

Source: own elaboration.

bite rate (BR) without evident substitution effects from supplementation with 2–8 kg day^{-1} of concentrate. In such conditions, BR averages 58 bites min^{-1}, ranging from 45 to 78 bites min^{-1} when grazing on ryegrass (Bargo *et al.* 2003) or alfalfa in the Mexican highlands, promoting voracious pasture consumption (Tarazona *et al.* 2012).

For African star grass, forage intake increases with pasture height due to a strong correlation between height, availability, and quality. Cows harvest tender tops with greater bite mass (GBM, g DM bite^{-1}), enabling high intake rates (IR, g DM min^{-1}) (Treydte *et al.* 2009; Soares *et al.* 2021). However, BR decreases when cows encounter lush, tall materials exceeding withers height, such as hybrid Brachiaria (Mulato, Toledo) or Guinea grasses (Tanzania). These conditions are likely to reduce forage intake despite larger bites due to the increased difficulty of coarse mastication and swallowing (Treydte *et al.* 2009; Jiménez & Améndola 2022; Rodrigues *et al.* 2024).

The 10 cm height difference between the FC and FCH pastures did not affect BR but did influence dry matter (DM) intake and grazing time in FCH. The lower forage density and stem toughness of African star grass in FCH critically restricted forage consumption, reducing the harvested DM and requiring longer grazing periods. In contrast, younger star grass pastures encourage higher BR and longer grazing time until rumen fill is achieved (Jiménez & Améndola 2022).

Chewing activity and rumination behavior (ChB)

Regardless of the environment, cows walked to the pastures after waiting in the milking parlor, crouched, and engaged in vigorous grazing. Generally, cows spent two to four hours filling their rumen, after which the grazing rate decreased, and rumination began. Rumination occurred consistently whether cows were standing, lying, walking, urinating, or defecating.

The average number of chews per bolus (ruminations) per minute was 49 ± 2 in FC and 47 ± 2 in FCH, with no significant differences observed (table 4). The onset of rumination depends on the mechanical effect of coarse forage rubbing against the reticulorumen atrium, triggering regurgitation (Church 1993). This process occurs effectively only when the rumen is full and contains coarse forage (Van Soest 1994), typically following intense post-milking grazing.

Monitoring chewing cadence and rumination time serves as a useful tool to assess herd health (Paudyal 2021). Variations in forage quality and rumen retention time (degradation in the rumen) may explain observed differences in rumination patterns (Hernández *et al.* 2024).

Dry matter intake and its relationship with milk production

The mathematical models evaluated demonstrated varying levels of accuracy in predicting average dry matter intake (DMI) in kg DMI/day per animal. The models ranked from highest to lowest prediction accuracy as follows: the National Research Council 2001 (NRC) model, the double-sampling model (2M), and the Mertens model (Mer) (table 5).

The gross biological efficiency of milk production for FC and FCH, calculated using the average data for milk production and DMI (table 6), showed that two of the three evaluated models closely approximated the reference values reported in the literature for each farm. Specifically, the

NRC model yielded efficiencies of 1.16 and 1.08 kg of milk per kg of DMI, while the 2M model showed efficiencies of 1.22 and 1.12 kg of milk per kg of DMI. These values align with those reported by Bargo *et al.* (2003) and Hutjens (2005), who identified efficiencies of approximately 1.20 liters for grazing systems in high-altitude tropics and higher values, ranging from 1.40 to 1.50 liters, for confinement systems.

Biological efficiency in grazing systems is primarily influenced by the quality of pasture at the time of intake. It was evident that even a difference of 600 meters in altitude significantly affected forage supply, quality, and availability (Morales & Ortiz 2018).

Looking ahead, under the unpredictable pressures of climate change, the rapid development of innovative agricultural technologies offers significant potential to advance grazing management in the digital era. However, as Horn and Isselstein (2022) emphasized:

The rapid development of innovative agricultural technologies can improve and elevate grazing management to the next level in the digital era. However, no holistic system adequately integrates innovative technology into precision livestock grazing management and the precision monitoring of pastures in a system that can be used to monitor and manage all driving factors within the grazing system.

TABLE 5. Dry matter intake estimated by three prediction models for Jer-Hol cows in CF and CHF

DESCRIPTOR	CF			CHF		
	NRC	2M	Mer	NRC	2M	Mer
DMI Stargrass	-	10.2	8.9	-	9.9	7.5
DMI Concentrate	-	4.5	4.5	-	2.7	2.7
DMI Corn silo	-	0.0	0.0	-	0.6	0.6
DMI TOTAL	15.5	14.7	13.4	13.7	13.2	10.8

Where: CF=Canada Farm. CHF=Chiquique Farm; DMI= dry matter intake (kg.d^{-1}); Models NRC=National Research Council, 2001, 2M= Double Sampling , Mer= Mertens.

Source: own elaboration.

TABLE 6. Relationship between dry matter intake, milk production and efficiency ratio for Jer-Hol cows

DESCRIPTOR	CF			CHF		
	NRC	2M	Mer	NRC	2M	Mer
DMI (kg/day)	15.5	14.7	13.4	13.7	13.2	10.8
Milk production (kg/day)	18.0	18.0	18.0	14.8	14.8	14.8
Gross biological efficiency	1.16	1.22	1.34	1.08	1.12	1.37

Where: FC=Canadá Farm. FCH=Chiquique Farm; DMI= dry matter intake (kg.d^{-1}); Models NRC=National Research Council, 2001, 2M= Double Sampling , Mer= Mertens.

Source: own elaboration.

CONCLUSIONS

The cows in this study allocated an average of 35.10% of their daily time to grazing, 31.10% to rumination, 22.60% to resting (neither grazing nor ruminating), 1.75% to drinking, and 9.45% to milking, including the time spent traveling to the milking parlor.

The gross biological efficiency of Jer-Hol cows under grazing conditions ranged between 1.16 and 1.08 kg of milk per kg of DMI. This efficiency is influenced by pasture quality and is closely tied to the increased time dedicated to grazing and rumination compared to other activities.

CONFLICT OF INTERESTS

This manuscript was prepared and reviewed with the participation of all authors, who declare that there are no conflicts of interests that could compromise the validity of the results presented.

FUNDING SOURCES

This work was carried out using personal resources.

USE OF ARTIFICIAL INTELLIGENCE

No artificial intelligence tools were used in the development of this work.

ACKNOWLEDGMENTS

We extend our gratitude to the owners and workers of the Canadá and Chiquique farms for their hospitality and support in the field, and to the Universidad Nacional de Colombia, Palmira campus, for their academic support.

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Forma de citación del artículo:

Solarte–Bacca, C. C., Morales–Vallecilla, F., Ortiz–Grisales, S. (2025). Supplemented grazing in Jersey-Holstein cows on two farms in the middle tropics of Valle del Cauca, Colombia. Rev Med Vet Zoot. 72(1): e116207. https://doi.org/10.15446/rfmvz.v72n1.116207

https://doi.org/10.15446/rfmvz.v72n1.114348

Compositional, hygienic and sanitary quality of bovine milk in three regions of Ecuador

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Recibido: 09/05/2024 Aprobado: 22/11/2024

ABSTRACT

Milk quality creates a scenario in which producers are required to meet standards established by regulations to ensure the production of a safe food product. This study aimed to characterize the compositional, hygienic, and sanitary quality of bovine milk in three regions of Ecuador: The Sierra (S), Coast (C), and Amazon (A). A total of 831 samples were analyzed following the LCL-INS-01 protocol to determine fat, protein, lactose, and total solids (TS) percentages, as well as total bacterial count (TBC), total and differentiated somatic cell counts (SCC and DSCC). The results indicated the following averages: **Compositional quality**: fat content—A: 4.05%, S: 3.87%, and C: 3.46%; protein content—C: 3.35%, A: 3.25%, and S: 3.22%; lactose content—S: 4.61%, A: 4.59%, and C: 4.29%; and TS—A: 12.61%, S: 12.47%, and C: 11.74%. **Hygienic quality**: TBC—A: 5.7 x 10^6, S: 5.5 x 10^6, and C: 2.4 x 10^5. **Sanitary quality**: SCC/ml—S: 354 x 10^3, C: 110 x 10^4, and A: 759 x 10^3; DSCC—S: 67.52%, C: 67.62%, and A: 78.21%. These findings indicate the presence of mastitis in all three regions.

In conclusion, all three regions met the compositional quality standards established by INEN. In terms of hygienic quality, compliance was observed in the Coast and Sierra regions, whereas sanitary quality requires improvement strategies across all regions. Addressing milk quality is a significant contribution to promoting better understanding and improvement in dairy production, consumer health, and the development of the dairy sector.

Keywords: milk, hygiene, somatic cells.

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Calidad composicional, higiénica y sanitaria de la leche bovina en tres regiones del Ecuador

RESUMEN

La calidad de leche forja un escenario donde los productores están obligados a cumplir estándares bajo normativas para generar un alimento salubre. El objetivo de este estudio fue caracterizar la calidad composicional, higiénica y sanitaria de la leche bovina en tres regiones del Ecuador: Sierra (S), Costa (C) y Amazonia (A). Se utilizaron 831 muestras bajo el protocolo LCL-INS-01 para determinar el porcentaje de grasa, proteína, lactosa y sólidos totales (ST), y el conteo de bacterias totales (CBT), conteo de células somáticas (CCS) totales y diferenciadas (CCSD). Los resultados indican los siguientes promedios: Calidad composicional, grasa, A: 4,05%, S: 3,87% y C: 3,46%. Proteína, C: 3,35%, A: 3,25%, y S: 3,22%. Lactosa, S: 4,61%, A: 4,59% y C: 4,29%. ST, A: 12,61%, S: 12,47% y C: 11,74%. Calidad higiénica, CBT, A: 5,7 x 10^6, S: 5,5 x 10^6 y C: 2,4 x 10^5. Calidad sanitaria: 354 x 10^3; 110 x 10^4; y 759 x 10^3 CCS/ml y 67,52, 67,62, y 78,21 %CCSD para la Sierra, Costa y Amazonia, para ambos parámetros, donde se determinó la existencia de mastitis. Se concluye que las tres regiones cumplieron con la calidad composicional según el Servicio Ecuatoriano de Normalización (INEN); en lo higiénico, la Costa y la Sierra, y en lo sanitario, las tres deben abordar estrategias en la producción. Estudiar la calidad de la leche resulta un aporte significativo para promover el entendimiento y la mejora en la producción lechera, así como la salud del consumidor y el desarrollo del sector lácteo.

Palabras clave: leche, higiene, células somáticas.

INTRODUCTION

In Ecuador, milk production systems (MPS) have significantly increased their yield, reaching a total of 6.1 million liters of milk per day at the national level by 2023 (Salguero *et al.* 2023). According to the Ecuadorian Dairy Observatory, milk production in 2022 amounted to 5,502,787 liters per day, highlighting its importance as a sector that generates both direct and indirect employment (Chanaluisa 2016). In this context, characterizing and understanding milk production and its compositional, hygienic, and sanitary quality is essential for implementing actions and developing strategies for decision-making regarding integral parameters that ensure a safe food supply for human consumption (Jurado *et al.* 2021; Valdivia *et al.* 2021). Raw milk must meet quality standards that require it to be homogeneous, free of contaminants, and possess a pleasant sensory profile (Vallejo *et al.* 2018), as stipulated by the Ecuadorian Standards Institute (INEN 2012).

