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The Arch. Vet. Sci. started in 1996 and is one of the oldest scientific academic journals of Brazil. Following the steps of our previous editors, we are implementing a few changes in the layout and the editing of the articles. This **anniversary edition** marks the beginning of a new phase, with the look and energy of the future focused on the dissemination of science.

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ARCHIVES OF VETERINARY SCIENCE

Arch. Vet. Sci. is the official scientific journal associated with the graduate program of Veterinary Sciences of the Federal University of Paraná, UFPR, based in Curitiba, PR, Brazil. AVS is a gold open access with no article processing charges, peer-review online journal, published quarterly. High quality full articles, invited reviews, and short communications will be considered for publication in Veterinary Medicine and other related areas of science.

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Tribute to Prof. Metry Bacila: 30 Years of AVS Journal

Celebrating the Archives of Veterinary Science (AVS) journal's three decades is, above all, honoring the vision and legacy of Professor Metry Bacila. By conceiving and founding this journal within the Federal University of Paraná, Prof. Bacila did not just create a vehicle for scientific dissemination; he erected a fundamental pillar for the strengthening of Brazilian Veterinary Medicine. His initiative reflected a life dedicated to science and academic excellence, always driven by the belief that knowledge must be shared and perpetuated to transform reality.

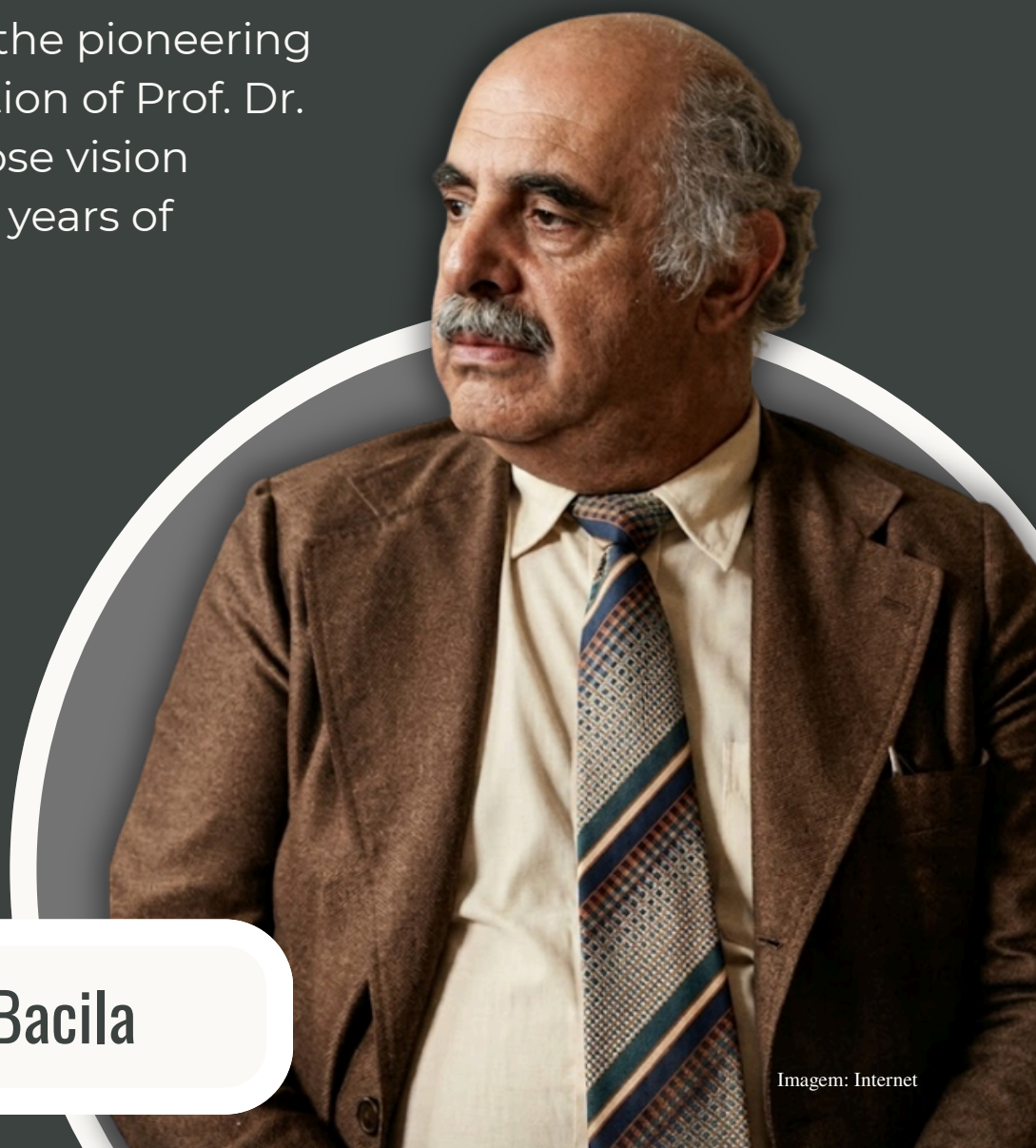
Today, as we look back at the trajectory of Arch. Vet. Sci., we express our deepest affection and gratitude. Gratitude for his courage in blazing trails when the editorial landscape was uncertain, and affection for every seed of intellectual curiosity he planted in his colleagues and students. Prof. Bacila personified the spirit of UFPR: the relentless pursuit of truth through rigorous research.



Tribute to Prof. Metry Bacila: 30 Years of AVS Journal

This thirtieth anniversary is a tribute to his persistence. Thanks to his starting point, generations of researchers have found in AVS a prestigious space for their discoveries. His name remains alive on every published page, in every citation, and in the international prestige the journal has achieved. Thank you, Professor Metry Bacila, for believing in veterinary science and for leaving us this invaluable gift, which continues to shine as a beacon of knowledge and inspiration.

In recognition of the pioneering spirit and dedication of Prof. Dr. Metry Bacila, whose vision brought to life 30 years of science at AVS.



Prof. Dr. Metry Bacila

SCIENTIFIC ARTICLES

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Quantitative analyses of polyphenol extracts from inflorescences of *Musa x paradisiaca* against free-living stages of *Haemonchus contortus*

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Abstract: Extracts from *Musa x paradisiaca* inflorescences were chemically characterized and tested *in vitro* on ruminant parasite egg hatch (EHT) and larval migration (LMT) tests. The aqueous (EHW-I) and hydroalcoholic (EOH-I) extracts of the plant were analyzed by using colorimetric assays, high-performance liquid chromatography (HPLC), and electrospray tandem mass spectrometry (ESI-MS/MS). EHW-I and EOH-I had similar phenolic contents. However, EOH-I had a higher flavonoid content than EHW-I. Both extracts presented low contents of condensed tannins. HPLC analysis showed that EHW-I contained phenolic acids (chlorogenic acid > coumaric acid > ferulic acid), other than rutin (glycosylated flavonoid). EOH-I contained rutin as the predominant compound. EHW-I and EOH-I showed an inhibitory effect on EHT, reaching almost 100% at a concentration of 1000 µg/mL. The inhibitory concentration of 50% (IC₅₀) of egg hatching was 62.58 and 137.79 µg/mL of EHW-I and EOH-I, respectively. Based on the quantification data of phenolic and flavonoid compounds in the extracts, chlorogenic acid and rutin were selected for the EHT. Chlorogenic acid presented high activity, with an IC₅₀ of 17.55 µg/mL. Rutin, however, did not affect egg hatching under the conditions tested. It can be inferred that chlorogenic acid may be a candidate with ovicidal activity. EHW-I and EOH-I did not affect the migration of third-stage nematode larvae, which may be related to the low tannin content. The inflorescences of *Musa x paradisiaca* can be further explored to develop a plant-derived anthelmintic product for ruminants and other animals.

Keywords: Phytotherapy, Biofriendly products, principal components, gastrointestinal parasites, *in vitro* assays.

1. Introduction

World ruminant production is economically affected by gastrointestinal parasite infections. One of the most important gastrointestinal nematode parasites affecting livestock is *Haemonchus contortus*. This hematophagous parasite can cause severe anemia, which reduces weight gain, and, in more severe infections, can lead to animal death (Kumarasingha et al., 2016). Conventional parasite control relies almost exclusively on the regular use of broad-spectrum anthelmintics. However, such treatments present major disadvantages, including the selection of resistant populations, the risk of environmental pollution, high costs, and, in addition, the unavailability of these medicines in some developing countries (Fortes and Molento, 2013).

The use of natural plant extracts to treat parasitic infections in livestock and humans is a long-standing practice. This therapeutic approach may offer sustainable and environmentally acceptable alternatives (Athanasiadou et al., 2007; Molento, 2009; Pavela and Benelli, 2016). Classes of secondary metabolites, such as terpenoids (essential oils) and polyphenols (phenolic acids, flavonoids, and condensed tannins), are sources of chemical components responsible for the broad therapeutic effects of several medicinal plants, including anthelmintic activity (Molan et al., 2002; Hoste et al., 2015). *In vitro* tests serve as a preliminary step for validating novel extracts, as they enable the characterization of active compounds present in plants and the evaluation of their biological properties (Pavela and Benelli, 2016).

Musa paradisiaca L., a plant in the family Musaceae, is a large shrub that grows in tropical regions, and its fruit is known as a banana (Padam et al., 2012; Jaleel et al., 2024). This plant is a functional and medicinal plant of high therapeutic potential, and its parts, including peels, leaves, pseudostem, and inflorescence, have various applications (Padam et al., 2012; Jaleel et al., 2024). They serve as alternative sources of macronutrients and micronutrients, are also used in nutraceuticals and livestock feed, and function as natural fibers and sources of bioactive compounds (Padam et al., 2012; Jaleel et al., 2024; Senevirathna et al., 2024). Studies on the phytochemical composition of banana (*Musa* spp.) inflorescences have increased due to the growing interest in

utilizing agricultural by-products as sustainable sources of bioactive compounds (Padam et al., 2012; Senevirathna et al., 2024). Many studies have identified and characterized metabolites in *M. paradisiaca* inflorescences, including phenolic compounds such as flavonoid-like quercetin, epicatechin (Bashkar et al., 2012, Bovo et al., 2013, Nisha and Mini, 2013, Amornlerdpison et al., 2021, Ramírez-Bolaños et al., 2021), anthocyanins (Pazmiño-Duran et al., 2001, Senevirathna et al. 2024), tannins (Mahmood et al., 2011, Amornlerdpison et al., 2021), terpenoids, such as tetracyclic triterpenoids (Dutta et al., 1983), saponins, steroids (Mahmood et al., 2011), alkaloids (Mahmood et al., 2011), and phenolic acids, such as gallic acid (Nisha and Mini, 2013, Amornlerdpison et al., 2021).

Most studies have evaluated the anthelmintic activity of plant extracts based on qualitative data from various classes of metabolites, using techniques such as phytochemical screening. Quantitative studies on the components, however, are lacking (Athanasiadou et al., 2007; Kumarasingha et al., 2016; Pavela and Benelli, 2016). For example, the aqueous extracts of the leaves, stems, and inflorescences of *Musa* spp. showed high efficacy in the inhibition of egg hatching ($IC_{50} = 190 \mu\text{g/ml}$) (Nogueira et al., 2012). In another study, the hydroalcoholic extract of *M. paradisiaca* (banana bracts), characterized by the presence of polyphenols, condensed tannins, and flavonoids, exhibited *in vitro* anthelmintic activity against bovine nematodes, inhibiting 88% of larval hatching at 2.5 mg/mL and 67.56% of larval migration at 5 mg/mL, while showing no effect in sheep nematodes (Kakimori et al., 2019). However, the study did not provide detailed chemical profiling of the phenolic compounds nor establish which compounds were associated with the anthelmintic effects (Kakimori et al., 2019). Díaz et al. (2017) demonstrated that chlorogenic acid, present in the extracts of *T. filifolia*, is one of the phenolic compounds responsible for the ovicidal activity against important sheep parasites. The present study aimed to analyze the anthelmintic effects of chemically characterized polyphenol-rich extracts from *M. paradisiaca* inflorescences in *in vitro* egg-hatch and larval-migration tests. We have also tested the isolated compounds, chlorogenic acid and rutin, using the same methodology.

2. Materials e Methods

Chemicals and reagents

Coumaric acid, chlorogenic acid, ferulic acid, gallic acid, sinapic acid, syringic acid, epicatechin, quercetin, and rutin were obtained from Sigma-Aldrich (St. Louis, USA). Methanol-grade HPLC was obtained from JT Baker (Xalostoc, Mexico). Trifluoroacetic acid (TFA) was obtained from Merck (Darmstadt, Germany). Water was purified using an ultra-purifier model MS 2000 from Gehaka (São Paulo, Brazil). Ivermectin (Ivomec 1 % w/v, Merial Limited, Duluth, USA) and albendazole sulfoxide (Ricoben 13.6 % w/v, Noxon Limited, Cravinho, Brazil). Folin-Ciocalteu reagent was obtained from Chromate (São Paulo, Brazil). All other reagents were of analytical grade.

Plant material and obtention of the aqueous and hydroalcoholic extracts of the inflorescences

The *M. paradisiaca* L. inflorescences were collected in Maringa, in the state of Paraná, Brazil. The plant was botanically identified, and a voucher specimen (number: 367379) was retained at the Municipal Botanical Museum of Curitiba. Fresh inflorescences (40 g) were cut into small pieces and ground in a blender (Philips Walita Co, Varginha, MG, Brazil) with water (100 mL) for 10 min. The mixture was then placed in a water bath at 70 °C for 60 min. The aqueous extract, designated EHW-I, was vacuum-filtered, concentrated in a rotary evaporator under reduced pressure at 40 °C, freeze-dried, and stored at -4 °C in the dark. For preparing the hydroalcoholic extract, fresh plant material (40 g) was mixed with 70% aqueous ethanol (v/v) (100 mL). After 10 min of grinding and stirring, the mixture was poured into glass beakers and stored at 25 °C, in the dark, for 10 days. The extract, designated EOH-I, was vacuum-filtered, concentrated in a rotary evaporator under reduced pressure at 40 °C, and then freeze-dried. It was stored at -4 °C in the dark. Yields, calculated relative to plant fresh mass, were 1.8% and 2%, respectively, for EHW-I and EOH-I.

Colorimetric methods

The total phenol content was determined using a microassay with the Folin–Ciocalteu reagent, adapted from Singleton and Rossi Jr. (1965). The calibration curve was prepared using gallic acid (GA) as a standard. The flavonoid content was determined using the aluminum chloride method (Vennat et al., 1992), with rutin (RUT) as the standard. Condensed tannin (CT) content was determined by the sulfuric-vanillin method (Queiroz et al., 2002). The results are expressed as μg epicatechin equivalents (EPI) per mg dry extract.

High-performance liquid chromatography analysis

The phenolic analysis was conducted using an Agilent 1200 Series high-performance liquid chromatography (HPLC) system (Agilent Co., St. Clara, USA) equipped with a vacuum degasser (G1322A), quaternary pump (G1311A), manual injector (Rheodyne, 7725i), and a multi-UV-VIS wavelength detector (G1365D) operating at a wavelength of 254, 280, 300, 325, and 375 nm, using an Agilent Eclipse XDB-C-18 column (150 mm $\times$ 4.6 mm, 5 μm particle size). The mobile phase used was water:trifluoroacetic acid (TFA; 0.1 %) (A) and methanol:TFA (0.1 %) (B) set with the following gradient: 0–10 min 40 % B, 10–22 min 60 % B, 22–28 min 30 % B, 28–30 min 40 % B at a flow rate of 1 mL/min (Pavei et al., 2010). The standards were prepared individually in methanol at a concentration of 100 $\mu\text{g/mL}$. Twenty microliters of the sample were injected through a manual injector. The samples were diluted in a methanol:water (1:9 v/v) solution to a concentration of 5 mg/mL then filtered through a 0.22- μm membrane filter (JetBiofil, Guangzhou, China) before injection. The phenolic compounds were identified by comparing their retention times with

standards. Sensitivity parameters of the analysis, including limits of detection (LOD) and limits of quantification (LOQ), were calculated for rutin and chlorogenic acid based on the standard deviation of the areas in sextuplicate at the minimum detectable concentration. The EZChrom Elite program, running on Windows 7, was used for system control and data analysis.

Mass spectrometry analysis

The tandem mass spectrometry (MS/MS) system used for quantitative analysis was a Quattro LC triple-quadrupole mass spectrometer (Micromass, Manchester, UK) fitted with a Z-electrospray (ESI) interface and operated in positive-ion mode. The temperatures of the source block and the desolvation gas were set at 100°C and 350°C, respectively. Nitrogen was used as both the desolvation gas (approx. 360 L/h) and the nebulizer gas (approx. 40 L/h), while argon was used as the collision gas. The voltages used in the ESI source during the analysis were 20 V for the cone, 3 kV for the capillary, and 4 V for the extractor. Ion detection was performed in the multiple reaction monitoring (MRM) mode, utilizing a collision energy range of 15–25 eV. The analytical data were processed by MassLynx software (Micromass, Manchester, UK). The target analytes were chlorogenic acid, ferulic acid, and rutin. The identification of these compounds was carried out based on mass spectra and by comparing the results with reference compounds from the literature (Jong et al., 2006; Horai et al., 2010).

Fecal culture analysis

The presence of *Haemonchus contortus* was monitored using fecal samples that were obtained from naturally infected sheep with gastrointestinal nematodes. Feces were collected directly from the rectum of the animals and maintained under 4° C. Samples were used to determine the fecal egg count per gram of feces (Gordon and Whitlock, 1939). To analyze which species was predominant, the fecal culture analysis was performed from a pool of feces. The data revealed that *H. contortus* (strain HcUFPR2015) was the most abundant (95%) nematode species, followed by *Trichostrongylus* spp. (4%).

Egg Hatch Test

The egg hatch test (EHT) was performed using the methodology described by Coles et al. (1992), adapted by Bizimenyera et al. (2006). Eggs were recovered from the feces of sheep naturally infected with gastrointestinal nematodes. The feces were homogenized in water at 30 °C and then filtered successively through sieves with mesh sizes of 1 mm, 150 µm, 75 µm, 56 µm, and 25 µm. The eggs retained in the final filter were collected and centrifuged for 2 min at 140 x g. The supernatant was removed, and sucrose solution (0.9 M, d = 0.375 g/mL, 25 °C) was added. In this condition, the eggs floated, and any unwanted waste precipitated in the tube, allowing their easy collection using a Pasteur pipette. The eggs obtained from this process were transferred to a 12-mL plastic tube, washed again with warm distilled water to remove sucrose, and then re-suspended in distilled water. The material was then centrifuged (1x) for 2 min at 140x g. Supernatants (eggs) were put together and subsequently washed with distilled water (2 x) and centrifuged under the same conditions, as described before, to collect the clean eggs. The egg concentration was counted via an optical microscope (SM-LUX model, Ernst Leitz GmbH, Wetzlar, Germany). The egg suspension was distributed into 24-well plates (100 eggs/well) and then mixed with the plant extract prepared in distilled water at final concentrations of 25, 50, 100, 500, 1000, 1500, and 2000 µg/mL, in a total volume of 1 mL. Test samples for the negative control (distilled H₂O) and a positive control (albendazole sulfoxide, at final concentrations of 10 to 1000 µg/mL) were obtained in parallel. Following this, the plates were maintained in a BOD-type incubator (Biological Oxygen Demand, Eletrolab, São Paulo, Brazil) at 25 °C for 24 h. Egg hatch was blocked by the addition of Lugol's iodine solution, which also facilitated the readings using an inverted microscope (INV 100 model, Bel Engineering, Monza, Italy). The number of eggs and first-stage larvae per well was determined. Three experiments, each with three replicates for the control group and each concentration, were performed. The results are expressed as percentages of inhibition of egg hatch, determined by the formula: (number of eggs / (number of eggs + number of L1) × 100, considering (L1) = L1 larvae; (eggs) = eggs not hatched. Photos of eggs and larvae were taken using an inverted microscope (Axiovert 40 CLF, Carl Zeiss, Jena, Germany).

Larval migration test (LMT)

The effect of plant fractions on the migration of third-stage larvae (L3) of helminths was determined using the method described by Molento and Prichard (2001), modified by Demeler et al. (2010). For this experiment, L3 (30-day-old) were incubated in a 0.3% (v/v) sodium hypochlorite solution for 1 h at 25 °C to remove the cuticle. After that, they were washed (3 x) with distilled water and were quantified and distributed into 24-well plates (200 larvae/well). The plant extracts, with final concentrations of 25, 50, 100, 500, 1000, 1500, and 2000 µg/mL, were prepared as previously described and added to the plates, which were then incubated in a BOD at 25 °C for 6 h. Tests using a negative control (distilled water) and a positive control (Ivermectin at a final concentration of 100 µg/mL) were performed in parallel. After the first incubation, the material was transferred to plates with an apparatus containing a mesh size 22 µm nylon membrane, and incubated under the same conditions, but with a source of light (150 Watts) situated below the plates to stimulate L3 migration. After 18 h, the apparatus was removed, and the L3 that had migrated were counted using an inverted microscope. The L3 that did not migrate was also included in the count. Three experiments, each with three replicates for the control group and each concentration, were performed. The average number of L3 that migrated at each tested concentration was transformed into a percentage.

Statistical Analysis

The results of the quantitative phytochemical measurements were subjected to One-Way analysis of variance (ANOVA), followed by post-hoc Fisher's LSD multiple comparison tests to compare means. Results are expressed as mean $\pm$ standard deviation of at least three experiments. The values were considered statistically significant if the p -value was ≤ 0.05 . The results of the EHT and LMT tests were analyzed using ANOVA followed by Tukey's multiple comparison test, as performed with GraphPad Prism 5. The differences were considered statistically significant at $p \leq 0.05$. The concentrations of the extract that inhibited 20, 50, and 90% of egg hatching, expressed as IC_{20} , IC_{50} , and IC_{90} , were calculated using linear equations ($R^2 \geq 0.95$) obtained by linearizing the activity curves with the GraphPad Prism 5 program.

3. Results and Discussion

Chemical characterization of *M. paradisiaca* inflorescence extracts

Table 1 presents the results of the quantitative analysis of phenolics, flavonoids, and CT for the EHW-I and EOH-I. The phenolic contents of the *M. paradisiaca* inflorescence extracts (35 and 26 mg GA equivalent/g dry sample for EHW-I and EOH-I, respectively) were higher than those reported for the extracts of the other parts of the species, such as the fruit aqueous extract (0.94 mg GA equivalent/g dry extract) (Shodehinde and Oboh, 2013) and the pseudostem methanolic extract (12 mg GA equivalent/g dry extract) (Loganayaki et al., 2010). Studies have demonstrated that inflorescence extracts are rich in phenolic acids and flavonoids, with total polyphenol contents typically between 10 and 30 mg GAE/g dry weight (Bashkar et al., 2012; Ramírez-Bolaños et al., 2021; Senevirathna et al., 2024; Gayathry et al., 2024). The content of flavonoids in EOH-I (25 mg RUT equivalent/g dry sample) was significantly higher than that in EHW-I (3 mg RUT equivalent/g dry sample), as expected, since the mixture of ethanol and water is more efficient in extracting flavonoids. The content of flavonoids, as described in the literature for *Musa* spp., varies according to the type of extraction and the type of plant material used. For example, the flavonoid contents were 0.73 mg quercetin equivalent/g dry extract and 75 mg rutin equivalent/g dry extract for the aqueous and methanolic extracts from the fruit pulp and peel, respectively (Shodehinde and Oboh 2013; Singhal and Ratra 2013). The content of CT was 0.8 mg EPI equivalent/g dry extract for EOH-I and 2 mg EPI equivalent/g dry extract for EHW-I, respectively. By comparing the values described for other parts of the plant (*Musa* spp.), such as fruit (3.7 to 25.9 %) and leaves (2.54 %), it can be inferred that EHW-I (0.08 %) and EOH-I (0.2 %) had a low content of CT (Bennett et al., 2010; Marie-Magdeleine et al., 2014).

	EHW-I	EOH-I
Yield (%) ¹	1.9	1.8
Phenolic (mg GA/g dry extract) ²	35 $\pm$ 6.9 ^a	26 $\pm$ 5.4 ^a
Flavonoid (mg RUT/g dry extract) ³	3 $\pm$ 0.9 ^b	25 $\pm$ 0.3 ^c
Condensed tannins (mg EPI per g dry extract) ⁴	0.8 $\pm$ 0.005 ^c	2 $\pm$ 0.004 ^c

Table 1 – Chemical data of the aqueous (EHW-I) and hydroalcoholic (EOH-I) extracts obtained from the inflorescences of *Musa x. paradisiaca*.

¹Calculated as dried weight in relation to the original material. ²Determined according to Singleton and Rossi Jr. (1966); expressed as gallic acid equivalents (GA). ³Determined according to Vennat et al. (1992); expressed as rutin acid equivalents (RUT). ⁴Determined according to Queiroz et al. (2002); expressed as epicatechin equivalents (EPI). Values are expressed as mean $\pm$ standard deviation of three experiments. Different letters in each column represent a significant difference between the samples, as determined by the Fisher LSD test ($p \leq 0.05$).

Figure 1 shows the HPLC chromatogram profile of the phenolics in *M. paradisiaca* inflorescence extracts. Comparison between the retention times of the standard peaks with the retention times of the peaks of the extracts led to the identification of phenolic acids: chlorogenic acid (present in EHW-I), coumaric acid (present in EHW-I), ferulic acid (present in EHW-I), and sinapic acid (present in EOH-I), and flavonoids: rutin (present in both extracts). The identification of other unnamed peaks was not possible because their retention points did not match any of the standards used. LOD and LOQ values were 0.55 and 1.67 μ g/mL for rutin and 0.61 and 1.85 μ g/mL for chlorogenic acid. The concentrations of phenolic and flavonoid compounds in the extracts were calculated using the equations obtained from the standard curves for rutin and chlorogenic acid (Table 2). The identification of phenolic acids and flavonoids was based on ESI-MS/MS analysis, using data such as the molecular ion (MI) and fragment ions (FIs) observed in the MS/MS spectra reported in the literature (Jong et al., 2006; Horai et al., 2010). The presence of chlorogenic acid ($[M + H]^+ = 355$, for MI), rutin ($[M + H]^+ = 611$ and 303, for MI and FI, respectively), and ferulic acid ($[M + H]^+ = 195$ and 177, for MI and FI respectively) was also suggested by ESI-MS/MS analysis.

HPLC analysis of the inflorescence extract demonstrated the presence of phenolic compounds, such as gallic acid, chlorogenic, ferulic, gallic, and p-coumaric acids, and rutin, quercetin, isoquercetin, catechin, and epicatechin (Bashkar et al., 2012; Ramírez-Bolaños et al., 2021; Amornlerdpison et al., 2021; Senevirathna et al., 2024; Gayathry et al., 2024). The cultivar and solvent polarity strongly influence differences in chemical composition, and the extraction methodology applied (Senevirathna et al., 2024). The phenolic compounds identified in *M. paradisiaca* inflorescences, such as rutin, chlorogenic acid, ferulic acid, coumaric acid, and

sinapic acid, are commonly found in fruits, vegetables, and cereals and have numerous beneficial health effects (Yang et al., 2008; Tajik et al., 2017). These beneficial health effects are associated with their antioxidant properties (Crozier 2010). The consumption of plant-based foods rich in nutrients and bioactive compounds has been linked to a lower incidence of chronic diseases in humans (Crozier 2010). In livestock nutrition, it has been proposed that consuming plant-based foods or adding bioactive compounds to the animal diet can be an alternative approach to improving animal health and enhancing anti-parasitic activity in the gastrointestinal system (Hoste et al., 2015). In addition, it has been speculated that the anthelmintic activity of plant extracts against nematodes may be attributed to phenolic compounds, particularly CT (Molan et al., 2002; Athanasiadou et al., 2007). The overall action of the extracts is a sum of the activities of their constituents (Adedapo et al., 2005). Díaz et al. (2017) have demonstrated that chlorogenic acid, present in the extracts of *T. filifolia*, is one of the phenolic compounds responsible for the ovicidal activity against *H. contortus*.

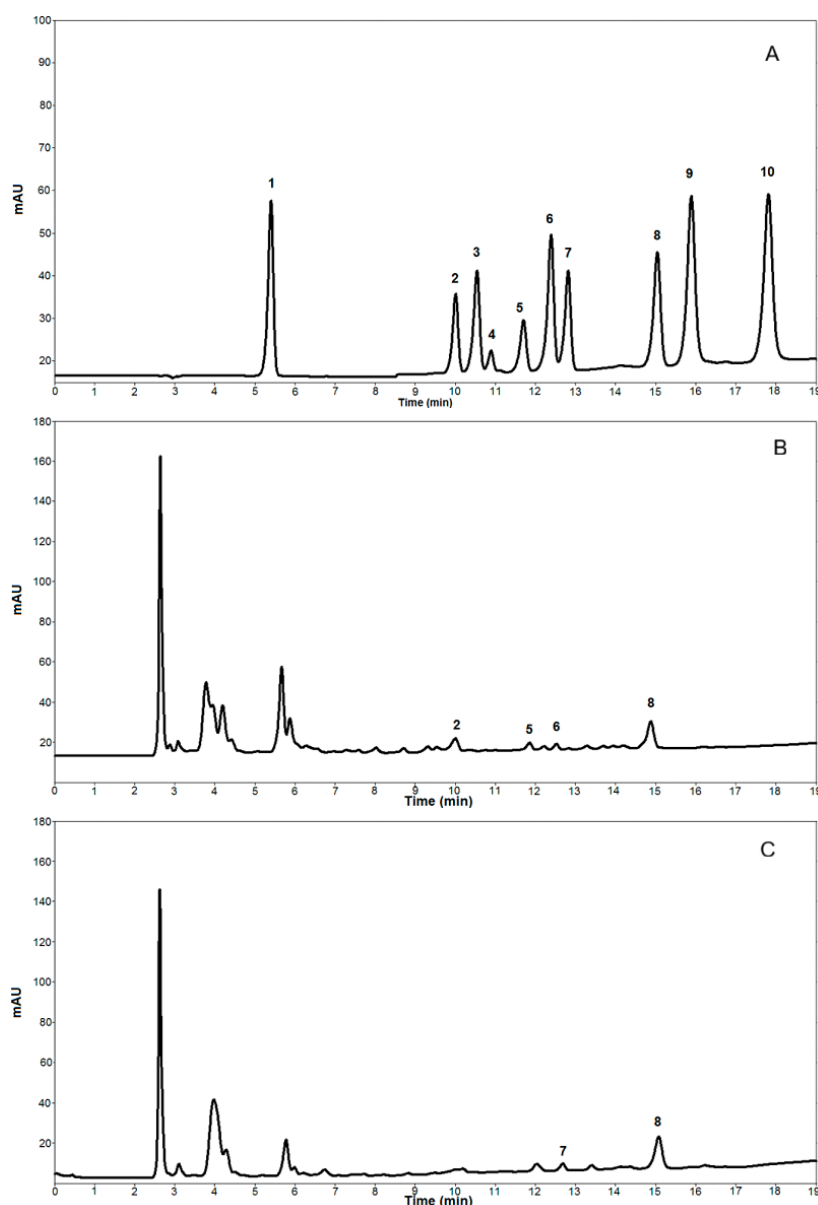


Figure 1 – HPLC Chromatogram of phenolic standards (A), of the aqueous (EHW-I) (B) and hydroalcoholic (EOH-I) (C) extracts obtained from the inflorescences of *Musa x. paradisiaca*.

The UV detector was set to an absorbance wavelength of 254 nm. Numbers in the chromatogram refer to the phenolic standards compounds: (1) gallic acid; (2) chlorogenic acid; (3) syringic acid; (4) epicatechin; (5) coumaric acid; (6) ferulic acid; (7) sinapic acid; (8) rutin; (9) myricetin; (10) quercetin. The phenolic compounds of EHW-I (B) and EOH-I (C) were identified by comparing their retention times with standards.

Phenolic acids or flavonoids ($\mu\text{g}/\text{mg}$ dry sample)	EHW-I	EOH-I
Chlorogenic acid	0.8 ± 0.04	-
Coumaric acid	0.17 ± 0.63	-
Ferulic acid	0.16 ± 0.58	-
Sinapic acid	-	0.21 ± 0.14
Rutin	1.00 ± 0.067^a	2.35 ± 0.08^a

Table 2 – Quantification of phenolic and flavonoid compounds in the aqueous (EHW-I) and hydroalcoholic (EOH-I) extracts obtained from the inflorescences of *Musa paradisiaca*.

Data are presented as mean $\pm$ standard deviation of three analyses. Different letters in each column represent statistically significant differences between samples, as determined by the Fisher LSD test ($p \leq 0.05$). (-) = not detected. The quantification of chlorogenic acid, coumaric acid, ferulic acid, and synapic acid was calculated in relation to the standard curve of chlorogenic acid, and the concentration of rutin was calculated in relation to the curve of rutin.

Egg hatch activity

EHW-I and EOH-I extracts showed a dose-dependent inhibitory action (Fig. 2A and 2B). Egg hatch inhibition increased to close to 100 % at the highest concentrations tested (1000 to 2000 $\mu\text{g}/\text{mL}$). The control groups, negative (H_2O) (7.4%) and positive control (Albendazole sulfoxide, 10 to 1000 $\mu\text{g}/\text{mL}$), presented different results with respect to egg hatch inhibition. In the case of the group treated with a commercial anti-anthelmintic, which was used as the positive control, at concentration tested the egg hatch inhibition was higher than 99.9% (Fig. 2D). The concentrations of EHW-I that could inhibit the hatching of eggs of *H. contortus* by 20, 50, and 90 % (IC_{20} , IC_{50} , and IC_{90}) were 7.99, 62.58, and 972.98 $\mu\text{g}/\text{mL}$, respectively (Fig. 2A and 2B). EOH-I exhibited IC_{20} , IC_{50} , and IC_{90} values of 34.89, 137.79, and 860.26 $\mu\text{g}/\text{mL}$, respectively. Figure 3 shows the general aspects of nematode eggs and/or larvae after 24 h of treatment in the Egg Hatch Test (EHT). Increasing concentrations of the plant extracts (EHW-I and EOH-I) resulted in a higher number of eggs that did not hatch after 24 h of incubation (Figure 3A–D). At the concentration of 1000 $\mu\text{g}/\text{mL}$ of EHW-I, most eggs remained at the morula stage (Figure 3C). Figures 3E and 3F show the inhibition of egg hatching by albendazole sulfoxide (60 $\mu\text{g}/\text{mL}$), used as the positive control, in which eggs were mainly observed at the morula or larvated stages. In contrast, the negative control (H_2O) did not affect egg hatching, as shown in Figures 3G and 3H, where hatched L1 larvae were observed. The literature data concerning the effect of *Musa* spp. extracts on egg hatch inhibition are contradictory. Some authors reported no significant activity (Marie-Magdeleine et al., 2014), which differs from the results reported by others, who described high efficacy for the aqueous extracts in egg hatch inhibition, with IC_{50} and IC_{90} values of 190 $\mu\text{g}/\text{mL}$ and 840 $\mu\text{g}/\text{mL}$, respectively (Nogueira et al., 2012). Another study, which presented the hydroalcoholic extract of *M. paradisiaca* bracts, showed 88% inhibition of hatching at 2.5 mg/mL against bovine nematodes (Kakimori et al., 2019). However, the quantitative information concerning the bioactive compounds was also insufficient to identify the compound(s) responsible for the tested activity.

Considering the data on phenolic and flavonoid compounds in EHW-I and EOH-I, chlorogenic acid and rutin were selected for testing in the *in vitro* anthelmintic assays (Fig. 2C). Figure 2C shows the efficacy of chlorogenic acid against egg hatch inhibition, with $\text{IC}_{20} = 3.55$ $\mu\text{g}/\text{mL}$, $\text{IC}_{50} = 17.55$ $\mu\text{g}/\text{mL}$, and $\text{IC}_{90} = 147.52$ $\mu\text{g}/\text{mL}$. In contrast, rutin (a glycosylated flavonoid) did not show any effect in the EHT under the tested conditions. Our results were similar to those published by Seyfried et al. (2022), which also demonstrated the efficacy of chlorogenic acid against egg hatch inhibition, with IC values as $\text{IC}_{20} = 1.81$ $\mu\text{g}/\text{mL}$, $\text{IC}_{50} = 8.66$ $\mu\text{g}/\text{mL}$, and $\text{IC}_{90} = 69.70$ $\mu\text{g}/\text{mL}$, and no effect on the EHT by rutin.

Several reports suggest that the anthelmintic activity of plant extracts, such as quebracho (*Schinopsis* sp.) and various forage species (*Lotus pedunculatus*, *Lotus corniculatus*, *Onobrychus viciifolia*, *Rumex obtusifolius*), is related to their content of CT (Molan et al. 2002; Athanasiadou et al. 2007). In the present study, the tested extracts, EHW-I and EOH-I, presented 0.08 and 2 % of CT, respectively, which was considerably less in comparison to the results reported for the aqueous extracts of quebracho bark, which contained 73 % of CT (Athanasiadou et al., 2007), suggesting that other chemical constituents were responsible for the ovicidal activity of *M. paradisiaca*. The ovicidal activity of plant extract with low-tannin content has been demonstrated (Díaz et al., 2017; Seyfried et al., 2022). The methanolic extract of *T. filifolia* exhibited significant ovicidal activity against *H. contortus*, reaching 99% efficacy at its highest concentration (10 mg/mL) after 48 h of incubation. For the extract of *Polygonum acre* (EHW-PA and ETOH-PA), the presence of phenolic acids (ferulic acid, rosmarinic acid, and sinapic acid) and aglycone flavonoid (quercetin) was speculated as relevant for influencing the observed egg hatch activity (Seyfried et al., 2022). It had been demonstrated that the chlorogenic acid presented a relevant activity against egg hatch inhibition, and in contrast, the glycosylated flavonoids (quercetagitrin and rutin) showed no effect (Díaz et al., 2017; Seyfried et al., 2022).

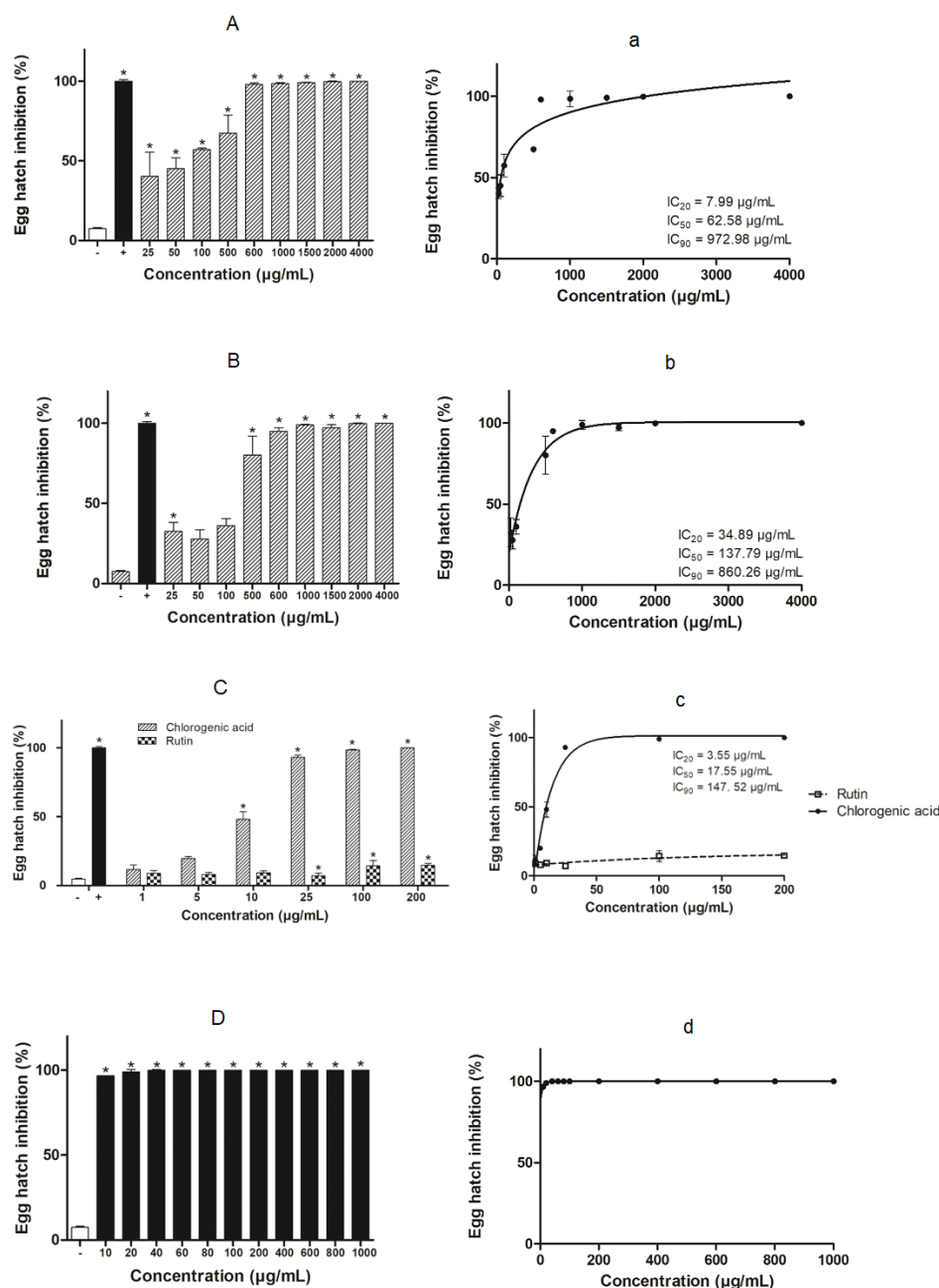


Figure 2 – Inhibition of the nematode egg hatch and the inhibition concentration (IC) determination of the aqueous (EHW-I) and hydroalcoholic (EOH-I) extracts obtained from the inflorescences of *Musa paradisiaca*, phenolic commercial standards and albendazole sulfoxide using the Egg Hatching Test. (A, a) EHW-I; (B, b) EOH-I; (C, c) Chlorogenic acid and rutin); (D, d) Albendazole sulfoxide; (-) H₂O; (+) Albendazole sulfoxide (60 µg/mL). Different letters in each column represent significant difference compared to negative control (H₂O) determined using Tukey's and t test ($p \leq 0.05$).

The nematocidal activity of chlorogenic acid has been described against *Pratylenchus scribneri* (grass nematode) and *Caenorhabditis elegans* (free-living nematode). However, the mechanism of the nematocidal or ovicidal activities needs to be elucidated. Some possibilities could be inferred from consideration of its properties described in other biological tests, for example, as an enzyme inhibitor (Tajik et al., 2017). Thus, the mechanism could involve inhibition of the so-called “hatching enzymes,” including proteases, lipases, glycosidases, and leucine aminopeptidases, as well as cyclic AMP-dependent protein kinase (Rogers and Brooks 1977).

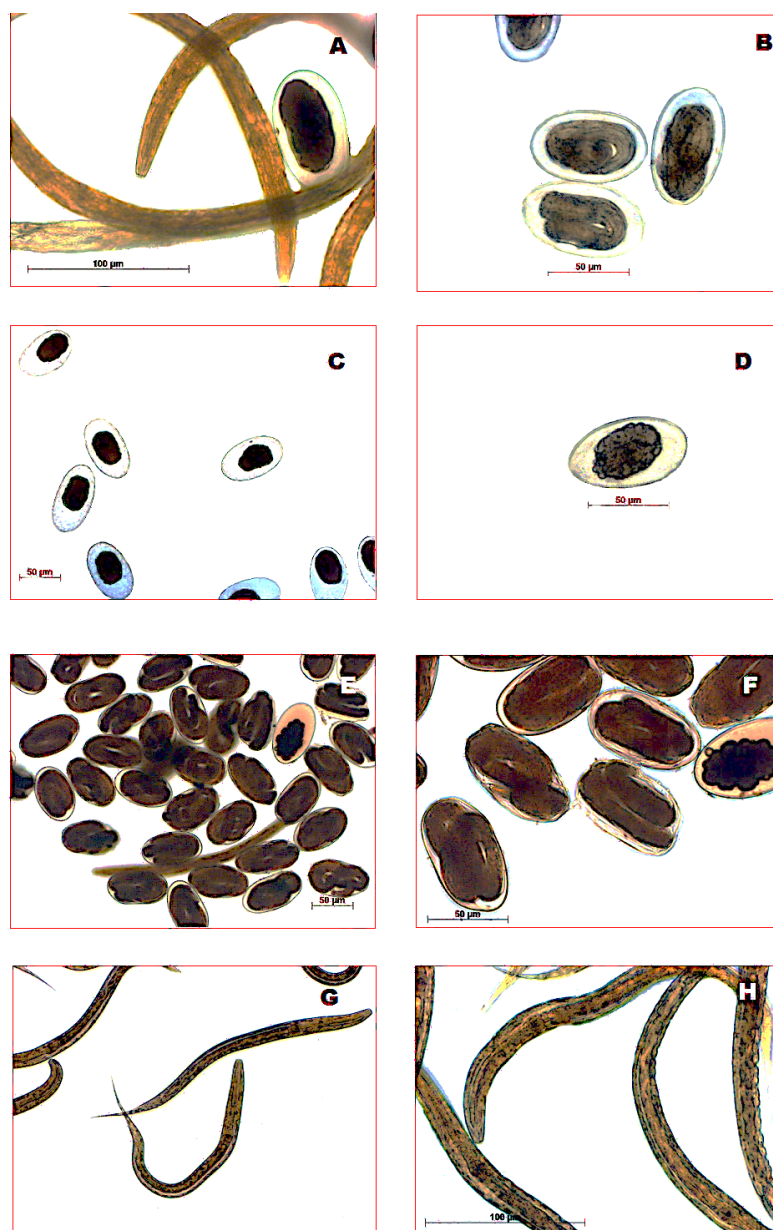


Figure 3 – The general appearance of nematode eggs and/or larvae from the Egg Hatching Test groups after 24 h of treatment with of the aqueous (EHW-I) and hydroalcoholic (EOH-I) extracts obtained from the inflorescences of *Musa paradisiaca*, Albendazole sulfoxide (positive control) and H₂O (negative control). Images: A: Group test with EHW-I at 200 µg/mL (40x magnification), B: Group test with EHW-I at 600 µg/mL (40x magnification), C: Group test with EHW-I at 1000 µg/mL (40x magnification), D: Group test with EOH-I at 1000 µg/mL (20x magnification), E: Group test with albendazole sulfoxide (positive control) (60 µg/mL) (20x magnification), F: Group test with albendazole sulfoxide (positive control) (60 µg/mL) (40x magnification), G: Group test with H₂O (negative control) (20x magnification), H: Group test with H₂O (negative control) (40x magnification).

Larval migration test

EHW-I and EOH-I did not affect L3 motility at the concentrations tested (Fig. 4). The percentages of L3 migration calculated for the tested extracts were similar to those calculated for the negative control (H₂O). As expected, in the negative control, 92.27 % of the larvae migrated (Fig. 4). However, the same observation was made for the positive control group (Ivermectin, 100 µg/mL), where the migration of approximately 82 % of the larvae occurred. In theory, this would not be expected for ivermectin, since its mechanism of action is related to a change in the motility of larvae, by acting as an agonist of the neurotransmitter, GABA, in the nervous system cells and of ligands of the glutamate-chlorine channels in neuron and muscle cells (Laing et al., 2017). The results suggested that the parasitic population was resistant to the drug at the concentration tested (Campos et al., 2017). Several authors have reported the current drug resistance situation, relating to the macrocyclic lactone family of anthelmintics worldwide (Gaudin et al., 2016).

The hydroalcoholic extract of *M. paradisiaca* bracts presented 67.56% of inhibition of larval migration at 5 mg/mL against bovine nematodes, but no effect was found against sheep nematodes (Kakimori et al., 2019). The extracts of the leaves and stems of *M. paradisiaca*, with low tannin content, also showed no significant effect on the migration of L3 from *H. contortus*, exhibiting activity similar to the negative control. However, these fractions could suppress the development of L3 of *H. contortus* from L1 to L3, in a dose-dependent manner (300 and 2400 µg/mL) (Marie-Magdeleine et al., 2014).

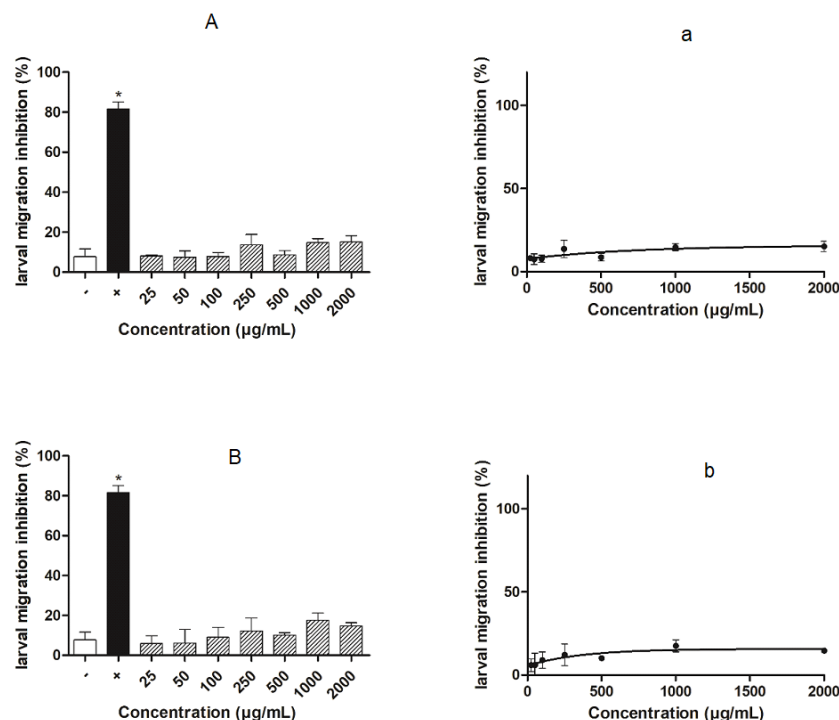


Figure 4 – Larval migration inhibition and the inhibition concentration (IC) determination of the aqueous (EHW-I) (A, a) and hydroalcoholic (EOH-I) (B, b) extracts obtained from the inflorescences of *Musa paradisiaca* on the larval migration test.

(-) H₂O; (+) Ivermectin (100 µg/mL). Different letters in each column represent a significant difference compared to the negative control (H₂O) determined using Tukey's test ($p \leq 0.05$).

The results presented in this study indicate that extracts obtained from the inflorescences of *Musa paradisiaca* L. show promising potential for further exploration as plant-derived anthelmintic agents. Although the activity was evaluated through *in vitro* assays, these approaches represent an important first step in the investigation of new bioactive products. Despite their inherent methodological limitations, *in vitro* assays offer several advantages, as they provide rapid, reproducible, and cost-effective tools for the initial screening and validation of plant extracts, allowing the identification of promising candidates for subsequent *in vivo* studies. The *in vitro* assays allow the preliminary identification of promising extracts and enable the evaluation of biological activity under controlled laboratory conditions (Pavela & Benelli, 2016). In recent years, *in vitro* studies investigating plant-derived products against *Haemonchus contortus* have increased considerably, reinforcing the interest in alternative strategies for controlling gastrointestinal nematodes in livestock (Molento et al., 2020). Several studies have shown that plant extracts can affect different developmental stages of the parasite, including egg hatching and larval viability (Molento et al., 2020; Davuluri et al., 2020; Rojas-Morales et al., 2021; Chishti et al., 2024). Among the available methods, the Egg Hatch Test (EHT) is widely considered the standard approach for initial screening, while the Larval Migration Test (LMT) evaluates larval motility and viability. These assays are particularly relevant for medicinal plants, whose activity often results from the combined effects of compounds within the phytochemical biocomplex (Athanasiadou et al., 2007). In the present study, plant extracts were chemically standardized to obtain reliable and reproducible data for future investigations by using other biological approaches.

4. Conclusion

It has been demonstrated that the ovicidal activity of extracts with low CT content. This is related to the presence of phenolic acids, whereas the reported larvicidal activity is associated with tannin content, which appears to be a relevant factor. Additionally, the glycosylated flavonoids did not affect egg hatching. However, it is interesting to note that using different models of anthelmintic activity, such as migration and development of L3 and adult larvae, may provide a better understanding of the effect of isolated plant compounds. Therefore, parts of the plant may act in different sites in the parasite stages. The inflorescences of *Musa paradisiaca* L. represent a promising banana by-product for the development of plant-derived anthelmintic agents, particularly when supported using chemically characterized extracts associated with active phytochemicals.

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Pós-Graduação em Ciências Veterinárias: 40 anos ajudando a construir o Setor de Ciências Agrárias

Volnei Pauletti

O Setor de Ciências Agrárias teve início em 1973 com a reforma universitária de 1968 (Lei Nº 5.540, de 28 de novembro de 1968) juntando a escola de Agronomia (curso iniciado em 1915) e Veterinária (curso iniciado em 1931) do Paraná com a Escola Nacional de Florestas (curso iniciado em 1960). Com o fim das cátedras e criação dos departamentos, nossos alunos tinham aulas em vários campi da UFPR (Batel, Politécnico, Centro, Cabral e Agrárias). Após sua criação, se juntaram ao Setor os cursos de Engenharia Industrial Madeireira (criado em 1998) e de Zootecnia (criado em 2000), completando este importante Setor da Universidade Federal do Paraná. Um Setor de excelência em tudo que faz.

A área das Ciências Veterinárias sempre foi um pilar para o Setor. Com a existência do Curso de Medicina Veterinária desde 1931 e a construção do Hospital Veterinário em 1950, faltava ainda a consolidação de sua liderança científica, com a implantação da pós-graduação. Por isso, em 1986, por iniciativa e esforço de pioneiros e visionários, cria-se o Programa de Pós-Graduação em Ciências Veterinárias. Completando, portanto, neste ano de 2026, 40 anos de existência. Nesta história foi gerado muito conhecimento e houve muita contribuição para a UFPR e para a sociedade, com a formação de mestres e doutores de elevadíssima qualificação e que foram e são transformadores dos ambientes onde trabalham.

Foram até agora 766 concluintes de mestrado e 123 concluintes de doutorado. Essa história de dedicação de seus docentes e técnicos, fez com que o PPGCV alcançasse nota atual 6 na CAPES, sendo a nota máxima 7. Enchendo a comunidade do Setor Agrárias de orgulho e comprovando sua excelência e vanguarda.

Este sucesso da Pós-Graduação também tem consequências positivas no ensino na Graduação pois contribuiu para que o Curso de Medicina Veterinária do Setor AG, fosse considerado o melhor do Brasil em 2025, na avaliação pelo exame nacional de desempenho dos estudantes (ENADE).

Portanto, o ensino e pesquisa precisam andar juntos. A pesquisa dentro da Universidade e dentro dos Cursos mobiliza professores, técnicos e estudantes pela vanguarda do conhecimento. Gerando e aprendendo novos resultados, novos saberes e determinando novos rumos para profissionais em formação e para a sociedade.

E ainda, não poderia deixar de citar, a existência e manutenção pelo PPGCV desde 1996 do periódico científico Archives of Veterinary Science. Uma importante ferramenta de integração com a comunidade científica nacional e internacional e de divulgação da ciência gerada dentro de nossos laboratórios.

Parabéns ao Programa de Pós-Graduação em Ciências Veterinárias por seus 40 anos de vida. Obrigado aos docentes e técnicos do passado e atuais pela dedicação e pela excelência no que fazem, transformando e formando pessoas.

Vida longa ao PPGCV!!!



Volnei Pauletti

Professor Titular na UFPR, Engenheiro Agrônomo, MSc em Ciência do Solo e Dr em Agronomia: Produção Vegetal. Atua no Departamento de Solos e Engenharia Agrícola e no Programa de Pós-graduação Ciência do Solo, na área de Nutrição de Plantas. Atualmente é Diretor do Setor de Ciências Agrárias da UFPR.



Graduate Program in Veterinary Sciences: 40 years shaping the Sector of Agricultural Sciences

Volnei Pauletti

The Sector of Agricultural Sciences began in 1973 following the 1968 university reform (Law No. 5,540 of November 28, 1968), merging Paraná's School of Agronomy (program started in 1915) and Veterinary Medicine (program started in 1931) with the National School of Forestry (program started in 1960). With the abolition of the chair system and the creation of departments, our students attended classes across several UFPR campuses (Batel, Politécnico, Centro, Cabral, and Agrárias). After its creation, the programs in Wood Industrial Engineering (established in 1998) and Animal Science (established in 2000) joined the Sector, completing this important division of the Federal University of Paraná. A Sector of excellence in everything it does.

The field of Veterinary Sciences has always been a pillar for the Sector. While the Veterinary Medicine program had existed since 1931 and the Veterinary Hospital was built in 1950, the consolidation of its scientific leadership through the implementation of graduate studies was still missing. Therefore, in 1986, through the initiative and efforts of pioneers and visionaries, the Graduate Program in Veterinary Sciences (PPGCV) was created. Consequently, it completes 40 years of existence this year, in 2026. Throughout this history, a vast amount of knowledge has been generated, contributing significantly to UFPR and society by training highly qualified master's and doctoral graduates who have been, and continue to be, agents of transformation in their workplaces.

To date, there have been 766 master's graduates and 123 doctoral graduates. This history of dedication from its faculty and technical staff has enabled the PPGCV to currently achieve a score of 6 out of a maximum of 7 from CAPES, filling the Agricultural Sciences Sector community with pride and proving its excellence and vanguard position.

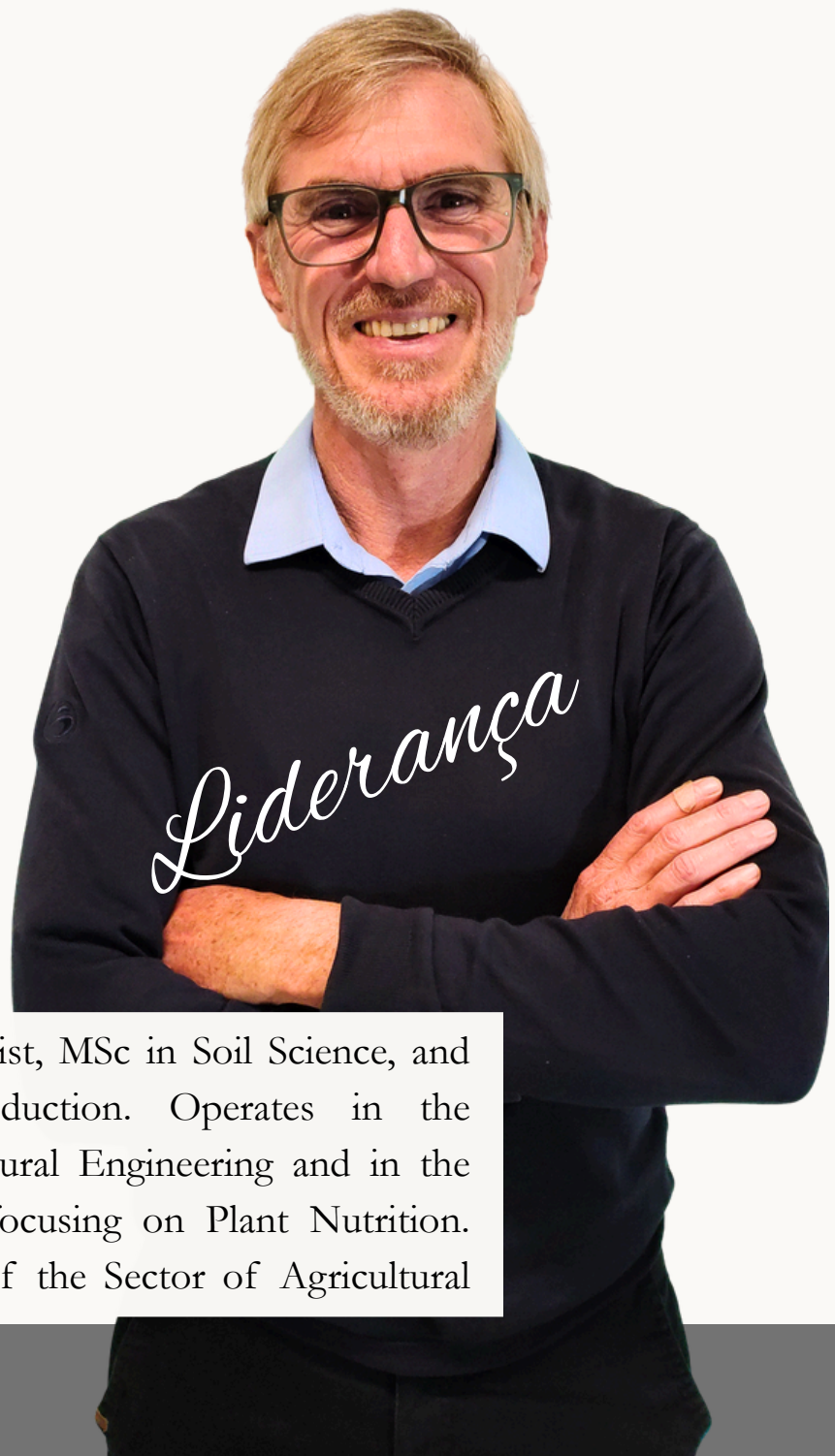
This success in Graduate Studies also yields positive impacts on Undergraduate education, contributing to the Veterinary Medicine program of the Agricultural Sciences Sector being ranked as the best in Brazil in 2025 by the National Student Performance Exam (ENADE).

Therefore, teaching and research must go hand in hand. Research within the University and its programs mobilizes professors, technical staff, and students toward the forefront of knowledge—generating and learning new results, new insights, and defining new directions for future professionals and society.

Furthermore, it is essential to mention the existence and maintenance by the PPGCV, since 1996, of the scientific journal Archives of Veterinary Science. This is an important tool for integration with the national and international scientific communities and for disseminating the science generated within our laboratories.

Congratulations to the Graduate Program in Veterinary Sciences on its 40th anniversary. Thank you to past and present faculty and technical staff for their dedication and excellence in what they do, transforming and educating individuals.

Long live PPGCV!!!



Volnei Pauletti

Full Professor at UFPR, Agronomist, MSc in Soil Science, and PhD in Agronomy: Crop Production. Operates in the Department of Soils and Agricultural Engineering and in the Soil Science Graduate Program, focusing on Plant Nutrition. Currently serves as the Director of the Sector of Agricultural Sciences at UFPR.

From Bench to Diagnosis: The Use of MALDI-TOF in Veterinary Microbiology

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Abstract: Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) has transformed routine diagnostic microbiology by enabling rapid, accurate, and cost-effective identification of microorganisms from cultured isolates. This qualitative narrative review critically synthesizes evidence on the use of MALDI-TOF MS in veterinary microbiology published between January 2000 and January 2026, focusing on principles, diagnostic applications, performance, limitations, and future perspectives. An active literature search was conducted in SciELO, PubMed, Virtual Health Library (VHL), Scopus, and Web of Science using controlled descriptors and free keywords in English, Spanish, and Portuguese related to MALDI-TOF MS, veterinary microbiology, microbial identification, and One Health. Overall, 162 records were retrieved; after deduplication and screening, 46 articles were assessed in full text, and 24 met eligibility criteria for qualitative synthesis. The included studies were categorized by microorganism group (bacteria, yeasts, filamentous fungi), animal host (companion animals, livestock, wildlife, exotic species), geographic region, and diagnostic application. Evidence indicates that MALDI-TOF MS is most robust for bacterial identification, frequently achieving high species-level accuracy with substantially reduced turnaround time compared with conventional biochemical methods, and supporting high-throughput workflows relevant to clinical practice, food safety, and antimicrobial resistance surveillance. Yeast identification also shows favorable performance when supported by curated spectral libraries, whereas filamentous fungi remain more technically challenging due to extraction and database constraints. Adoption is geographically uneven, with most studies originating from Europe and North America, while implementation in underrepresented regions remains limited. Continued expansion of veterinary-specific databases, protocol standardization, and integration with molecular approaches are essential to maximize global diagnostic and One Health impact.

Keywords: MALDI-TOF MS, veterinary microbiology, bacterial identification, spectral databases, One Health.

1. Introduction

In recent decades, advances in diagnostic technologies have profoundly transformed microbiological diagnostics, particularly by enabling faster, more accurate, and cost-effective identification of infectious agents. Within this context, Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) has emerged as a revolutionary tool in clinical microbiology, redefining routine workflows for microbial identification in both human and veterinary medicine (SENG et al., 2009; CLARK et al., 2013).

Originally adopted in human clinical laboratories, MALDI TOF MS has progressively gained relevance in veterinary microbiology due to its ability to rapidly identify a wide range of microorganisms, including bacteria, yeasts, and filamentous fungi, directly from cultured isolates. By generating unique protein spectral fingerprints, primarily derived from ribosomal proteins, this technology allows reliable species-level identification within minutes, significantly reducing turnaround time when compared with conventional phenotypic and biochemical methods (PATEL, 2015; CROXATTO; PROD'HOM; GREUB, 2012).

The application of MALDI TOF MS in veterinary diagnostics is particularly valuable given the diversity of animal species, sample types, and pathogens encountered in diagnostic laboratories. Veterinary microbiology routinely deals with zoonotic agents, emerging pathogens, and microorganisms that are underrepresented in commercial biochemical systems, often leading to misidentification or delayed diagnosis. In this setting, MALDI TOF MS offers a robust alternative, enhancing diagnostic accuracy and supporting timely clinical decision making in companion animals, livestock, wildlife, and exotic species (RANDALL et al., 2015; BIZZINI; GREUB, 2010).

Despite its advantages, MALDI TOF MS in veterinary microbiology is not without limitations. Challenges include incomplete or human-biased reference databases, difficulty in distinguishing closely related species, and reduced performance for certain fastidious organisms, parasites, and viruses, which remain beyond the direct scope of this technology (BISWAS; ROLAIN, 2013; CLARK et al., 2013). Additionally, standardization of sample preparation protocols and the expansion of databases tailored to veterinary pathogens are essential to fully realize the diagnostic potential of MALDI TOF MS in animal health settings.

From a One Health perspective, the rapid and accurate identification of veterinary pathogens is critical for disease surveillance, antimicrobial stewardship, and zoonotic risk mitigation. MALDI-TOF MS contributes to these goals by facilitating early detection of infectious agents, improving outbreak investigations, and supporting integrated approaches to animal, human, and environmental health (PATEL, 2015; WHO, 2023). Therefore, the objective of this review is to discuss the principles, applications, limitations, and future perspectives of MALDI-TOF MS in veterinary microbiology, emphasizing its role in diagnostic workflows and its relevance within a One Health framework.

2. Development

2.1. Type of study

This study is a qualitative narrative review that compiles and critically analyzes scientific evidence on the use of MALDI-TOF MS in veterinary microbiology. The review covers publications from January 2000 to January 2026. It aims to describe the principles, diagnostic applications, performance, limitations, and future perspectives of MALDI TOF MS for the identification of veterinary pathogens. The methodological approach was designed to synthesize evidence on the application of MALDI TOF MS

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across veterinary contexts, including companion animals, livestock, wildlife, and exotic species. Although this is a narrative review, recommendations from the PRISMA 2020 guidelines were adopted and adapted, as described by Page et al. (2021), to enhance transparency, consistency, and reproducibility in the literature selection process. Additional methodological guidance was based on the frameworks proposed by Peters et al. (2020) and Haddaway et al. (2022), ensuring a structured and rigorous synthesis of the available evidence.

2.2. Descriptors and Database

An active literature search was conducted in the Scientific Electronic Library Online (SciELO), PubMed (National Library of Medicine), Virtual Health Library (VHL), Scopus, and Web of Science. Peer-reviewed articles published in English, Spanish, and Portuguese between January 2000 and January 2026 were considered eligible for inclusion. The search strategy employed controlled descriptors and free keywords in English, Spanish, and Portuguese, including MALDI TOF, MALDI TOF MS, Veterinary Microbiology, Bacterial Identification, Fungal Identification, Diagnostic Microbiology, and One Health. These terms were combined with specific keywords related to target microorganisms and diagnostic contexts, such as zoonotic pathogens, veterinary diagnostic laboratories, antimicrobial resistance, and wildlife pathogens. Boolean operators, primarily “AND”, were used to restrict search results to studies containing all relevant terms.

2.3. Search Strategy

The literature search strategy was designed to ensure comprehensive identification of studies on the use of MALDI TOF MS in veterinary microbiology. A total of 162 records were initially retrieved from the selected databases (SciELO, PubMed, Virtual Health Library, Scopus, and Web of Science) using predefined descriptors and Boolean combinations. After removal of duplicate records and initial screening based on title and abstract relevance, 46 articles were selected for full-text evaluation. Following a detailed assessment of methodological quality, diagnostic relevance, and alignment with the scope of this review, 24 studies met all eligibility criteria and were included in the final qualitative synthesis. The study selection process was conducted in accordance with the adapted PRISMA 2020 framework (PAGE et al., 2021).

3. Summary of Results

The selected studies were organized and summarized using a PRISMA flowchart to illustrate the literature selection process. After independent evaluation by two reviewers, the included studies were categorized according to microorganism type (bacteria, yeasts, filamentous fungi), animal host group (companion animals, livestock, wildlife, and exotic species), geographic region, and diagnostic application. Key variables systematically extracted from each study included year of publication, authors, animal species, country of origin, target pathogens, sample type, diagnostic methodology, database used, and reported performance metrics, including accuracy, sensitivity, and limitations. Additionally, figures were generated to illustrate temporal trends in scientific production and the expanding application of MALDI TOF MS in veterinary diagnostic laboratories worldwide. Comparative analyses were conducted to highlight differences in performance across microbial groups and diagnostic contexts.