As emphasized by Chuquín *et al.* (2016), it is evident that milk production without Good Livestock Practices (GLP) in Master Plan Production (MPP) leads to elevated levels of colony-forming units (CFU) and somatic cell counts (SCC), which pose risks to industrial processing and inclusion in the population's diet (Luigi *et al.* 2013). Compositional quality is evaluated based on nutritional content, including fat, protein, lactose, total solids (TS), and non-fat solids (NFS) (Vázquez *et al.* 2014; Moreira *et al.* 2020). Hygienic quality, as noted by Contero *et al.* (2019), involves total bacterial count (TBC) and CFU, both critical parameters for ensuring food safety. Meanwhile, sanitary quality involves analyzing somatic cell counts, both total (Contero *et al.* 2021) and differential (DSCC) (Orozco & Santana 2022), which are associated with inflammatory processes in the mammary gland, reflecting the internal health of the udder (Vallejo *et al.* 2018).

The primary causes of poor milk quality among producers are the lack of technical guidance and reliance on incorrect empirical practices from production to milk handling during storage and transportation, leading to increased microbial loads (Guevara *et al.* 2019). Additionally, the compositional quality of milk can be influenced by factors such as the number of calvings, lactation period, diseases, genetics, and climatic conditions (Ramírez *et al.* 2019).

This issue represents a global concern, particularly in Ecuador, where ensuring high-quality food and providing a foundation for the industrialization of raw materials to produce premium dairy derivatives remain priorities (Arrieta *et al.* 2019). Compositional, hygienic, and sanitary milk quality can lead to variations in production processes and impact the sensory and nutritional characteristics of the final product. As a result, the dairy industry has compelled primary producers to meet standards enforced by each nation (Jiménez *et al.* 2020).

Although studies on this topic have been conducted in Ecuador, they have been fragmented. Given this situation, the present study was conducted in three regions (Sierra, Coast, and Amazon), with the objectives to: 1. Analyze the compositional quality of bovine milk samples from production units using mid-infrared spectrophotometry. 2. Determine the hygienic quality of bovine milk samples through TBC using flow cytometry. 3. Assess the sanitary quality of bovine milk samples by analyzing SCC and DSCC using flow cytometry.

MATERIALS AND METHODS

Study area

The study was conducted in the main milk collection centers and dairy production units of Ecuador, considering small (1-5 ha), medium (5-20 ha), and large producers (> 20 ha) based on the percentages of milk production across the country's three regions. According to Márquez (2021), the distribution of milk production is as follows: 77.2% in the Sierra, 17.9% in the Coast, and 4.8% in the Amazon. Dairy farms in these regions primarily raise breeds such as Holstein, Jersey, Brown Swiss, F1 (H+J), (H+B), Girolando, Gyr, and Brangus, with most systems employing rotational grazing practices.

The initial sampling distribution comprised 750 milk samples (100%), determined by the budget allocated to the research and proportionally divided according to the regional milk production percentages. However, during characterization, a total of 831 samples were collected to obtain more comprehensive data on milk quality.

Milk sample collection

Milk samples were collected from various areas across ten provinces within the three regions of Ecuador (figure 1), which are the primary zones of bovine milk production. In the Sierra (Andean region), samples were obtained from Pichincha, Cotopaxi, Imbabura, Tungurahua, Carchi, Azuay, and Cañar (n = 570). In the Coast (Littoral region), samples were collected from Santo Domingo de los Tsáchilas and Manabí (n = 135). In the Amazon (Eastern region), samples were taken from Napo (n = 126).

Milk samples were collected from ten provinces across Ecuador's three primary bovine milk production regions (figure 1). In the Sierra (Andean region), samples were obtained from Pichincha, Cotopaxi, Imbabura, Tungurahua, Carchi, Azuay, and Cañar (n = 570). In the Coast (Littoral region), samples were collected from Santo Domingo de los Tsáchilas and Manabí (n = 135). In the Amazon (Eastern region), samples were taken from Napo (n = 126).

For the evaluation of milk quality across the three criteria (compositional, hygienic, and sanitary), each was assessed in two stages, described as follows:

Sampling procedure

Sampling followed the LCL-INS-01 protocol established by the Milk Quality Laboratory (LCL, by its initials in Spanish) at Universidad Politécnica Salesiana (UPS) (Alvear *et al.* 2021), with each sample coded and identified according to the producer or sampling area.

For compositional and sanitary quality evaluation, milk samples were collected from 40-liter milk cans using 40 ml plastic vials with white caps containing a preservative (8 ml bronopol and 0.3 mg natamycin tablets) provided by the laboratory. The samples were stored in a cooler maintained at 4 °C to 6 °C using gel refrigerants and transported to the LCL at the UPS Cayambe campus (Alvear *et al.* 2021).

For hygienic quality evaluation, sterile 40 ml vials with red caps were used. At the end of the collection process, 4 drops of azidiol bacteriostatic solution were added to preserve the samples. These were also transported in coolers maintained at 4 °C to 6 °C until they arrived at the laboratory (Alvear *et al.* 2021).

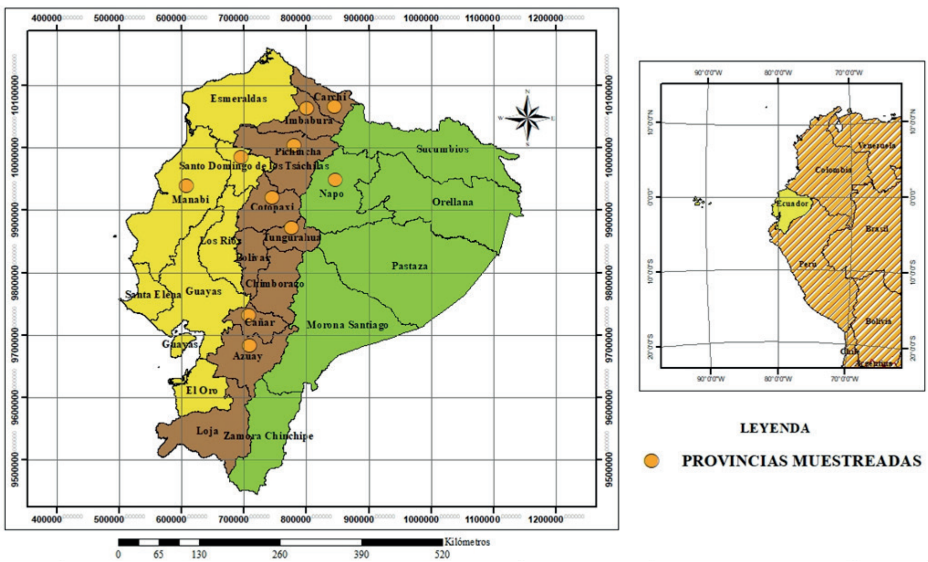

FIGURE 1. Milk sampling areas for characterizing milk quality in three regions of Ecuador.

Source: own elaboration.

Analytical procedures

Compositional analysis
The compositional analysis was conducted at the LCL using mid-infrared spectrophotometry (MILKOSCAN) in accordance with the ISO 9622-IDF 141/2013 method, Guideline for the Application of Mid-Infrared Spectrometry for Milk (LCL-PE-01) at UPS Cayambe (Alvear *et al.* 2021).

Total bacterial count (TBC)
TBC was analyzed using the BACTOSCAN FC equipment following the protocol Bacterial Count Protocol for the Evaluation of Alternative Methods, ISO 16297 IDF 161 / LCL-PE03 through flow cytometry. Results were initially expressed as TBC and subsequently converted to CFU/ml.

Sanitary analysis
Sanitary analysis was conducted using the FOSSOMATIC 7 DC equipment, based on the method Enumeration of Somatic Cells-Part 2: Guidance on the Operation of Fluoro-Opto-Electronic Counters, ISO 13366-2:2006(E) IDF 148-2:2006 / LCL-PE02 through flow cytometry (Orozco & Santana 2022). This analysis provided total somatic cell counts (SCC) and differentiated somatic cell counts (DSCC). Samples with SCC >700,000 cells/ml were classified as positive for mastitis, while samples with SCC <200,000 cells/ml were classified as originating from healthy cows (Quevedo 2018).

Statistical analysis
Microsoft Excel 2023 (Microsoft 365) was used to calculate averages for all analyzed components. Descriptive analysis was performed using Minitab 20.3 (64-bit, 2021), where the data dispersion of DSCC% relative to SCC/ml in the milk samples was examined to determine the presence of mastitis.

Regulatory review

The results were compared with the Ecuadorian Technical Standard INEN 9:2012 (INEN 2012). TBC analysis was further benchmarked against Ministerial Agreement 394 MAG, issued on September 4, 2013 (MAGAP 2013).

Ethical considerations

This project did not involve animal handling. Milk samples were collected from 40-liter milk cans at production units after producers from the three regions had gathered the milk. Sampling adhered to the internal guidelines of the UPS Milk Quality Laboratory (INS-01) and followed the regulations established by the Agency for Phytosanitary and Zoosanitary Regulation and Control (Agrocalidad) in the Instruction Manual for Sampling of Raw Milk and Whey (INT/CL/01).

RESULTS AND DISCUSSION

Compositional quality

The compositional quality of milk can vary depending on multiple factors and appropriate management practices, which are essential for ensuring the nutritional quality of the final product (Juárez *et al.* 2015; Bustamante *et al.* 2023). Regular analyses of milk composition are therefore crucial to preserving its nutritional value and suitability for human consumption.

Fat content

The average fat percentage in the Sierra region was 3.87% g/100 g (table 1), exceeding the standards for raw milk established by INEN 9:2012. These findings are consistent with those reported by Alvear *et al.* (2021), who recorded a fat percentage of 3.9% in Ecuador's Andean region in 2021. Similarly, the results align with data from two cantons in the province of Pichincha, where Salguero *et al.* (2023) found fat percentages of 4.0% in milk collection centers and 3.8% on dairy farms.

In the Coast region, the average fat percentage was 3.46% (table 1), which is also above the parameters established by INEN 9:2012 for raw milk. These values are comparable to those reported by Alvear *et al.* (2021), who evaluated the concentration of fatty acids nationwide and recorded a fat percentage of 3.55% for the Ecuadorian Coast. Vallejo *et al.* (2018) observed fat percentages ranging from 3.97% to 4.03% in Flavio Alfaro canton, 2.67% in Pedernales, and 2.71% in El Carmen. The variation in these results can be attributed to the type of cattle sampled. In this study, milk was predominantly sourced from single-purpose dairy breeds such as Holstein, Jersey, and Brown Swiss, whereas in Manabí, the samples came from dual-purpose breeds such as Girolando, Gyr, and Brangus (Vallejo *et al.* 2018).

In the Amazon region, the average fat content was 4.05% g/100 g, making it the region with the highest percentage of this physicochemical component among the three regions analyzed (table 1). These findings are consistent with those of Alvear *et al.* (2021), who reported a fat content of 4.13%. Similarly, Culqui (2022) observed fat percentages ranging from 3.79% to 4.16% across various milk collection centers in the Amazon region of Peru, which showed minimal differences from the present study.

The differences in fat percentages can be attributed to individual management practices within production units, which include increased production and improved

TABLE 1. Average values of compositional quality parameters in three regions of Ecuador

	Components			
Region	Fat (% g/100 g)	Protein (% g/100 g)	Lactose (% g/100 g)	Total solids (% g/100 g)
Highland	3.87	3.22	4.61	12.47
Coast	3.46	3.35	4.29	11.74
Amazon	4.05	3.25	4.59	12.61
Average				
Ecuador	3.79	3.27	4.50	12.27
Reference value a				
INEN	3.0*	2.9*	-	11.2*

Note: * VMP = Minimum permitted value. Norma Técnica Ecuatoriana INEN 9:2012. [a] (INEN 2012; Laboratorio Calidad de Leche-UPS 2023) There is no reference value.

Source: own elaboration.

handling of high-quality forage (Bernal *et al.* 2007; Requelme & Bonifaz 2012) and enhanced genetics through artificial insemination (Huanca 2001).

Calderón *et al.* (2006) indicated that fat content can be influenced by diet composition, specifically higher fiber concentrations, which lead to increased fat content in milk due to the compensatory effect of volatile fatty acids produced in the rumen. Fat quality is also affected by factors such as lactation stage, genetics, age, health, milking integrity, and seasonality (Harvatine *et al.* 2009; Belage *et al.* 2017).

Protein

Table 1 presents an average protein percentage of 3.22% in the Sierra region, the lowest among the analyzed regions. Nevertheless, this value exceeds the minimum standard established by INEN 9:2012. These averages are comparable to those reported by Alvear *et al.* (2021) for the Sierra region (3.22%) and by Chuquín *et al.* (2016) in the province of Carchi (3.2%).

According to regional averages (table 1), the Coast region exhibited a protein content of 3.35%, surpassing the INEN 9:2012 standard and standing out among the regions of Ecuador. These findings align with data from Suárez (2023), who reported protein percentages ranging from 3.2% to 3.4% in raw milk sold by five vendors in Chone. Similarly, Roncallo *et al.* (2012) observed protein percentages between 3.31% and 3.46% in dry Caribbean production systems, comparable to Suárez's (2023) results, which show minimal differences from this study.

However, the findings differ from the 3.15% protein reported by Alvear *et al.* (2021) for the Coast in 2021. In contrast, Vallejo *et al.* (2018) reported a higher protein percentage of 3.76% in Flavio Alfaro canton, likely due to superior nutritional management practices that enhance amino acid availability for protein synthesis, resulting in increased production.

In the Amazon region, the average protein percentage was 3.25% (table 1). This value exceeds the minimum threshold established by INEN 9:2012, classifying the milk as having good protein quality. These findings are consistent with Alvear *et al.* (2021), who reported a protein content of 3.2% for the region. Similarly, Culqui (2022) found comparable protein percentages in the Amazon region of Peru, ranging from 2.84% to 3.98% across 11 milk collection plants.

The differences in protein percentages are primarily attributed to the genetic variability of the cattle breeds managed within production units. In this region, the majority of producers raise Holstein cows, which produce milk with a lower protein content (3.0%) compared to other breeds such as Jersey or Guernsey cows, which have an average protein content of 3.9% (Penn State Extension 2022; Cajamarca 2022).

Additionally, feed management and quality play a significant role in milk composition, particularly its protein content. Inadequate nutrition can result in deficiencies in both milk production and composition (Cahuascanco *et al.* 2019). Another influencing factor is the presence of diseases in the dairy herd, specifically mastitis, which can lead to a reduction in the protein percentages of milk composition (Campabadal 1999).

Lactose

The Sierra region exhibited the highest lactose percentage, averaging 4.61%

g/100 g (table 1). This result is close to the findings of Cajamarca (2022), who reported a value of 4.78%, representing a difference of 0.17% from this study. Similarly, in an evaluation of the influence of milking on cheese quality within the Inti Churi organization, which assessed bovine milk, lactose percentages ranging from 4.53% to 4.71% were reported (Vallejo 2020). These values align with those of Huillca (2020), who recorded averages of 4.56% and maximum values of 4.80% in milk samples from 162 producers in the Urinsaya region.

For the Coast region, the lactose percentage averaged 4.29% (table 1). This value aligns with one of the results reported by Roncallo *et al.* (2012), where the lowest lactose percentage was 4.44%. However, in the same study, other treatments yielded higher lactose percentages, ranging from 4.59% to 4.65% (Roncallo *et al.* 2012). These latter findings are consistent with those of Aveíga *et al.* (2021), who reported values of 4.61%, 4.68%, 4.60%, and 4.62% in milk from four vendors in the Chone market —values higher than those obtained in this study.

The Amazon region had a lactose percentage of 4.59%, as shown in table 1. Santillan and Frías (2023) reported an average lactose percentage of 5.01%, reflecting a difference of 0.42% compared to this study. Similarly, Culqui (2022) found values ranging from 4.95% (the lowest across different plants) to 5.47% (the highest across 11 milk collection plants), which are higher than those observed in this investigation. Conversely, Ambuludi *et al.* (2017) reported lower averages of 4.36%, 4.30%, and 4.38%. Ortiz *et al.* (2023) documented averages of 4.26% and 4.58%, which are also lower than the values found in this study.

One factor contributing to reduced lactose percentages is product adulteration through water addition to increase volume, which ultimately lowers quality. Additionally, the presence of mastitis (marked by high somatic cell counts) negatively impacts lactose production (Campabadal 1999). Milking frequency also affects milk composition and quality, particularly lactose content. For instance, with once-daily milking, production decreases, while fat and protein concentrations increase, and lactose levels decline (Nandan & Kumar 2022).

Moreover, lactose percentages are influenced by the nutrition of lactating cows. Adequate glycogen availability allows for the physiological processes that synthesize lactose by forming glucose and galactose molecules. The relationship between blood glucose absorption and milk production is fundamental for nutrient concentration (Kittivachra *et al.* 2007).

Total solids

The average total solids (TS) percentage in the Sierra region was 12.47% g/100 g (table 1), exceeding the standard established by INEN (2012), which is 11.2%. This result aligns with data from Vallejo (2020), who identified minimum and maximum TS values ranging from 10% to 15%, and with Huamaní and Morales (2022), who reported an average of 12.62%. Similarly, Padilla *et al.* (2020) highlighted total solids percentages of 12.9% and 12.8% in a district in Peru, observed in two treatments: one without supplementation and another using nutritional blocks.

For the Coast region, the average TS percentage was 11.74%, the lowest among the regions analyzed (table 1); however, this value still exceeds the regulatory standard. These results are comparable to those reported by Sandoval *et al.* (2023), who found an average TS percentage of 12.11%. Similar values were observed by Romero *et al.* (2018) in their evaluation of milk in the department of Sucre, Colombia, with TS averages of 12.23% in the Subregion Sabanas, 11.71% in San Jorge, and 11.95% in the Gulf of Morrosquillo.

In the Amazon region, the average TS percentage was 12.61% (table 1), the highest among the three regions, indicating milk with superior total solids content. These findings differ from those of Ortiz *et al.* (2023), who reported averages of 11.29% in a control treatment and 12.68% in a supplementation treatment. The consistency of these results may be attributed to the presence of *Bos taurus* and *Bos indicus* crosses (Martínez & Gómez 2013), which are commonly managed in the Amazon region of Ecuador.

Total percentages can vary depending on the diet provided to the animals. Higher levels of protein and energy intake lead to increased levels of milk's physicochemical components (Linn 1988). Additionally, seasonal variations can influence TS levels. During the dry season, pastures typically lack the quality observed in the rainy season when they are more abundant and nutritious (Campabadal 1999).

Total solids are a critical factor influencing milk's suitability for processing and its sensory properties, such as flavor and consistency. This is particularly important when the raw material is used for dairy product transformation. Consequently, total solids are a key determinant of milk's nutritional and economic value (Yang *et al.* 2020).

Hygienic quality

Globally, regulations established by authorities and control entities determine permissible limits for total bacterial count (TBC) or colony-forming units (CFU). These parameters vary depending on the country, type of milk (requirements), production process, and storage conditions (Bustamante et al. 2023). Generally, high-quality milk production exhibits a low total bacterial count, whereas elevated TBC levels indicate potential contamination within the milk value chain (Kabera *et al.* 2020).

TABLE 2. Average TBC values that evaluate the hygienic quality of raw milk in the three regions of Ecuador

Region	TBC/ml	CFU/ml
Highland	5.5×10^6	1.5×10^6
Coast	2.4×10^5	6.6×10^4
Amazon	5.7×10^6	1.6×10^6
Average		
	3.8×10^6	1.1×10^6
Reference value [a]		
Ministerial agreement 394 MAG [b]	$3{,}0 \times 10^5$ ***	$3{,}0 \times 10^5$ ***
INEN 2012 [a]		$1{,}5 \times 10^6$

Note: *** VMP = Maximum allowed value. [a] (INEN 2012; Laboratorio Calidad de Leche-UPS, 2023) [b] Ministerial agreement 394 MAG (MAGAP 2013).

Source: own elaboration.

Table 2 shows that the Sierra region has an average value of $5.5\times1065.5\times10^6$ TBC/mL or $1.5\times1061.5\times10^6$ CFU/mL. A comparison with the INEN (2012) standard reveals compliance with the established regulations in this region. However, this average exceeds the maximum permissible value set by Ministerial Agreement 394, suggesting that these samples are not eligible for quality-based payment.

This result aligns with data from the Sierra Centro region, where a CFU/mL value of 1.5 million was reported up until 2013. By 2018, the average had dropped to 200×10^3 CFU/mL, indicating improved milk quality, largely attributed to the involvement of formal producers obligated to meet the standards of nationally recognized companies (Contero *et al.* 2021).

In a collection center in Tungurahua Province, Ecuador, results showed 1.22×10^8 CFU/mL for mesophilic aerobic bacteria during the rainy season, while the dry season recorded values of 8.07×10^6 CFU/mL (Albuja *et al.* 2021). Similarly, Guevara *et al.* (2019) reported mesophilic aerobic counts of 758×10^5 and 802×10^5 CFU/mL for two companies. Such elevated counts may stem from improper milking practices, such as using non-disposable towels shared among cows, using calves near the cows to stimulate milk release, and other practices (Aguilera *et al.* 2014).

In the Costa region, the average TBC was 2.4×10^5 TBC/mL or 6.6×10^4 CFU/mL, as shown in table 2. These values comply with both INEN (2012) and Ministerial Agreement 394 MAG standards, remaining well below the maximum permissible limit and indicating hygienic milk quality.

These results are consistent with those of Contero *et al.* (2021), who reported variable CFU/mL values from 2009 to 2018, ranging from high to low bacterial counts, culminating in an average of 2×10^5 CFU/mL in 2018. In Manabí Province, Ecuador, Arteaga *et al.* (2021) identified an average of $6.25\log_{10}$ $106.25\log_{10}$ CFU/mL or 1,778,279.41 CFU/mL, exceeding regulatory limits and reflecting poor milk quality.