3.1. Active Search Flowchart

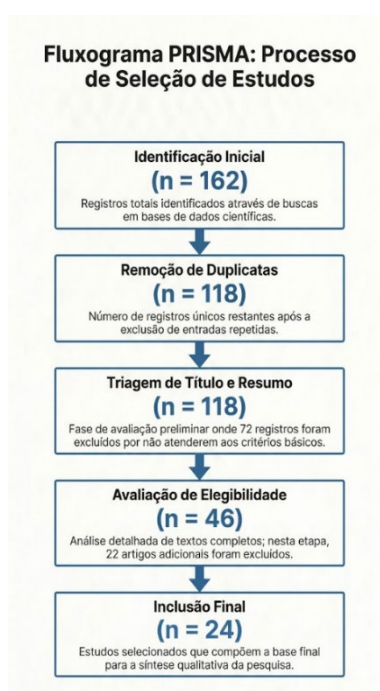


Figure 1 – Systematic Review Flowchart of Study Selection.

3.2. Geographic distribution of emerging fungal diseases in wildlife

The analysis of the 48 studies included in this qualitative narrative review reveals a marked geographical concentration of research on the application of MALDI-TOF MS in veterinary microbiology, with most studies originating from temperate regions of the Northern Hemisphere, particularly Europe and North America (Figure 2).

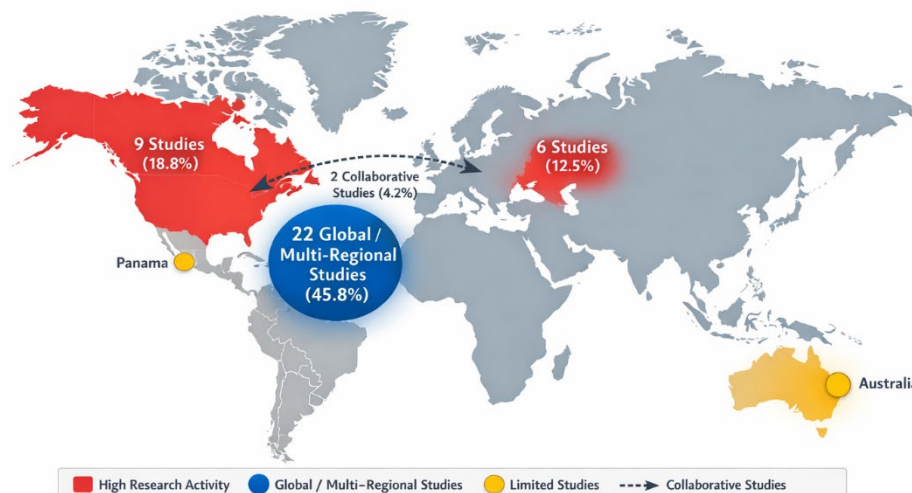


Figure 2 – Global distribution of studies evaluating the use of MALDI-TOF MS in veterinary microbiology.

The map summarizes the geographical origin and scope of the 48 studies included in this review. Europe and North America represent the main centers of research activity, reflecting early adoption and routine implementation of MALDI-TOF MS in veterinary diagnostic laboratories. Studies with a global or multi-regional scope are highlighted separately, emphasizing methodological validation, database development, and interlaboratory standardization. Dashed arrows indicate collaborative studies between Europe and North America. Regions in the Southern Hemisphere remain underrepresented, where MALDI-TOF MS use is largely restricted to academic or reference laboratory settings rather than routine diagnostics. The map reflects the study origin rather than the pathogen prevalence.

Of the 48 studies analyzed, 22 (45.8%) had a global or multi-regional scope, primarily addressing methodological validation, database development, and interlaboratory standardization of MALDI-TOF MS for veterinary pathogens (Seng et al., 2010; Clark et al., 2013). Nine studies (18.8%) were conducted exclusively in North America, six studies (12.5%) originated solely from Europe, and two studies (4.2%) involved collaborative efforts between European and North American institutions, highlighting transcontinental cooperation in veterinary diagnostic standardization (Randall et al., 2015; Croxatto et al., 2012). The remaining studies were geographically limited, with individual reports from Central America and Australia and a small number from other regions with mixed or underrepresented populations.

In Europe, studies predominantly focused on the routine diagnostic application of MALDI-TOF MS in veterinary laboratories serving companion animals and livestock. Frequently identified bacterial pathogens included *Staphylococcus pseudintermedius*, *Staphylococcus aureus*, *Streptococcus* spp., *Enterococcus* spp., *Escherichia coli*, *Salmonella enterica*, *Pasteurella multocida*, *Mannheimia haemolytica*, and *Trueperella pyogenes* (Clark et al., 2013; Randall et al., 2015). Across these studies, MALDI-TOF MS consistently achieved species-level identification rates exceeding 90%, with substantially shorter turnaround times than conventional biochemical methods (Seng et al., 2010; Croxatto et al., 2012).

In North America, similar patterns were observed, with MALDI-TOF MS increasingly integrated into the routine workflows of veterinary diagnostic laboratories. North American studies emphasized large-scale bacterial identification from clinical samples obtained from respiratory, gastrointestinal, urinary, reproductive, and integumentary systems (Randall et al., 2015; Clark et al., 2013). Several investigations reported the analysis of hundreds to thousands of bacterial isolates, demonstrating high concordance between MALDI-TOF MS results and reference molecular or sequencing-based methods (Croxatto et al., 2012; Bizzini et al., 2010). In addition to routine diagnostics, MALDI-TOF MS supported outbreak investigations and surveillance activities, particularly for *Salmonella enterica*, *Escherichia coli*, and *Staphylococcus* spp., reinforcing its role within One Health surveillance frameworks (Randall et al., 2015).

Studies with a global or multi-regional scope primarily addressed methodological aspects, including spectrum reproducibility, database completeness, interlaboratory comparability, and the inclusion of veterinary-specific bacterial species underrepresented in human clinical libraries (Clark et al., 2013; Seng et al., 2010). These studies consistently emphasized that database coverage was a key determinant of identification performance, particularly for host-adapted and less common veterinary pathogens. In contrast, studies originating from the Southern Hemisphere were markedly underrepresented. In South America, only a limited number of studies have described the use of MALDI-TOF MS in veterinary microbiology, and these have been largely restricted to academic or reference laboratory settings rather than routine diagnostic practice. Most studies in this region relied predominantly on molecular diagnostics, reflecting limitations in equipment availability, database access, and maintenance costs (Croxatto et al., 2012). Similar

constraints were observed in the few studies conducted in Central America and Australia, where MALDI-TOF MS was mainly used in exploratory or validation contexts.

Although the primary strength of MALDI-TOF MS across all regions was bacterial identification, a small proportion of studies also evaluated its performance for veterinary-relevant yeasts, such as *Candida* spp. and *Cryptococcus* spp., with generally favorable results (Bizzini et al., 2010; Clark et al., 2013). In contrast, filamentous fungi, particularly wildlife-associated pathogens, were rarely included in MALDI-TOF MS-based analyses and continued to rely on molecular and morphological methods as diagnostic standards. Overall, the quantitative distribution of studies highlights a pronounced geographical imbalance in the adoption and evaluation of MALDI-TOF MS in veterinary microbiology. While the technology is firmly established in Europe and North America as a routine tool for rapid and accurate bacterial identification, its implementation remains limited in biodiversity-rich regions of the Southern Hemisphere. Expanding diagnostic infrastructure, veterinary-specific databases, and international collaboration will be essential to realize the global diagnostic potential of MALDI-TOF MS fully and to strengthen veterinary and One Health surveillance worldwide (Seng et al., 2010; Clark et al., 2013; Randall et al., 2015).

3.3. Brazil in the Global Context of MALDI-TOF MS Implementation in Veterinary Microbiology

Brazil is a key country in global veterinary microbiology due to its extensive livestock production, large companion animal population, and relevance within One Health frameworks. Despite this strategic importance, the routine implementation of MALDI-TOF MS in Brazilian veterinary diagnostic laboratories remains limited when compared with Europe and North America, where the technology is widely established (Seng et al., 2010; Clark et al., 2013; Randall et al., 2015).

In Brazil, MALDI-TOF MS has been applied mainly in academic institutions and reference laboratories, often for method validation, training, or complementary identification rather than as a primary tool in routine diagnostic workflows. Studies conducted in Brazil demonstrate its application in the identification of veterinary pathogens, particularly those associated with bovine mastitis, including *Staphylococcus* spp. and other bacteria (Oliveira et al., 2021; Lopes et al., 2022; Lopes et al., 2023). These studies show that MALDI-TOF MS provides high accuracy and rapid identification when compared with conventional biochemical and molecular methods.

From a veterinary bacteriology perspective, Brazilian laboratories process a high volume of clinically and epidemiologically relevant bacterial isolates from livestock, companion animals, food safety programs, and antimicrobial resistance surveillance. Common pathogens include *Escherichia coli*, *Salmonella enterica*, *Staphylococcus* spp., *Streptococcus* spp., *Enterococcus* spp., *Pasteurella multocida*, *Mannheimia haemolytica*, and *Trueperella pyogenes*. These organisms are well suited for MALDI-TOF MS identification, as demonstrated by international validation studies reporting species-level accuracy rates exceeding 90% (Seng et al., 2009; Clark et al., 2013; Randall et al., 2015). In addition, applied studies have confirmed high identification performance for clinically relevant pathogens such as *Salmonella*, *Staphylococcus* spp., and *Escherichia coli* across food safety, environmental, and veterinary contexts (Kang et al., 2017; Manukumar et al., 2017; Sulaiman et al., 2023; Ashfaq et al., 2022; Van den Beld et al., 2022), further supporting the robustness and reliability of MALDI-TOF MS as a routine diagnostic tool in veterinary microbiology.

The limited expansion of MALDI-TOF MS in Brazil is primarily attributed to high initial investment costs, maintenance requirements, restricted access to veterinary-specific spectral databases, and regional disparities in laboratory infrastructure. Nevertheless, Brazil represents a high-potential environment for broader MALDI-TOF MS implementation, particularly through database customization using regional veterinary isolates and international collaboration to strengthen diagnostic capacity and surveillance.

Reference	Field / Host	Biome / Region	Location	Key Findings / Species
Seng et al., 2009 (<i>Clin. Infect. Dis.</i>)	General microbiology	Global	Multinational	Established MALDI-TOF MS as a routine method for rapid and accurate bacterial identification (<i>Escherichia coli</i> , <i>Salmonella enterica</i> , <i>Staphylococcus</i> spp.), serving as a benchmark for veterinary applications.
Clark et al., 2013 (<i>Clin. Microbiol. Rev.</i>)	Clinical & veterinary microbiology	Global	Multinational	Demonstrated high species-level accuracy for clinically relevant bacteria, supporting application in veterinary bacteriology.
Randall et al., 2015 (<i>J. Microbiol. Methods</i>)	Veterinary (livestock & companion animals)	Europe / North America	Multiple countries	Validated MALDI-TOF MS for identification of animal bacterial isolates, supporting transfer to veterinary diagnostic laboratories.
Croxatto et al., 2012 (<i>FEMS Microbiol. Rev.</i>)	General microbiology	Global	Multinational	Reviewed applications and limitations, including infrastructure and database challenges relevant to developing countries.
Patel, 2015 (<i>Clin. Chem.</i>)	Clinical microbiology	Global	Multinational	Highlighted reduced turnaround time and cost-effectiveness in high-throughput diagnostic settings.

Reference	Field / Host	Biome / Region	Location	Key Findings / Species
Welker & Moore, 2011 (<i>Syst. Appl. Microbiol.</i>)	Environmental & veterinary microbiology	Global	Multinational	Emphasized need to expand databases to include environmental and veterinary isolates.
Oliveira et al., 2021 (<i>J. Dairy Research</i>)	Veterinary (livestock – cattle)	Latin America	Brazil	High accuracy (~95%) for identification of bovine mastitis pathogens (<i>Staphylococcus spp.</i> , <i>Streptococcus spp.</i>).
Lopes et al., 2022 (<i>Pesq. Vet. Bras.</i>)	Veterinary (livestock – cattle)	Latin America	Brazil	Identification of <i>Staphylococcus spp.</i> from bovine mastitis using MALDI-TOF MS in research settings.
Lopes et al., 2023 (<i>J. Appl. Microbiol.</i>)	Veterinary (livestock – cattle)	Latin America	Brazil	Rapid subtyping of <i>Staphylococcus aureus</i> in subclinical mastitis using MALDI-TOF MS combined with molecular methods.

Table 1 – Studies contextualizing Brazil within the global application of MALDI-TOF MS in veterinary microbiology.

3.4. Temporal Trends in the Use of MALDI-TOF MS in Veterinary

The temporal analysis of the studies included in this qualitative narrative review reveals a clear and progressive increase in the use of MALDI-TOF MS in veterinary microbiology over the past two decades. This evolution reflects both technological maturation and the growing demand for rapid, accurate, and cost-effective diagnostic tools in veterinary diagnostic laboratories. In the early 2000s, MALDI-TOF MS applications in microbiology were largely restricted to experimental and proof-of-concept studies, primarily focused on evaluating the feasibility of proteomic fingerprinting for microbial identification. During this initial phase, veterinary pathogens were rarely the primary focus, and studies were typically conducted within academic or research-oriented settings (Lay, 2001; Fenselau and Demirev, 2001; Welker and Moore, 2011). The transition toward routine clinical application began in the late 2000s, driven by improvements in database development, sample preparation protocols, and instrument standardization, which enabled reliable identification of clinically relevant bacteria (Seng et al., 2009; Bizzini and Greub, 2010).

Between approximately 2005 and 2010, advances in instrumentation, spectral resolution, and data processing facilitated the gradual transition of MALDI-TOF MS from experimental use to clinical and diagnostic applications. During this period, the technology began to be adopted in clinical microbiology laboratories, with veterinary-related studies often embedded within broader evaluations that included both human and animal pathogens (Seng et al., 2010; Bizzini and Greub, 2010; Croxatto et al., 2012; Patel, 2013). A major shift occurred after 2010, when MALDI-TOF MS became increasingly integrated into routine diagnostic workflows in veterinary microbiology, particularly in Europe and North America. Studies published during this period consistently demonstrated high diagnostic accuracy, reduced turnaround times, and improved standardization when compared with conventional biochemical identification methods, establishing MALDI-TOF MS as a reference tool for routine bacterial identification in veterinary diagnostic laboratories (Clark et al., 2013; Randall et al., 2015; Bizzini et al., 2011; Neville et al., 2011; Carbonnelle et al., 2011; Biswas and Rolain, 2013; Schmitt et al., 2013; Couturier et al., 2016).

In more recent years, the application of MALDI-TOF MS in veterinary microbiology has expanded beyond routine species-level identification to include database expansion, veterinary-specific spectral libraries, and integration into surveillance programs for foodborne pathogens and antimicrobial resistance. These temporal trends in the adoption and diversification of applications are illustrated in Figure 3.

Despite these advances, temporal trends also reveal persistent global disparities in MALDI-TOF MS adoption. While the technology is now firmly established in veterinary diagnostic laboratories in high-income regions, its implementation in low- and middle-income countries has progressed more slowly, often remaining confined to academic or reference laboratory settings.

Overall, the temporal progression of MALDI-TOF MS in veterinary microbiology reflects a transition from an experimental proteomic approach to a routine diagnostic platform with expanding applications in surveillance and One Health initiatives. This evolution, summarized in Figure 3, highlights the growing importance of MALDI-TOF MS as a cornerstone technology in modern veterinary diagnostic microbiology.

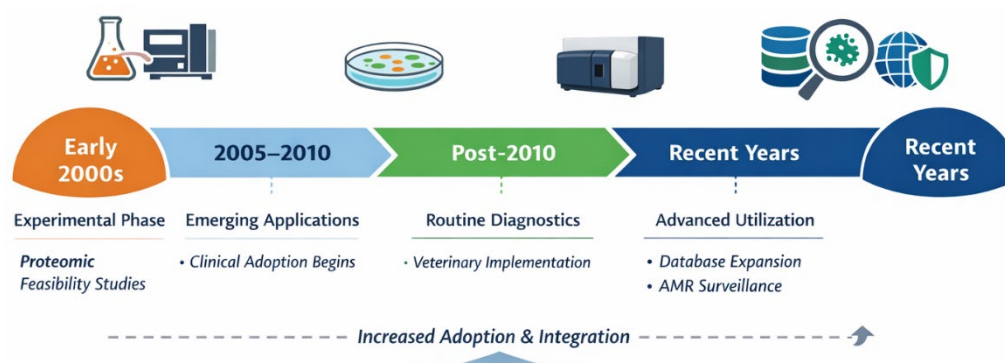


Figure 3 – Temporal trends in the adoption of MALDI-TOF MS in veterinary microbiology.

The figure illustrates the progressive evolution of MALDI-TOF MS from an experimental proteomic approach in the early 2000s to a routinely implemented diagnostic tool in veterinary microbiology after 2010. Expanded database development, veterinary-specific applications, and integration into surveillance programs for foodborne pathogens and antimicrobial resistance have been characterized in the past few years. The timeline reflects trends in methodological adoption rather than pathogen prevalence.

3.5. MALDI-TOF MS for the Identification of Veterinary Pathogens

MALDI-TOF MS has become one of the most impactful technologies for the identification of veterinary pathogens due to its speed, accuracy, and cost-effectiveness compared with conventional phenotypic and biochemical methods (Bizzini and Greub, 2010; Clark et al., 2013). In veterinary microbiology, MALDI-TOF MS enables rapid species-level identification from cultured isolates, significantly reducing diagnostic turnaround times and supporting timely clinical and epidemiological decision-making (Seng et al., 2010; Patel, 2015).

The application of MALDI-TOF MS in veterinary diagnostics has expanded alongside its adoption in human clinical microbiology, with increasing validation for animal-derived bacterial and fungal isolates (Clark et al., 2013; Randall et al., 2015). However, MALDI-TOF MS performance is strongly influenced by the quality and coverage of reference spectral databases, organism-specific growth characteristics, and standardized sample preparation protocols (Welker and Moore, 2011; van Belkum et al., 2012). Consequently, while MALDI-TOF MS is well established for bacterial identification, its application to fungal pathogens, particularly filamentous fungi, remains more limited and technically challenging (Patel, 2015).

3.5.1. Bacterial Identification

Bacterial identification represents the most mature and widely validated application of MALDI-TOF MS in veterinary microbiology (Seng et al., 2010; Clark et al., 2013). Numerous studies have demonstrated that MALDI-TOF MS provides rapid, accurate, and reproducible identification of bacterial pathogens isolated from companion animals, livestock, wildlife, and food-producing animals (Randall et al., 2015; Patel, 2015).

Common veterinary bacterial pathogens routinely identified using MALDI-TOF MS include *Escherichia coli*, *Salmonella* spp., *Staphylococcus* spp., *Streptococcus* spp., *Enterococcus* spp., *Pasteurella multocida*, *Mannheimia haemolytica*, *Trueperella pyogenes*, and *Campylobacter* spp. (Seng et al., 2010; Clark et al., 2013; Randall et al., 2015). Across multiple validation studies, MALDI-TOF MS achieved species-level identification accuracies exceeding 90% for most clinically relevant veterinary bacteria, frequently outperforming conventional biochemical testing methods (Bizzini and Greub, 2010; Patel, 2015).

In addition to routine diagnostics, MALDI-TOF MS has been increasingly incorporated into veterinary surveillance programs, including food safety monitoring and antimicrobial resistance (AMR) surveillance, due to its ability to process large numbers of isolates rapidly (Clark et al., 2013; Randall et al., 2015). Nevertheless, challenges persist in identifying closely related species, fastidious organisms, and bacterial taxa underrepresented in commercial databases, reinforcing the need for continuous expansion of veterinary-specific reference spectra (Welker and Moore, 2011; van Belkum et al., 2012).

3.5.2. Fungal and Yeast Identification

The application of MALDI-TOF MS for fungal identification in veterinary microbiology is more heterogeneous and organism-dependent compared with bacterial diagnostics (Welker & Moore, 2011; Patel, 2015). For yeasts, MALDI-TOF MS has demonstrated robust performance, with high identification accuracy reported for clinically relevant species such as *Candida* spp. and *Cryptococcus neoformans/Cryptococcus gattii* isolated from animals (Bizzini & Greub, 2010; Clark et al., 2013).

Several studies have shown that, when supported by appropriate reference spectra and standardized protein extraction protocols, MALDI-TOF MS can reliably differentiate veterinary-relevant yeast species, offering faster results than conventional biochemical or morphological methods (Seng et al., 2010; Patel, 2015). As a result, MALDI-TOF MS has been increasingly adopted for routine yeast identification in veterinary diagnostic laboratories with access to curated databases (Clark et al., 2013; Randall et al., 2015). In contrast, the identification of filamentous fungi using MALDI-TOF MS remains technically challenging and less standardized (Welker & Moore, 2011; Van Belkum et al., 2012). Factors such as complex hyphal morphology, variable growth conditions, and difficulties in protein extraction contribute to lower identification rates compared with bacteria and yeasts (Patel, 2015).

Consequently, molecular methods, including PCR and sequencing, continue to represent the reference standards for the identification of filamentous fungal pathogens in veterinary microbiology (Welker & Moore, 2011).

Despite these limitations, recent efforts to expand fungal reference databases and improve extraction protocols have demonstrated promising results for selected molds, including *Aspergillus* spp., *Fusarium* spp., and dermatophytes of veterinary relevance (Clark *et al.*, 2013; Patel, 2015). In this context, MALDI-TOF MS is best regarded as a complementary diagnostic tool for fungal identification, supporting but not replacing molecular diagnostics, particularly for complex or rare veterinary pathogens (Van Belkum *et al.*, 2012).

3.5.3. Rare, Fastidious, and Emerging Pathogens

The identification of rare, fastidious, and emerging pathogens represents one of the most challenging aspects of veterinary diagnostic microbiology. These organisms are often characterized by slow growth, demanding nutritional requirements, atypical biochemical profiles, or close phylogenetic relatedness to other species, which complicates accurate identification using conventional phenotypic and biochemical methods (Welker and Moore, 2011; van Belkum *et al.*, 2012). MALDI-TOF MS has demonstrated significant potential to improve the identification of these pathogens, particularly when appropriate reference spectra are available. Several studies have shown that MALDI-TOF MS can reliably identify fastidious bacteria and rare species that are frequently misidentified or remain unidentified using traditional diagnostic approaches (Seng *et al.*, 2010; Clark *et al.*, 2013).

In veterinary microbiology, rare and fastidious pathogens of clinical relevance include *Brucella* spp., *Mycoplasma* spp., *Ureaplasma* spp., *Actinobacillus* spp., *Haemophilus* spp., *Erysipelothrix rhusiopathiae*, and *Francisella* spp. These organisms are associated with significant impacts on animal health, zoonotic disease, and the economy. MALDI-TOF MS has been shown to successfully identify several of these taxa at the genus and species levels, provided that biosafety requirements are met and that reference databases contain validated spectra (Clark *et al.*, 2013; Randall *et al.*, 2015; Patel, 2015).

For emerging pathogens, MALDI-TOF MS is increasingly important as a complementary diagnostic tool during outbreak investigations and surveillance activities. Although newly emerging or previously uncharacterized organisms may not be immediately identifiable using standard commercial databases, MALDI-TOF MS can facilitate rapid preliminary classification and guide subsequent molecular confirmation. The continuous expansion and customization of spectral databases are therefore critical to improving MALDI-TOF MS performance for emerging veterinary pathogens (Welker and Moore, 2011; van Belkum *et al.*, 2012).

Despite these advantages, limitations remain in the application of MALDI-TOF MS to rare and fastidious organisms. Identification accuracy is highly dependent on database completeness, standardized culture conditions, and optimized protein extraction protocols. In the absence of representative reference spectra, MALDI-TOF MS may yield low-confidence scores or incorrect identifications, necessitating confirmatory molecular methods such as PCR or sequencing (Patel, 2015; Croxatto *et al.*, 2012). Overall, MALDI-TOF MS represents a valuable addition to the diagnostic toolbox for rare, fastidious, and emerging veterinary pathogens. When integrated with molecular diagnostics and supported by curated veterinary-specific databases, MALDI-TOF MS can enhance diagnostic resolution, reduce time to identification, and strengthen surveillance and response efforts within veterinary and One Health frameworks (Clark *et al.*, 2013; Randall *et al.*, 2015).

3.6. Database Development and Spectral Libraries in Veterinary MALDI-TOF MS

The performance of MALDI-TOF MS in veterinary microbiology is intrinsically dependent on the quality, completeness, and representativeness of reference spectral databases. Unlike molecular methods that rely on conserved genetic targets, MALDI-TOF MS identification is based on protein spectral fingerprints, making database coverage a critical determinant of identification accuracy and confidence (Welker and Moore, 2011; Clark *et al.*, 2013).

Commercial MALDI-TOF MS systems were initially developed and optimized for human clinical microbiology, leading to reference libraries heavily biased toward human-associated pathogens. Although these databases include many bacterial species of veterinary relevance, host-adapted strains, animal-specific pathogens, and regionally prevalent organisms remain underrepresented. This limitation can lead to low-confidence scores or misidentification when veterinary isolates differ significantly from reference spectra derived from human clinical strains (Randall *et al.*, 2015; Van Belkum *et al.*, 2012). In veterinary bacteriology, studies have consistently demonstrated that database expansion with animal-derived isolates significantly improves identification accuracy, particularly for closely related species and fastidious organisms. The inclusion of multiple strains per species, representing different hosts, geographic regions, and ecological niches, enhances spectral robustness and interlaboratory reproducibility. As a result, customized or locally curated databases have emerged as an effective strategy to optimize MALDI-TOF MS performance in veterinary diagnostic laboratories (Welker and Moore, 2011; Seng *et al.*, 2010).

The importance of database development is even more pronounced for fungal pathogens. While MALDI-TOF MS performs well for yeasts when appropriate reference spectra are available, the identification of filamentous fungi remains challenging due to limited spectral representation and variability in protein expression under different culture conditions. Ongoing efforts to expand fungal spectral libraries and standardize extraction protocols are therefore essential to improve diagnostic reliability in veterinary mycology (Clark *et al.*, 2013; Patel, 2015).

3.6.1. Challenges and Future Perspectives for Veterinary Spectral Libraries

Despite substantial progress, several challenges continue to limit the effectiveness of MALDI-TOF MS databases in veterinary microbiology. One major constraint is the lack of standardized criteria for spectrum acquisition, curation, and validation across laboratories. Variations in culture media, incubation time, sample preparation, and protein extraction protocols can introduce spectral variability, complicating database harmonization and data sharing (van Belkum *et al.*, 2012; Croxatto *et al.*, 2012).

Biosafety considerations also influence database development, particularly for zoonotic and high-risk pathogens such as *Brucella* spp. and *Francisella* spp. In these cases, generating reference spectra requires appropriate containment facilities and standardized inactivation protocols, which may not be available in all veterinary laboratories. These constraints further contribute to gaps in reference libraries for rare, fastidious, and emerging veterinary pathogens (Patel, 2015; Randall et al., 2015). Future advances in veterinary MALDI-TOF MS are expected to focus on collaborative database development, interlaboratory data sharing, and integration with molecular and genomic data. Regional and international initiatives to build veterinary-specific spectral repositories could substantially improve identification performance, particularly in underrepresented regions and for host-adapted pathogens. The incorporation of metadata related to host species, geographic origin, and antimicrobial resistance profiles may further enhance the epidemiological utility of MALDI-TOF MS within One Health surveillance frameworks (Clark et al., 2013; Welker and Moore, 2011).

Overall, continued investment in database expansion, standardization, and validation is essential to fully realize the diagnostic potential of MALDI-TOF MS in veterinary microbiology. Strengthening spectral libraries tailored to veterinary pathogens will not only improve routine diagnostic accuracy but also support surveillance, outbreak investigation, and preparedness for emerging diseases at the animal–human–environment interface.

3.7. Comparison of MALDI-TOF MS with Conventional and Molecular Diagnostic Methods

MALDI-TOF MS occupies a strategic intermediate position between conventional phenotypic methods and molecular diagnostic techniques in veterinary microbiology. Conventional culture-based identification, which relies on colony morphology, staining characteristics, and biochemical reactions, remains essential for microbial isolation but is inherently time-consuming and often limited in discriminatory power, particularly for closely related or fastidious organisms (Bizzini and Greub, 2010; Clark et al., 2013). In contrast, MALDI-TOF MS enables rapid, standardized species-level identification within minutes of microbial growth, significantly reducing diagnostic turnaround time and minimizing subjective interpretation (Seng et al., 2010).

When directly compared with conventional biochemical methods, MALDI-TOF MS consistently demonstrates superior accuracy, reproducibility, and cost-efficiency in high-throughput veterinary diagnostic laboratories. Its ability to correctly identify taxa that frequently yield ambiguous biochemical profiles represents a major diagnostic advantage, while still requiring conventional culture as a prerequisite (Patel, 2015; Randall et al., 2015). This comparative relationship between speed, accuracy, and workflow integration is illustrated in Figure 4, which highlights the operational advantages of MALDI-TOF MS over traditional phenotypic approaches.

In comparison with molecular diagnostic methods such as PCR, quantitative PCR, and sequencing, MALDI-TOF MS provides a fundamentally different diagnostic contribution. Molecular techniques offer superior sensitivity, enable direct detection from clinical samples, and allow detailed genetic characterization, including strain typing and detection of antimicrobial resistance genes (Croxatto et al., 2012; van Belkum et al., 2012). However, these approaches are typically associated with higher costs, greater technical complexity, and the need for organism-specific targets, which can limit their routine application for broad-spectrum identification. MALDI-TOF MS, by contrast, offers a rapid, hypothesis-free approach that does not require prior knowledge of the pathogen and is therefore particularly well suited for routine veterinary bacteriology and yeast identification once culture is established. Although its performance depends on comprehensive reference databases and it lacks the genomic resolution provided by sequencing-based methods, MALDI-TOF MS achieves an optimal balance between speed, accuracy, and cost. This complementary positioning relative to molecular diagnostics is summarized in Figure 4, which shows that MALDI-TOF MS bridges the gap between conventional and molecular methodologies.

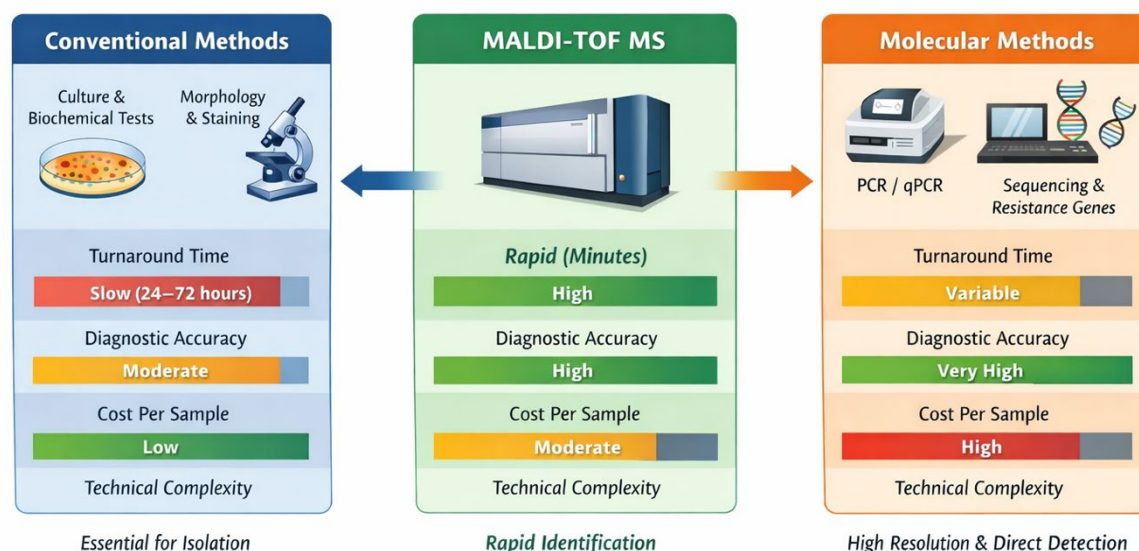


Figure 4 – Comparative overview of MALDI-TOF MS, conventional phenotypic methods, and molecular diagnostic techniques used in veterinary microbiology, highlighting differences in turnaround time, diagnostic accuracy, cost-effectiveness, and applicability in routine diagnostics.

<https://doi.org/10.5380/avs.v31i2.102904>

Overall, MALDI-TOF MS does not replace conventional culture or molecular diagnostics but functions as a central integrative tool within modern veterinary diagnostic workflows. The combined use of conventional isolation methods, MALDI-TOF MS for rapid identification, and molecular techniques for confirmation and advanced characterization maximizes diagnostic efficiency and accuracy. As illustrated in Figure 4, this integrated approach reinforces MALDI-TOF MS's role as a cornerstone technology in contemporary veterinary microbiology and One Health surveillance frameworks (Randall et al., 2015).

3.8. Applications of MALDI-TOF MS Beyond Identification

Beyond its established role in rapid species-level identification, MALDI-TOF MS has increasingly been explored for advanced applications in veterinary microbiology. These emerging uses extend the value of MALDI-TOF MS from routine diagnostics to antimicrobial resistance detection, strain-level discrimination, and epidemiological surveillance, reinforcing its relevance within integrated One Health frameworks. One of the most actively investigated applications of MALDI-TOF MS beyond identification is the detection of antimicrobial resistance (AMR). Several studies have demonstrated that MALDI-TOF MS can detect specific resistance mechanisms by identifying characteristic spectral peaks, protein modifications, or enzymatic activities associated with antimicrobial resistance, particularly β -lactamases and carbapenemases (Seng et al., 2010; van Belkum et al., 2012; Patel, 2015).

In veterinary contexts, these approaches have been explored primarily as rapid screening tools rather than definitive resistance tests. While MALDI-TOF MS does not replace phenotypic susceptibility testing or molecular detection of resistance genes, its ability to provide early indications of resistance can support timely clinical decision-making and guide further confirmatory testing (Clark et al., 2013). MALDI-TOF MS has also shown potential for strain typing and discrimination, particularly when combined with advanced data analysis approaches such as peak-based comparison, spectral clustering, and machine learning algorithms. Studies have reported that MALDI-TOF MS can differentiate closely related strains within the same species, including epidemiologically relevant clones of *Staphylococcus*, *Salmonella*, and *Escherichia coli* (Welker and Moore, 2011; Croxatto et al., 2012). Although the discriminatory power of MALDI-TOF MS is generally lower than that of whole-genome sequencing, it offers a faster and more cost-effective alternative for preliminary strain-level analysis, especially in high-throughput veterinary diagnostic laboratories (Randall et al., 2015).

In epidemiology and laboratory surveillance, MALDI-TOF MS contributes to large-scale monitoring by enabling rapid, standardized processing of large numbers of isolates. Its integration into veterinary diagnostic workflows facilitates real-time data generation for foodborne pathogen surveillance, outbreak investigation, and monitoring of zoonotic agents across animal populations (Clark et al., 2013; Randall et al., 2015). When combined with laboratory information systems and shared databases, MALDI-TOF MS data can support regional and international surveillance networks, enhance the early detection of emerging pathogens, and shift in pathogen distribution. Despite these promising applications, the use of MALDI-TOF MS beyond identification remains subject to important limitations. Interpreting resistance-associated spectral features and strain-level differences requires standardized protocols, robust reference datasets, and advanced bioinformatics tools. Moreover, interlaboratory variability and database dependency continue to constrain reproducibility, particularly for surveillance applications that rely on data comparability across institutions (van Belkum et al., 2012; Patel, 2015).

Overall, while MALDI-TOF MS is firmly established as a cornerstone technology for microbial identification in veterinary microbiology, its expanding applications in antimicrobial resistance detection, strain typing, and epidemiological surveillance highlight its broader diagnostic and public health potential. Continued methodological refinement, database expansion, and integration with molecular and genomic approaches will be essential to fully leverage MALDI-TOF MS as a multifunctional tool within veterinary diagnostics and One Health surveillance systems.

3.9. Challenges, Limitations, and Technical Constraints of MALDI-TOF MS

Despite its widespread adoption and proven utility in veterinary microbiology, MALDI-TOF MS presents several challenges and technical constraints that limit its universal applicability. One of the primary limitations is its reliance on high-quality, comprehensive reference spectral databases. Identification accuracy is strongly influenced by database coverage, and veterinary-specific, host-adapted, rare, and emerging pathogens remain underrepresented in many commercial libraries, leading to low-confidence scores or misidentification (Welker and Moore, 2011; Clark et al., 2013).

Another significant constraint is the requirement for cultured isolates, as MALDI-TOF MS is not routinely applicable for direct pathogen detection from most clinical samples. This dependency limits its usefulness for slow-growing, fastidious, or unculturable organisms and necessitates the continued use of conventional culture and molecular diagnostic methods (Croxatto et al., 2012; Patel, 2015). Additionally, variability in culture conditions, sample preparation, and protein extraction protocols can introduce spectral inconsistencies, affecting reproducibility across laboratories (Van Belkum et al., 2012).

Technical and infrastructural barriers also influence MALDI-TOF MS implementation in veterinary settings. High initial equipment costs, ongoing maintenance requirements, and the need for trained personnel restrict access, particularly in low- and middle-income regions. Biosafety considerations further complicate database development for zoonotic and high-risk pathogens, as spectrum generation requires appropriate containment facilities and standardized inactivation protocols (Randall et al., 2015). Collectively, these challenges highlight that, while MALDI-TOF MS is a powerful diagnostic tool, it cannot function as a standalone solution and must be integrated with complementary diagnostic approaches to ensure accurate and reliable veterinary pathogen identification.

3.10. Future Perspectives of MALDI-TOF MS in Veterinary Diagnostics

Future advancements in MALDI-TOF MS are expected to expand its diagnostic scope beyond routine microbial identification and address current technical limitations. One of the most critical priorities is the development of comprehensive veterinary-specific spectral libraries that incorporate diverse host species, geographic regions, and ecological contexts. International collaboration and interlaboratory data sharing will be essential to achieve standardized, high-quality databases capable of supporting global veterinary diagnostics (Clark et al., 2013; Welker and Moore, 2011).

Technological innovations are also anticipated to enhance the analytical capabilities of MALDI-TOF MS. Improvements in instrumentation sensitivity, software algorithms, and machine learning-based spectral analysis may increase discriminatory power, enabling more reliable strain-level differentiation and resistance-associated pattern recognition. These advances could strengthen the role of MALDI-TOF MS in antimicrobial resistance screening, outbreak investigation, and epidemiological surveillance (van Belkum et al., 2012; Patel, 2015).

Additionally, integrating MALDI-TOF MS with molecular, genomic, and bioinformatic tools represents a promising direction for veterinary diagnostics. Hybrid workflows combining rapid proteomic identification with targeted molecular confirmation or whole-genome sequencing could optimize diagnostic efficiency while preserving high-resolution epidemiological insight. As diagnostic infrastructure evolves, MALDI-TOF MS is expected to play an increasingly central role in One Health surveillance frameworks, supporting coordinated monitoring of zoonotic, foodborne, and emerging pathogens across animal, human, and environmental interfaces (Randall et al., 2015).

3.11. Limitations of the Present Review

This narrative review has several inherent limitations that should be acknowledged. First, although efforts were made to conduct a comprehensive literature search across multiple databases, the review may not capture all relevant studies, particularly unpublished data, non-English publications, or reports from regions with limited research output. As a result, certain geographic areas and veterinary contexts may be underrepresented.

Second, the heterogeneity of study designs, laboratory protocols, and outcome measures limited direct quantitative comparison of MALDI-TOF MS performance across studies. Differences in database composition, sample preparation methods, and validation criteria further contributed to variability in reported identification accuracy and diagnostic outcomes.

Finally, as a narrative review, this study emphasizes qualitative synthesis rather than formal meta-analysis. While methodological frameworks were applied to enhance transparency and rigor, the conclusions drawn reflect trends and patterns in the available literature rather than statistically pooled estimates. Future systematic reviews and meta-analyses incorporating standardized performance metrics may provide more precise quantitative insights into the diagnostic performance of MALDI-TOF MS in veterinary microbiology.

4. Conclusion

This qualitative narrative review synthesized scientific evidence on the use of MALDI-TOF MS in veterinary microbiology published between January 2000 and January 2026. The findings demonstrate that MALDI-TOF MS has become a cornerstone technology in veterinary diagnostics, particularly for bacterial identification, by providing rapid, standardized, and cost-effective species-level results once culture is obtained. The technology also shows consistent performance with veterinary-relevant yeasts when supported by curated spectral databases, while applications for filamentous fungi remain more limited and technically challenging. Despite its proven diagnostic value, MALDI-TOF MS adoption in veterinary microbiology is unevenly distributed worldwide, with routine implementation concentrated in Europe and North America and limited use in regions of the Southern Hemisphere. This disparity reflects differences in diagnostic infrastructure, investment capacity, and access to veterinary-specific reference libraries. Overall, the evidence indicates that continued database expansion, methodological standardization, and integration with complementary molecular approaches will be essential to fully realize the global diagnostic and surveillance potential of MALDI-TOF MS within veterinary and One Health frameworks.

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40 ANOS DE EXCELÊNCIA: A TRAJETÓRIA DA PÓS-GRADUAÇÃO EM CIÊNCIAS VETERINÁRIAS DA UFPR

A celebração dos 40 anos do **Programa de Pós-Graduação em Ciências Veterinárias (PPGCV) da Universidade Federal do Paraná** marca quatro décadas de um compromisso inabalável com a ciência, a saúde animal e o desenvolvimento agrário brasileiro. Desde a sua fundação, o programa consolidou-se como um dos pilares da produção de conhecimento no Sul do país, evoluindo de uma iniciativa pioneira para um centro de excelência reconhecido nacional e internacionalmente.

A linha do tempo desta história é pautada por conquistas graduais e sólidas:

- **O Início:** Nos anos iniciais, o foco residia na consolidação do corpo docente e na estruturação de laboratórios fundamentais, estabelecendo as bases para a pesquisa científica rigorosa.
- **Expansão e Qualificação:** Com o passar das décadas, o PPGCV expandiu sua atuação, elevando seus conceitos na CAPES e diversificando suas áreas de concentração, que hoje abrangem desde a patologia e clínica cirúrgica até a epidemiologia e biotecnologia.
- **Internacionalização:** A maturidade do programa trouxe parcerias com instituições globais, permitindo o intercâmbio de pesquisadores e a publicação de estudos em periódicos de alto impacto, elevando o nome da UFPR ao cenário mundial.
- **Inovação Tecnológica:** Nos anos mais recentes, o programa incorporou tecnologias de ponta, como a medicina regenerativa e diagnósticos moleculares avançados, respondendo aos desafios contemporâneos da "Saúde Única" (One Health).



40 ANOS DE EXCELÊNCIA: A TRAJETÓRIA DA PÓS-GRADUAÇÃO EM CIÊNCIAS VETERINÁRIAS DA UFPR

O legado desses 40 anos reflete-se na formação de centenas de mestres e doutores que hoje lideram universidades, centros de pesquisa e grandes empresas do setor agropecuário. Mais do que números, o PPGCV entrega à sociedade soluções práticas para o controle de zoonoses, melhoria da produtividade animal e bem-estar, garantindo que o conhecimento gerado dentro da universidade ultrapasse os muros acadêmicos e transforme a realidade do campo e da cidade. Ao olhar para o futuro, o programa reafirma sua vocação para a liderança, preparando as próximas gerações para os desafios biotecnológicos e éticos que o próximo século reserva à Medicina Veterinária.



Fernando Andrade Souza

Coordenador do Programa de Pós-Graduação em
Ciências Veterinárias da Universidade Federal do Paraná

Fisiopatologia e Biotecnologia da Reprodução Animal
T.E. em equinos, IATF em bovinos e uso de antioxidantes
na criopreservação espermática.



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Autologous serum in surgical wound healing of dogs (*Canis lupus familiaris*): preliminary efficacy data

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Abstract: This study evaluated the efficacy of autologous serum (AS) in promoting the healing of primarily closed abdominal surgical wounds in female dogs using a modified Bates-Jensen Wound Assessment Tool (BWAT). Thirty dogs were equally divided into Control, Treatment 1 (once-daily AS), and Treatment 2 (Twice-daily AS) groups. Wounds were assessed every other day for 10 days postoperatively, focusing on parameters including epithelialization, exudate, skin color, edema, necrosis, and wound edges. Findings revealed a strong positive correlation between AS application frequency and epithelialization rate, with treatment 2 showing a significant difference compared with the other groups. Repeated measures ANOVA confirmed a highly significant difference among groups ($p < 0.001$). These results indicate that autologous serum not only accelerated wound healing but also promoted a more efficient inflammatory response without increasing the risk of complications. This study highlights Autologous Serum as a biologically active, cost-effective adjunct to conventional wound management in veterinary surgery, offering practical benefits for clinical application.

Keywords: Autologous serum, modified Bates-Jensen Wound Assessment Tool, wound healing.

1. Introduction

Surgical wound healing is a vital aspect in veterinary medicine, particularly in facilitating the dog's recovery and well-being following surgical procedures. There are four major stages of wound healing. The first stage is hemostasis, in which blood vessels constrict (vasoconstriction) to reduce blood flow, and platelets gather to form a temporary plug at the wound site, initiating clot formation. The second is inflammation, which begins shortly after hemostasis is complete and is characterized primarily by vasodilation (dilation of blood vessels) that facilitates the recruitment of immune cells and bioactive molecules to the wound site. During these phase, leukocytes, particularly neutrophils and macrophages, along with various enzymes and inflammatory mediators, infiltrate the wound area to eliminate pathogens, remove cellular debris, and release cytokines that regulate subsequent healing phases; the third stage is proliferation wherein the temporary wound matrix that emerges during the hemostasis phase is then replaced by granulation tissue, characterized by a substantial presence of granulocytes, fibroblasts, blood vessels, macrophages, together with collagen bundles and this process also serves to partially restore both the structure and functional aspects of the wound; lastly, the remodeling stage as the collagen fibers that were recently deposited shift along the tension lines (Winker, 2023).

Autologous serum (AS) and platelet-rich plasma (PRP) are effective and are widely used across various fields of medicine. In musculoskeletal conditions, autologous serum has been used to mediate inflammation, pain, and tissue destruction. Moreover, studies using autologous serum in eye drops have demonstrated variable efficacy in treating ocular surface disease (Evans et al., 2016).

Several studies have highlighted the therapeutic role of blood-derived products in promoting wound repair. Growth factors such as platelet-derived growth factor (PDGF), epidermal growth factor (EGF), and transforming growth factor- β (TGF- β) play important roles in stimulating fibroblast proliferation, collagen synthesis, and epithelial cell migration during tissue repair. Experimental studies have shown that PDGF and EGF significantly enhance epithelial and keratocyte proliferation, both of which are essential for wound healing (Haber et al., 2003). Similarly, research on platelet-derived biological products has demonstrated improved wound-healing outcomes through increased angiogenesis, collagen deposition, and reduced inflammation. For example, a study in veterinary regenerative medicine reported that platelet-rich fibrin significantly accelerated skin wound healing by enhancing growth factor expression and collagen formation, while simultaneously reducing inflammatory cytokine levels (Zhang et al., 2024).

Furthermore, experimental work in canine wound models showed that growth factor-enhanced stromal cell therapies promoted faster epithelialization and increased granulation tissue formation compared with control treatments, highlighting the role of platelet-derived growth factors in improving tissue repair and regeneration (Kim et al., 2020).

Hence, the potential of autologous serum for surgical wound healing is well-supported by studies exploring its therapeutic applications across diverse medical contexts, and the established biological properties of AS may promote faster, safer recovery following routine surgical procedures.

2. Materials and Methods

A total of 30 female dogs, aged between 7 months and 2 years, scheduled for elective or routine procedures (such as spaying or a scheduled cesarean), were selected to participate in the study. Each group, treatment 1, treatment 2, and the control group, consisted of 10 female dogs. All selected dogs were required to be apparently healthy, weigh at least 5 kilograms, and have no pre-existing conditions that could affect wound healing. As part of pre-surgical assessment, each dog underwent a physical examination and a complete blood count. Before surgery, 5–10 mL of blood was collected from each patient via venipuncture into red-top tubes. The blood was allowed to clot at room temperature for 20 minutes, then centrifuged at $300 \times g$ for 10 minutes. The resulting serum was collected, transferred to a sterile tube, and stored at 4°C. Since refrigerated autologous serum remains viable for up to 7 days, repeat blood collection, preferably on the 5th to 6th day after the initial collection, was necessary to ensure a consistent, fresh supply of serum throughout the 10-day treatment period.

The data gathering form served as the primary instrument for collecting information and records for this study. It included sections to document patient signalment, deworming and vaccination status, history of allergies or concurrent diseases, tick/flea preventives, and diet. The form incorporated survey topics from Hill et al. (2006), covering clinical signs, etiological categories, diagnoses, diagnostic procedures, and prescribed treatments for skin disorders.

Surgical animals were administered specific medications for pain management, antibiotics, and pre-anesthetic purposes. Atropine sulfate was used as the pre-anesthetic, while Zoletil (a combination of Zolazepam and Tiletamine) served as the anesthetic agent. Before surgery, food was withheld for 8–12 hours. The ventral abdomen area was clipped and aseptically prepared for the procedure. The length of the surgical incision was recorded.

Thirty dogs undergoing elective or abdominal surgery were randomly assigned to two treatment groups that received autologous serum and a control group that received the conventional method. An adequate amount of autologous serum, approximately 3–5 drops (0.15–0.25 mL), was applied to the surgical wounds of animals in treatment groups 1 and 2 for ten consecutive days. Co-amoxiclav was administered twice daily for seven days as the antibiotic of choice. For pain management, prednisone was given once daily for three days. Postoperative care was primarily managed at home by the pet owners, while follow-up treatments and evaluations were conducted at Whizler Animal Clinic. To ensure consistent daily use of autologous serum, the owners were regularly monitored and guided through follow-up via chat.

Wound healing was assessed using the modified Bates-Jensen assessment tool. The primary modification involved the "Size" characteristic (Item 1). Unlike open wounds, which are measured by area (length $\times$ width in cm²), primarily closed surgical incisions maintain a relatively constant linear dimension throughout the healing process, provided there are no complications such as dehiscence. Therefore, "Size" was redefined to specifically measure the longest linear length of the incision in millimeters (mm). Subsequently, the standard BWAT depth scale was adapted to more precisely reflect the anatomical layers encountered in abdominal wounds and to better distinguish between various levels of tissue involvement in a surgical incision or potential dehiscence.

Furthermore, the granulation tissue category, which assesses the presence and quality of granulation tissue filling a wound bed, is primarily relevant for wounds healing by secondary intention (open wounds). In a healthy, primarily closed surgical incision, significant external granulation tissue is not expected and, if present, would indicate a complication such as infection or partial dehiscence (Johnston & Tobias, 2017). Therefore, this item was omitted from the modified assessment tool as it did not provide relevant information for the specific wound type under investigation. In primarily closed surgical incisions, epithelialization is the process by which epidermal cells migrate across the approximated wound edges to re-establish the skin barrier. Rather than assessing the "percentage of wound covered," as is typically done for open wounds, this item was modified to quantify the length of the apposed (healed) wound edge along the incision line. This measured length was then converted to a percentage of the total incision length, serving as a direct approximation of the extent of epithelial healing along the surgical incision. The scoring was designed to reflect the increasing proportion of the incision length that was successfully epithelialized. This method aligns with the principles validated by Bloemen et al. (2012), suggesting that well-structured subjective clinical assessments can reliably reflect the progress of wound healing. While digital tools provide precise measurements, the study concluded that clinical evaluation remains a valid, practical, and time-efficient method for assessing epithelialization in both clinical and research contexts. Therefore, the use of a modified, length-based scoring approach to assess epithelialization in this study is not only appropriate for surgical wounds but also supported by validated methodologies in the existing literature.

Wound depth was excluded from the modified assessment tool due to the practical limitations in evaluating this parameter in primarily closed surgical wounds. Once sutured, the wound edges are approximated, concealing internal layers, and making direct depth measurement impractical and unreliable during early healing. Moreover, the biological timeline of tissue repair further supports this decision. According to Gurtner et al. (2008), while the skin and subcutaneous tissues typically achieve visible closure within 10 to 14 days through epithelialization and initial collagen deposition, deeper structures such as the rectus abdominis muscle heal more slowly. Muscle tissue undergoes fibrous scar formation rather than true regeneration, with significant functional recovery occurring over four to six weeks and full scar maturation requiring two to four months. Given that the current study spanned only 10 days, meaningful changes in muscle layer healing would not yet be apparent. Therefore, excluding depth from the assessment is both methodologically sound and biologically appropriate.

The remaining BWAT categories (edges, undermining, necrotic tissue type, necrotic tissue amount, exudate type, exudate amount, skin color surrounding wound, and peripheral tissue edema) were retained with their original scoring rubrics, as they provide valuable insights into the inflammatory response, integrity, and potential complications of surgical wounds.

Statistical Analysis

All analyses were performed using Microsoft Excel and R software version 4.4.2. Descriptive statistics, such as frequency, mean, and standard deviation, were used to summarize the data. One-way repeated-measures Analysis of Variance (ANOVA) was used to test differences among means across multiple time points and treatment variables. This analysis was used since the observations were measured at more than two time points. If the equality of means for treatment and/or time variables was rejected, the Bonferroni Test was used for pairwise comparison of means. All analyses were performed at the 5% significance level.

3. Results

This study was conducted at Whizler Animal Clinic from January 2024 to January 2025. The selected animals met the criteria for elective surgery, and all the dogs passed the physical exam; they are all apparently healthy.

Table 1. Mean wound length and standard deviation among treatment groups

VARIABLE	CATEGORY	MEAN	STANDARD DEVIATION
Treatment	Control	51.20	10.91
	Treatment 1	47.73	11.45
	Treatment 2	52.20	11.50
F		0.405	
p-value		≤0.673	
Day	Day 0	51.2	11.40
	Day 2	51.2	11.40
	Day 8	51.2	11.40
	Day 10	51.2	11.40
F		1.000	
p-value		≤0.429	

Table 1 – shows that wound length remained consistent across all treatment groups and time points. Mean values for the control (51.20 mm), treatment 1 (47.73 mm), and treatment 2 (52.20 mm) groups showed minimal variation, with no statistically significant difference ($F = 0.405$, $p = 0.673$). Likewise, wound length stayed constant from day 0 to Day 10 ($F = 1.000$, $p = 0.429$), confirming no significant change over time.

VARIABLE	CATEGORY	MEAN	STANDARD DEVIATION
Treatment	Control	41.66 ^a	36.38
	Treatment 1	46.60 ^b	36.33
	Treatment 2	52.16 ^c	38.65
F		89.119	
p-value		≤0.001* (if 2 decimal places, <0.01*)	
Day	Day 0	0	0.00
	Day 2	8.427	1.45
	Day 4	20.474	1.62
	Day 6	46.783	3.85
	Day 8	74.267	3.40
	Day 10	100	0.00
F		4187.383	
p-value		≤0.001* (if 2 decimal places, <0.01*)	
Control Group	Day 0	0.00 ^{ac}	0.00
Control Group	Day 2	8.4 ^b	1.45
Control Group	Day 4	20.47 ^{ac}	1.62
Control Group	Day 6	46.78 ^{ac}	3.85
Control Group	Day 8	74.27 ^{ac}	3.40
Control Group	Day 10	100.00 ^{ac}	0.00
Treatment 1	Day 0	0.00 ^a	0.00
Treatment 1	Day 2	10.26 ^b	1.15
Treatment 1	Day 4	33.06 ^{bc}	1.53
Treatment 1	Day 6	56.98 ^{bc}	5.62
Treatment 1	Day 8	79.30 ^{abc}	7.39
Treatment 1	Day 10	100.00 ^{ac}	0.00
Treatment 2	Day 0	0.00 ^b	0.00
Treatment 2	Day 2	10.95 ^b	1.15
Treatment 2	Day 4	39.69 ^{ac}	1.80
Treatment 2	Day 6	71.63 ^{ac}	4.36
Treatment 2	Day 8	90.67 ^{abc}	8.70
Treatment 2	Day 10	100.00 ^{ac}	0.00
F		33.46	
p-value		≤0.01*	

p-values are from repeated measures ANOVA. Superscript letters are based on the of Bonferroni test. Means with the same letters are not significantly different ($p\text{-value} > 0.05$)

*indicates statistical significance at 0.05 level ($p\text{-value} \leq 0.05$).

Table 2 – mean wound epithelialization and standard deviation by treatment and day post-operation.

The results presented in Table 2 indicate a statistically significant enhancement in epithelialization among dogs treated with autologous serum, with the most notable improvement observed in the treatment 2 group. Although all groups achieved complete epithelialization (100%) by day 10, the rate of progression varied substantially across groups. The mean epithelialization percentages over the 10 days were highest in treatment 2 (52.16%), followed by treatment 1 (46.60%), and lowest in the Control group (41.66%). Analysis of variance (ANOVA) confirmed a highly significant difference among treatments ($F = 89.119$, $p < 0.001$), indicating that increased serum application frequency positively influenced epithelialization.

Temporal analysis revealed a consistent upward trend in epithelial coverage beginning on day 2. Epithelialization advanced to 46.78% by day 6 and reached 74.27% by day 8, demonstrating that epithelial cell migration and wound surface coverage were well underway before complete closure. This progression was also statistically significant across time points ($F = 4187.383$, $p < 0.001$), underscoring a sustained and accelerating healing response.

Wound edge assessment provides insight into how well the wound margins are approximated and whether healing by primary intention is taking place. As shown in Figure 2, all treatment groups, control, treatment 1, and treatment 2 consistently demonstrated a wound edge score of 1 across all days of observation. This uniformity suggests that surgical closure was properly achieved and that the incision margins remained intact and closely apposed throughout the healing process. This finding is consistent with expectations for surgical wounds closed by primary intention, in which the wound edges are intentionally aligned using sutures, staples, or surgical adhesive.

From days 0 to 6, a notable amount of exudate was present across all treatment groups. This finding aligns with the expected early inflammatory response following surgical incision. The volume of exudate gradually decreased by day 8 and was minimal or absent by day 10, suggesting progression into the proliferative and remodeling phases of healing.

As for the exudate type, it was primarily serosanguinous during the first few days, indicating a healthy wound environment with minimal bleeding and no signs of infection. In some instances, sanguineous or slightly bloody exudate was observed within the first 24–48 hours, particularly in wounds with greater tissue disruption or vascularity. This transitioned appropriately to a clearer, more fluid state as healing progressed. No cases of purulent or infected exudate were recorded throughout the observation period, supporting the conclusion that all wounds were healing in an orderly, non-infected manner.

All treatment groups had a skin color rating of 2 (erythema $<50\%$) on days 0 and 2, indicating the expected inflammatory response immediately following surgery. By day 4, some wounds began to improve, particularly in the treatment 2 group, where some animals already had a rating of 1, indicating resolution of erythema and reduced inflammation. From day 6 onward, all groups showed complete normalization of skin color (score 1 across all treatments), indicating that the acute inflammatory phase had subsided and that the wounds were progressing into the proliferative phase of healing. This pattern is consistent with the normal progression of surgical wound healing, as the inflammatory phase usually lasts 2 to 4 days postoperatively, depending on wound severity and management.

All groups, including the Control, Treatment 1, and Treatment 2, consistently scored 1 for peripheral tissue edema from Day 0 through Day 10. This score denotes no visible edema, suggesting that swelling around the wound site was either absent or minimal throughout the monitoring period. This is particularly relevant in this study, where standardized surgical procedures were employed, and healing occurred by primary intention, reducing the likelihood of excessive fluid accumulation in the peri-wound tissues.

The results for a variable that consistently yielded a mean of 0.00 with a standard deviation of 0.00 across all treatment groups (control, treatment 1, and treatment 2) and observation days (day 0 to day 10). This indicates that there was no variation in this variable during the study. As a result, ANOVA was not applicable, and therefore, no F-values or p-values were generated. The uniformity of the data suggests that the variable in question remained constant, unaffected by either the treatment administered or the passage of time. This outcome may reflect the absence of the specific condition or characteristic being measured in all cases, or a uniform response across all subjects and conditions.

Throughout the study, both the type and amount of necrotic tissue consistently registered scores indicating the absence of necrosis in all surgical wounds across all treatment groups.

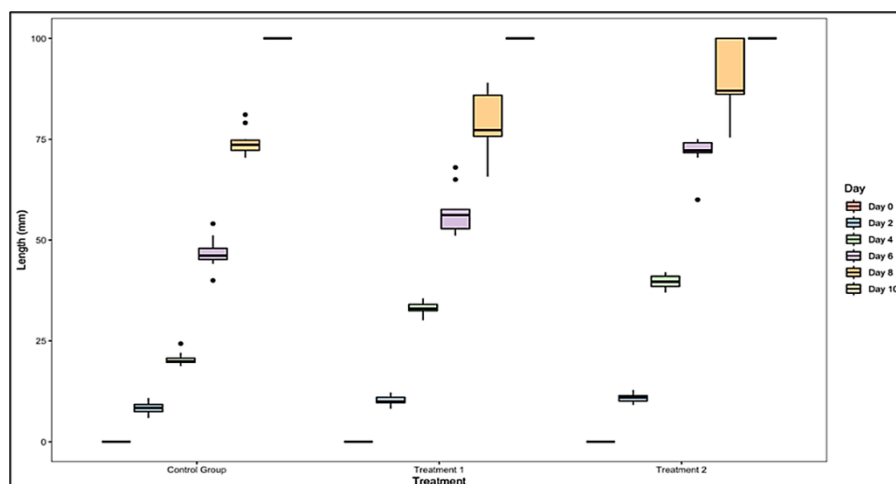


Figure 7 – Boxplot of treatment and time variable of wound healing.

The width of the boxplot from its center indicates the data's variability (dispersion). A wider boxplot indicates greater variability, suggesting that the data are more widely distributed. In contrast, a narrower boxplot suggests that the data values are more closely clustered. In the present study, the granulation (% of wound covered) remained at 0 at the initial time point and progressively increased over the subsequent days.

Notably, for treatments 1 and 2, greater variability was observed on day 8, as indicated by the wider boxplot spread compared to other days. This visual representation is supported by the corresponding standard deviation values, with day 8 showing the highest (3.40), although day 6 recorded the highest overall value. On days 0 and 10, the boxplots appear as flat lines, indicating zero variability because the data values are uniform across all observations.

The central line in each boxplot denotes the median, the midpoint of the dataset when values are arranged in ascending or descending order. This measure provides a reliable indication of the central tendency at each observation point.

4. Discussion

In surgical wound healing, particularly with primary closure techniques, the wound length remains relatively constant throughout the healing process. Measuring wound length alone is not a sensitive indicator of healing, as it does not reflect underlying biological processes such as granulation or epithelialization. In surgically closed wounds, the dimensions remain largely unchanged throughout healing, making length a static parameter. Instead, assessing the quality and extent of tissue regeneration, particularly through granulation and epithelialization, provides a more accurate and clinically relevant measure of healing progression (Landa et al., 2014).

The results of the study support the hypothesis that autologous serum accelerates wound repair, primarily by enhancing epithelialization. This effect was especially noticeable in the group that received treatment twice daily, suggesting that both the frequency and consistency of application play important roles in improving healing outcomes. The likely reason behind this benefit is the presence of naturally occurring biological components in autologous serum, such as growth factors, cytokines, and immunoglobulins. These substances are known to stimulate keratinocyte activity, support tissue regeneration, and help create a balanced wound environment that promotes faster and more effective healing. Comparable results were reported by Jee et al. (2014), who observed improved epithelialization and more organized collagen formation in dogs treated with platelet-rich plasma. This product also comes from the animal's own blood. By day 14, they noted significant improvements in wound closure and tissue structure. Together, these findings strengthen the growing body of evidence showing that autologous blood-derived products, including autologous serum, can be effective, natural options for supporting wound healing in veterinary patients.

Granulation tissue formation was not evaluated in this study because the surgical wounds were closed primarily with sutures. In wounds healing by primary intention, the wound edges are closely approximated with minimal tissue loss, thereby reducing the biological requirement for granulation tissue formation. Healing in such cases primarily occurs through rapid epithelialization and collagen deposition beneath the approximated skin edges, rather than through the development of granulation tissue on the wound surface. The absence of visible granulation tissue in these wounds is consistent with the expected pattern of healing by primary intention. The presence of granulation tissue on the surface of the sutured might indicate delayed closure, a wound starting to reopen, or even a local infection, any of which could lead the wound to shift toward secondary healing. These findings suggest that both the administered treatment and the duration of the healing process had a significant effect on the measured outcome, with treatment 2 showing the most favorable response and a consistent progression of values over time.

When such closure is performed correctly and without disruption, the edges remain well-approximated until epithelialization is complete, as supported by Fossum (2019). Furthermore, Guo and DiPietro (2010) emphasized that minimal edge separation and early epithelialization are indicators of proper inflammatory control and early transition into the proliferative phase of healing. The results of this study reinforce the findings that no complications, such as wound dehiscence, tension, or infection, disrupted the

integrity of closure in any group. The presence of exudate during the early inflammatory phase of wound healing is a normal and essential response. Following tissue injury, increased vascular permeability allows fluid, proteins, and immune cells to enter the wound site. This exudate maintains moisture, aids in debridement, delivers nutrients and oxygen, and supports immune defense to prevent infection (Spear, 2012).

The wound depth parameter was excluded from the modified assessment tool due to inherent limitations in measuring it in primarily closed surgical wounds. Following suturing, the wound edges are approximated, and the internal layers are no longer visible or readily accessible for evaluation, making direct depth measurement impractical and unreliable in the early postoperative period. Moreover, the biological timeline of tissue repair further supports this decision. According to Gurtner et al. (2008), the skin and subcutaneous tissues typically exhibit visible closure within 10 to 14 days through epithelialization and initial collagen deposition. However, deeper structures such as the muscle layer, specifically the rectus abdominis, undergo a much slower healing process. Muscle tissue heals predominantly through fibrous scar formation rather than true regeneration, with initial repair occurring within 10 to 14 days but functional restoration taking approximately four to six weeks. Full scar maturation may take 2 to 4 months. Given that the present study monitored healing over 10 days, it is expected that substantial remodeling and tissue deposition in the deeper layers, particularly muscle, would not yet have occurred. As such, depth would remain largely unchanged during this time frame. In contrast, more dynamic and observable parameters, such as epithelialization and granulation tissue formation, are more appropriate indicators of early healing progression. Therefore, the exclusion of depth from the wound assessment is both methodologically and physiologically justified.

5. Conclusion

This study demonstrates that autologous serum significantly improves epithelialization in primarily closed abdominal surgical wounds in female dogs. A dose-dependent effect was observed, with more frequent applications yielding better outcomes. The absence of infection across all groups suggests additional potential in supporting wound hygiene. Overall, autologous serum shows promise as a practical and accessible adjunct to enhance wound healing in veterinary surgical practice.

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Briefing notes: In accordance with the ethical policies and guidelines approved by Central Luzon State University, this study was submitted to an ethics application to the Ethics Review Committee (ERC) with the ERC Code 2024-674

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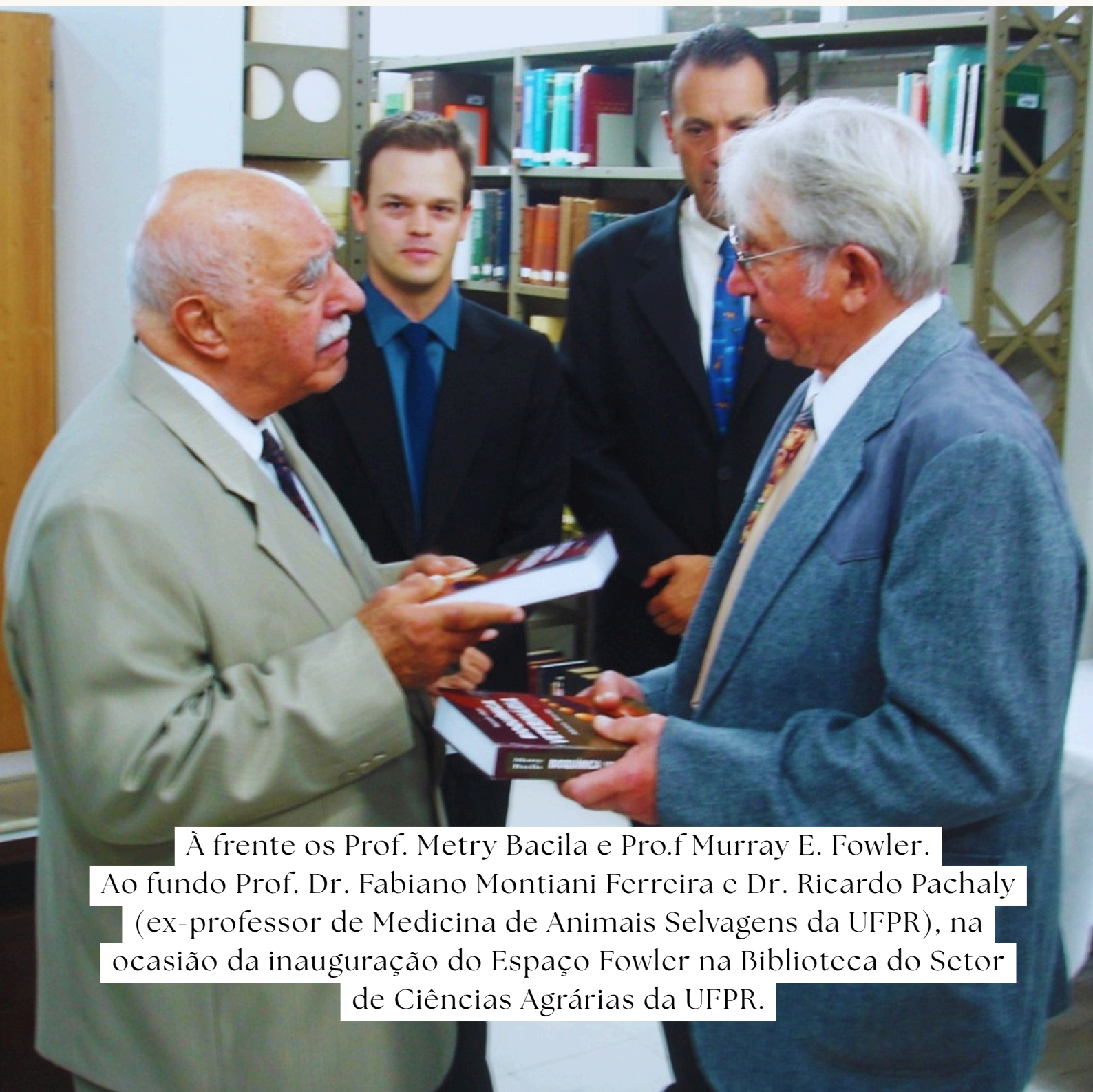
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PPGCV-UFPR: Ciência, Pioneirismo e Gerações que construíram um legado

Em homenagem ao Professor Lysimaco Ferreira da Costa, pioneiro da educação agrária paranaense e inspiração presente na trajetória de nossa família e da UFPR.