In the Amazonian region, the average TBC was 5.7×10^6 TBC/mL or 1.6×10^6 CFU/mL, representing the highest TBC and CFU values in this analysis (table 2). These values surpass the maximum permissible limits established by INEN 9:2012 and Ministerial Agreement 394 MAG, indicating that production systems in this region do not meet hygienic quality standards.

Similar findings were reported by Contero *et al.* (2021), who documented CFU/mL values of 1×10^6 in 2009, though these decreased significantly to an average of $<400\times10^3$ CFU/mL by 2018. In contrast, values reported by Ambuludi *et al.* (2017) ranged from 3.93×10^4 to 3.43×10^4 CFU/mL, which are not directly comparable.

Several factors can contribute to variations in TBC levels during milk production, including inadequate cleaning and sanitation during milking (Bonifaz & Requelme 2011). Carrillo *et al.* (2004) emphasized that failure to adhere to proper hygiene protocols within production units can lead to milk contamination, as bacteria are ubiquitous (Orozco & Santana 2022).

Additional contributing factors include improper temperature management, such as failure to cool and store milk immediately after milking, animal health issues (e.g., mastitis), water quality, cleanliness of production units and equipment, and cross-contamination caused by operators (Aguilera *et al.* 2014; Motta *et al.* 2014).

Although these challenges persist, producers in the region are implementing strategies and forming associations to enhance production quality through

TABLE 3. Sanitary quality in the three regions of Ecuador

Region	SCC/ml	% DSCCx LYMPHOCYTES + PMN
Highland	354 x 10^3	67.52
Coast	110 x 10^4	67.62
Amazon	759 x 10^3	78.21
Average		
	734 x 10^3	71.12
Reference value [a]		
INEN 2012	700 x 10^3	

Note: *** VMP = Maximum allowed value. [a] (INEN 2012; Laboratorio Calidad de Leche-UPS 2023) [b] Ministerial agreement 394 MAG (MAGAP 2013).

Source: own elaboration.

training, advisory services, and regulatory compliance (Motta *et al.* 2014). Consequently, efforts are being made to promote milk production aligned with best practices for milking, handling, and management (Deddefo *et al.* 2023).

Sanitary quality

Elevated somatic cell counts (SCC) indicate udder health problems in cows, such as infections (e.g., mastitis), which compromise the sanitary quality of milk (Orozco & Santana 2022). Sanitary quality is closely linked to hygienic quality, both of which are regulated by the Ministry of Agriculture and Livestock as well as by the companies to which producers deliver their milk. These regulations are based on standards designed to control and monitor milk production (Bustamante *et al.* 2023). The findings from this characterization are presented in table 3.

Differential somatic cell count (DSCC) refers to the proportion of white blood cells, including polymorphonuclear leukocytes (PMN), lymphocytes, and macrophages. Using advanced technology, FOSS has developed a method that simultaneously

TABLE 4. Frequency of DSCC for the identification of the presence of mastitis in the three regions of Ecuador

Quadrant [c]	Parameter [c] DSCC %–SCC/ml	N	Percentage	Reference [c]
A	<65–< 200,000	118	14.2	Healthy
B	>65–< 200,000	165	19.9	Sensitive
C	>65–> 200,000	511	61.5	Subclinical mastitis
D	<65–> 200,000	37	4.5	Chronic–removal

Note: [c] (Orozco & Santana 2022).

Source: own elaboration.

determines SCC and DSCC (Schwarz 2017).

This parameter incorporates four quadrants, each based on the percentage of DSCC and the total SCC value (×1000/mL×1000/mL) to reflect disease status, enabling more precise diagnoses (Orozco & Santana 2022).

Somatic cell count has been pivotal in detecting mastitis. According to Contero *et al.* (2021), an increased SCC is associated with inflammation caused by the non-specific immune response of phagocytosis, a key component of the animal's immune system. Contributing factors include the number of lactations, irritants affecting the udder, postpartum periods, and other variables (Gonçalves *et al.* 2018).

In the Sierra region, an SCC of 354×10^3 cells/mL was recorded (table 3), which complies with the INEN 2012 standard. However, when this value is analyzed alongside the DSCC parameter, with values of 354×10^3 cells/mL and 67.52% DSCC (lymphocytes + PMNs), this region falls into quadrant C (figure 2), indicating the presence of subclinical mastitis.

These findings are consistent with previous studies in the Sierra region, where SCC values below 400×10^3 cells/mL were reported in 2018 (Contero *et al.* 2021). Similar results were reported by Orozco and Santana (2022) in studies of mastitis across various sectors of the Ecuadorian Sierra, with values ranging from 200×10^3 to 500×10^3 cells/mL. According to mastitis dispersion quadrants, these values were classified under quadrant C, corresponding to subclinical and clinical mastitis.

In the Costa region, the average SCC was 110×10^4 cells/mL (table 3), exceeding the maximum permissible value set by INEN standards. This indicates non-compliance with regulatory parameters. An interpretation of the quadrants shows that most samples fall within the >65% DSCC (lymphocytes + PMNs) and >200,000 SCC/mL range, corresponding to cases of subclinical mastitis.

These results align with data from Sucre, Colombia, where an SCC of 1,002,382 cells/mL was reported (Arrieta *et al.* 2019). Similarly, Jurado *et al.* (2019) documented a maximum SCC of 111×10^4 cells/mL,

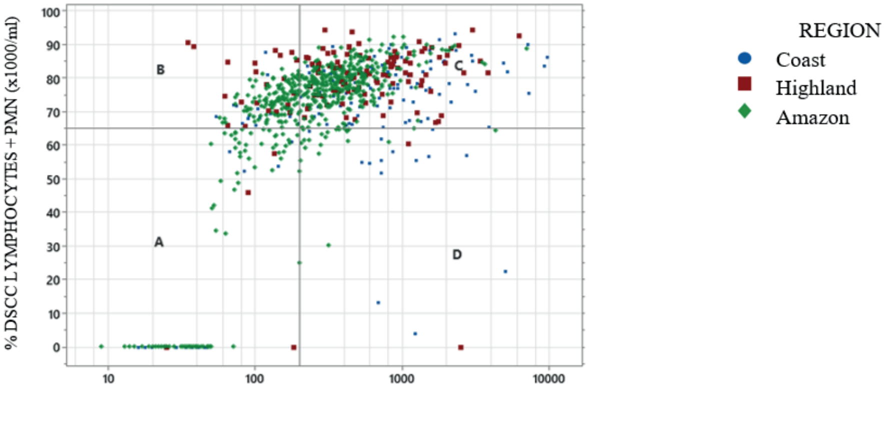

FIGURE 2. Dispersion of SCC and DSCC data in four quadrants for identification of mastitis type.

Source: own elaboration.

recommending that milk with such levels should neither be consumed directly nor used in industrial processes. In an assessment of milk quality among small- and medium-scale producers in Havana, Remón *et al.* (2019) reported an SCC of 1×10^6 cells/mL.

In the Amazonian region, the average SCC was 759×10^3 cells/mL (table 3), exceeding the INEN maximum permissible limit and failing to meet quality standards. Similarly, grouping cases into their respective quadrants showed that most samples fell into quadrant C (figure 2), corresponding to milk from cows with mastitis.

These results are consistent with those of Culqui (2022), who reported high SCC values of up to 705,823.5 cells/mL. Contero *et al.* (2021) noted that SCC values in the Amazonian and Sierra regions of Ecuador were similar, with values below 400×10^3 cells/mL in 2018. However, these values differ from those found in this study. Contrastingly, Santillan & Frías (2023) reported an average SCC of 210×10^3 cells/mL, associating this with milk of average quality suitable for various industrial processes.

In Ecuador, the majority of milk producers face issues with subclinical mastitis, as shown in table 4 and figure 2, with quadrant C classification (>65% DSCC lymphocytes + PMNs and >200,000 SCC/mL).

According to Vallejo *et al.* (2018), high SCC levels in raw milk affect the production of dairy products, primarily due to increased enzymatic activity, such as lipase and plasmin. These enzymes result in unstable curds, reduced cheese yields, bitterness in derivatives (Alhussien & Dang 2018), and decreased shelf life of final products (Krekelberg 2023).

Variations in SCC can be attributed to differences in plant-level regulations, many of which require producers to minimize somatic cell counts while maintaining sufficient production to sustain profitable systems (Ruegg & Pantoja 2013).

CONCLUSIONS

According to the Ecuadorian Technical Standard INEN 9:2012, Requirements for Raw Milk, all three regions of Ecuador meet the regulatory criteria for raw milk to be considered suitable for human consumption and nutritionally adequate.

In terms of hygienic quality, the Costa region complies with the parameters established by regulatory authorities, with a total bacterial count (TBC) of 2.4×10^5 CFU/mL or 6.6×10^4 CFU/mL. In contrast, the Sierra and Amazon regions showed average values of 5.5×10^6 TBC/mL or 1.5×10^6 CFU/mL and 5.7×10^6 TBC/mL or 1.6×10^6 CFU/mL, respectively, reflecting high bacterial content. This highlights the transitional state of production systems in these regions, which are adopting improved hygiene and management practices. The government and dairy industries have implemented stricter inspections, including periodic testing, to ensure milk meets quality and safety standards. Such analyses also inform milk pricing strategies, emphasizing quality-based payments to producers.

Regarding sanitary quality, the average somatic cell counts (SCC) of 354×10^3, 110×10^4, and 759×10^3 SCC/mL, along with differential somatic cell counts (DSCC, lymphocytes + PMNs) of 67.52%, 67.62%, and 78.21%, were recorded for the Sierra, Costa, and Amazon regions, respectively. Based on these values, the Sierra region meets the SCC regulatory standards. However, analyzing the relationship between

SCC and DSCC (lymphocytes + PMNs) reveals that milk samples from various provinces in Ecuador predominantly fall into quadrant C (>65% DSCC and >200,000 cells/mL). This classification indicates that most producers face issues with subclinical mastitis.

Subclinical mastitis is a common disease in production systems, leading to a decline in both the quality and quantity of milk produced. Severe cases may result in the culling of affected cows, causing significant economic losses for producers. These losses are further compounded by increased production costs associated with treating affected cows and by the broader impact on the value chain of this essential raw material.

CONFLICT OF INTEREST

The authors declare no conflict of interest in relation to this research.

FUNDING SOURCES

This study was supported by competitive research funding from the NUNKUI WAKAN group at Universidad Politécnica Salesiana, which facilitated the processes required for this characterization.

ACKNOWLEDGMENTS

The authors extend their gratitude to the dairy producers, collection centers, and farms who provided access to milk samples for analysis.

DECLARATION OF ARTIFICIAL INTELLIGENCE USE

No artificial intelligence was used in the conduct of this research.

DATA AVAILABILITY

The data are available to the authors; however, all information remains the intellectual property of Universidad Politécnica Salesiana.

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Forma de citación del artículo:

Arias–Sandoval, C. A., Bonifaz–García, N. F., Simbaña–Díaz, P. E., Argüello–Argüello, A. F. (2025). Compositional, Hygienic and Sanitary Quality of Bovine Milk in Three Regions of Ecuador. Rev Med Vet Zoot. 72(1): e114348. https://doi.org/10.15446/rfmvz.v72n1.114348

https://doi.org/10.15446/rfmvz.v72n1.116681

Prevalence of methicillin-resistant *Staphylococcus aureus* in surgeon gloves of a mobile veterinary surgical sterilization unit in Bogotá D.C.

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Recibido: 20/09/2024 Aprobado: 26/01/2025

ABSTRACT

Mobile Surgical Units (MSUs) facilitate canine and feline sterilization in vulnerable areas of Bogotá, Colombia, expanding access to veterinary surgical services. Methicillin-resistant Staphylococcus aureus (MRSA) is a significant veterinary pathogen due to its role in animal morbidity and mortality, as well as its zoonotic potential. This study aimed to determine MRSA prevalence on surgeons' gloves during MSU sterilization campaigns in Bogotá in May 2024. A survey was administered, and swab samples were collected from the internal (palmar) and interdigital glove surfaces after every five animals treated across 10 sterilization campaigns in different Bogotá localities. Samples were microbiologically processed using enriched, selective, and differential media, including CHROMagar MRSA. Data were recorded in an Excel database and analyzed using a chi-square test in RStudio to assess associations between MRSA presence and factors such as animal type (canine or feline), socioeconomic stratum, surgeon's sex, and glove changes between procedures. The results indicated a 20% MRSA prevalence, with no significant correlations observed between MRSA presence and the studied variables.

Keywords: *Staphylococcus aureus,* prevalence, surgical procedures, Bogotá.

Prevalencia de *Staphylococcus aureus* meticilino-resistente en guantes de cirujano de unidad móvil quirúrgica veterinaria de esterilización en Bogotá D.C.

RESUMEN

Las Unidades Móviles Quirúrgicas (UMQ) son empleadas para procedimientos quirúrgicos de esterilización canina y felina en zonas vulnerables de la ciudad de Bogotá, Colombia, ampliando el acceso a estos servicios quirúrgicos veterinarios. *Staphylococcus aureus* meticilino-resistente (MRSA) es un patógeno de importancia en medicina veterinaria por su capacidad de causar morbilidad y mortalidad en animales, además de su

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potencial zoonótico. Este estudio buscó determinar la prevalencia de MRSA en guantes de cirujanos de las UMQ durante las jornadas de esterilización en Bogotá durante mayo de 2024. Se diligenció una encuesta y se obtuvieron hisopados de la porción interna (palma) e interdigital a los guantes de los cirujanos cada 5 animales intervenidos en 10 jornadas de esterilización de diferentes localidades de Bogotá. Las muestras se procesaron microbiológicamente utilizando medios enriquecidos, selectivos y diferenciales como el CHROMagar MRSA. Los datos se registraron en una base de datos en Excel y se realizó un análisis estadístico de chi-cuadrado por medio del programa RSTUDIO, el cual permitió evaluar la asociación entre la presencia de MRSA y factores como tipo de población (canina o felina), estrato socioeconómico, sexo del cirujano y cambio de guantes entre procedimientos. Los resultados indicaron una prevalencia de MRSA del 20% en la población analizada. No se evidenciaron correlaciones significativas entre la presencia de MRSA y las variables estudiadas.
Palabras clave: *Staphylococcus aureus*, prevalencia, procedimientos quirúrgicos, Bogotá.

INTRODUCTION

MSUs are specially equipped vehicles designed to perform surgical procedures, such as canine and feline sterilization, in vulnerable areas of Bogotá, Colombia. The ability to bring these services directly to underserved locations underscores the significance of MSUs within the One Health framework, which recognizes the interconnectedness of human, animal, and environmental health. By contributing to public health, reducing the stray dog and cat population in high-density areas, and mitigating the spread of zoonotic diseases, MSUs play a crucial role in veterinary and public health interventions. Mass sterilization procedures conducted in MSUs provide extensive coverage and accessibility to the communities of Bogotá. However, due to the nature of these procedures and the conditions in which they are performed, there is a risk of contamination of the gloves used by surgeons with pathogenic microorganisms such as *Staphylococcus aureus*, posing a potential health risk to both medical personnel and animals (Denamiel *et al.* 2009).

MRSA is a bacterial pathogen that poses a significant threat due to its resistance to multiple antibiotics. Initially identified as a predominant nosocomial pathogen in humans (García 2011), MRSA has also emerged as a leading bacterial agent in veterinary hospitals (Loeffler *et al.* 2010). Colonized veterinary patients and medical personnel may serve as sources of infection, facilitating bacterial transmission through contaminated hands. This transmission is particularly concerning in hospital and clinical settings, where inadequate hygiene practices contribute to bacterial spread, underscoring the importance of proper disinfection and personal protective equipment (Boerlin *et al.* 2001).

Previous studies have documented MRSA in various veterinary facilities, including clinics and hospitals, with prevalence rates ranging from 2% to 14%, depending on hygiene practices and the management of infected animals (Morris *et al.* 2006; Weese & Van Duijkeren 2010; Tarazi *et al.* 2015). However, no specific data are available on MRSA prevalence in MSUs, a critical factor in implementing effective infection control measures for surgical procedures performed outside traditional veterinary operating rooms.

MRSA's ability to cause severe infections in both companion and livestock animals underscores the need for studies on its prevalence and control in veterinary environments (Porrero 2014). Methicillin-resistant strains have emerged as a growing concern, particularly in small animals and equines (Chaparro *et al.* 2005). Transmission occurs through direct contact with infected animals, contaminated surfaces, and personal protective equipment, such as surgical gloves. Studies in human hospitals have shown that MRSA can persist on gloves and other medical equipment, suggesting these items may serve as infection sources if not properly handled (García 2011).

Additionally, MRSA causes severe infections in both humans and animals, including skin infections, pneumonia, endocarditis, and septicemia, with significant morbidity and mortality. In humans, MRSA infections account for approximately 19,000 deaths annually in the United States (Klevens *et al.* 2007). In animals, infections can be fatal (Leonard & Markey 2008), particularly in veterinary settings with inadequate infection control. MRSA-associated disease is a major concern for both human and animal health due to its high morbidity and mortality (Weese *et al.* 2006). In animals, MRSA can also cause post-surgical infections, wound infections, and urinary tract infections, further increasing morbidity and mortality (Leonard & Markey 2008).

Methicillin resistance in *Staphylococcus aureus* arises primarily from the acquisition of the *mecA* gene, which encodes penicillin-binding protein 2a (PBP2a) (Kakooza *et al.* 2024). This modified protein has a low affinity for β-lactam antibiotics, allowing the bacterium to continue synthesizing its cell wall despite the presence of methicillin. As a result, MRSA strains are particularly difficult to treat, exhibiting resistance not only to methicillin but also to other β-lactam antibiotics (Kakooza *et al.* 2024). Due to this threat, the World Health Organization (WHO) has designated MRSA as a high-priority pathogen (WHO 2024).

Given these concerns, this study aimed to determine the prevalence of MRSA on surgeons' gloves during procedures in Mobile Surgical Units (MSUs) across 10 of Bogotá's 20 localities. To date, no studies have specifically examined the presence of this pathogen in MSUs.

MATERIALS AND METHODS

Study type and population

This cross-sectional study employed convenience sampling. Samples were collected from 10 localities in Bogotá, chosen based on accessibility and availability. Given that the number of animals sterilized daily in MSUs varied, glove swab sampling was performed after every five animals, resulting in a variable number of swabs per sampling day.

Simultaneously with sample collection, relevant data were recorded, including the animal species (canine or feline), sex (male or female), locality, socioeconomic stratum, predominant climate, sample sequence, glove changes between procedures, and the surgeon's sex (male or female).

Sample collection

Samples were collected between June and July 2024 during sterilization procedures conducted in MSUs across 10 localities in Bogotá, D.C. (figure 3). Glove swab sampling was performed after every five animals treated, using a swab with CE Class IIa transport medium (MDD)TM (Girovet, Italy). The swabbing procedure involved rubbing the gloves used by the surgeon—including the interdigital spaces, palm, and edges—ensuring bilateral sampling. Samples were labeled, identified, and transported in Amies transport medium under refrigeration to the facilities of Universidad Javeriana.

Sample processing

All samples underwent primary isolation on blood agar base (Oxoid, England) supplemented with 5% sheep blood and incubated for 24 hours at 37 ± 2 °C. Subsequent purification steps were performed. Based on the phenotypic characteristics of the colonies, established protocols for Gram-positive microorganisms—particularly *Staphylococcus* spp.—and/or Gram-negative microorganisms were followed (OMSA 2024).

Confirmatory biochemical tests for *Staphylococcus* spp. were conducted, including Gram staining, a catalase test, and culture on Baird-Parker agar, Mannitol Salt agar, and DNase agar (Oxoid). Once the genus was confirmed, isolates were plated on CHROMagar™ MRSA (CHROMagar Microbiology, France) for specific MRSA identification (Diederen *et al.* 2005).

Other bacterial genera present in the samples were identified using selective and differential culture media, along with additional biochemical tests, including MacConkey agar, oxidase test strips (Oxoid), and the API 20E™ panel (bioMérieux, USA).

Data analysis

Data collected from the survey conducted during the sampling of 100 specimens (annex 1), along with laboratory results, were recorded in an Excel® database. Descriptive statistical analyses were performed, and associations were assessed using a chi-square test in RStudio (Lastre *et al.* 2019). This analysis evaluated the relationship between MRSA presence and factors such as animal population type (canine or feline), socioeconomic stratum, surgeon's sex, and glove changes between procedures.

RESULTS AND DISCUSSION

In this study, 100 surgeon glove swabs were analyzed from MSUs during sterilization procedures conducted in 10 localities of Bogotá, D.C.

Microbial growth was observed in 48% of the samples (figure 1). Of these, 22.9% (11/48) exhibited mixed growth (more than one microorganism), while the remaining 77.1% (37/48) showed pure or axenic cultures (figure 2).

These findings are consistent with previous studies that have reported MRSA prevalence in veterinary clinics and other healthcare settings, albeit on a smaller scale (Yamauchi & Santorelli 2005).

When compared to other studies involving animals and humans, the prevalence of MRSA in clinical and surgical environments has shown an increasing trend, highlighting the need for improved infection control practices (Weese *et al.* 2006; Tarazi *et al.* 2015). In this study, the overall MRSA prevalence in the analyzed localities was estimated at 20%. Independent analysis revealed differences among localities, with Usme exhibiting the highest positivity rate at 66.7%, while Rafael Uribe Uribe had the lowest at 6.3% (figure 3). This variability may be influenced by several factors, including

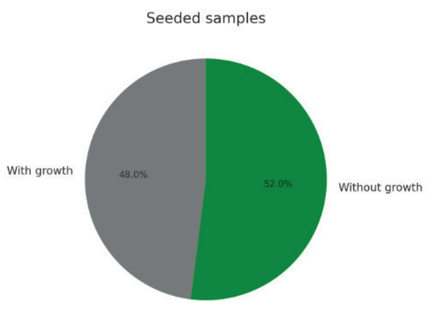

FIGURE 1. Graphical representation of growth obtained after seeding.
Source: own elaboration.