Fabiano Montiani Ferreira



À frente os Prof. Metry Bacila e Pro.f Murray E. Fowler.
Ao fundo Prof. Dr. Fabiano Montiani Ferreira e Dr. Ricardo Pachaly
(ex-professor de Medicina de Animais Selvagens da UFPR), na
ocasião da inauguração do Espaço Fowler na Biblioteca do Setor
de Ciências Agrárias da UFPR.

A história do Programa de Pós-Graduação em Ciências Veterinárias da Universidade Federal do Paraná (PPGCV-UFPR) é resultado da visão de pioneiros pesquisadores e educadores que ajudaram a construir as bases da ciência veterinária moderna no Paraná e no Brasil. Desde sua criação oficial em 1986, o programa consolidou-se como referência nacional e internacional em formação científica, produção de conhecimento e inovação em Medicina Veterinária.

Muito antes do surgimento formal do PPGCV, já existia no Paraná um ambiente acadêmico fortemente influenciado pelo ideal de desenvolvimento científico e universitário. Nesse contexto, destaca-se a importância histórica do Professor Lysimaco Ferreira da Costa, educador, intelectual e primeiro diretor da Escola Agrônômica do Paraná, fundada em 1918. A Escola Agrônômica do Paraná, posteriormente incorporada à então Universidade do Paraná, passou a integrar a instituição que, após sua federalização em 1950, tornou-se a Universidade Federal do Paraná (UFPR).

A atuação de Lysimaco Ferreira da Costa também ocorreu em um dos períodos mais importantes da história econômica paranaense: o ciclo da erva-mate. A prosperidade gerada pela produção e comercialização do mate foi decisiva para o desenvolvimento urbano, científico e educacional do Paraná no final do século XIX e início do século XX.

A erva-mate integrava cadeias comerciais que conectavam o Paraná aos estados do sul do país e países vizinhos, especialmente Argentina, Uruguai e Paraguai. A necessidade de conhecimento técnico e modernização agrícola ajudou a consolidar o papel das Ciências Agrárias no estado, ambiente acadêmico que, décadas mais tarde, permitiria o fortalecimento do PPGCV-UFPR.



Lysimaco Ferreira da Costa foi meu bisavô, enquanto seu filho, Carlos Franco Ferreira da Costa, meu avô, tornou-se médico, professor titular e uma das figuras importantes da medicina paranaense. Carlos participou ativamente do processo de criação e consolidação inicial da Faculdade de Ciências Médicas da então Universidade Católica do Paraná, posteriormente PUCPR.

A tradição acadêmica e o vínculo familiar com a Universidade Federal do Paraná também se estenderam à área da Educação. Meus pais, Professor Evaldo Montiani Ferreira e Professora Susana da Costa Ferreira, ambos docentes da UFPR no Departamento de Educação, dedicaram suas carreiras à formação universitária e ao ensino público de qualidade. Sua atuação reforçou, em nossa família, os valores da educação, da ciência e do compromisso institucional com a universidade pública brasileira.

Embora esta narrativa dialogue com minha própria trajetória familiar, ela representa sobretudo a história coletiva de docentes, técnicos, estudantes e servidores que construíram o PPGCV-UFPR ao longo de quatro décadas.

Outro nome incontornável na história do programa é o Professor Metry Bacila, que tive a oportunidade de ter como professor de Bioquímica. Em 1984, Bacila presidiu a comissão responsável pela estruturação do então denominado Curso de Pós-Graduação em Ciências Veterinárias da UFPR (CPGCV), iniciativa que representou um marco decisivo para a consolidação da pós-graduação veterinária na instituição.



Na entrada principal do Campus Ciências Agrárias, destaca-se a exibição do busto de Lysimaco Ferreira da Costa, cuja memória e legado também são homenageados com outro busto exposto no Memorial de Curitiba. A obra celebra o ilustre professor e engenheiro, fundamental para a história da instituição e do ensino agrônomo. Posicionado em local de destaque, o monumento saúda a comunidade acadêmica e preserva sua grande dedicação à educação.



HERMA DE LYSÍMACO FERREIRA DA COSTA

(1883-1941) – engenheiro, professor da Universidade do Paraná

João Aquino | 1983 | bronze
Praça Santos Andrade | Centro



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Em 1995, estudantes do programa criaram o Núcleo de Desenvolvimento Científico Metry Bacila (NDCMB), voltado à promoção de palestras e debates científicos periódicos.

Entre as diversas lideranças que contribuíram para essa trajetória, merece destaque a professora Rosângela Locatelli-Dittrich. O caminho até a excelência acadêmica do atual PPGCV-UFPR, entretanto, não ocorreu sem desafios. Em determinado momento de sua trajetória, o programa enfrentou risco real de descontinuidade em decorrência de avaliações desfavoráveis da CAPES. A reação institucional liderada por docentes e coordenadores – com destaque para a gestão da professora Rosângela Locatelli-Dittrich – permitiu uma profunda reestruturação acadêmica e administrativa. Esse processo resultou na elevação do conceito CAPES de 3 para 4, possibilitando a implantação do curso de doutorado e consolidando definitivamente a transição do antigo curso para um programa de pós-graduação estruturado e fortalecido.

Posteriormente, tive a honra de assumir a coordenação do PPGCV-UFPR entre 2013 e 2017, período marcado por iniciativas voltadas à modernização e internacionalização do programa. Durante essa gestão, passaram a ser permitidas defesas de teses em língua inglesa, foi criado o atual logotipo institucional do PPGCV e o programa avançou do conceito 4 para o conceito 5 na avaliação da CAPES. Também nesse período ocorreu a implantação do sistema Sucupira, plataforma nacional que reformulou os mecanismos de acompanhamento e avaliação da pós-graduação brasileira.

Após minha atuação como coordenador, continuei colaborando diretamente com os processos nacionais de avaliação da pós-graduação, participando de duas avaliações quadrienais da CAPES e integrando, em duas ocasiões, a comissão julgadora do Prêmio CAPES de Tese.

A trajetória consistente de crescimento acadêmico, internacionalização e fortalecimento institucional culminou, anos mais tarde, na conquista do conceito 6 da CAPES, já sob a coordenação da professora Carla F. M. Molento.

O PPGCV acompanhou as transformações da Medicina Veterinária contemporânea, expandindo-se de áreas tradicionais da produção e patologia animal para temas estratégicos globais como Saúde Única, medicina translacional, bem-estar animal e conservação de espécies selvagens.

Ao longo de sua trajetória, o PPGCV também desenvolveu forte vocação internacional. Nesse aspecto, merece destaque a contribuição do Professor Murray Elwood Fowler, uma das maiores referências mundiais em medicina de animais selvagens. Sua primeira visita ao Brasil ocorreu justamente na UFPR, em 1985, por convite do Professor Metry Bacila.

A realização desse curso contou também com participação ativa do Professor Pedro Ribas Werner, que auxiliou na organização logística do evento, incluindo a obtenção de espaço físico, estrutura de tradução simultânea, sonorização e projeção audiovisual. O curso, inicialmente planejado como uma atividade de menor porte, acabou atraindo participantes de diversas regiões do Brasil, tornando-se um marco para o desenvolvimento da medicina de animais selvagens no país. Conforme destacado pelo próprio Professor Pedro Werner, a concepção original e o mérito da iniciativa pertencem integralmente ao Professor Metry Bacila.

A vinda do Professor Murray Elwood Fowler ao então embrionário CPGCV da UFPR representou muito mais do que a realização de um curso internacional. Sua presença serviu como inspiração e referência de excelência acadêmica, internacionalização e qualidade científica, características que passaram a marcar profundamente a identidade do programa e cujos reflexos permanecem evidentes até os dias atuais.

A vocação internacional do recém-criado Curso de Pós-Graduação em Ciências Veterinárias (CPGCV) manifestou-se também em 1986, ano de criação do programa, por meio da visita do Professor Dr. John King, da Cornell University (Nova York, EUA), uma das mais prestigiadas instituições de Medicina Veterinária do mundo. A convite dos Professores Metry Bacila e Pedro Ribas Werner, o Prof. King ministrou um curso de Patologia que obteve ampla repercussão nacional, atraindo participantes de diferentes regiões do Brasil. Assim como ocorrera com Murray Fowler, esta iniciativa evidenciou a visão estratégica de seus idealizadores, que compreendiam precocemente a importância da inserção internacional e da aproximação com centros de excelência para a formação científica de recursos humanos no país.

Essas iniciativas internacionais demonstram que a internacionalização do programa antecedeu em décadas sua incorporação aos critérios formais da CAPES.

A partir desse encontro, estabeleceu-se uma relação duradoura de intercâmbio científico e colaboração acadêmica que durou por muitos anos. Em 2002, estudantes e professores interessados em animais selvagens, incluindo diversos estudantes e docentes do PPGCV, sob liderança do Professor Rogério Ribas Lange, criaram o Grupo Fowler.

Hoje, mestres e doutores formados pelo PPGCV atuam em universidades, laboratórios, órgãos governamentais e centros de pesquisa no Brasil e no exterior.

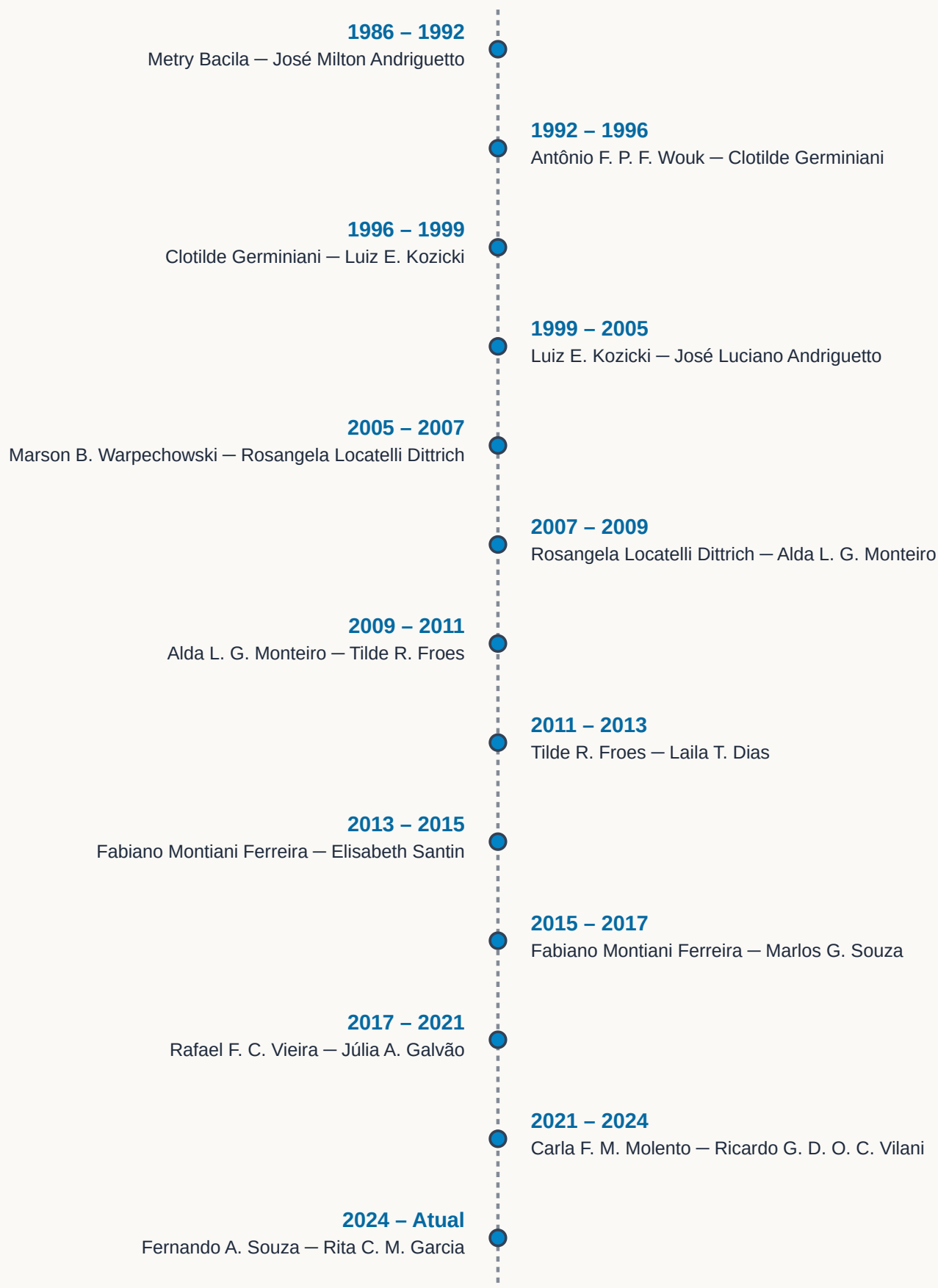
A tradição internacional do PPGCV-UFPR permanece ativa e em constante renovação por meio das colaborações científicas estabelecidas por seus docentes com instituições de excelência no exterior. Na área de Oftalmologia Veterinária, destacam-se as pesquisas desenvolvidas em parceria com a Michigan State University, sob coordenação do Professor Simon Petersen-Jones, envolvendo principalmente doenças hereditárias da retina. Essas colaborações incluem intercâmbio científico e formação de recursos humanos, possibilitando que estudantes do PPGCV desenvolvam parte de suas atividades de pesquisa em centros internacionais de referência. A parceria possui ainda significado pessoal e institucional, uma vez que foi na Michigan State University que realizei meu doutorado pleno (PhD).

De forma semelhante, pesquisas conduzidas em colaboração com a University of Miami Miller School of Medicine, sob liderança da Dra. Carolyn Cray, têm contribuído para o avanço do conhecimento sobre a infecção por *Encephalitozoon cuniculi*, incluindo suas manifestações sistêmicas e oculares.

Mais do que um programa de pós-graduação, o PPGCV-UFPR representa a continuidade de uma tradição científica construída ao longo de gerações, do pioneirismo agrário de Lysimaco Ferreira da Costa à internacionalização promovida por Metry Bacila, Pedro Ribas Werner e Murray Fowler e tantos outros pesquisadores brilhantes do presente que ajudaram a transformar a Medicina Veterinária da UFPR em referência nacional e internacional.

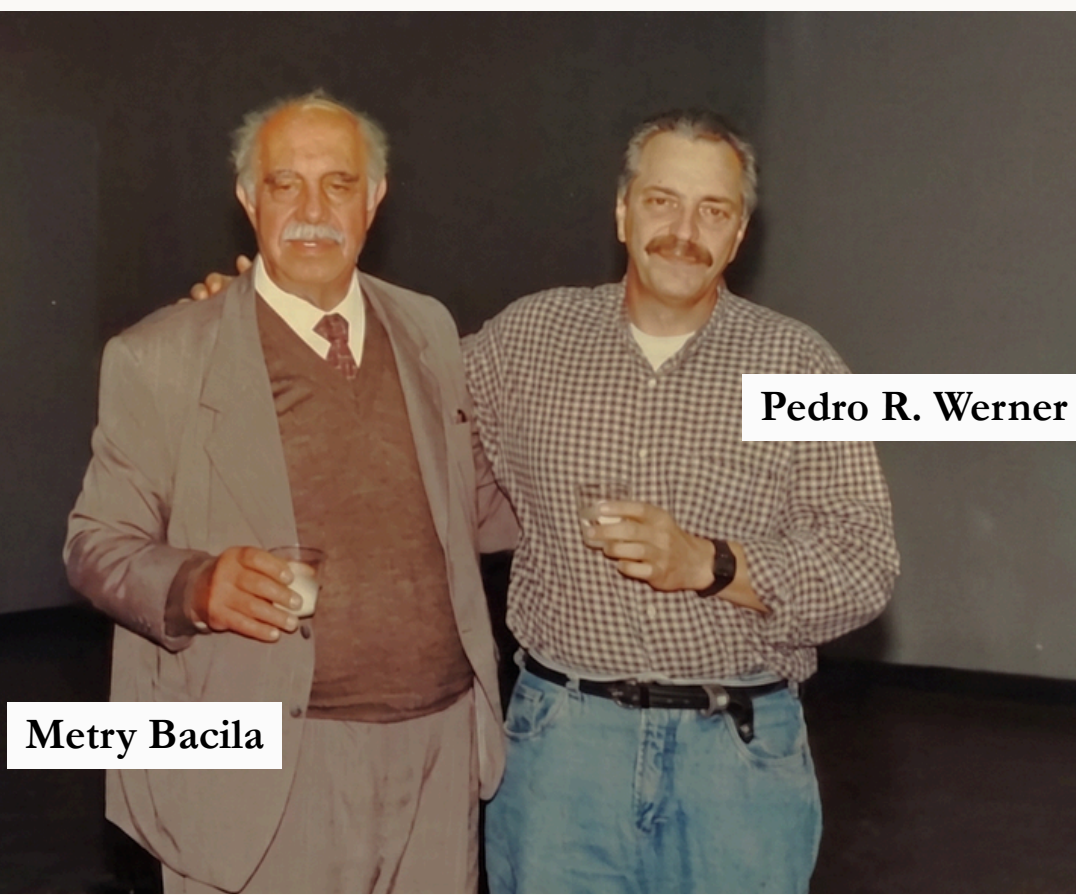
A Figura 1 apresenta uma retrospectiva histórica das coordenações e vice coordenações do PPGCV-UFPR entre 1986 e 2026, destacando nomes que participaram diretamente da construção institucional do programa.

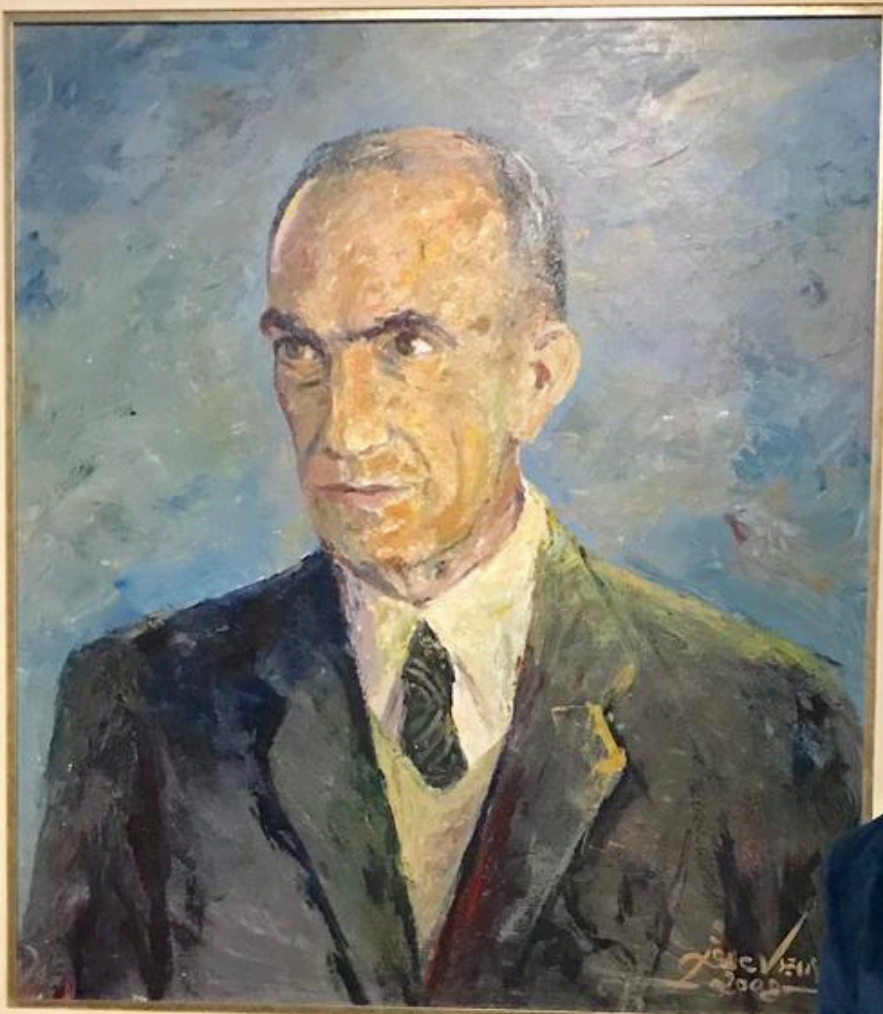
Linha histórica das coordenações do PPGCV-UFPR (1986–2026)



Nenhuma dessas conquistas teria sido possível sem a dedicação de docentes, técnicos administrativos, estudantes e servidores que, ao longo de décadas, contribuíram para a consolidação do programa.

Celebrar a trajetória do PPGCV-UFPR é reconhecer que instituições científicas são construídas por gerações. Dos pioneiros que lançaram as bases do ensino agrário no Paraná aos pesquisadores que hoje lideram redes internacionais de colaboração, permanece o mesmo compromisso: formar pessoas, produzir conhecimento e devolver à sociedade os frutos da universidade pública. A história do PPGCV demonstra que excelência acadêmica não é resultado de esforços isolados, mas da continuidade de valores compartilhados ao longo do tempo. Curiosidade científica, compromisso institucional e dedicação à formação das futuras gerações.





Auditório Carlos Costa

Carlos Franco Ferreira da Costa (1914-2001).
Médico e professor universitário.
Foi fundador da Faculdade de Ciências Médicas da
Universidade Católica do Paraná e primeiro
Vice-Reitor da Instituição.



Ao lado da pintura de meu avô, Carlos Franco Ferreira da Costa (1914-2001), filho de Lysimaco, que integra o espaço do Auditório Carlos Costa na PUCPR. Médico, professor universitário, fundador da Faculdade de Ciências Médicas da instituição e seu primeiro vice-reitor. Um legado que continua inspirando gerações.

Fabiano Montiani Ferreira

É Professor Titular MV, MSc, PhD no Departamento de Medicina Veterinária e Programa de Pós-graduação em Ciências Veterinárias. Sua área de atuação é Oftalmologia veterinária, doenças hereditárias de retina, doenças sistêmicas com manifestação ocular e modelos translacionais.



Archives of Veterinary Science

Tradição

Fabiano Montiani Ferreira

Bisneto de Lysimaco Ferreira da Costa

Preferences for sheep traits among male keepers in El Oued, Algeria: socio-cultural and breeding insights

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Abstract: Identifying farmer trait preferences is fundamental to designing participatory breeding programs adapted to local agroecological constraints, yet such data remain scarce for Algeria's arid zones. This study investigated sheep trait preferences and their socioeconomic determinants among male sheep keepers in El Oued Province, southeastern Algeria. A mixed-method approach combining structured questionnaires and qualitative interviews was applied to 200 randomly selected male sheep keepers registered in the 2023 national sheep census, covering eight municipalities across the province. Multivariate analysis of variance (MANOVA) and chi-square tests were used to assess the influence of age, education, experience, and occupation on trait prioritization. For rams, breed identity (27%), body conformation (12.5%), and apparent health status (12%) emerged as the most valued selection criteria. For ewes, breed (22%), drought tolerance (16%), and udder size (10%) were ranked highest, reflecting the paramount importance of environmental adaptability and reproductive functionality under arid conditions. Locally adapted breeds, particularly Ouled Djellal, were strongly favored. A significant three-way interaction among education, experience, and occupation (Pillai's Trace = 0.070, $p = 0.009$) indicated that farmers with higher education, greater experience, and full-time breeding status prioritized long-term resilience traits, while less experienced or less educated keepers favored readily observable morphological attributes. Farmer age and education level were independently associated with drought tolerance preference in ewes ($p = 0.036$ and $p = 0.004$, respectively). These findings provide a socio-ecological evidence base to support the design of context-specific, participatory breeding programs targeting genetic resilience and sustainable livestock production in North African drylands.

Keywords: Breeding programs, Livelihoods, Sheep Keepers, Trait selection, Productivity.

1. Introduction

Sheep production is vital to Algeria's agricultural economy, particularly in arid and semi-arid regions such as El Oued, where livestock farming is the primary source of income for rural families. Environmental constraints, drought, fodder scarcity, and climatic fluctuations threaten the sustainability and productivity of this important sector. Under such difficult conditions, genetic improvement and well-planned selection are essential tools to strengthen herd resilience and performance (Berihulay et al., 2019; Siad et al., 2022).

In Algeria, sheep breeding accounts for a major share of animal production, with the national flock estimated at around 28–31 million heads, the majority of which are located in steppe and semi-arid regions (FAO, 2017; Laoun et al., 2026; Meziane et al., 2024). The sector supports the livelihood of a significant portion of the rural population and holds important economic and cultural value (Laoun et al., 2026). Production is primarily oriented toward red meat, supplying a substantial share of domestic consumption, though the country is increasingly dependent on imports due to rising demand and production shortfalls (Meziane et al., 2024; Mouffok et al., 2026). However, rising prices, rural migration, and recurrent droughts have made this sector less profitable and less accessible, increasing dependence on imported meat (Hadbaoui et al., 2023; Meziane et al., 2024). Beyond meat, sheep contribute significantly to rural livelihoods by providing manure, supporting mixed farming, and helping families diversify their income (Meziane et al., 2024).

Understanding sheep keepers' preferences for certain traits is therefore crucial for developing suitable, sustainable breeding programs adapted to local environments. Many studies across African countries have shown that farmer preferences vary by production system, cultural background, and ecological conditions. For example, Ethiopian farmers prefer large body size, good mothering ability, and litter size, while in Nigeria, disease resistance is the top priority (Duguma et al., 2011; Gizaw et al., 2010; Hemacha et al., 2022a; Welay et al., 2022; Yakubu et al., 2020). Despite these valuable findings, information about trait preferences in Algeria's arid regions, especially El Oued, remains very limited.

This study fills this gap by identifying, for the first time, the preferred traits of sheep keepers in the El Oued region of southeastern Algeria, with attention to differences in selection criteria between males and females. Using quantitative questionnaires and qualitative interviews, this research analyses decision-making processes and the factors influencing farmers' breeding choices. The results are expected to provide policymakers, agricultural extension services, and development programs with practical insights to inform the design of more sustainable and participatory breeding strategies. The novelty lies in integrating environmental, social, and genetic dimensions to better understand how traditional knowledge can inform breeding strategies adapted to arid, harsh conditions.

2. Materials and Methods

2.1. Description of the Study Area

This investigation was conducted in the El Oued region of Southeast Algeria from June 2023 to January 2024. The state is located at 33°22'16.823" north latitude and 65°52'6.866" east longitude.

2.2. Sampling Procedure

Sheep keepers were selected from the study area (El Oued) based on sheep production and farmers' willingness to participate in this research. A total of 200 male sheep keepers were randomly sampled in the study area, covering 08 municipalities: El Oued, Debila, Guemar, Hassi Khelifa, Mih Ouensa, Ourmes, Robbah, and Taleb Larbi (Figure 1). This sampling ensured representative coverage of the region's main sheep-producing zones, reflecting variations in management practices, flock sizes, and environmental conditions.

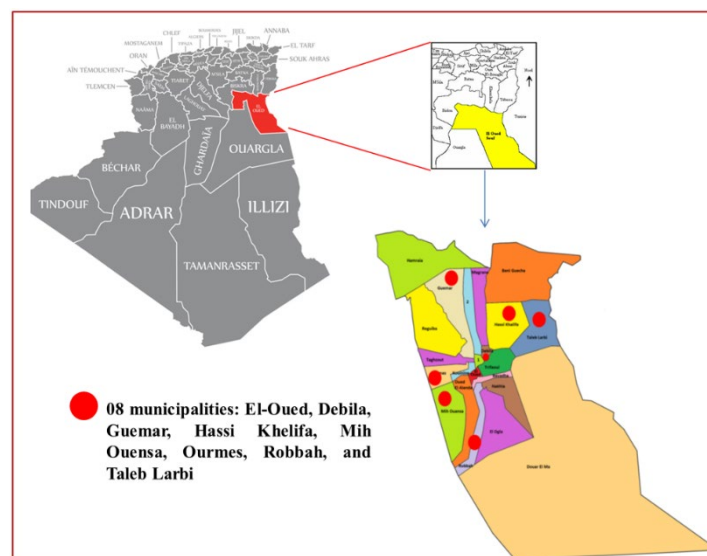


Figure 1 – Map of the study area of El Oued, southeastern Algeria.

2.3. Data Collection Procedure

Before the main survey, the semi-structured questionnaire was pre-tested with 10 sheep keepers to ensure clarity, relevance, and adaptation of the questions to local conditions, as recommended by Dossa et al. (2015). The pre-test data were not included in the final analysis and were used solely to refine the questionnaire. The finalized questionnaire combined open-ended and closed-ended questions to obtain comprehensive data on several aspects: (a) the socioeconomic characteristics of sheep keepers, (b) flock size, and (c) specific trait preferences for male and female sheep. For the main study, sheep keepers were randomly selected from those registered in the government's 2023 national sheep numbering campaign across eight municipalities in El Oued (El Oued, Debila, Guemar, Hassi Khalifa, Mih Ouensa, Ourmes, Robbah, and Taleb Larbi), ensuring that only officially recognized farmers were included. Each selected participant managed a flock ranging from fewer than 20 to more than 100 animals, with categories defined as <20, 20–50, 50–100, and >100. All respondents operated under grazing-based systems typical of the region. Data collection involved in-person interviews and field observations to obtain detailed information on trait preferences. The questionnaire structure allowed sheep keepers to elaborate on their preferences and farming practices, while also including multiple-choice options to ensure data consistency and comparability across responses.

Statistical Analysis

A multivariate analysis of variance (MANOVA) was conducted to examine the effects of age, education, experience, and occupation on trait preferences among sheep keepers. The analysis was performed using IBM SPSS Statistics version 25.0. Pillai's trace was chosen as the primary multivariate test statistic because of its robustness to violations of assumptions, such as unequal sample sizes and heterogeneity of covariance matrices (Olson, 1976; Tabachnick & Fidell, 2019). The significance level was set at $\alpha = .05$. Partial eta squared (η^2) was used as a measure of the effect size to quantify the proportion of variance in the dependent variables explained by each independent variable or interaction. To further explore specific associations between individual socioeconomic variables and particular trait preferences, chi-square tests of independence were performed. When significant relationships were identified, Cramér's V was calculated to assess the strength of the associations. In addition, data visualization techniques, such as heatmaps and pie charts, were used to illustrate the relationships among the studied parameters and the distribution of trait preferences among respondents. These graphical representations were generated using R (version 4.4.2), enabling effective visual exploration of correlations and frequency patterns within the dataset.

3. Results

3.1. 3.1 Socio-economic characteristics

The socio-economic characteristics of respondents are presented in Table 1. The sample comprised exclusively male participants with heterogeneous educational backgrounds, ranging from primary education or less to university level, with basic schooling representing the predominant category. Age distribution spanned young to older adulthood, with the majority exceeding 25 years of age and middle-aged participants (36–45 years) forming the largest demographic cohort. Sheep breeding was the primary occupation for most respondents, while others engaged in it alongside civil service, trading, or other professional occupations. Years of experience demonstrated marked variability, encompassing novice practitioners with limited tenure to highly skilled breeders with extensive careers exceeding two decades.

Parameters	Category	No. Of respondents	Percentage (%)
Sex	Male	200	100%
	Female	0	0%
Age	Younger than 25	22	11.0
	From 26 to 35	27	13.5
	From 36 to 45	58	29.0
	From 46 to 55	50	25.0
	55 or older	43	21.5
Education level	Primary school or less	77	38.5
	Middle school	47	23.5
	Secondary school	53	26.5
	University	23	11.5
Occupation	Breeder	142	71.0
	Civil job	13	06.5
	Free occupation	28	14.0
	Trading	17	08.5
Experience years	Less than 5 years	32	16.0
	From 5 to 10 years	25	12.5
	From 10 to 15 years	44	22.0
	From 15 to 20 years	46	21.5
	More than 20 years	56	28.0

Table 1 – Socio-economic characterization of sheep keepers in El Oued, Algeria.

Figure 2 illustrates the cross-tabulation between educational attainment and occupational status among respondents. The data reveal a distinct pattern in which participation in sheep breeding as a primary occupation predominates among individuals with lower educational levels, with progressively diminishing engagement among those with higher academic qualifications. This distribution suggests that sheep farming in the region operates predominantly as a traditional vocation transmitted through inherited practical knowledge rather than through formal educational channels. Conversely, higher educational attainment appears to be correlated with occupational diversification, as university-educated respondents demonstrate a greater propensity for civil service or self-employment. The relative paucity of representation in commercial and professional categories across all educational strata indicates that livestock production remains a dominant economic activity, particularly among less formally educated cohorts. Collectively, these findings demonstrate an inverse association between educational level and direct involvement in sheep husbandry, underscoring education's role as a determinant of professional trajectory and economic diversification within this agrarian context.

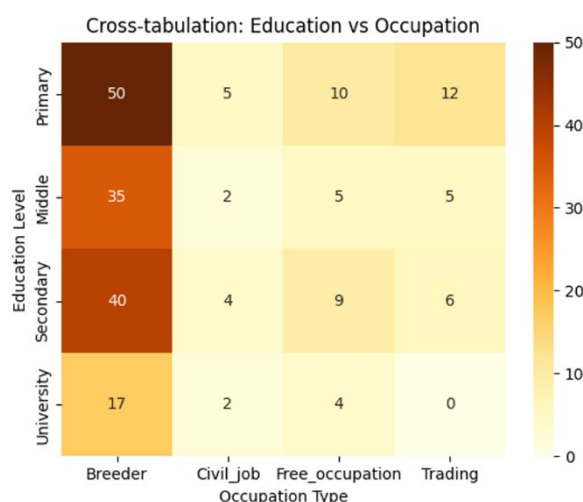


Figure 2 – Heatmap shows the relationship between education level and occupation type among male sheep keepers in El Oued, Algeria.

3.2. Characterization of Studied Farms

The farms studied were distributed throughout the El Oued region under homogeneous breeding conditions and comparable resource availability and infrastructure. All producers employed traditional grazing systems adapted to the arid climate, utilizing sparse vegetation surrounding oases and desert peripheries to sustain their herds. Geographic distribution exhibited spatial concentration within specific localities, particularly Robbah and Taleb Larbi, establishing these areas as principal centers of ovine production activity (Table 2). Flock demographics were predominantly small- to medium-sized operations, with most breeders maintaining moderate-sized herds, while large-scale enterprises remained notably scarce. The principal mechanism for flock establishment involved integrated strategies that combined hereditary transfer with market acquisition, reflecting a mixed approach to livestock procurement.

Parameters	Category	No. Of respondents	Percentage (%)
Location	El Oued	11	5.5
	Debila	06	3.0
	Guemar	19	9.5
	Hassi Khalifa	15	7.5
	Mih Ouensa	24	12.0
	Ourmes	19	9.5
	Robbah	57	28.5
	Taleb Larbi	49	24.5
Flock size	Less than 20 heads	77	38.5
	From 20 to 50	82	41.0
	From 50 to 100	28	14.5
	More than 100	13	6.50
Source of the herd	Inherited	21	10.5
	Purchase from the market	33	16.5
	Both	146	73.0

Table 2 – Characterization of studied farms of sheep keeper in El Oued, Algeria.

Figure 3A illustrates the spatial distribution of the surveyed sheep keepers across the eight municipalities of the El Oued region, showing both the number of respondents and their corresponding percentages for each site. Consistent with the data presented in Table 2, the figure clearly shows a concentration of sheep keepers in Robbah (28.5%) and Taleb Larbi (24.5%), confirming these two municipalities as the main centers of sheep production in the region. Moderate representation is observed in Mih Ouensa (12%), Guemar (9.5%), and Ourmes (9.5%), while El Oued (5.5%) and Debila (3%) record the lowest proportions of breeders. This spatial pattern underscores the unequal distribution of livestock activity across the study area, reflecting differences in rangeland availability, water resources, and grazing opportunities. Overall, the results indicate that sheep farming is primarily concentrated in the southern and border zones, where pastoralism remains a dominant livelihood, and traditional management practices continue to play a crucial role in sustaining rural economies.

Figure 3B presents the distribution of sheep keepers by flock size, highlighting the predominance of small- to medium-scale herds in the study area. The data, consistent with Table 2, show that the majority of respondents manage flocks containing 20 to 50

heads (41%), followed closely by those with fewer than 20 heads (38.5%). These two categories together account for nearly four-fifths of the total sample, reflecting the dominance of smallholder production systems in El Oued. By contrast, medium-sized flocks of 50 to 100 heads represent 14.5%, while only 6.5% of sheep keepers own herds exceeding 100 animals. This structure indicates that extensive large-scale breeding remains limited and that sheep farming in the region is primarily a family-based, traditional activity relying on modest herd sizes adapted to the arid environment and local resource constraints.

Figure 3C illustrates the main sources of herd acquisition among sheep keepers in the El Oued region. The results reveal that the majority of respondents (73%) acquired their herds through a combination of inheritance and market purchases, reflecting a mixed strategy that balances family continuity with market-based expansion. A smaller proportion, 16.5%, rely exclusively on purchasing animals from local markets, while only 10.5% reported that their herds were entirely inherited. These findings, in line with Table 2, indicate that most breeders maintain their flocks through both generational transfer and periodic renewal via market exchanges. This practice enhances herd diversity and resilience within the region's traditional livestock systems.

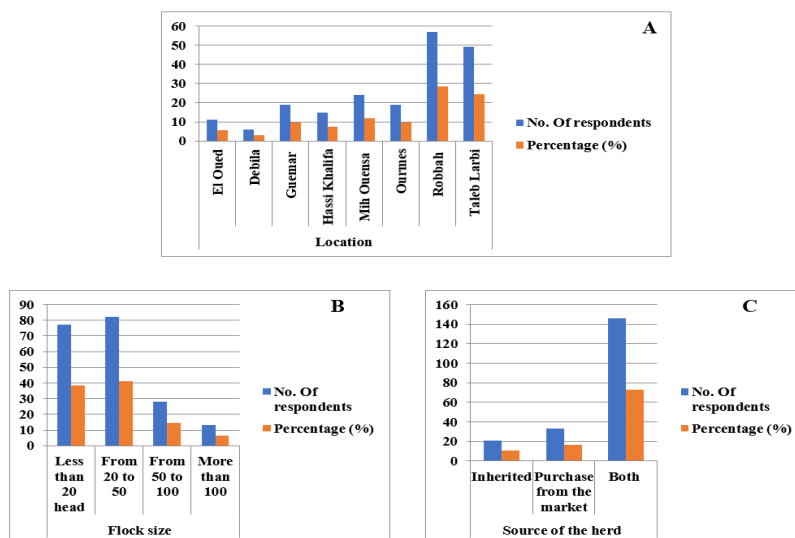


Figure 3 – Socioeconomic characteristics of male sheep keepers in El Oued, Algeria: (A) Spatial distribution of surveyed sheep keepers across the eight municipalities of El Oued, showing the number of respondents and their corresponding percentages per location. (B) Distribution of respondents according to flock size, highlighting the predominance of small and medium flocks (less than 50 heads). (C) Sources of herd acquisition indicate that most flocks originate from both inheritance and market purchases.

3.3. Selection of male and female sheep breeds

Selection criteria for breeding rams are illustrated in Figure 4. Breed identity emerged as the predominant consideration among respondents, substantially outweighing all other attributes. In particular, the Ouled Djellal breed was consistently cited as the preferred choice, reflecting its recognized adaptation to arid environments and its suitability for meat production systems in southern Algeria. Physical conformation and robustness constituted secondary priorities, with structural characteristics and apparent health status ranking prominently in the decision-making hierarchy. Reproductive and behavioral traits, including temperament and testicular size, represented intermediate preferences. In contrast, aesthetic features such as coat color and horn morphology, along with production-related attributes like growth rate and disease resistance, received comparatively modest emphasis. Notably, chronological age was accorded minimal priority compared with morphological and genetic qualities, suggesting that phenotypic superiority and breed integrity supersede youth in ram selection criteria in this production context.

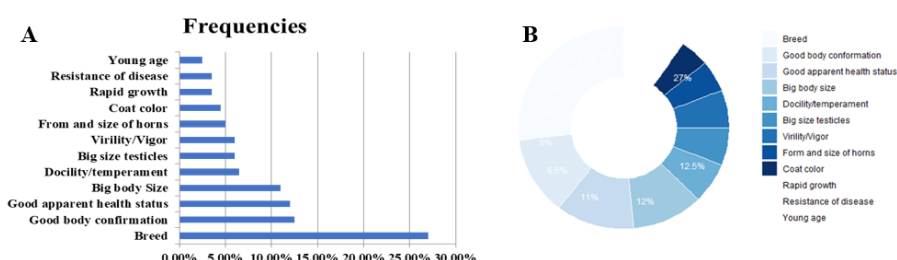


Figure 4 – Male selection traits preferred by male sheep keepers in El Oued, Algeria.

The breed selection criteria are illustrated in Figure 5. Breed identity constituted the foremost priority among respondents, substantially outweighing other considerations. In particular, the Ouled Djellal breed was predominantly preferred, reflecting its well-established adaptation to arid conditions and its suitability for extensive production systems in southern Algeria. Environmental adaptation emerged as a prominent secondary factor, with drought tolerance ranking highly in the selection hierarchy, reflecting the ecological constraints of the arid production environment. Udder morphology and apparent health status were significant intermediate priorities, underscoring the importance of reproductive function and vitality in the female stock. Physical conformation and temperament ranked relatively high in importance, while structural attributes such as body size and disease resistance, along with aesthetic characteristics including coat color, occupied lower positions in the preference ranking. Production-oriented traits such as growth rate and feeding efficiency, alongside chronological age, received comparatively modest emphasis, indicating that genetic integrity, adaptive capacity, and reproductive soundness supersede accelerated development or ease of management in ewe selection within this pastoral context.

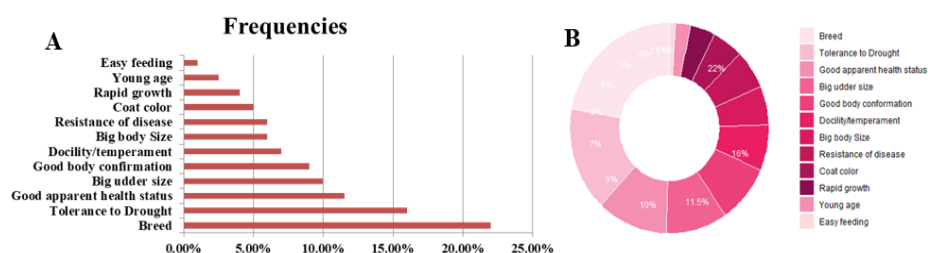


Figure 5 – Female selection traits preferred by sheep keepers in El Oued, Algeria.

3.4. Effect of socio-economic characterization on trait preferences

Multivariate analysis revealed a significant three-way interaction between education, experience, and occupation (Pillai's Trace = 0.070, $F(2, 128) = 4.833$, $p = .009$, partial $\eta^2 = .070$). No other main effects or interactions were significant (all $p > .05$). This suggests that the combined effects of education, experience, and occupation significantly influenced trait preferences among sheep keepers (Table 3).

Effect	Value	F	Sig.	Partial Eta Squared
Intercept	.648	117.760 ^b	.000	.648
Main effect				
Age	.038	.631	.751	.019
Education	.029	.642	.696	.015
Experience	.049	.814	.591	.025
Occupation	.053	1.182	.316	.027
Two-way effect				
Age / Education	.122	1.401	.165	.061
Age / Experience	.120	1.369	.181	.060
Age / Occupation	.084	1.419	.189	.042
Education / Experience	.128	.980	.484	.064
Education / Occupation	.058	.637	.810	.029
Experience / Occupation	.065	1.075	.381	.032
Three-way effect				
Age / Education / Experience	.052	1.722	.145	.026
Age / Education / Occupation	.000	. ^b	.	.
Age / Experience / Occupation	.000	. ^b	.	.
Education / Experience / Occupation	.070	4.833 ^b	.009	.070

Table 3 – Effect of socio-economic characterization on trait preferences in El Oued, Algeria.

3.5. Socioeconomic Determinants of Trait Preferences

Chi-square analysis revealed two statistically significant associations between farmer characteristics and trait preferences (Table 4). Farmer age significantly influenced the selection of drought tolerance in ewes ($\chi^2(4)=10.26$, $p=0.036$, Cramer's $V=0.227$). Post-hoc analysis of standardized residuals indicated that farmers aged 56 or older selected drought tolerance significantly less frequently than expected (2.3% vs. 16.0% overall; standardized residual = -2.24). Conversely, the youngest farmers (<25 years) showed the

highest preference for drought tolerance (27.3%), though this did not exceed the critical threshold for statistical significance in residual analysis (standardized residual = +1.32). The effect size was small to medium.

Education level showed a significant association with drought-tolerance preference ($\chi^2(3)=13.37$, $p=0.004$, Cramer's $V=0.259$). Farmers with secondary education exhibited significantly higher selection rates for drought tolerance than expected (30.2% vs. 16.0% overall; standardized residual = +2.58). In contrast, farmers with only primary education showed significantly lower selection rates (7.8%; standardized residual = -1.80). University-educated farmers demonstrated intermediate preferences (21.7%). This represents a medium effect size.

No significant associations were detected between socioeconomic variables and male trait preferences, nor between experience/occupation and female trait preferences. However, marginal associations were observed between education and preferences for good body confirmation ($p=0.087$) and udder size ($p=0.090$) in females.

Trait (Selection Type)	Predictor	χ^2	df	p-value	Cramer's V	Effect Size
Female: Drought Tolerance	Age	10.26	4	0.036*	0.227	Small-Medium
Female: Drought Tolerance	Education	13.37	3	0.004**	0.259	Medium
Female: Drought Tolerance	Experience	4.17	4	0.383	0.144	Small-Medium
Female: Drought Tolerance	Occupation	4.61	3	0.203	0.152	Small-Medium
Female: Good Body Confirmation	Education	6.56	3	0.087	0.181	Small
Female: Big Udder Size	Education	6.48	3	0.090	0.180	Small
Male: Good Apparent Health Status	Experience	7.71	4	0.103	0.196	Small
Female: Breed	Age	7.39	4	0.117	0.192	Small

* $p < 0.05$, ** $p < 0.01$ (chi-square test of independence)

Table 4 – Effect of socio-economic characterization on trait preferences in El Oued, Algeria.

4. Discussion

The dominance of male interviewees in the present study aligns with findings from Laoubi et al. (2011) in the Laghouat region (94.3%) and from Kadi et al. (2014) in the Kabyle mountainous area of Algeria (86.2%). This gender disparity is likely due to the traditional and cultural structure of society, where men are typically the primary decision-makers in livestock management and are less likely to allow women to participate in interviews. This cultural dynamic may limit women's perspectives and experiences in shaping breeding practices and trait preferences, despite their potential contributions to household livestock management. The exclusive male composition of the sample constitutes a methodological limitation of this study. Future research should employ gender-responsive sampling strategies to capture women's trait preferences and decision-making roles, particularly given documented evidence that women manage substantial proportions of small-ruminant herds in North African agropastoral systems (Gizaw et al., 2010). The proportion of illiterate individuals in this study (44.34%) was lower than that reported by Ouchene-Khelifi et al. (2021) among goat farmers across various Algerian regions. However, the relatively low literacy level in El Oued can be attributed to the remoteness of schools and limited access to educational infrastructure, particularly in livestock-breeding areas. This lack of education may hinder sheep keepers' ability to adopt advanced breeding practices, record pedigrees, and monitor animal performance. Educated sheep keepers are more likely to prioritize traits that enhance productivity and resilience, such as growth rate and disease resistance. In contrast, less-educated keepers may rely more on traditional knowledge and prioritize visible traits such as body size or coat color (Gizaw et al., 2010).

The low percentage of breeders under 30 years old (11%) indicates a lack of interest among young people in sheep farming. This trend may be driven by the pursuit of professions with faster, easier revenue streams, such as business or trading. The aging population of sheep keepers raises concerns about the future sustainability of sheep farming in the studied region, as younger generations may not continue traditional practices. This finding aligns with observations by Meziane et al. (2024) in the Batna region of Eastern Algeria, where the younger population views sheep farming as less profitable than other career options due to its physical demands and economic challenges. A similar trend was reported by Amare et al. (2021) in pastoral areas in Ethiopia, where young people are increasingly moving away from livestock farming due to economic and social factors. This demographic trend underscores an urgent need for targeted policy interventions, including youth-oriented incentive schemes, vocational training in modern animal husbandry, and access to microcredit, to attract younger entrants and ensure the intergenerational transfer of indigenous breeding knowledge before it is irretrievably lost.

The majority of respondents (71%) were full-time breeders, reflecting the central role of sheep farming in their livelihoods. This aligns with findings from Meziane et al. (2024) in Batna, Algeria, where only a small proportion (10%) of the surveyed sheep keepers pursued other professions such as teaching or commerce. Similar results from Yakubu et al. (2020) in Nigeria showed that full-time breeders prioritized traits such as growth rate and meat quality to maximize economic returns. However, the study also found that cultural and religious significance influenced trait preferences regardless of occupation, suggesting that occupation status may not heavily influence specific preferences. For example, in the studied region, the high demand for sheep meat during religious festivals may drive preferences for traits such as body size and health status, regardless of the sheep keepers' primary occupation.

Experience also plays a critical role in shaping trait preferences. In the study region, 28% of respondents had more than 20 years of experience, while 16% had less than 5 years of experience. Experienced sheep keepers are more likely to prioritize traits that ensure long-term flock productivity and resilience, such as drought tolerance and health status. This is consistent with findings from Amare et al. (2021), who reported that experienced sheep keepers in Afar, Ethiopia, prioritized fast growth and trekking ability to

cope with arid conditions. In contrast, less experienced sheep keepers may focus on short-term gains, such as rapid growth or market value.

The geographical distribution of respondents shows that Robbah and Taleb Larbi are the most prominent areas for sheep farming in El Oued wilaya. This concentration is likely driven by improved access to oasis resources, groundwater, and crop residues for supplementary feeding, proximity to local markets, and long-standing farming traditions in these districts. The predominance of small- to medium-sized flocks (fewer than 50 sheep) aligns with recent findings from arid and steppe regions of Algeria, where smallholder systems prevail due to limited capital, feed resources, and infrastructure constraints (Faradji et al., 2022; Meziane et al., 2024; Ouali et al., 2023). Such modest flock sizes are typical in resource-scarce arid environments, limiting many keepers' ability to expand operations. However, the presence of a small percentage of respondents with flocks exceeding 100 sheep indicates that some breeders are gradually scaling up, often linked to improved access to resources, market integration, or supplementary income sources. Most respondents (73%) acquired their herds through a combination of inheritance and market purchases. This mixed strategy reflects the interplay between traditional practices and modern market dynamics in flock building. Inheritance remains a cornerstone in many Algerian pastoral and agropastoral systems, ensuring the continuity of family-owned livestock, the preservation of locally adapted breeds, and the transmission of indigenous knowledge across generations (Amare et al., 2021; Bencherif et al., 2021; Meziane et al., 2024). Market purchases, on the other hand, enable keepers to introduce new genetic material, potentially enhancing traits such as growth rate, disease resistance, and reproductive performance. This dual approach demonstrates the adaptive capacity of sheep keepers in the El Oued region, allowing them to balance cultural continuity with innovation amid environmental and economic pressures.

The survey results indicate that breed, good body conformation, and large body size were the most important criteria for selecting male sheep (rams) in the El Oued region. Among the breeds mentioned by respondents, the Ouled Djellal breed was predominantly preferred, reflecting its well-known adaptation to arid conditions and desirable productivity traits. These findings both support and contrast with previous studies on sheep breeding preferences in various African regions, where physical appearance and reproductive potential are often highly valued. However, the relative importance of specific traits varies by production system and environment. In the present study, the top-ranked trait, "breed," reflects the value placed on locally adapted types that ensure survival and productivity under arid conditions. This aligns with broader patterns in smallholder systems, where observable physical traits linked to market value and flock expansion are prioritized.

For instance, Abebe et al. (2020) reported that body size and coat color were among the primary criteria for ram selection in the northwest highlands of Ethiopia, with body size receiving the highest index. Similarly, in southern Ethiopia, Hemacha et al. (2022) found that farmers placed strong emphasis on body size and conformation when selecting breeding rams. These preferences are consistent with the current study's focus on good body conformation and large body size, suggesting that sheep keepers in arid environments such as El Oued are commonly selected for strong structural features that are assumed to improve breeding success, meat yield, and overall flock resilience. The importance attributed to "good apparent health status" (12%) further underscores the need for robust animals that can thrive in challenging conditions. This finding aligns with Yakubu et al. (2020) in Nigeria, who identified disease resistance and overall health as top breeding priorities among sheep farmers. Additionally, the emphasis on "big size testicles" (6%) highlights attention to reproductive capacity, echoing results from pastoral systems where traits related to fertility and growth potential receive notable consideration (Amare et al., 2021; Meziane et al., 2024). Overall, these preferences illustrate how sheep keepers in the El Oued region balance adaptation to harsh arid environments with economic and productive goals, while revealing both commonalities and contextual differences with other African smallholder systems.

In the present study, the relatively low importance given to docility/temperament (6.5%) contrasts with broader research on sheep behavior, which shows that temperament traits can be consistently selected for and may influence ease of handling and welfare (Beausoleil et al., 2012). This difference could stem from differing livestock management systems or cultural perspectives, in which physical and health-related traits are prioritized over behavioral traits in harsh, arid environments such as El Oued. In such contexts, observable attributes linked to survival, productivity, and market value often take precedence over temperament. Similarly, rapid growth (3.5%) and disease resistance (3.5%) received lower rankings compared to breed, body conformation, and size. This pattern differs from several Ethiopian studies, where smallholder farmers frequently prioritize growth rate and survival traits to cope with environmental stresses (Abebe et al., 2020; Hemacha et al., 2022b). The lower emphasis in El Oued may reflect that these traits are implicitly assumed within preferred local breeds or are less immediately visible to keepers under traditional extensive systems, where short-term observable physical features dominate decision-making. The minimal preference for young age (2.5%) further indicates that sheep keepers place greater weight on physical and genetic attributes than on age at selection. This aligns with findings from Duguma et al. (2011), who reported that preferences centered more on physical and reproductive characteristics. In contrast to studies in Ethiopia and Botswana, where coat color was sometimes ranked highly for cultural or market reasons (Bolowe et al., 2022), this trait received little emphasis in the current study. This variation likely reflects the unique environmental pressures and functional priorities in the arid El Oued region, where adaptation and productivity traits outweigh aesthetic ones.

Finally, horn shape and size (5%) were considered of low importance, consistent with research showing that such secondary morphological traits rank below body size, conformation, and health in ram selection (Abebe et al., 2020; Hemacha et al., 2022b). Overall, these results highlight how breeding preferences in the study area are shaped by the demanding arid conditions, favoring traits that support flock resilience and economic returns.

The results from the studied region highlight the importance of genetic quality, adaptability to harsh environmental conditions, health, and milk production potential in the selection of female sheep (ewes). The strong prioritization of breed attributes in our study aligns with findings from Abebe et al. (2020), who reported that smallholder sheep keepers in the northwest highlands of Ethiopia placed a high emphasis on genetic and physical traits, such as body size and coat color, when selecting breeding ewes. However, body size received greater prominence in Ethiopian highlands, suggesting regional differences in the breeding objectives shaped by local environmental and production challenges. In the arid context of El Oued, keepers appear to prioritize the breed's overall adaptability to drought and resource scarcity over individual morphological traits. The high value placed on drought tolerance (16%) is consistent with the recognized importance of adaptive traits in smallholder systems facing environmental stress. Similar emphasis on resilience-related traits (such as trekking ability and survival under harsh conditions) has been documented in pastoral areas (Amare et al., 2021; Meziane et al., 2024). This underscores the critical need for breeding programs in arid zones like El Oued to target traits that enhance long-term survival and productivity amid recurrent drought and fodder scarcity.

The preference for “good apparent health status” (11.5%) and “big udder size” (10%) reflects a clear focus on animal robustness and milk production potential, both essential for lamb survival and overall flock productivity. This finding corresponds with studies in Nigeria, where disease resistance and health were ranked among the top breeding priorities (Yakubu et al., 2020), and with research in Ethiopian pastoral systems, where milk yield and mothering ability were highly valued (Amare et al., 2021). The moderate emphasis on “good body conformation” (9%) and “docility/temperament” (7%) is consistent with Hemacha et al. (2022), who identified body size and mothering ability as key criteria for ewe selection in southern Ethiopia. However, the relatively low importance of temperament in our study contrasts with research showing that behavioral traits can be reliably selected for and may improve handling and welfare (Beausoleil et al., 2012). This discrepancy likely arises from differences in extensive management practices and cultural attitudes toward animal behavior in arid versus other production systems. Notably, the emphasis on udder morphology in the present study is particularly relevant under arid conditions where colostrum availability and early lamb nutrition are critical determinants of neonatal survival during heat stress and feed scarcity; this suggests that participatory breeding indices for the El Oued region should formally incorporate udder conformation as a quantifiable selection criterion. Overall, these preferences illustrate how sheep keepers in the El Oued region adapt selection criteria to the specific demands of arid environments, balancing genetic quality, resilience, and productive traits.

For the design of sustainable genetic improvement programs, a thorough understanding of existing reproductive performance, community trait preferences, and the relative importance of different production constraints is essential (Baker & Gray, 2004). Education also plays a pivotal role, as it influences household income, adoption of improved technologies, demographic patterns, health outcomes, and the overall socio-economic status of livestock-keeping communities (Meziane et al., 2024; Tabachnick & Fidell, 2021). The present study's statistical results further demonstrate the combined influence of education, experience, and occupation on trait preferences. Although the main effects of these socio-demographic factors were not statistically significant, their three-way interaction was significant ($p = 0.009$, partial $\eta^2 = 0.070$). This indicates that sheep keepers with higher education levels, extensive practical experience, and full-time involvement in breeding tend to prioritize traits associated with long-term flock productivity and resilience (e.g., Adaptability and health). In contrast, less educated or less experienced keepers often focus on more immediately visible traits such as body size or coat color, which align with traditional knowledge and short-term economic returns. These findings highlight the need to account for the complex interplay between education, experience, and occupation when developing participatory breeding programs, ensuring that interventions are tailored to the diverse priorities and capacities of sheep keepers in arid regions such as El Oued.

The findings from the present study reveal both similarities and differences in sheep trait preferences compared to other African regions. For male sheep (rams), the strong emphasis on breed and physical traits such as body size and conformation aligns with studies in Ethiopia and Botswana, reflecting a widespread preference for traits that enhance reproductive performance, meat yield, and economic value (Abebe et al., 2020; Bolowe et al., 2022). However, the lower priority assigned to coat color in El Oued highlights the influence of regional environmental challenges and cultural practices, where functional adaptation often outweighs aesthetic traits.

For female sheep (ewes), the high value placed on drought tolerance and udder size (linked to milk production potential) contrasts with the greater emphasis on body size and twinning ability observed in many Ethiopian highland and pastoral systems (Abebe et al., 2020; Amare et al., 2021; Hemacha et al., 2022b). These differences underscore the importance of developing context-specific breeding objectives tailored to local agro-ecological conditions.

The observed generational difference in drought tolerance preference is consistent with findings reported in similar arid and semi-arid production systems. For instance, Meziane et al. (2024) highlighted that younger sheep farmers in eastern Algeria exhibit greater sensitivity to climate-related constraints and are more inclined toward adaptive management practices. In contrast, older farmers tend to rely on traditional, visually assessable traits. Comparable patterns have also been documented in Ethiopian pastoral systems, where younger and more educated farmers place higher importance on resilience-related traits such as trekking ability and survival under environmental stress (Amare et al., 2021; Hemacha et al., 2022). Furthermore, the significant influence of education observed in the present study aligns with Abebe et al. (2020), who demonstrated that higher education levels are associated with increased consideration of functional and adaptive traits in livestock selection. However, the pronounced effect at the secondary education level in this study suggests a context-specific dynamic in arid regions such as El Oued, where this level of education may provide sufficient awareness of climate challenges without distancing farmers from practical livestock engagement.

These findings reveal two key dynamics shaping trait preferences in the El Oued region. First, there is a clear generational shift toward greater awareness and valuation of climate resilience among younger farmers. Second, education emerges as a critical driver of adaptive decision-making, with secondary schooling playing a particularly influential role. In contrast, older and less-educated farmers continue to prioritize readily observable traits such as breed, body size, and conformation, likely reflecting reliance on accumulated experiential knowledge and immediate productivity needs in a resource-constrained environment.

Several limitations of the present study should be acknowledged. First, the exclusively male sample restricts the generalizability of findings. It omits the perspectives of women sheep keepers, whose trait preferences and household decision-making roles may differ substantially in this agropastoral context. Second, the cross-sectional design captures preferences at a single point in time and cannot account for temporal shifts in breeding objectives driven by interannual climate variability or changing market conditions. Third, self-reported trait rankings obtained through questionnaire surveys may be subject to social desirability bias, particularly for culturally prestigious traits such as breed identity. Fourth, the study was confined to eight municipalities within El Oued Province, limiting direct extrapolation to other arid zones of Algeria. Future research should adopt gender-inclusive and longitudinal designs and employ conjoint-based discrete choice experiments to derive the relative economic weights of preferred traits—an essential step toward constructing formal breeding objective indexes applicable to the Ouled Djellal and other locally adapted breeds in North African drylands.

Overall, while certain traits such as reproductive performance, health, and body conformation remain universally important across smallholder systems, others are strongly influenced by local environmental pressures, market demands, and farmer priorities; therefore, breeding and extension programs in arid regions like El Oued should prioritize resilience traits (e.g., drought tolerance and adaptability) alongside productivity, while incorporating farmers' economic, cultural, and traditional knowledge and remaining sensitive to socioeconomic differences, particularly age and education to tailor communication and interventions that enhance the adoption of resilience-oriented selection criteria and ultimately support long-term genetic resilience and sustainability livestock production under increasing climate variability. Practically, this implies that national breeding programs and extension services in Algeria should move beyond top-down genetic improvement schemes and instead co-design community breeding programs that anchor selection indexes on the trait priorities documented here, breed identity and drought tolerance for both sexes, udder conformation for ewes, and body conformation for rams progressively incorporating objective performance recording as farmer literacy and infrastructure improve. Sustained investment in participatory genetic resource management, coupled with genomic characterization of locally adapted breeds such as Ouled Djellal, will be essential to safeguarding the productive and adaptive capital of Algerian sheep populations under accelerating climate change.

5. Conclusion

In conclusion, this study provides valuable insights into the socioeconomic characteristics and breeding preferences of sheep keepers in El Oued Province, Algeria. The community is predominantly male, with many keepers over 36 and substantial experience in the sector. Preferred traits such as breed, body conformation, health status, and drought tolerance reflect a strategic emphasis on sustainability and adaptation to the region's harsh environmental conditions. The prioritization of resilience-related traits in both male and female sheep underscores the importance of long-term viability over short-term productivity. Interestingly, the relatively low focus on rapid growth and early maturity indicates that sheep keepers prioritize herd stability and genetic integrity over immediate commercial returns. The emphasis on breed quality, virility, and docility demonstrates a practical understanding of the need for manageable, productive, and robust flocks in resource-limited environments.

Furthermore, the predominance of herds obtained through both inheritance and market acquisition reveals the coexistence of traditional knowledge and economic adaptation, contributing to the preservation of genetic diversity. Collectively, these findings highlight the deep-rooted adaptive strategies employed by local breeders to sustain sheep production under arid and semi-arid conditions. Looking forward, integrating traditional selection practices with modern breeding programs, farmer education, and genetic improvement initiatives will be crucial for enhancing productivity, sustainability, and resilience. Future research should focus on genomic characterization of local breeds and the development of tailored breeding strategies to ensure the long-term sustainability of sheep farming in El Oued and comparable dryland regions.

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Doutorado em Ciências Veterinárias

Rosangela Locatelli Dittrich

Iniciei minha jornada no PPGCV em 2003, como docente permanente, orientadora e pesquisadora. Quando fiz o credenciamento na pós-graduação, o programa era denominado “Curso de Pós-graduação em Ciências Veterinárias (CPGCV)”, com a capacitação de pesquisadores a nível de mestrado.

O CPGCV foi criado em 1986 e durante muitos anos os objetivos foram sendo alcançados, com a formação de Mestres e pesquisadores em diversas áreas, cumprindo seu papel com excelência no desenvolvimento da pesquisa, formação de docentes e capacitação de profissionais.

Entretanto, por algumas razões, no triênio de 2001-2003 o CPGCV recebeu o conceito 3, na avaliação da CAPES. O conceito 3 é o padrão mínimo, com recomendação de “aspectos a melhorar”. Esse conceito não poderia repetir ou diminuir, porque o curso seria descredenciado! Dessa forma, a preocupação foi grande por parte dos docentes e servidores, porque o CPGCV não poderia fechar, seria uma perda imensa.

A partir de 2005 resolvi colaborar no CPGCV, participando e exercendo cargo administrativo no curso. De 2005 a 2007 fui vice coordenadora, sendo o Prof. Marson B. Warpechowski o coordenador, e de 2007 a 2009 assumi a Coordenação.

Houve grande empenho do colegiado do curso, coordenadores e docentes em realizar várias modificações para elevar o conceito para 4 e enviar a proposta de criação do doutorado à CAPES. Recebemos os avaliadores da CAPES em 2006, e houve ampla discussão sobre os indicadores do curso. Em 2007 assumi a coordenação do curso, com a Professora Alda Lúcia Gomes Monteiro como vice coordenadora, e o colegiado constituído pelos docentes Geraldo Camilo Alberton, Fabiano Montiani Ferreira, Tilde Froes, Elizabeth Santin, Alex Maiorka, Marson B. Warpechowski, João Ricardo Dittrich, Paulo Rossi Júnior; e representantes discentes Ronald P.M. Gonçalves e Amanda Schutzemberg.

Continuamos o árduo trabalho com os objetivos principais de elevar o conceito do curso para 4 e enviar a proposta de criação do Doutorado. Entre as principais realizações, destaca-se a Reforma Curricular em 2007, com alterações na grade curricular, sendo mantidas algumas disciplinas, outras extintas e várias foram criadas. Em dezembro de 2007 a reformulação curricular foi apreciada e aprovada no Conselho de Ensino e Pesquisa - CEPE. Em 2008 foi implementada a grade curricular totalmente reformulada para atender os alunos e acompanhar o desenvolvimento científico nas cinco linhas de pesquisa do curso.



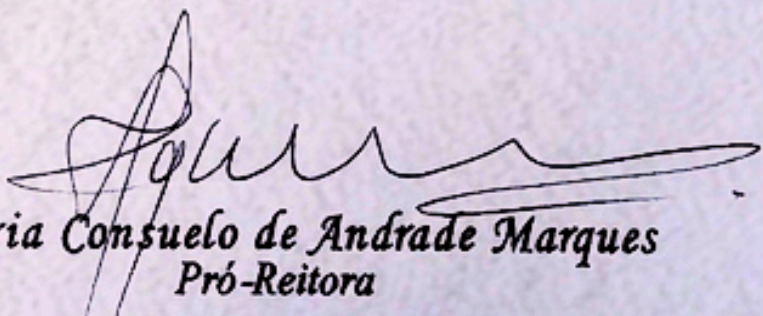
Archives of Veterinary Science

Várias modificações foram implementadas no Regimento Interno do Curso para aumentar o conceito, como: (1) aplicação de índices de produtividade de participação responsável nas atividades do curso para a manutenção do credenciamento do docente; (2) controle rígido dos planos de trabalho dos discentes; (3) regulamentação da disciplina de prática de docência; (4) alterações no processo seletivo; (5) instituição dos comitês de orientação; (6) alterações no formato da prova de qualificação e da dissertação, para obter aumento das publicações no curso e (7) instituição de critérios de produtividade para renovação anual das bolsas de mestrado pelos alunos.

E o resultado apareceu!! Na avaliação trienal da CAPES de 2004-2007 o curso recebeu o conceito 4, demonstrando o grande potencial do corpo docente, atribuído ao desempenho e dedicação de professores e alunos, atingindo, um dos principais objetivos propostos.

No período verificou-se a consolidação dos pesquisadores, com aumento das publicações, número de projetos de pesquisa com financiamento nas agências de fomento, melhoria de infraestrutura dos laboratórios e das instalações do curso.

Trabalhei com os professores e colegiado para implementar o Curso de Doutorado. Organizamos o processo, com os formulários e documentações necessárias e o envio do projeto de criação à CAPES foi em 28/03/2008.



Maria Consuelo de Andrade Marques
Pró-Reitora

É importante ressaltar que em maio de 2008 foi realizada uma solenidade no Setor de Ciências Agrárias da UFPR, em Homenagem ao **Prof. Dr. Metry Bacila**, pesquisador brilhante que durante grande parte da sua vida investigou, estudou, buscou soluções, introduziu novos conhecimentos, enfim, pesquisou e inovou. Na cerimônia estavam presentes o Prof. Metry Bacila e sua família, convidados da Pró Reitoria de Pós-Graduação da UFPR, do curso de Pós-graduação, professores, alunos ex-orientados, técnicos administrativos e discentes. O Prof. Dr. Bacila recebeu um agradecimento e homenagem em nome do Curso, por sua imensa contribuição a ciência e à pós-graduação, por ser exemplo de dedicação, doação e de dignidade.