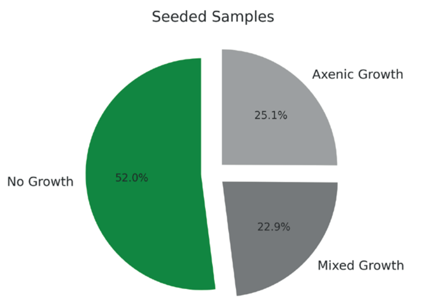

FIGURE 2. Detail of mixed axenic growth obtained from seeded samples.
Source: own elaboration.

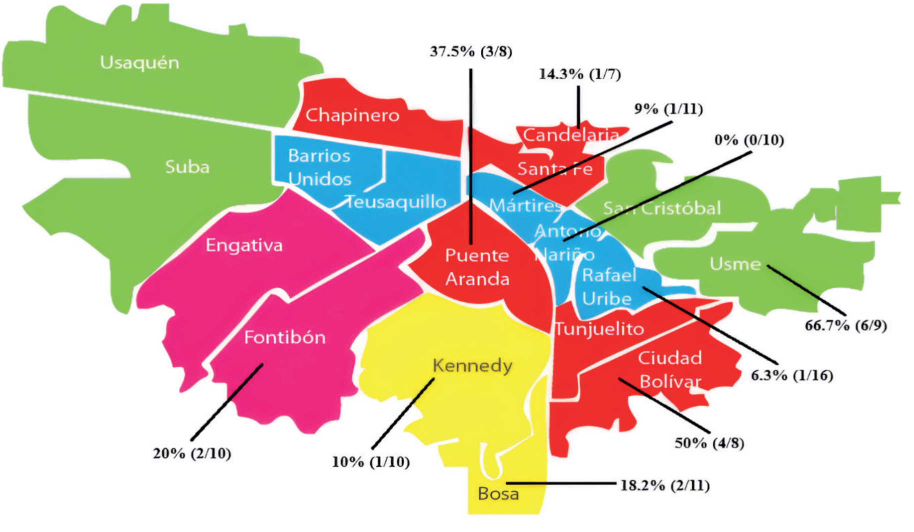

FIGURE 3. Estimated prevalence in 10 localities of Bogotá.
Source: http://www.lacandelaria.gov.co/mi-localidad/mapas

the less controlled working conditions in MSUs compared to traditional veterinary hospitals. Previous studies suggest that inadequate glove rotation and potential cross-contamination between animals and surgeons could contribute to these differences (Otter *et al.* 2013; Rubin *et al.* 2011).

The findings of this study align with the global trend of increasing MRSA infections in both human and veterinary medical settings (Weese *et al.* 2006). The lack of specific studies on MRSA prevalence in surgical gloves used in MSUs represents a gap in the literature that this study seeks to address.

Chi-square test results indicated no statistically significant difference in MRSA presence between using new gloves and reusing gloves (p = 0.24). Additionally, no significant correlation was found between

MRSA presence and the recorded study variables, including surgeon sex (male/female) ($p = 0.82$), animal species (dog/cat) ($p = 0.33$), weather conditions (rainy/sunny) ($p = 0.14$), animal sex (male/female) ($p = 0.99$), and socioeconomic stratum (1/2/3) ($p = 0.86$). However, a significant difference was observed across localities ($p = 0.00199$), suggesting that local context and specific practices within each area may influence MRSA prevalence.

Few studies report on MRSA prevalence in veterinary clinics and hospitals; however, previous research indicates a prevalence ranging from 2% to 16% (Yamauchi & Santorelli 2005). Outside hospital settings, reported prevalence rates include 5.3% in canines and 5.0% in humans who had contact with infected dogs, with strong associations observed between isolates from these hosts (Tarazi *et al.* 2015). These findings underscore the zoonotic and anthropozoonotic potential of *S. aureus* and highlight the critical need to enhance infection control measures, regardless of the setting.

The presence of MRSA can be inferred given that MSUs operate under less controlled conditions compared to traditional veterinary hospitals, potentially increasing the risk of cross-contamination. Additionally, hygiene practices and glove rotation between procedures may not be optimal, facilitating the persistence and transmission of MRSA (Otter *et al.* 2013). Similar studies have indicated that improper handling of personal protective equipment, such as gloves, can serve as a significant vector for MRSA spread (Rubin *et al.* 2011). Reports suggest that MRSA infections in both humans and animals are associated with high morbidity and mortality rates, underscoring the importance of effective preventive measures. According to the Centers for Diseases Control and Prevention (CDC 2018), MRSA infections can be severe and challenging to treat due to their antibiotic resistance, increasing the risk of serious complications and community transmission.

In relation to the findings of this study, previous research has highlighted how environmental factors and management practices influence MRSA prevalence in shared human-animal environments. Bullone *et al.* (2024) found that frequent antimicrobial treatments in stables significantly increase the risk of carrying these pathogens in both animals and caretakers, while appropriate environmental conditions, such as proper ventilation, act as protective factors. This perspective underscores the necessity of implementing surveillance programs and control strategies under the One Health approach to mitigate the risks associated with MRSA transmission.

MRSA transmission not only affects animal health but also poses a zoonotic threat to humans, particularly in environments with frequent human-animal contact (Weese *et al.* 2006; CDC 2018). The presence of other pathogens, such as *Acinetobacter* spp. and *Klebsiella* spp., further emphasizes the urgency of enforcing strict aseptic protocols in MSUs to prevent the spread of these infections in both animals and humans (WHO 2024). The recent update by the WHO of its priority pathogen list highlights the significance of these microorganisms due to their high resistance to critical antibiotics (WHO 2024).

Although identifying the accompanying microbiota was not an objective of this study, the analysis of all collected samples revealed the presence of additional microorganisms in the following proportions: α-hemolytic *Streptococcus* (12%), *Acinetobacter* spp. (7%), *Micrococcus* spp. (5%), *Pseudomonas* spp. (4%), coryneform Gram-positive bacilli (1%), and *Escherichia coli* (3%). Additionally, β-hemolytic *Streptococcus*, *Klebsiella* spp., *Staphylococcus* spp., and *Candida* spp. were each detected in 1% of the samples (figure 4).

As previously mentioned, antimicrobial resistance (AMR) complicates the treatment of infectious diseases, increasing healthcare costs for both animal and human patients and negatively impacting public health. The detection of other pathogens, such as *Acinetobacter* spp., although not initially within the scope of this study, warrants attention given the recent findings by WHO. The 2024 update to the WHO priority pathogen list classifies *Acinetobacter baumannii*, *Enterobacterales* (including *Escherichia coli* and *Klebsiella* spp.), and *Pseudomonas aeruginosa* as critical- and high-priority pathogens due to their resistance to carbapenems and third-generation cephalosporins. Additionally, Group A and B *Streptococcus* are classified as medium-priority pathogens due to their resistance to macrolides (WHO 2024).

The findings of this study provide valuable data that could inform the development of public health policies aimed at reducing the transmission of MRSA and other pathogens in these environments.

CONCLUSION

The prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) was found to be 20%; however, no statistically significant association was observed between the presence of the pathogen and the studied variables.

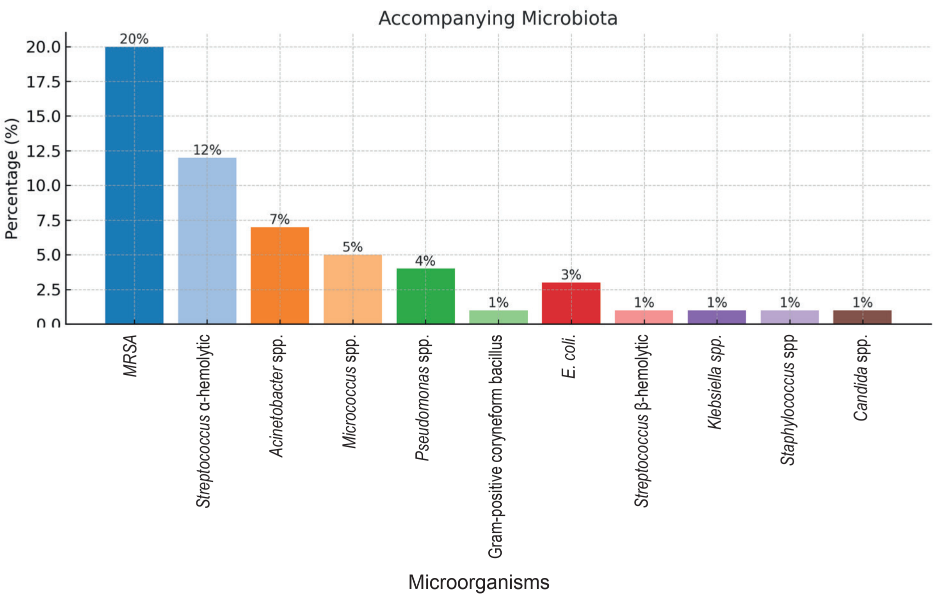

FIGURE 4. Identified accompanying microbiota.
Source: own elaboration.

AUTHOR CONTRIBUTIONS

All authors made equal contributions to data analysis and manuscript preparation.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ETHICS COMMITTEE APPROVAL

Since no direct interaction with animals occurred during this study, ethics committee approval was not required.

DECLARATION ON THE USE OF ARTIFICIAL INTELLIGENCE

Artificial intelligence (AI) tools were used to facilitate data visualization and develop the graphs presented in this study.

FUNDING SOURCES

This research was conducted using personal funds without external funding. However, academic and experimental support was provided by the Agricultural Research Unit (UNIDIA) at Pontificia Universidad Javeriana.

DATA AND MATERIAL AVAILABILITY

All data and materials are available from the corresponding author upon request. However, the sampling methods are proprietary to Pontificia Universidad Javeriana.

ACKNOWLEDGMENTS

We extend our sincere gratitude to Dr. Adriana and Dr. Moisés for their invaluable guidance, dedication, and support throughout the development of this research project. Their expertise, patience, and continuous encouragement were instrumental to the successful completion of this study.

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Forma de citación del artículo:

Salazar–Mahecha, A.C., Gallo–Álvarez, M.C., Hernández–Gallo, N., Pulido–Villamarín, A.P., Aranda–Silva, M. (2025). Prevalence of Methicillin-resistant Staphylococcus aureus in surgeon gloves of a mobile veterinary surgical sterilization unit in Bogotá D.C. Rev Med Vet Zoot. 72(1): e116681. https://doi.org/10.15446/rfmvz.v72n1.116681

https://doi.org/10.15446/rfmvz.v72n1.117262

Renal amyloid protein deposition in a Shar Pei dog

J.S. Masiero[1], *F.G. Sousa*[2]*, *S.L. Beier*[3]

Recibido: 29/10/2024 Aprobado: 02/01/2025

ABSTRACT

Renal amyloidosis is a glomerular disease with a familial predisposition, particularly common in Shar Pei dogs, and is associated with clinical manifestations of chronic kidney disease (CKD). This case report describes a female Shar Pei diagnosed with renal amyloidosis, similar to her father and brother. A nine-year-old female Shar Pei presented with a history of vomiting, halitosis, hyporexia, polyuria, and polydipsia. Structural and functional renal abnormalities were observed, including azotemia, cortical hyperechogenicity, and a mild reduction in corticomedullary differentiation. Given the patient's clinical condition, euthanasia was performed, and renal tissue samples were submitted for histopathological analysis. The presence of eosinophilic fibrillar material within the glomeruli and some tubules confirmed the diagnosis of renal amyloidosis.