Segundo o Prof. Dr. Bacila, o Curso de Doutorado “era um sonho a ser realizado”. E o sonho se tornou realidade...

Em julho de 2008 recebemos a excelente notícia que o Conselho Técnico Científico da CAPES decidiu recomendar o Curso de Pós-Graduação em Ciências Veterinárias, nível Doutorado, atribuindo-lhe a nota 4. Onze docentes foram recomendados para orientarem no doutorado, sendo: Alda Lúcia Gomes Monteiro, Alex Maiorka, Antonio Felipe Paulino de Figueiredo Wouk, Antonio Ostrensky Neto, Elizabeth Santin, Fabiano Dalhke, Fabiano Montiani Ferreira, João Ricardo Dittrich, Marcelo Beltrão Molento, Paulo Rossi, Rosangela Locatelli Dittrich.

Houve a renomeação de “Curso” para **“Programa de Pós-Graduação em Ciências Veterinárias”**, e em **2009 iniciou a primeira turma de pós-graduandos no DOUTORADO.**



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Curitiba, 07 de agosto de 2008.

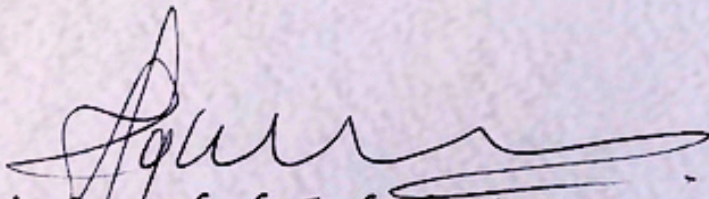
Ílma. Prof.ª

Dr.ª Rosângela Locatelli Dittrich

Coordenadora do Programa de Pós-Graduação em Ciências Veterinárias

Em nome da equipe desta Pró-Reitoria a parabenizo pelo tão esperado credenciamento através da Capes - Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, do curso de pós-graduação, nível de Doutorado, que passa a agregar o Programa de Pós-Graduação em Ciências Veterinárias.

De maneira extensiva, cumprimento os integrantes do Corpo Docente, Corpo Discente e Servidores Técnico-Administrativos deste Programa de Pós-Graduação pelo esforço e dedicação no alcance deste objetivo que, sobremaneira, contribui para elevar ainda mais o prestígio desta instituição em níveis nacional e internacional.


Maria Consuelo de Andrade Marques
Pró-Reitora

Foi uma conquista muito importante à Pós-graduação em Ciências Veterinárias, com os objetivos de incrementar a produção de conhecimento científico na ampla área de concentração, formar pesquisadores e profissionais, e capacitar, de maneira interdisciplinar, profissionais para o exercício da docência, da pesquisa e da atividade autônoma.

O trabalho foi árduo, mas muito gratificante, com conquistas essenciais e imprescindíveis ao Programa de Pós-graduação em Ciências Veterinárias da UFPR e à ciência. Os frutos estão sendo colhidos até a presente data, junho de 2026, com o atual conceito 6 da CAPES e os inúmeros pesquisadores, mestres e doutores entregues à sociedade.

Obrigada!



Rosangela Locatelli Dittrich

É Professora Titular, MV, MSc, PhD
no Departamento de Medicina Veterinária e no
Programa de Pós-graduação em Ciências
Veterinárias sua área de atuação é Patologia
Clínica Veterinária – Hematologia e Bioquímica
clínica de mamíferos, aves, répteis e peixes.
Neosporose. Toxoplasmose.

Vencemos!

Rosangela Locatelli Dittrich



Understanding the social dynamics of exotic pet ownership in Brazil: insights from an online survey on legal and illegal trade

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Abstract: Illegal wildlife trade is a multi-billion-dollar industry with profound ecological and social consequences worldwide. Recognizing that a multidisciplinary approach is essential to address this issue, this study aimed to understand the social dynamics of exotic pet consumption in Brazil, focusing on consumer preferences, motivations, and education. A cross-sectional anonymous online survey collected 1,706 responses. Results showed that 88.3% of respondents knew someone who owned or currently owns an exotic pet, while 48.9% had owned or currently own one. Moreover, 63.6% expressed interest in owning an exotic pet in the near future. Men, individuals aged 30–49, and respondents with higher education were more likely to own or desire to own exotic pets, both legally and illegally. Among those who owned illegal pets, most reported that the animal died or was surrendered, while about 10% were released or the animal had escaped. The price gap between legal and illegal pets significantly influenced acquisition decisions: although 40% chose legal pets despite higher costs, when the gap reached 10-fold, preferences split evenly. About 50% of the responders answered questions about wildlife legislation correctly, reflecting limited legal awareness and the cultural normalization of wildlife trade in Brazil. These findings indicate that the scarcity of affordable legal pets and the high compliance costs for breeders drive consumers toward illegal markets, while low reporting rates hinder enforcement. Strengthening environmental education, promoting legal awareness, and reducing the economic gap between legal and illegal markets may foster responsible ownership and support multidisciplinary strategies to mitigate domestic illegal wildlife trade.

Keywords: Conservation, responsible pet ownership, traffic, wildlife.

1. Introduction

Wildlife trade represents a multi-billion-dollar global industry, involving the annual trade of millions of fungi, plants, and animals for timber, medicines, fashion items, food, and pets worldwide. Brazil, one of the most biodiverse countries and a global hotspot for wildlife, plays a central role in both the supply and demand sides of wildlife trade, reflecting its ecological richness and strong domestic market for fauna. Among these activities, illegal wildlife trade stands out as one of the most lucrative forms of illicit commerce worldwide (Herrel & van der Meijden, 2014; Hughes, 2021; Nijman, 2010; Robinson et al., 2015; Smith et al., 2009). The global value of this illegal trade—encompassing hunting, logging, fishing, and trafficking in wild fauna and flora—is estimated at USD 91–258 billion annually (Hughes, 2021; Sas-Rolfes et al., 2019; Scheffers et al., 2019; South & Wyatt, 2011). Moreover, it has been increasingly linked to other forms of transnational organized crime (Felbab-Brown, 2018; Massé et al., 2020; van Uhm, 2021).

Beyond its economic magnitude, illegal wildlife trade has profound ecological and social consequences, posing a severe threat to biodiversity, particularly in Africa, Asia, and South America (Alves et al., 2013; Challender et al., 2014b; Karesh et al., 2005; Scheffers et al., 2019; Smith et al., 2019; Sutherland et al., 2019). It is estimated that one in four species on Earth is affected by the wildlife trade. Vertebrates are the most traded animal group, with fish accounting for more than half of these species, primarily as food. Amphibians, reptiles, and birds are also widely traded as pets, with amphibians being the most threatened vertebrate group (Scheffers et al., 2019).

The illegal or unsustainable trade of exotic species (i.e., non-domesticated animals kept as pets) undermines global conservation efforts (Altherr & Lameter, 2020; Auliya et al., 2016; Nijman et al., 2018; UNODC, 2016). The pet trade has expanded substantially in recent decades, both internationally and domestically (ABINPET, 2023; Bush et al., 2014), raising concerns over animal welfare (Peng & Broom, 2021; Sollund, 2011), biological invasions (Lockwood et al., 2019), and zoonotic disease risks (Chomel et al., 2007). Although international trade has shifted from wild-sourced to captive-sourced products, driven by trade treaties, import bans, and breeding programs (Harfoot et al., 2018; Jepson & Ladle, 2005; Robinson et al., 2015), millions of wild animals are still traded each year. Meso- and Latin America remain major exporting regions, with large proportions of wild-caught and ranched individuals (Hughes, 2021; Robinson et al., 2015). In Brazil alone, at least 38 million animals are captured from the wild annually, and around four million are traded illegally, mainly in domestic markets (Destro et al., 2012; Maximo et al., 2021; RENCITAS, 2001).

From a social perspective, illegal domestic wildlife trade in Brazil is typically characterized as disorganized crime composed of transient networks rather than hierarchical organizations. These involve catchers or breeders, resellers, and consumers (Oliveira et al., 2020; Wyatt et al., 2020), often relying on opportunistic intermediaries—such as truck drivers, border officials, or couriers—who facilitate trade without belonging to structured criminal groups (Pires & Moreto, 2011; Wyatt et al., 2020). While this market is mainly in rural areas and small towns, its products often reach large urban centers such as São Paulo, where demand is higher

(Alves et al., 2013; Magalhães & São-Pedro, 2012; Oliveira et al., 2020). Although CITES regulates high-value international wildlife trade, a large portion of the domestic pet market remains unregulated and largely uninspected (Hughes, 2021).

In Brazil, despite robust regulations on exotic pet ownership, illegal trade in native and non-native reptiles, amphibians, and birds remains widespread (Alves et al., 2019; Magalhães & São-Pedro, 2012; Maximo, 2021; Pistoni & Toledo, 2010). Many authors emphasize the need for a multidisciplinary approach to combat illegal wildlife trade, engaging government agencies, NGOs, researchers, managers, and local communities (Challender et al., 2014a, 2014b; Fonseca et al., 2021; Magalhães & São-Pedro, 2012; Moorhouse et al., 2017; Siriwat, 2019; van Uhm, 2021; Veríssimo et al., 2012; Wyatt et al., 2020).

Understanding consumer behavior remains pivotal for effective conservation policies (Challender et al., 2015; Veríssimo et al., 2020). Specifically, analyzing social dynamics of exotic pet consumption—including preferences, demand, social marketing, and education—is essential for developing strategies to mitigate domestic illegal trade in Brazil (Abensperg-Traun, 2009; Challender, 2014b; Drury, 2011; Phillip et al., 2009; Veríssimo et al., 2012). However, few quantitative studies have examined the demographic and attitudinal profiles of exotic pet consumers in Brazil, despite Brazil's relevance to both regional and global wildlife trade. Empirical data on consumer motivations and market behavior remain scarce, limiting the development of evidence-based demand-reduction strategies. Since motivations shape behavior and responses to conservation strategies (Thomas-Walters et al., 2020a, 2020b), identifying these factors is crucial.

Therefore, this study aims to (1) characterize the profile of legal and illegal exotic pet owners in Brazil, (2) assess the population's knowledge of legislation on exotic pets, and (3) identify the primary motivations driving illegal exotic pet acquisition in the country. By addressing these points, the research contributes to ongoing efforts in enforcement, environmental education, and demand-reduction campaigns aligned with national biodiversity strategies and CITES objectives, providing actionable insights for interdisciplinary policy development.

2. Materials e Methods

An anonymous online survey was developed using Google Forms (Google Inc.) to collect data on exotic pet ownership and perceptions of wildlife trade in Brazil. The study was approved by the Research Ethics Committee and registered in the Brazilian National Research Ethics Platform (Plataforma Brasil) under protocol number 70587923.9.0000.0093/2023.

Survey design and distribution

The questionnaire was available in Portuguese from June to December 2021, took approximately ten minutes to complete, and was disseminated using the snowball sampling technique (Newing et al., 2011). Initial participants included exotic pet owners and individuals or organizations involved with wildlife trade, who were encouraged to share the survey link within their personal and professional networks. This approach enabled the recruitment of successive participants beyond the researchers' direct reach and facilitated access to a broad range of social and geographic groups across the country. The survey was initially shared through three main channels: experts and non-governmental organizations working with wildlife trade were invited to distribute it among known exotic pet owners and independent groups; administrators of online communities and exotic pet networks were contacted to circulate the link within their internal communication channels; and targeted social media posts were used to increase participation in underrepresented regions.

Participants and structure of the survey

To ensure geographic representation, screening questions were included regarding the respondents' state of residence, age, gender, and educational level. Only individuals aged 18 years or older and residing in Brazil were eligible to participate. The questionnaire began with questions about exotic pet ownership, including whether respondents themselves or someone they knew owned a legal or illegal exotic pet, and whether they were interested in owning one. Participants were also asked whether they had ever reported someone for owning illegal wildlife. They were presented with two questions to assess their knowledge of current legislation on the voluntary surrender or legalization of illegal pets. Those who reported having owned an illegal exotic pet were further asked about the fate of the animal (death, surrender, release, or escape) and their likelihood of acquiring another illegal pet. Respondents expressing interest in owning exotic pets were asked to rate, on a 10-point Likert-type scale (Likert, 1932), their preference for acquiring a legal or illegal animal according to relative acquisition costs. The first question assumed equal prices, followed by scenarios in which legal pets were twice, ten times, or twenty times more expensive than illegal ones. To assess motivations for exotic pet ownership, respondents indicated their agreement with 13 statements using a five-point Likert-type scale ranging from "strongly disagree" to "strongly agree." Participants who reported owning an illegal exotic pet were also invited to describe, in an open-ended response, their reasons for doing so. The complete questionnaire is available in the Supplementary Material.

Statistical analysis

Data were compiled and analyzed using IBM SPSS Statistics (version 26). Multiple logistic regression models were applied to identify factors predicting exotic pet ownership and knowledge of wildlife legislation. In the first models, variables related to pet ownership and the reporting of illegal wildlife trade were analyzed simultaneously, controlling for respondents' age, gender, and educational level. In the second model, only age and education were included to examine knowledge of legislation, as gender was not expected to influence this variable. For Likert-type scale data, the Shapiro-Wilk test was applied to assess normality. Since the data were not normally distributed, the Friedman test was used to compare median preferences across different relative price scenarios. Descriptive statistics, including absolute and relative frequencies, were used for all other categorical variables.

3. Results

A total of 1,706 valid responses were collected and included in the analysis. Respondents represented all 27 Brazilian states, with the highest participation originating from southeastern and southern Brazil (Figure 1). Detailed demographic characteristics, frequencies of legal and illegal exotic pet ownership, and results of the logistic regression analysis are presented in Table 1. Overall, exotic pet ownership was common among respondents, and men, individuals aged 30–49 years, and those with higher educational attainment were significantly more likely to report ownership of exotic pets.

Among respondents who had owned illegally acquired animals, the most frequently reported outcome was that the animal remained in their possession. Other commonly reported outcomes included animal death and voluntary surrender due to legal or ethical concerns. Additional reported outcomes are presented in Table 2.

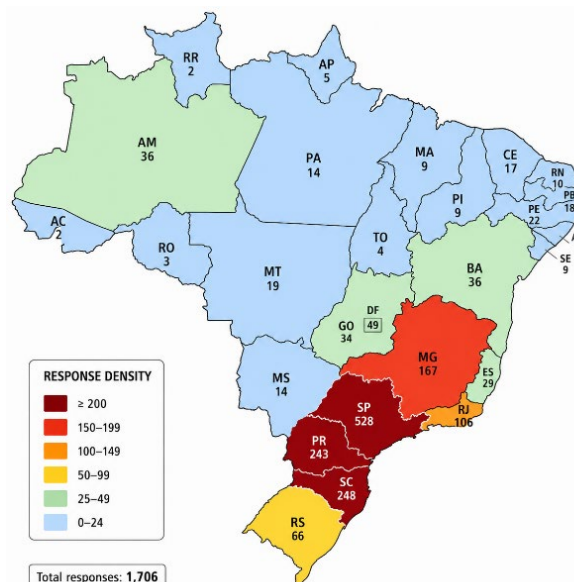


Figure 1 – Heat map showing the distribution of survey responses across the 27 Brazilian federal units. Colors represent response density by state, ranging from 0–24 responses (light blue) to ≥ 200 responses (dark red). A total of 1,706 responses were included in the analysis.

Variable		Do you own or ever owned a LEGAL exotic pet?				
		Total	Yes (n=508)	No (n=1094)	OR (95% CI)	p-value ^a
Age	18 - 29 years old	1167	348 (29.82%)	819 (70.18%)	Reference	----
	30 - 49 years old	372	146 (39.25%)	226 (60.75%)	1.304 (1.003-1.695)	0.047
	50+ years old	63	14 (22.22%)	49 (77.78%)	0.611 (0.329-1.135)	0.119
Gender	Female	943	262 (27.78%)	681 (72.22%)	Reference	----
	Male	648	243 (37.50%)	405 (62.50%)	1.577 (1.270-1.959)	0.000
	Other	11	3 (27.27%)	8 (72.73%)	1.131 (0.292-4.381)	0.858
Scholarity	Elementary School	172	44 (25.58%)	127 (74.42%)	Reference	----
	High School	751	219 (29.16%)	532 (70.84%)	1.248 (0.851-1.831)	0.256
	Superior or Postgraduation	680	245 (36.03%)	435 (63.97%)	1.641 (1.103-2.443)	0.015
Variable		Do you own or ever owned an ILLEGAL exotic pet?				
		Total	Sim (n=467)	No (n=1134)	OR (95% CI)	p-value ^a
Age	18 - 29 years old	1168	314 (26.88%)	854 (73.12%)	Reference	----
	30 - 49 years old	369	133 (36.04%)	236 (63.96%)	1.230 (0.937-1.614)	0.136
	50+ years old	64	20 (31.25%)	44 (68.75%)	1.112 (0.631-1.959)	0.714
Gender	Female	951	213 (22.40%)	738 (77.60%)	Reference	----
	Male	639	251 (39.28%)	388 (60.72%)	2.350 (1.877-2.941)	0.000
	Other	11	3 (27.27%)	8 (72.73%)	1.616 (0.415-6.293)	0.489
Scholarity	Elementary School	170	36 (21.18%)	134 (78.82%)	Reference	----
	High School	753	196 (26.03%)	557 (73.97%)	1.487 (0.984-2.247)	0.060
	Superior or Postgraduation	678	235 (34.66%)	443 (65.34%)	2.168 (1.415-3.321)	0.000

^a Significance of difference compared with reference category

Table 1 – Data of legal and illegal exotic pet ownership based on an online questionnaire in Brazil, stratified by age, gender and scholarity and presented by total and relative frequencies.

Fate of the animal	Responses
Still owned by respondent	160 (35%)
Animal died	108 (23%)
Surrendered due to legal restrictions	86 (19%)
Handed over for ethical reasons	82 (18%)
Donated to another person	70 (15%)
Released or escaped	59 (13%)
Surrendered due to welfare concerns	45 (10%)

Table 2 – Reported fate of illegally acquired exotic pets among respondents who had owned at least one illegally sourced animal (n = 461) in Brazil. Values are presented as total and relative frequencies of respondents. Multiple responses were allowed; therefore, the sum of percentages exceeded 100%.

Interest in owning exotic pets was high among respondents. Logistic regression analysis demonstrated significant effects of gender, age, and education level on this outcome (Table 3). In general, younger respondents, men, and participants with lower educational attainment were more likely to express interest in owning exotic pets. Motivations for acquiring illegally sourced exotic pets are presented in Table 4. Most respondents indicated that there was no justification for purchasing an animal illegally, whereas lower prices, perceived animal rescue, accessibility, and greater species availability were the most frequently cited motivations.

Variable		Would have n = 1084	Wouldn't have n = 598	OR (95% CI)	P-value ^a
Age	Total	1234	385	Reference	----
	18 - 29 years old	1234	385 (31.20%)	Reference	----
	30 - 49 years old	381	168 (44.09%)	0.642 (0.496-0.829)	0.001
	50+ years old	67	45 (67.16%)	0.264 (0.154-0.452)	0.000
Gender	Total	1004	412	Reference	----
	Female	1004	412 (41.04%)	Reference	----
	Male	665	181 (27.22%)	1.815 (1.460-2.256)	0.000
	Other	13	5 (38.46%)	0.961 (0.301-3.071)	0.970
Scholarity	Total	795	259	Reference	----
	Elementary School	183	39 (21.31%)	Reference	----
	High School	795	259 (32.58%)	0.627 (0.423-0.929)	0.020
	Superior or Postgrad	704	300 (42.61%)	0.494 (0.329-0.742)	0.001

^a Significance of difference compared with reference category

Table 3 – Results of respondents' interest in owning an exotic pet in Brazil. Data is stratified by age, gender and scholarity and presented by total and relative frequencies.

Motivation category	Description	Responses
Ethical motivations	Desire to rescue or save an animal	205 (19%)
Economic motivations	Lower purchase price	210 (19%)
Availability motivations	Easier accessibility	149 (14%)
Market motivations	Greater variety of species/mutations	124 (12%)
No justification	Respondents reporting no reason to acquire illegally	628 (58%)

Table 4 – Reported motivations for acquiring illegally sourced exotic pets in Brazil. Responses were grouped into thematic categories and presented as total and relative frequencies. Multiple responses were allowed; therefore, the sum of percentages exceeded 100%.

Among respondents interested in exotic pet ownership, willingness to acquire legally sourced animals decreased progressively as the price difference between legal and illegal animals increased (Figure 2). Although legal acquisition was strongly preferred when prices were similar, this preference declined substantially as the cost of legally sourced animals increased.

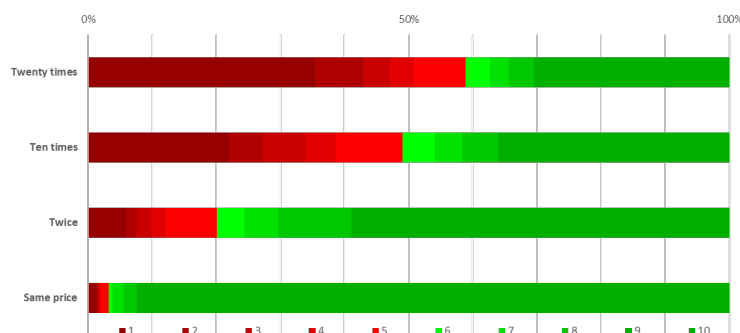


Figure 2 – Respondents likelihood to acquire legal (green) or illegal (red) pets according to the price gap – same price, legal pets twice, ten times and twenty times more expensive than illegal animals in Brazil. Data is presented in a Likert scale from 1 to 10, where 1 is extremely likely to acquire an illegal pet and 10 extremely likely to acquire a legal pet.

Knowledge of Brazilian wildlife legislation varied among respondents. Results of the legislation questions and the associated logistic regression analyses are presented in Table 5. Educational level was the main variable associated with correct responses, whereas age and gender showed limited influence.

Finally, only a small proportion of respondents reported having previously reported illegal exotic pet ownership. No significant associations were detected between reporting behavior and the demographic variables evaluated (Table 6).

Variable	What happens if you volunteer surrender an illegal pet?				
	Total	Correct (n=896)	Wrong (n=152)	OR (95% CI)	p-value ^a
Age					
18 - 29 years old	767	656 (85.53%)	111 (14.47%)	Reference	-----
30 - 49 years old	240	209 (87.08%)	31 (12.92%)	1.011 (0.632-1.617)	0.962
50+ years old	41	31 (75.61%)	10 (24.39%)	2.098 (0.977-4.503)	0.057
Scholarity					
Incomplete 1st degree	94	67 (71.28%)	27 (28.72%)	Reference	-----
High School	492	429 (87.20%)	63 (12.80%)	0.364 (0.216-0.613)	0.000
Superior or Postgraduation	462	400 (86.58%)	62 (13.42%)	0.367 (0.210-0.640)	0.000
Variable	Is it possible to legalize an illegal pet?				
	Total	Correct (n=801)	Wrong (n=389)	OR (95% CI)	p-value ^a
Age					
18 - 29 years old	892	594 (66.59%)	298 (33.41%)	Reference	-----
30 - 49 years old	252	179 (71.03%)	73 (28.97%)	1.13 (0.815-1.567)	0.476
50+ years old	46	28 (60.87%)	18 (39.13%)	0.7 (0.377-1.301)	0.290
Scholarity					
Incomplete 1st degree	125	83 (66.40%)	42 (33.60%)	Reference	-----
High School	561	364 (64.88%)	197 (35.12%)	1.079 (0.716-1.627)	0.716
Superior or Postgraduation	504	354 (70.24%)	150 (29.76%)	0.857 (0.555-1.323)	0.485

^a Significance of difference compared with reference category

Table 5 – Results of the legislation questions. Data is stratified by age and scholarity and presented by total and relative frequencies. Multiple logistic regression analysis was performed including all variables in the model.

Variable	Total	Yes (n=173)	No (n=1532)	OR (95% CI)	P-value ^a
Age					
18 - 29 years old	1241	127 (10.13%)	1127 (89.87%)	Reference	-----
30 - 49 years old	380	36 (9.38%)	348 (90.63%)	0.769 (0.508-1.166)	0.216
50+ years old	66	10 (14.93%)	57 (85.07%)	1.326 (0.648-2.710)	0.440
Gender					
Female	1004	98 (9.66%)	916 (90.34%)	Reference	-----
Male	670	73 (10.77%)	605 (89.23%)	1.170 (0.846-1.617)	0.342
Other	13	2 (15.38%)	11 (84.62%)	1.708 (0.363-8.024)	0.498
Scholarity					
Elementary School	183	18 (9.84%)	165 (90.16%)	Reference	-----
High School	796	69 (8.60%)	733 (91.40%)	0.923 (0.530-1.608)	0.778
Superior or Postgraduation	708	86 (12.04%)	628 (87.96%)	1.421 (0.807-2.502)	0.223

^a Significance of difference compared with reference category

Table 6 – Results if the respondents have ever reported illegal exotic animal ownership. Data is stratified by age, gender and scholarity and presented by total and relative frequencies. Multiple logistic regression analysis was performed including all variables in the model.

4. Discussion

The domestic wildlife trade in Brazil poses a significant threat to native fauna and remains a major enforcement challenge for national agencies. Understanding consumer demand is therefore central to designing effective responses to illegal wildlife trade. Brazil and other Latin American countries have a strong cultural tradition of keeping exotic pets; in our sample, nearly half of the respondents had owned one, and almost 90% knew someone who had. This is particularly concerning, given that one-third reported owning an illegal pet and two-thirds knew someone who had. Addressing this problem requires a comprehensive approach, as evidence indicates that national markets can operate independently of international trade flows (Daut et al., 2015). Characterizing domestic trade patterns is thus essential to support national interventions. Although domestic trade has historically been concentrated in the North and Northeast, it permeates the entire country and meets demand in major urban centers, notably São Paulo (RENTAS, 2001). Given that Southeastern states comprise a large share of our sample, these findings likely capture a substantial share of domestic demand. Moreover, the widespread familiarity with exotic pet ownership across the country suggests that wildlife keeping has become culturally normalized rather than perceived as an illicit or marginal practice, reinforcing the need to address this issue not only through enforcement but through social awareness strategies.

Consistent with prior literature, younger respondents expressed greater interest in exotic pet ownership (Cronin et al., 2022), and evidence suggests they show stronger affective responses toward animals (Kellert, 1993). One plausible explanation is the heightened exposure to exotic pets on social media, which may shape generational norms and preferences (Cronin, 2022; Moloney, 2021; Nekaris, 2013; Siriawat, 2019). Whether this interest declines with age cannot be confirmed by cross-sectional data; longitudinal designs would be required to test this hypothesis (Cronin, 2022; Kellert, 1993).

Although several studies report that women derive greater benefits from pet ownership and exhibit stronger affiliative attitudes toward animals (Czech, 2002; Herzog, 2007; Kellert, 1987; Mathew, 1997), men in our sample were more likely to show interest in

exotic pets. Prior work links male preferences to taxa associated with fear or predation—such as snakes and spiders (Cronin, 2022; Fredrikson, 1996; Kellert, 1987; Knight, 2008; Myers, 2004; Prokop & Tunnicliffe, 2008)—whereas this pattern is less evident for “friendlier” exotic species (Cronin, 2022). In Brazil, the popularity of reptiles—especially snakes—together with historical restrictions on legal trade for other groups (e.g., amphibians and many lizards) may help explain the observed male bias (Alves et al., 2019; Magalhães & São-Pedro, 2012). Men were also more likely to own illegal pets, a tendency reported among poachers and apparently extending to wildlife consumers (Wyatt et al., 2020). Interestingly, respondents with higher levels of education were also more likely to acquire illegal pets, despite the common assumption that education promotes environmental awareness. This paradox may reflect a perception of “controlled risk” or moral justification among more educated individuals who believe they can informally ensure welfare or legality, highlighting a disconnect between knowledge and compliance.

More than one-third of respondents expressed interest in owning exotic pets. Not all intentions will translate into ownership, but the popularity of non-traditional pets is increasing (Cronin et al., 2022; ABINPET, 2023), potentially driven by ease of maintenance, lower acquisition costs, reduced space and time demands, and amplified visibility on social media (Alves et al., 2019; Bush et al., 2014; Cronin et al., 2022). Conversely, the absence of a national positive “Pet List” (i.e., a list of species legally permitted as pets) constrains legal availability (Fonseca et al., 2021). In such contexts, consumers frequently turn to illegal markets (Oliveira et al., 2020; Wyatt et al., 2020). This tendency reinforces the finding that legal scarcity and affordability gaps, more than ignorance of the law, are central drivers of consumer behavior in Brazil.

Where legal and illegal markets coexist under weak regulation, demand can fuel illegality. Some consumers knowingly purchase illegal animals, underscoring that blanket bans alone are insufficient (Rivalan et al., 2007; Siriwat et al., 2019). As in other studies, price emerged as a dominant driver (Alves et al., 2019; Burivalova et al., 2017; de Oliveira et al., 2020): with each increment in the legal–illegal price ratio, a proportion of consumers shifted toward illegal options. When prices were equal, >95% preferred legal pets, yet ~5% still preferred illegal sources. Prior research suggests that beliefs about the superiority of wild-caught animals (genetics, morphology, behavior) and perceptions that laws overprotect species may sustain illegal demand irrespective of price (Brooks et al., 2010; Burivalova et al., 2017; Oliveira et al., 2020; Siriwat et al., 2019; Sung & Fong, 2018). Accessibility, rarity, and diversity of species/morphs also motivate illegal purchases globally and were frequently cited here (Altherr & Lameter, 2020; Courchamp et al., 2006; Gault et al., 2008; Hall et al., 2008; Johnson et al., 2010; Lyons & Natusch, 2013; Siriwat, 2019). Notably—and to our knowledge, not previously emphasized—almost 20% reported keeping illegal pets to “save” animals (e.g., rescuing from the wild or from previous owners, or accepting donations). This may be partly driven by respondents with biological or veterinary backgrounds who perceive a duty or capacity to help despite lacking official authority. On the other hand, the majority (58%) of respondents who claimed to have “no reason” for acquiring illegal pets reveals a normalization of this behavior—suggesting that, for many, illegality itself is no longer perceived as a barrier or moral conflict, but rather as a socially tolerated practice. This result reinforces the interpretation that wildlife trade in Brazil operates within a permissive cultural environment.

When legal pets cost no more than twice as much as illegal counterparts, over 80% of consumers favored legal, captive-bred animals. However, in Brazil, the bureaucratic and financial barriers to establishing licensed breeding facilities can render legal animals ten times more expensive than wild-caught ones (Alves et al., 2013), a tipping point at which preferences split roughly evenly. Even at a twenty-fold price differential, >40% still preferred the legal option, indicating that normative and legal considerations can outweigh cost, accessibility, or rarity for a substantial subset of consumers. Conversely, scarcity of legal, affordable pets—combined with the ease of illegal acquisition—directly sustains illegal trade (Ding et al., 2008; McNeely et al., 2009; Thach et al., 2018). Expanding access to affordable captive-bred animals tends to shift demand toward legal markets (Brooks-Moizer et al., 2009; Jepson & Ladle, 2005; Oliveira et al., 2020). These findings highlight a pragmatic policy opportunity: balancing regulation with economic accessibility could be more effective than stricter prohibitions alone, particularly when demand is already socially embedded.

Education remains a key tool for countering domestic illegal trade (Fonseca et al., 2021). Campaigns emphasizing legal consequences appear more effective at reducing demand than those focused solely on ethics (e.g., welfare or extinction risks) (Moorhouse et al., 2017). Roughly half of our respondents answered legal questions correctly, but more granular knowledge (e.g., penalties, fines, imprisonment) was not assessed and merits future investigation. Interestingly, respondents with elementary education performed better in legal knowledge questions than those with higher education, suggesting that awareness may derive from exposure to enforcement narratives rather than formal schooling. This underscores that educational campaigns should target behavioral drivers and information gaps rather than relying solely on educational attainment as a proxy for awareness.

Only 10% reported having ever reported an illegal wildlife practice—far below the 78.5% willingness to report poaching observed in a U.S. study (Leavitt et al., 2021). Several factors could explain this gap. Urban residents are more likely to report than rural residents and are more inclined to contact government rather than nonprofit channels; crimes against pets are also perceived as more serious than those against wildlife (Leavitt et al., 2021; Wagner et al., 2019). Additionally, individuals in areas with frequent violations, higher tolerance to associated risks, and/or precarious economic conditions may be less likely to report and may distrust criminal justice institutions (Brown & Benedict, 2002; Leavitt et al., 2021). These dynamics are likely salient in a developing-country context such as Brazil. Importantly, our question captured past reporting behavior—not willingness to report—so discrepancies between stated intentions and actions should be expected. Although men often engage in higher-risk, direct interventions while women favor indirect actions (e.g., calling authorities) (Eagly & Crowley, 1986; Nicksa, 2014; Salminen & Glad, 1992), we detected no gender differences in reporting illegal exotic pets. The combination of widespread awareness of illegal

ownership and extremely low reporting rates suggests a high level of social tolerance and a lack of deterrence—one of the most important behavioral barriers to reducing illegal wildlife trade.

Given that environmental agencies in Brazil and elsewhere are typically understaffed, public participation is a critical pillar of multi-pronged strategies against illegal trade. The relatively high proportion of Brazilian breeders with biological or veterinary training—compared with Europe and the United States—presents an opportunity to strengthen discourse and environmental awareness in schools, universities, and communities, particularly among children and future wildlife professionals (Fonseca et al., 2021; Alves et al., 2019; Awasthy et al., 2012; Beery & Jørgensen, 2016).

Abandonment remains a central concern in exotic pet ownership. Owners may relinquish animals due to regret, loss of interest, or fear of legal consequences (Souza et al., 2007). Brazilian law permits the voluntary surrender of exotic animals, legal or illegal, to environmental agencies at any time without penalty. Over half of respondents recognized this option, and interestingly, those with elementary education were most likely to answer correctly, suggesting that legal knowledge is not strictly correlated with formal education. Nevertheless, abandonment persists among respondents who owned illegal pets; more than 10% stated that animals had escaped or been released. Whether accidental or intentional, releases contribute to invasion risks (Pyšek et al., 2010; Romagosa, 2015). Not every release leads to establishment; outcomes depend on complex species–environment interactions. Priorities for future work include identifying the main taxa being released in Brazil to better assess and manage invasion risks (Fonseca et al., 2021; Stohlgren & Schnase, 2006; Thuiller et al., 2006).

Finally, the study's cross-sectional, online snowball design entails limitations. Snowball sampling can amplify homophily, and online recruitment may under-sample populations with limited internet access (Leighton et al., 2021). Selection bias is also plausible if individuals involved with exotic pets were more motivated to participate. Social desirability bias may have influenced responses to sensitive questions about illegal ownership. Even so, the large nationwide sample ($n = 1,706$) spanning all Brazilian states likely mitigates some biases and provides valuable insight into consumer dynamics within the domestic market.