Keywords: chronic kidney disease, glomerulopathies, amyloid protein.

Deposição de proteína amiloide renal em cão da raça Sharpei

RESUMO

A amiloidose renal é uma doença glomerular de características familiares, especialmente para Sharpeis e está associada com manifestações clínicas de doença renal crônica (DRC). O objetivo do presente relato é descrever o caso de uma fêmea, da raça Sharpei, diagnosticada com amiloidose renal, de forma semelhante aos seu pai e irmão. Cão, fêmea, nove anos, Sharpei, foi atendida mediante histórico de vômitos, halitose, hiporexia, poliúria e polidipsia. Alterações estruturais e funcionais dos rins, tais como azotemia, hiper ecogenicidade cortical e redução discreta da definição corticomedular foram encontradas. Diante do quadro clínico, a paciente foi submetida à eutanásia e fragmentos renais foram encaminhados para análise histopatológica. Observou-se a presença de material de características eosinofilicas e fibrilares no interior dos glomérulos e em alguns túbulos, sendo o diagnóstico definitivo de amiloidose renal.

Palavras-chave: doença renal crônica, glomerulopatias, proteína amiloide.

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INTRODUCTION

Amyloidosis is characterized by the deposition and accumulation of amyloid protein in tissues and organs, such as the kidneys and liver (DiBartola *et al.* 1990) and is frequently observed in the renal region (Sonne *et al.* 2008; Woldemeskel *et al.* 2012; Segev *et al.* 2012; Serakides & Silva 2023). This condition is associated with glomerular dysfunction and, consequently, renal impairment due to structural and/or functional alterations, leading to glomerular loss and fibrosis. Amyloid protein deposition can result from primary causes, such as immunoglobulin involvement, or occur secondary to chronic inflammation (Jones *et al.* 1997; Serakides & Silva 2023; Giaretta & Barros 2023). The disease is commonly diagnosed in breeds predisposed to amyloidosis, such as Shar Peis and Beagles, although its occurrence is not exclusive to these breeds (Sonne *et al.* 2008; Segev *et al.* 2012; Jung *et al.* 2014). Notably, animals with familial relationships to affected individuals have an increased risk of developing the disease, emphasizing the genetic component associated with its pathogenesis (Sonne *et al.* 2008; Segev *et al.* 2012; Jung *et al.* 2014).

Renal amyloidosis is characterized by structural and/or functional glomerular lesions, which may present an acute or chronic course. These conditions are classified as glomerulopathies and generally carry a guarded to poor prognosis (Vaden 2011). Furthermore, a hereditary component has been identified in some cases (Sousa *et al.* 2024). The investigation of glomerular diseases, including amyloidosis, relies on the assessment of pathophysiological characteristics, enabling a more comprehensive clinical evaluation and a more accurate determination of therapeutic options (IRIS 2013). Among hereditary renal disorders, amyloidosis is particularly concerning due to its potential for genetic transmission, facilitating the propagation of affected genes across generations.

The clinical manifestations of amyloidosis in dogs are consistent with renal dysfunction and often include azotemia and functional abnormalities. As the disease progresses, chronic kidney disease (CKD) is frequently observed, although nonspecific clinical signs may also be present (Sousa *et al.* 2024). A definitive diagnosis requires histopathological evaluation of affected tissues, revealing amyloid protein deposition in glomerular and/or tubular regions (Sonne *et al.* 2008; Segev *et al.* 2012; Sousa *et al.* 2024). Once diagnosed, amyloidosis is considered incurable, and treatment primarily focuses on managing disease progression and associated complications, though therapeutic interventions often remain ineffective (Sousa *et al.* 2024). This report describes a case of renal amyloid deposition in a female Shar Pei with a familial history of amyloidosis.

CASE DESCRIPTION

A 9-year-old female Shar Pei dog, weighing 25 kg, was presented to a veterinary clinic in the metropolitan region of Belo Horizonte, MG, with a history of vomiting, halitosis, hyporexia, polyuria, and polydipsia. The owner reported that the patient was the daughter and sibling of two other Shar Pei dogs that had died with a definitive diagnosis of renal amyloidosis. On clinical examination, the patient had a heart rate of 132 bpm, a respiratory rate of 24 breaths per minute, and normal mucous membrane coloration; however, reduced brightness and moisture were noted, consistent with approximately 8% dehydration. The lymph nodes were of normal size, volume, and appearance, with a capillary refill time of 3 seconds and a body temperature of 38.9 ºC. Moderate dental tartar and early-stage periodontal disease were present, along with bilateral necrosis near the lingual frenulum. No significant abnormalities were detected in other examined regions.

Given the strong suspicion of familial amyloidosis, laboratory tests, including a complete blood count, serum biochemistry, and urinalysis, were performed, along with abdominal ultrasonography and a canine visceral leishmaniasis screening test using enzyme-linked immunosorbent assay (ELISA) and total dilution. Laboratory analysis revealed leukocytosis due to neutrophilia without a left shift, significant azotemia, hyperphosphatemia, decreased total protein levels due to hypoalbuminemia, elevated alkaline phosphatase, hypocalcemia, hypercholesterolemia, and increased creatine phosphokinase levels (table 1). The ELISA test and total dilution for visceral leishmaniasis were non-reactive. Urinalysis revealed proteinuria (++), a urine pH of 5, and a specific gravity of 1.018.

Ultrasonographic evaluation (ESAOTE, MyLab X8) showed that both kidneys were of normal size (7 cm × 4 cm × 4.6 cm; 8 cm × 4.8 cm × 5.5 cm) but exhibited structural alterations, including moderate cortical hyperechogenicity and a slight reduction in corticomedullary differentiation, suggestive of CKD. No significant abnormalities were detected in other abdominal structures (figure 1).

TABLE 1. Laboratory findings of the patient in the case described

	Value obtained	Reference values
Leukocytes	17,470 mil/µL	6 a 16 mil/µL
Neutrophils	14,675 mil/µL	3,300 – 12,800 µL
Urea	201.80 mg/dl	15 a 40 mg/dL
Creatinine	6.04 mg/dl	0.5 a 1.5 mg/dL
UPCR	33.41 mg/dl	
Phosphorus	14.40 mg/dl	2.7 a 5.4 mg/dL
Total proteins	4.94 g/dl	5.30 a 7.80 g/dl
Albumin	1.13 g/dl	2.30 a 3.80 g/dl
Alkaline phosphatase	128.80 U/L	10 a 96 U/L
Calcium	8.15 mg/dl	9 a 11.3 mg/dl
Cholesterol	570.40 mg/dl	125 a 270 mg/dl
CPK	333.30 U/L	20 a 200 U/L

Consider: UPCR – urinary protein creatinine ratio; CPK–creatine phosphokinase

Source: own elaboration.

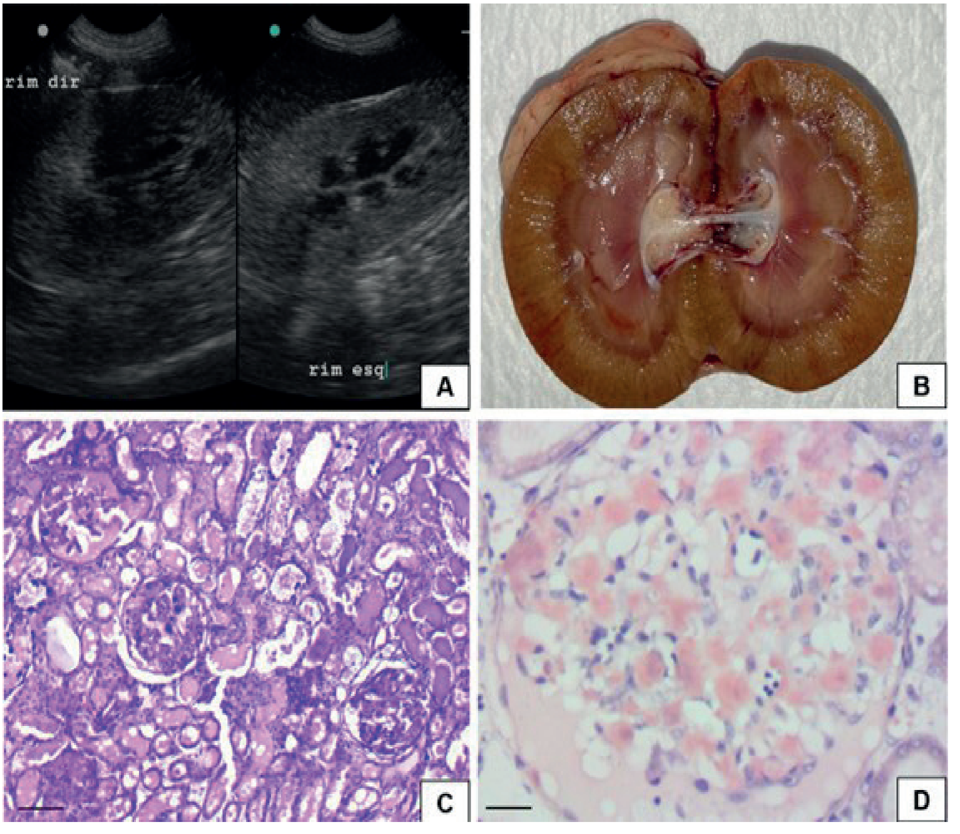

FIGURE 1. A: Ultrasound images of the right and left borders, showing changes in echogenicity. B: Left border, sectioned, for histopathological evaluation, showing mild nephromegaly, loss of the corticomedullary relationship, and dilation of the renal pelvis. C: Photomicrograph of the cortical region, showing the position of the amyloid protein within the glomeruli and tubules. ELE, 20x. D: Glomerulus stained with Congo red, showing the presence of amyloid protein. Congo red, 40x.

Source: own elaboration.

As the patient's clinical condition deteriorated and due to a delay in seeking veterinary care, she arrived in critical condition. Following discussions with the owner, euthanasia was elected. Necropsy was not authorized; however, permission was granted for the removal of the kidneys for diagnostic evaluation.

Fragments of the right and left kidneys, measuring approximately 6.8 × 4.7 × 4.4 cm and 7.2 × 4.0 × 5.0 cm, respectively, exhibited diffuse whitish striations in the cortex, mild dilation of the renal pelvis, and a tense-elastic consistency. These samples were preserved in 10% formalin and submitted to an outsourced laboratory for histopathological analysis.

Histological examination of hematoxylin-eosin-stained sections revealed enlarged glomeruli with extensive deposition of eosinophilic, homogeneous, and slightly fibrillar material. Congo red staining confirmed amyloid deposition, affecting large areas of the glomeruli and interspersed with mineralized regions. Glomerulosclerosis was also observed. In the tubules, multifocal basophilic, fragmented material indicative of mineralization was noted, along with a marked accumulation of intratubular proteinaceous material, occasionally associated with mild dilation. The interstitial space exhibited a mild multifocal lymphoplasmacytic inflammatory infiltrate.

Based on these findings, a diagnosis of renal amyloidosis was confirmed (figure 1). The owner was informed of the diagnosis and advised against breeding animals from the same lineage, given the hereditary nature of the disease.

DISCUSSION

Renal amyloidosis, as previously described, is characterized by the accumulation of amyloid-type protein in tissues and organs, often in response to inflammatory processes (DiBartola *et al.* 1990; Sousa *et al.* 2024). Inflammatory conditions lead to increased production of amyloid proteins, resulting in their deposition through aggregation of isoforms in organs such as the kidneys (DiBartola *et al.* 1990; Johnson *et al.* 1996). Amyloid protein is insoluble, highly resistant to proteolytic degradation, and composed of fibrils derived from amyloidogenic proteins (Serakides & Silva 2023). The extent of amyloid protein deposition varies depending on factors such as clinical condition and protein composition (Woldemeskel *et al.* 2012). Deposition may involve heterogeneous protein types, including amyloid A (AA), light chain (AL), and familial forms (Serakides & Silva 2023; Giaretta & Barros 2023). The kidneys are among the most commonly affected organs, as demonstrated in this case. However, in this patient, the presence of amyloid deposits in other organs, such as the liver, was not assessed.

This disease is frequently reported in predisposed breeds, such as the Shar Pei, where it is primarily transmitted through familial inheritance, allowing affected individuals to pass the disease to subsequent generations. In the present case, there was a strong suspicion of familial amyloidosis, as the affected patient belonged to a predisposed breed and had direct familial ties to other dogs that had been diagnosed with renal amyloidosis. Previous studies have also reported familial amyloidosis in Shar Pei dogs (DiBartola *et al.* 1990; Lee *et al.* 2007). Given the hereditary nature of the disease, animals diagnosed with renal amyloidosis should be spayed or prevented from breeding, as they are potential carriers of the causative genetic variants (Sonne *et al.* 2008). In this case, the recommendation to neuter affected animals was not followed, as the patient's sire had also been diagnosed with renal amyloidosis, indirectly suggesting that breeding with a diseased or carrier animal had occurred. Spaying affected animals is essential to reduce the risk of genetic transmission, even in cases where they do not exhibit clinical symptoms (Sousa *et al.* 2024).

Owner education is critical in ensuring an understanding of the disease and its associated complications. Additionally, providing emotional support and guidance following the diagnosis of a progressive and life-threatening condition is essential (Sousa *et al.* 2024). Given the severe and irreversible nature of amyloidosis, this stage can be particularly challenging for owners, especially those with a strong emotional attachment to their pets (Sousa *et al.* 2024). They must be informed that animals diagnosed with amyloidosis, whether clinically affected or identified as carriers, should not be used for breeding to prevent further transmission of the disease (Sousa *et al.* 2024).

In a study conducted on Shar Pei dogs, amyloid protein deposition in the glomerular region was detected in nearly 80% of the animals, highlighting the breed's strong predisposition to renal amyloidosis. However, the disease is not exclusive to Shar Pei dogs and has also been reported in breeds such as Beagle (Jung *et al.* 2014), Weimaraner (Loewen *et al.* 2018), and Italian Pointer (Inman *et al.* 2021). Regarding age-related susceptibility, glomerulopathies tend to manifest more frequently in middle-aged to older dogs (Vaden 2011), with amyloidosis typically reported in animals between four and eight years of age (Segev *et al.* 2012). However, some studies suggest a broader range of one to six years, with Shar Pei dogs potentially developing the disease at an earlier age (Sonne *et al.* 2008; Júnior *et al.* 2011; Loewen *et al.* 2018). In the present case, the patient was diagnosed at nine years of age, which aligns with available data (Vaden 2011). Nevertheless, although clinical signs manifested at this stage, amyloid deposition likely began earlier, with severe impairment of renal function occurring progressively over time. Other dogs from the same familial lineage were diagnosed at three and four years of age.

The clinical manifestations of amyloid deposition vary depending on factors such as proteinuria and the extent of renal dysfunction (Sousa *et al.* 2024). Renal amyloidosis is frequently associated with chronic kidney disease (CKD), as amyloid accumulation induces structural and functional damage in the glomeruli and tubules, impairing nephron function (Greco 2001; Woldemeskel *et al.* 2012). Consequently, the severity and extent of amyloid deposition are expected to correlate with the progression of clinical symptoms due to structural and functional damage in affected renal segments (Greco 2001). Amyloid deposition in the kidneys is directly associated with the frequency of clinical signs, which are commonly indicative of CKD or acute kidney injury, leading to significant organ dysfunction (Sousa *et al.* 2024).

As a result, dogs with renal amyloidosis typically exhibit progressive signs of CKD. The clinical presentation of CKD is often nonspecific in the early stages but becomes more pronounced as the disease advances. Affected dogs may exhibit lethargy, inappetence, vomiting, diarrhea, proteinuria, azotemia, impaired urine concentration and metabolite excretion, and ultrasonographic abnormalities indicative of renal dysfunction (Perondi *et al.* 2020). In the present case, the patient was evaluated due to clinical signs including vomiting, halitosis, hyporexia, polyuria, and polydipsia, which align with descriptions from previous studies on amyloidosis (DiBartola *et al.* 1990; Sonne *et al.* 2008; Júnior *et al.* 2011; Loewen *et al.* 2018; Inman *et al.* 2021). Additionally, the owner reported that other dogs from the same familial generation had exhibited clinical signs suggestive of CKD.

The laboratory characterization of patients with renal amyloidosis is highly variable and closely linked to the severity and progression of chronic kidney disease (CKD) (Sousa *et al.* 2024). Evidence suggests that affected patients may exhibit hematological and serum biochemical alterations, with the most pronounced findings including azotemia, proteinuria, electrolyte imbalances, and changes in urine specific gravity and the urine protein-to-creatinine ratio, among others (Lee *et al.* 2007; Sonne *et al.* 2008; Júnior *et al.* 2011; Loewen *et al.* 2018; Inman *et al.* 2021; Sousa *et al.* 2024). In this case, the patient exhibited proteinuria (2+), a urine pH of 5, and a specific gravity of 1.018. Patients with amyloidosis typically present with reduced urine specific gravity, approximately 1.015, along with proteinuria (generally exceeding 3+), findings that align with those observed in this case (Lee *et al.* 2007; Segev *et al.* 2012).

Renal amyloidosis is commonly associated with alterations in hematological and serum biochemistry parameters, particularly urea and creatinine levels, hypoalbuminemia, hypoproteinemia, and hyperphosphatemia (Lee *et al.* 2007; Sonne *et al.* 2008; Segev *et al.* 2012; Júnior *et al.* 2011; Loewen *et al.* 2018; Inman *et al.* 2021). In the discussed case, the patient exhibited leukocytosis due to neutrophilia without a shift, significant azotemia (urea: 201.80 mg/dL; creatinine: 6.04 mg/dL), a urea/creatinine ratio of 33.41 mg/dL, hyperphosphatemia, hypoalbuminemia, and hypoproteinemia, findings consistent with those reported in previous studies (Lee *et al.* 2007; Sonne *et al.* 2008; Segev *et al.* 2012; Júnior *et al.* 2011; Loewen *et al.* 2018; Inman *et al.* 2021; Sousa *et al.* 2024). Azotemia is frequently associated with glomerular disorders, occurring in approximately 90% of affected animals, with an average creatinine level of 5.5 mg/dL (Segev *et al.* 2012). Notably in this case, the patient's creatinine level exceeded the values reported in the literature (Segev *et al.* 2012).

In cases involving glomerular pathology, imaging abnormalities may be detected, particularly via abdominal ultrasonography. Chronic glomerular diseases, including amyloidosis, often present with ultrasonographic findings characteristic of CKD, such as irregular renal contours and size, altered cortico-medullary junction and differentiation, cortical and medullary hyper echogenicity, renal infarction, pyelectasia, and mineralization (Perondi *et al.* 2020). According to the International Renal Interest Society (IRIS), ultrasonographic abnormalities in CKD patients are stage-dependent, with the severity of findings increasing in proportion to disease progression (IRIS 2023). In this case, the patient exhibited ultrasonographic evidence of CKD, including increased cortical echogenicity and decreased cortico-medullary differentiation, consistent with previous reports (Perondi *et al.* 2020; Inman *et al.* 2021; Sousa *et al.* 2024).

Although renal amyloidosis is associated with non-specific pathological changes and commonly presents as part of CKD, histological evaluation of renal tissue remains the gold standard for definitive diagnosis. This approach enables the identification of amyloid deposits through specialized staining techniques. Diagnosis is based on the detection of eosinophilic material within glomerular and/or tubular structures, the presence of lymphocyte and plasma cell infiltrates, fibrosis, and, in some cases, protein casts (Sonne *et al.* 2008). Amyloid deposition may be focal or widespread, with the potential for severe systemic involvement (Segev *et al.* 2012). In the present case, histopathological analysis revealed eosinophilic, fibrillar material within the glomerular and tubular regions, as well as calcification and inflammatory infiltrates, findings consistent with those reported in previous studies (DiBartola *et al.* 1990; Lee *et al.* 2007; Sonne *et al.*

2012; Segev *et al.* 2012; Sousa *et al.* 2024). However, investigations for extra-renal amyloidosis and amyloid protein typing were not conducted due to constraints imposed by the patient's guardians.

The clinical presentation of renal amyloidosis was initially suspected based on a combination of clinical findings, the patient's familial history, and previous diagnoses in related individuals. Despite this suspicion, the severity of the disease led to an unfavorable prognosis. Therapeutic management of renal amyloidosis remains challenging, as no effective treatment strategies are currently available to significantly improve disease outcomes or prolong survival (Sousa *et al.* 2024).

CONCLUSION

Renal amyloidosis is a progressive and severe disease often associated with poor clinical outcomes. Characterized by the deposition of amyloid protein, the condition has a strong familial component, particularly in Shar Pei dogs, though it is not exclusive to this breed. In the discussed case, amyloid deposition in renal tissue was confirmed in a Shar Pei dog, a breed predisposed to this condition. Diagnosis was established through a combination of clinical assessment, imaging (ultrasonography), laboratory findings, and histopathological evaluation, supported by specialized staining techniques. The familial nature of renal amyloidosis in this case is strongly suspected, given the history of the disease in closely related dogs (sire and sibling). Future investigations will include immunoperoxidase staining for amyloid AA, kappa, and lambda to further characterize the protein deposits. It is crucial that owners are made aware of the risks of including affected animals in breeding programs, as the disease can be transmitted to subsequent generations.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

FUNDING SOURCES

The diagnosis and treatment were fully funded by the animal's owner.

USE OF ARTIFICIAL INTELLIGENCE

No artificial intelligence was utilized in the diagnosis, treatment, or preparation of this manuscript.

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Forma de citación del artículo:

Masiero, J.S., Sousa, F.G., Beier S.L. (2025). Renal amyloid protein deposition in a Shar Pei dog. Rev Med Vet Zoot. 72(1): e117262. https://doi.org/10.15446/rfmvz.v72n1.117262

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