5. Conclusion

This study demonstrates that a complex interplay of cultural normalization, economic asymmetries, and behavioral tolerance sustains legal and illegal wildlife trade in Brazil. Exotic pet ownership remains widespread, particularly among men and younger generations, reflecting both generational trends and a socially accepted perception of wildlife ownership. Higher levels of education do not necessarily reduce demand for illegal pets, suggesting that academic degrees alone are insufficient to ensure compliance when cultural and economic incentives prevail. Beyond legal considerations, keeping exotic pets also raises important ethical concerns regarding animal welfare, as many species have highly specialized behavioral, environmental, and social needs that are difficult to meet in captivity. Furthermore, the movement and maintenance of exotic animals may increase the risk of zoonotic disease transmission and expose owners and veterinarians to health challenges, particularly when species-specific medical knowledge and evidence-based treatment protocols remain limited or poorly established. The persistence of illegal trade is reinforced by limited access to affordable legal alternatives, weak law enforcement, and low rates of public reporting, which collectively reduce deterrence. Addressing these challenges requires coordinated actions that go beyond prohibition: strengthening environmental education focused on the legal and ecological consequences, establishing a national list of species authorized for trade, expanding accessible, stigma-free channels for voluntary surrender, and promoting public engagement in reporting environmental crimes. Such integrated measures are essential for aligning social behavior with conservation goals and mitigating the long-standing impact of the domestic wildlife trade on Brazil's biodiversity.

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Seis é só um número



Molento & ChatGPT, 2026, inspirado em Mucha

Carla Forte Maiolino Molento

À Universidade Federal do Paraná devo minha formação profissional e minha carreira – bem verdade que com algum esforço da minha parte também, claro. Primeiro vieram os diplomas de médica veterinária e de mestre em Ciências Veterinárias. Depois, após 11 anos fora de Curitiba, durante os quais fiz meu doutorado na Universidade McGill em Montreal e algumas incursões profissionais em outras cidades brasileiras, tive uma oportunidade de ouro: voltar à UFPR. Voltei como docente em julho de 2004 e imediatamente pedi meu credenciamento como pesquisadora no Programa de Pós-graduação em Ciências Veterinárias.

Tal vínculo vem sendo essencial para o desenvolvimento das pesquisas de nosso grupo nas áreas de diagnóstico de bem-estar animal e estratégias de melhoria; mais recentemente com um foco acentuado em métodos alternativos ao uso de animais para produção de alimentos. A fundação do Laboratório de Bem-estar Animal (LABEA/UFPR), também logo após meu ingresso como professora, marca o início de mais de duas décadas de pesquisa, ensino e extensão, que me permitiram uma inserção nos círculos internacionais de vanguarda na área. Mais recente, a fundação do Laboratório de Zootecnia Celular (Zoocel/UFPR) traz ao PPGCV um pioneirismo único em termos de América Latina.

Diante de tantos benefícios, vinha cultivando uma motivação para direcionar ao Programa alguns anos de dedicação focada em contribuições para além de meu trabalho como pesquisadora, pois para que os pesquisadores possam florescer há necessidade de uma certa manutenção da casa... A passagem pela vice-coordenação do curso de graduação em medicina veterinária havia me mostrado que o aprendizado inerente a tais cargos é interessante e a contribuição relevante.

De qualquer forma, foi mais um exemplo de como a vida pode reorganizar – ou melhor bagunçar – nossos planos, por que assumi o cargo de coordenação antes do que meus planos previam, sem ter passado pela vice-coordenação e, portanto, conhecendo somente a superfície das engrenagens do Programa e do funcionamento da Pós-graduação no Brasil.



A situação tomou um caráter um pouco assustador quando a excelente Gislaine Klemba, secretária do PPGCV à época, pediu transferência para outro campus. Ao mesmo tempo, a eleição para coordenação e vice-coordenação foi antecipada para um período anterior à entrega do temido relatório quadrienal para a CAPES, que é preenchido em uma plataforma chamada Sucupira e, assim, acaba sendo apelidado de ‘o Sucupira’. Assim nos vimos, o querido Pedro Paulo de Oliveira e eu, um tanto despreparados e com uma tarefa desafiadora. Nosso Programa vinha tentando melhorar suas notas nas avaliações da CAPES e a responsabilidade era grande, queríamos pelo menos manter as conquistas das coordenações que nos antecederam.

Pobre professor Newton Sucupira, que foi homenageado pela CAPES por ter estruturado o sistema da pós-graduação brasileira em 1965: mal podia adivinhar que a cada quatro anos coordenadores de programas de pós-graduação Brasil afora suariam frio ao ouvir ‘o Sucupiiiiira’. E não é sem motivo. A avaliação do quadriênio 2017 a 2020 foi especialmente difícil, ou pelo menos para mim assim pareceu. A portaria com a minha nomeação foi publicada em 09 de fevereiro de 2021, com a entrega do Sucupira prevista para março.

O professor Rafael Felipe da Costa Vieira, que me antecedeu, vinha fazendo um excelente trabalho tanto como coordenador quanto como pesquisador do PPGCV; vinha também compilando uma diversidade de materiais para o preenchimento do Sucupira e isso ajudou muito. Mesmo assim, foi uma tarefa difícil.

O horror da pandemia de COVID alargou consideravelmente o prazo de entrega, desestruturando-nos por outro lado. Nos vimos diante da necessidade de preencher um relatório com 14 campos de conteúdo, a maioria deles com 40.000 caracteres, dentro de um complexo sistema de pesos (Quadro 1), e mais dois anexos de tamanho livre. Tivemos muita ajuda, especialmente dos professores Marcelo Beltrão Molento e Ricardo Vilani, assim como a generosa contribuição do professor Rodrigo Moraes da Silva, da Escola de Administração. Importantíssima a presença marcante dos coordenadores de área da CAPES, professores Rinaldo Aparecido Mota e Carlos Eduardo Ambrósio, incansáveis nas explicações aos coordenadores de PPGs em todo o Brasil e na dedicação para a promoção da medicina veterinária dentro da CAPES.

Quadro 1. Quesitos e respectivos pesos da ficha de avaliação dos Programas de Pós-graduação, área da Medicina Veterinária da CAPES, quadriênio 2017 a 2020

Item	Quesitos / Itens	Peso Acadêmico	Peso Profissional
1	Programa	Acadêmico	Profissional
1.1	Articulação, aderência e atualização das áreas de concentração, linhas de pesquisa, projetos em andamento e estrutura curricular, bem como a infraestrutura disponível, em relação aos objetivos, missão e modalidade do programa.	30%	30%
1.2	Perfil do corpo docente, e sua compatibilidade e adequação à Proposta do Programa.	30%	30%
1.3	Planejamento estratégico do programa, considerando também articulações com o planejamento estratégico de instituição, com vistas à gestão do seu desenvolvimento futuro, adequação e melhorias da infraestrutura e melhor formação de seus alunos, vinculada à produção intelectual – bibliográfica, técnica/tecnológica e/ou artística.	25%	25%
1.4	Os processos, procedimentos e resultados da autoavaliação do programa, com foco na formação discente e produção intelectual.	15%	15%
2	Formação	Acadêmico	Profissional
2.1	Qualidade e adequação das teses, dissertações ou equivalente em relação às áreas de concentração e linhas de pesquisa de programa.	30%	30%
2.2	Qualidade da produção intelectual de discentes e egressos.	15%	20%
2.3	Destino, atuação e avaliação dos egressos do programa em relação à formação recebida.	10%	20%
2.4	Qualidade das atividades de pesquisa e da produção intelectual do corpo docente no programa.	30%	15%
2.5	Qualidade e envolvimento do corpo docente em relação às atividades de formação no programa.	15%	15%
3	Impacto na Sociedade	Acadêmico	Profissional
3.1	Impacto e caráter inovador da produção intelectual em função da natureza da programa.	40%	25%
3.2	Impacto econômico, social e cultural do programa.	30%	45%
3.3	Internacionalização, inserção (local, regional e nacional) e visibilidade de programa.	30%	30%

Começamos a calcular os índices de publicação dos professores do PPGCV, pois além da parte textual acima, precisávamos dos indicadores quantitativos. E os egressos? Como localizá-los para trazer as informações ao relatório? Impacto social, visibilidade, internacionalização – professores muito ativos, sempre dando palestras, entrevistas, organizando eventos, entre outras atividades. Mas colocávamos tudo que fazíamos no currículo Lattes? Não.

Encurtando uma história longa, juntamos pontas de vários novos diferentes e nos surpreendemos com o resultado. Começamos a ter esperanças concretas de uma boa avaliação, estávamos começando a ver e, conseqüentemente, a poder descrever um Programa de Pós-graduação robusto. Tratamos de transformar em indicadores numéricos as questões mais valorizadas pela CAPES para progressão de nível. Por exemplo, programas que são credenciados para o nível de doutorado, o que havíamos conseguido na gestão da professora Rosângela Locatelli Dittrich em 2009, devem orientar mais doutorados que mestrados.

Fizemos nossos cálculos e mostramos que estávamos indo na direção recomendada (Figura 1A). No quadriênio, recebemos alunos internacionais, da Colômbia e da Somália, e alunos de todas as regiões do Brasil (Figura 1B). Assim fomos descrevendo o Programa ao longo do relatório, com transparência e sem modéstia, que não era hora...

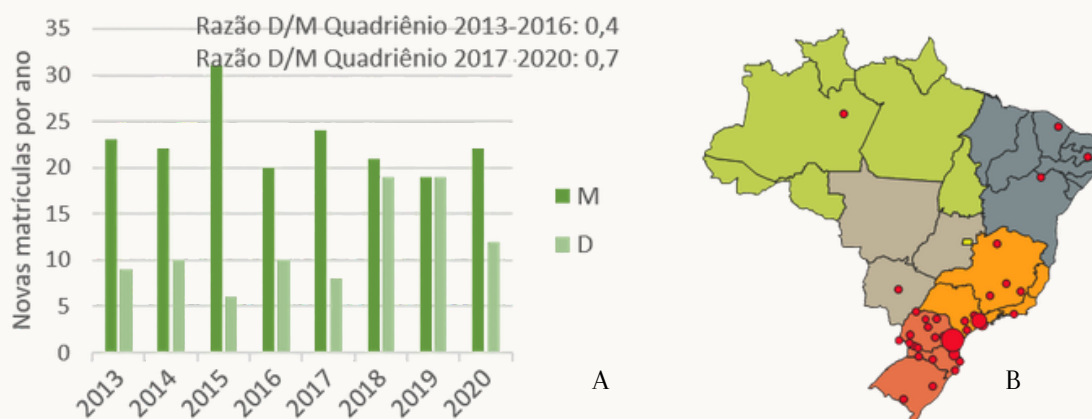


Figura 1. A. Novas matrículas de mestrandos e doutorandos por ano, no período de 2013 a 2020; B. Cidades de origem dos alunos matriculados no Programa de Pós-graduação em Ciências Veterinárias da Universidade Federal do Paraná, em abril de 2021.

Todos almejávamos a progressão para a nota 6, pois ela significaria entrar no grupo dos Programas considerados de excelência, o que em si já é bastante agradável. Ainda mais agradável, com o 6, há um aporte maior de recursos ao Programa pela CAPES, há mais possibilidade de concorrência dos pesquisadores permanentes em diferentes editais e, claro, é fortalecida uma percepção mais positiva do Programa, o que confere diversas vantagens. Mas era um querer meio comedido, como se não achássemos completamente correta uma tal expectativa, ou como se quiséssemos manter um pé no chão para, no caso de um tropeço, não cair. Só que o 6 veio. Que coisa, como um número pode trazer tanta alegria.

O 6 veio bem florido e sobre ombros de muitas pessoas, como acontecem todas as coisas que dependem do esforço de gerações. Para ele chegar, alguém teve que criar o Programa, alguém teve que segurar as pontas no momento de sucessivas notas 3, que ameaçavam a própria existência do Programa – de fato, passar de nota 3 para nota 4 é tão desafiador quanto passar de nota 5 para 6, embora por motivos diferentes. Alguém teve que propor o doutorado e obter sucesso na proposta. São marcos que contam a história da nossa avaliação pela CAPES (Figura 2).

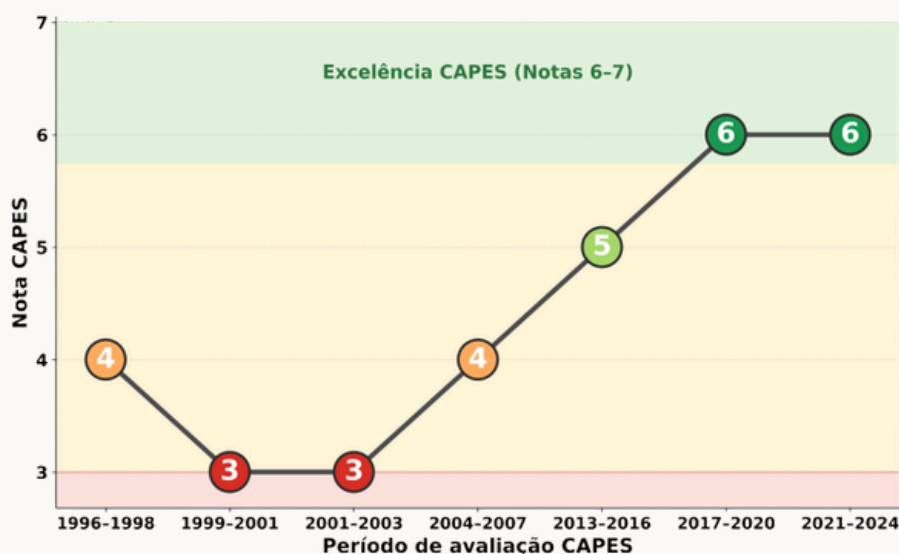


Figura 2. Evolução das notas trienais e quadrienais da CAPES para o Programa de Pós-graduação em Ciência Veterinárias da Universidade Federal do Paraná

Seis é só um número, sete também... O significado adicional depende do contexto e dos sonhos que nos permitimos sonhar. Este texto celebra o aniversário do Programa de Pós-graduação em Ciências Veterinárias da Universidade Federal do Paraná, com os sinceros parabéns pelo percurso ao longo de seus 40 anos de existência e votos de uma longa e bem-sucedida jornada pela frente.

Que o futuro traga mais conquistas na direção de avanços genuínos à ciência e à sociedade brasileira, contribuindo para um mundo mais justo para todos os seres sencientes, independentemente de espécie e posição em que cada um é colocado na hierarquia inventada pelos seres humanos.



Carla Forte Maiolino Molento

É Professora Titular, MV, MSc, PhD, coordenadora do Laboratório de Bem-estar Animal (LABEA/UFPR) e do Laboratório de Zootecnia Celular (Zoocel/UFPR), ambos lotados no Departamento de Zootecnia. No Programa de Pós-graduação em Ciências Veterinárias sua área de atuação é em Bem-estar Animal e Alternativas ao Uso de Animais.

Study of reproductive and productive traits in fourth-generation crossbred ewes (62.5% D'man blood and 37.5% Ouled Djellal blood): effect of lambing order and intergenerational overview

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Abstract: The aim of the present study was to: (1) evaluate the impact of lambing order (Ewe age) on the reproductive performance and productivity of fourth-generation (G4) crossbred ewes, with 62.5% D'man (D) blood and 37.5% Ouled Djellal (OD) blood; and (2) to compare the performance of G4 (all parity levels combined) with that of previous generations (G1-G3). Ninety (90) crossbred ewes were used, including 21 females from the first lambing order (1st L), 20 females from the second order (2nd L), 26 females from the third order (3rd L), and 23 females from the fourth order (4th L). All females underwent heat synchronization through the ram effect and were mated with purebred OD rams. At G4, the results obtained show an improvement in the fecundity of crossbred ewes in the 4th L ($147.83 \pm 12.37\%$; $p=0.051$) of 55%, 41% and 28%, in numerical productivity ($116.67 \pm 15.54\%$; $p=0.048$) by 88%, 56% and 38%, and weight productivity (18.66 ± 1.49 kg/ewe; $p=0.001$) by 94%, 7% and 62% compared to the first, second and third lambing. In addition, an improvement in reproductive and productive performance was observed across the four generations (G1-G4), particularly in G2 (50% OD blood and 50% D blood), compared with values reported in the literature for purebred OD ewes raised under Algerian conditions. The notable improvement recorded in the 4th L ewes of G4 highlights the importance of this factor in the strategies for the second phase of multiplication and selection in this crossbreeding program, with a view to increasing sheep production in Algeria.

Keywords: crossbred ewe, crossbreeding, lambing, productivity, reproductive.

1. Introduction

Sheep farming is an essential component of global agriculture, playing a crucial role in food and economic security in many countries, particularly in Algeria. Sheep are valued for their meat, milk, wool, and skins, contributing significantly to the livelihoods of rural populations and the national economy (Aouina et al., 2025). Nevertheless, enhancing sheep flocks' zootechnical performance remains a major challenge, especially in arid and semi-arid regions with limited resources and severe climatic conditions.

Genetic crossbreeding is a commonly used strategy to improve the productivity and profitability of sheep farms. Algerian herds, which are dominated by local breeds, are well adapted to their environment but have productivity that remains low (0.6-0.8 weaned lambs per breeding ewe per year) (Benyounes et al., 2013). This low productivity is due to several factors, including poor ewe reproductive performance and low lamb growth and survival rates (Sebkhi et al., 2024).

Among the sheep breeds found in Algeria, the OD and D breeds stand out for their distinctive characteristics. The OD breed is the most widespread sheep breed in Algeria, known for its robustness, ability to travel long distances, and adaptation to arid areas, but with low fecundity (91%). It is mainly used for meat production (Chellig, 1992; Adaouri et al., 2017, 2022; Sebkhi et al., 2024; Mouffok et al., 2025). In contrast, breed D is known for its exceptional fecundity (173%), early sexual maturity, and ability to reproduce outside the usual breeding season, but has low growth rates (Chellig, 1992; Boubekour et al., 2019). Crossbreeding between these two sheep breeds, OD and D, is of major interest as it creates a hybrid genotype that combines the genetic advantages of both breeds and is better suited to market requirements for improving sheep productivity in Algeria.

A previous study explored the reproductive and productive performance of the first three generations (G1 to G3) of these two complementary breeds, providing valuable data on earlier generations (Sebkhi et al., 2024). The present study, which follows on from the previous one, aims mainly to evaluate the effect of parity (from 1st to 4th lambing) on the reproductive and productive performance of crossbred ewes (62.5% D blood and 37.5% OD blood) used in the fourth generation (G4). An analysis of the reproductive and productivity results of G4 crossbred ewes was also carried out, compared with previous generations (G1 to G3) and the parental breeds (OD and D).

2. Materials e Methods

2.1. Study location

The study was conducted on the Baba Ali experimental farm of the Technical Institute of Breeding (ITELV, Algeria), located at 36° 48' N latitude and 2° 59' E longitude, near Algiers.

2.2. Animals and herd management

2.2.1. Animals

Ninety (90) crossbred ewes (62.5% D'man (D) blood and 37.5% Ouled Djellal (OD)) blood were used in this study to produce the fourth generation (G4), including 21 females from the first lambing (1st L), 20 females from the second lambing (2nd L), 26 females from the third lambing (3rd L) and 23 females from the fourth lambing (4th L) (Table 1). These females were from the last generation (G3) of alternating OD × D crossbreeding (Sebkhi *et al.* 2024). The breeding males (n=12) used in this study were purebred OD, approximately 4 years old, with an average live weight of 59.42±0.48 kg and an average body condition score of 3.15±0.08 (all lambings combined). They were in good reproductive health (Table 1).

Genetic type	Breeding females				Breeding males			
	37.5% OD blood and 62.5% D blood				100% OD blood			
Lambing order	n	Age	Live weight	Body condition score	n	Age	Live weight	Body condition score
1st L	21	1.29 ±0.10	37.62 ±1.31	2.71 ±0.04	3*	4 ±0.06	60 ±0.58	3.25 ±0.25
2nd L	20	2.15 ±0.18	41.40 ±1.66	2.76 ±0.07	3*	4.23 ±0.34	60.33 ±1.20	3 ±0
3rd L	26	2.27 ±0.23	44.38 ±1.59	2.74 ±0.06	3*	4.33 ±0.24	58.67 ±0.88	3.17 ±0.17
4th L	23	3.04 ±0.17	41.42 ±0.42	2.98 ±0.08	3	4.03 ±0.12	58.67 ±1.20	3.17 ±0.17
Total generation (G4)	90	2.22 ±0.11	41.38 ±0.70	2.80 ±0.03	12	4.15 ±0.10	59.42 ±0.48	3.15 ±0.08

Table 1 – Number of females and males used, their average age, weight and body score per lambing (average ± standard error). D: D'man; OD: Ouled Djellal; 1st L: first lambing order; 2nd L: second lambing order; 3rd L: third lambing order; 4th L: fourth lambing order; G4: fourth generation; *: 1 ram was shared (1st L – 2nd L); 1 ram was shared (2nd L – 3rd L).

Ewes in each lambing group were subdivided at the time of mating into three homogeneous groups of 6 to 9 females each to be mated with one ram per group.

2.2.2. Reproduction management

The estrus synchronization technique used in this study is known as the 'ram effect' technique. It is based on strict, complete isolation of males and females (physical, visual, olfactory, and auditory) for a period of one month, in our case. Subsequently, a single ram was introduced into each group of females by simple release (Tournadre *et al.*, 2002; Khiati, 2013; Castonguay, 2018; Adaouri *et al.*, 2022). The rams remained with the females for 25 days. The reproduction method adopted throughout the experimental period (2020-2024) was one lambing per year. All females were put into breeding during the autumn season. Gestation was confirmed by ultrasound approximately one month after mating.

All animals used in this experiment (females and males) were treated for external and internal parasites at the start of the trial, thus ensuring optimal sanitary conditions.

2.2.3. Feeding

The rams and ewes used in this study were fed a diet according to their physiological stage, including forage, concentrate, and multivitamin-mineral blocks (Table 2).

2.3. Calculation of reproduction and productivity parameters

Reproduction and productivity parameters were calculated using the following formulas:

Fertility = (Number of ewes that lambed / Number of ewes used for reproduction) × 100.

Fecundity = (Number of lambs born / Number of ewes used for reproduction) × 100.

Prolificacy or litter size at birth (LSB) = Number of lambs born / Number of ewes that lambed.

Litter size at 90 days (LSW) = Number of weaned lambs / Number of ewes that lambed.

Litter weight at birth (LWB) = Weight in kg of lambs at birth / Number of ewes that lambed.

Litter weight at 90 days (LWW) = Weight in kg of weaned lambs / Number of ewes that lambed.

Numerical productivity (NP) = Number of weaned lambs / Number of ewes used for breeding × 100.

Weight productivity (WP) = Weight of weaned lambs at 90 days / Number of ewes used for breeding.

Feeding Regimen for Breeding Stock					
Animal	Physiological Stage	Basal diet	Concentrate (g/animal/day)	Multivitamin-mineral blocks	Water
Ewes	Flushing	Oat hay (ad libitum, 20% refusal)	500	ad libitum	ad libitum
	First three months of gestation		300		
	Steaming (the fourth and fifth months of gestation)		400-500		
	Lactation		600		
Rams	Flushing	Oat hay (ad libitum, 20% refusal)	600	ad libitum	ad libitum
	Maintenance		-		

Table 2 – Feeding management of crossbred ewes (62.5% D and 37.5% OD) and purebred OD rams according to physiological stages; Common to all animals: - Concentrate: composed of corn, soybean meal, wheat bran, oats, and a commercial vitamin and mineral supplement; - The ration was distributed twice daily at 9:00 a.m. and 4:00 p.m.

The coding used for the reproduction parameters was formulated as follows:

Fertility: 100 = fertile female; 0 = infertile female;

Fecundity: 0 = zero lambs born; 100 = one lamb born; 200 = two lambs born;

Litter size at birth: 1 = single birth; 2 = twin birth; 3 = triplet birth.

2.4. Statistical analyses

Statistical analyses were performed using SPSS/PASW version 24 software. Descriptive statistics (means and standard errors) were calculated for all traits studied. Differences between subgroups were assessed using analysis of variance (ANOVA) and considered significant when $p < 0.05$.

3. Results

3.1. Effect of lambing order (parity) on reproduction in G4 crossbred females

The analyses showed an improvement in the fertility of G4 ewes (62.5% D blood and 37.5% OD blood) with the lambing order, increasing from $71.43 \pm 10.10\%$ in the 1st L to $90 \pm 6.88\%$ in the 2nd L, $80.77 \pm 7.88\%$ at 3rd L, and $95.65 \pm 4.35\%$ at 4th L, reflecting an overall improvement of 24.22 points. The differences between ranks were not statistically significant ($p = 0.133$) (Table 3).

The LSB followed an upward trend: 1.33 ± 0.13 lambs/female in the 1st L, 1.17 ± 0.09 in the 2nd L, 1.43 ± 0.11 in the 3rd L, and 1.55 ± 0.11 in the 4th L, representing an overall improvement of +0.22 points. The frequency of multiple births increased from 33.3% in the 1st lambing to 54.5% in the 4th lambing. The LWB recorded average values of 5.04 ± 0.37 kg, 5.68 ± 0.37 kg, 4.67 ± 0.31 kg, and 5.18 ± 0.36 kg, respectively, for the four lambing ranks ($p = 0.232$). Fecundity (fertility $\times$ prolificacy) increased from $95.24 \pm 16.15\%$ at first lambing to $105.00 \pm 11.41\%$ at second lambing, $115.38 \pm 14.35\%$ at the third, and $147.83 \pm 12.37\%$ at the fourth lambing, representing an improvement of 52.59 points ($p = 0.051$).

Lambing order	1st L	2nd L	3rd L	4th L	p-value	Overall variation (points)	Total generation (G4)
n	21	20	26	23	-	-	90
Fertility (%)	71.43 ± 10.10	90 ± 6.88	80.77 ± 7.88	95.65 ± 4.35	0.133	+24.22	84.44 ± 3.84
LSB (Lambs)	1.33 ± 0.13	1.17 ± 0.09	1.43 ± 0.11	1.55 ± 0.11	0.096	+0.22	1.38 ± 0.06
FMP (%)	33.3	40.0	49.0	54.5	-	+21.2	38.16
LWB (Lambs)	5.04 ± 0.37	5.68 ± 0.37	4.67 ± 0.31	5.18 ± 0.36	0.232	+0.14	5.11 ± 0.18
Fecundity (%)	95.24 ± 16.15	105 ± 11.41	115.38 ± 14.35	147.83 ± 12.37	0.051	+52.59	116.67 ± 7.11

Table 3 – Evolution of reproductive performance of fourth generation (G4) ewes according to lambing order (ewe age) (mean $\pm$ standard error); LSB: Litter size at birth; LWB: Litter weight at birth; FMP: Frequency of multiple births. 1st L: first lambing order; 2nd L: second lambing order; 3rd L: third lambing order; 4th L: fourth lambing order; G4: fourth-generation.

The LWW varied significantly ($p = 0.013$) from 16.45 ± 1.32 kg at the first lambing, 20.30 ± 1.85 kg at the second lambing, 15.17 ± 0.02 kg in the 3rd lambing, and 19.64 ± 1.18 kg in the 4th lambing, representing an overall increase of 3.19 points. Analyses of numerical productivity at weaning showed a significant difference ($p = 0.048$) between farrowing ranks ($61.90 \pm 14.60\%$ at the 1st L, $75.00 \pm 14.28\%$ in the 2nd L, $84.62 \pm 12.02\%$ in the 3rd L, and $116.67 \pm 15.54\%$ in the 4th L). The overall increase was 54.77 points. Weight productivity at 90 days (for 10 ewes) followed an upward trend: 96.3 ± 20.4 kg in the 1st L, 174.0 ± 25.2 kg in the 2nd L, 115.3 ± 14.9 kg in the 3rd L, and 186.6 ± 14.9 kg in the 4th L ($p = 0.001$). Relatively high standard errors were observed for certain

parameters (fertility, fecundity, numerical and weight productivity), probably due to individual variability and the limited sample size per lambing order.

3.2. Comparison of performance between generations (G4 vs. G1–G3) and parental breeds

The reproductive and productive performance of crossbred ewes from the four generations was compared to the purebred parental breeds, OD and D (Fig 1).

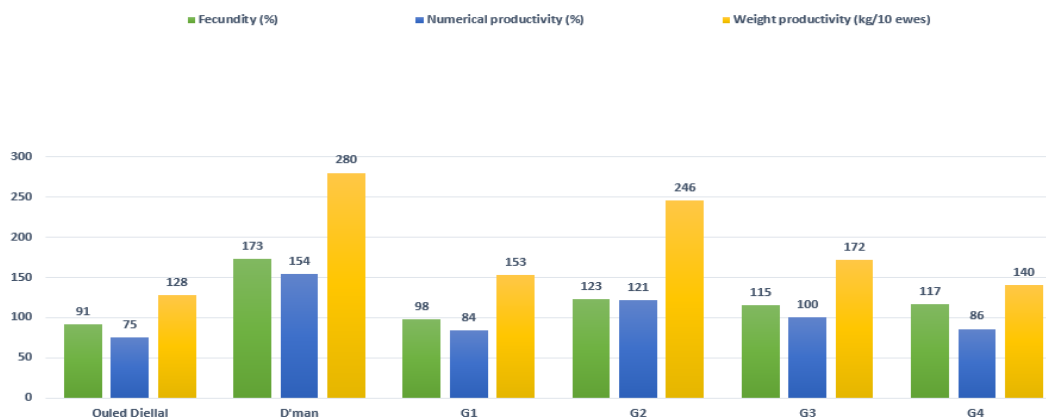


Figure 1 – Comparison of reproductive performance and productivity of the four generations (G4 vs. G1–G3) and parental breeds (OD and D). D: D'man ; OD: Ouled Djellal; G1: first generation (n=130) (100% OD blood); G2: second generation (n=71) (50% OD blood and 50% D blood); G3: third generation (n=87) (75% OD blood and 25% D blood); G4: fourth generation (n=90) (37.5% OD blood and 62.5% D blood).

Compared to the purebred OD (average fecundity $\approx$ 91%; NP $\approx$ 75%; WP $\approx$ 128 kg/10 ewes; Belkhirat and Bradai, 2021), all crossbred generations showed improvements. The average values were as follows: 98%, 123%, 115%, and 117% for fecundity; 84%, 121%, 100%, and 86% for numerical productivity; 153 kg/10 ewes, 246 kg/10 ewes, 172 kg/10 ewes (G3), and 140 kg/10 ewes for weight productivity, respectively for G1, G2, G3, and G4. Generation G2 recorded the best performance, with respective gains of +32 points in fecundity, +46 points in NP, and +118 points in WP compared to the pure OD breed. Compared to the pure D breed (fecundity $\approx$ 173%; NP $\approx$ 154%; WP $\approx$ 280 kg/10 ewes; Boubekeur et al., 2019), all crossbred generations performed less well.

The highest numerical productivity at weaning was observed in G2 (121%) and G3 (100%), compared to 84% in G1 and 86% in G4. WP (per 10 ewes) was also highest in G2 (246 kg) and G3 (172 kg). The comparison between generations remains indicative, as generations G1 to G3 were based on a reproduction rate of three lambings per generation, whereas G4 was based on four (due to logistical constraints).

4. Discussion

4.1. Effect of lambing order (parity) on reproduction and productivity

The results observed in fourth-generation ewes (G4; 62.5% D blood and 37.5% OD blood) indicate improved reproductive and productive performance across lambing orders, although the differences are not always statistically significant. Fertility increased from 71.43% to 95.65%, prolificacy from 1.33 to 1.55 lambs/ewe, fecundity from 95.24% to 147.83%, NP at weaning from 61.90 to 116.67%, and WP from 96.3 to 186.6 kg/10 females.

This trend could be explained mainly by the increase in the average age of ewes (from 1.29 years at first lambing to 3.04 years at fourth lambing; $p = 0.0001$), reflecting increased physiological maturity and better ovulatory capacity in multiparous ewes (Boujenane & Kansari, 2005; Abebe et al., 2023; Missoko Mabeki et al., 2024).

These observations are consistent with several previous studies that have highlighted a positive effect of ewe parity and age on sheep reproductive parameters (Dekhili & Benkhilif, 2005; Dekhili & Aggoun, 2006; Augas et al., 2010; Benjelloun et al., 2021). In Djallonké ewes, numerical productivity increases significantly with maternal age, from approximately 1.09 lambs in young mothers to 1.61 lambs in older mothers (Missoko Mabeki et al., 2024). Similarly, in crossbreeds (local crossbred ewes and Dorper x local) in Ethiopia, parity improves prolificacy and lamb survival (Abebe et al., 2023). However, other authors have not observed any significant effects of parity (Ceyhan et al., 2009; Boujenane et al., 2013; Dekhili, 2014).

The LWB remains higher than that of pure local breeds, Ouled Djellal ($\approx$ 4.25 kg) or Hamra ($\approx$ 3.68 kg), highlighting the advantages of crossbreeding through heterosis and breed complementarity: the D breed provides high prolificacy, while the OD breed confers rapid growth (Adaouri et al., 2017; Adaouri et al., 2022; Sebkhil et al., 2024). Weight productivity at weaning increased significantly ($p = 0.001$) by 90.3 kg/10 ewes in the fourth lambing compared to the first, reflecting the synthesis of all other zootechnical performances measured (fertility, prolificacy, fecundity, viability, and lamb weight) (Ajafar et al., 2022; Beshah et al., 2023).

The high reproductive performance observed in our study, regardless of the number of lambings, could be due to the type of crossbreeding used (crossbred ewes with 62.5% D blood and 37.5% OD blood $\times$ purebred OD rams). It is known that alternating

crossbreeding improves reproductive parameters, on the one hand through the continuous heterosis effect and, on the other hand, through the phenomenon of racial complementarity, which allows the complementary performances present in the two parental breeds to be combined in the same individual (Jakubec, 1977; Leymaster, 2002; Sebkhi et al., 2024).

4.2. Performance comparisons between generations (G4 vs G1-G3) and parental breeds (OD and D)

The intergenerational comparison shows that G4 performance is lower than G2 and G3 but higher than the pure OD breed. The G2 generation (50% OD blood and 50% D blood) shows the best results, with notable gains in fecundity (+34%), numerical productivity (+62%), and weight productivity (+92%) compared to the pure OD breed. This suggests an optimum around 50% D blood, beyond which performance declines due to reduced complementarity.

This decline observed in G4, particularly marked for weight productivity, is attributable to higher lamb mortality (20.2% in G4 vs. 12.1% in G1, 0% in G2, and 2.17% in G3) and slower lamb growth between birth and weaning (115.04 g/day in G4 vs. 143.1 g/day in G1, 185.15 g/day in G2, and 140.51 g/day in G3). These lower effects could be explained by the high proportion of D'man genes (62.5%) in G4, a breed known for its high mortality and lower growth, despite its high prolificacy. El Fadili (2005) reports that productivity increases with an increase in D'man genes up to 50%, but decreases beyond that, suggesting an optimal threshold at which the complementarity between prolificacy (D) and growth (OD) is maximized. This phenomenon is also corroborated by observations from Hight & Jury (1970), Ricordeau et al. (2020), and Oyieng et al. (2025), who highlight a gradual reduction in performance in advanced generations of crossbreeding due to a loss of heterosis and an imbalance in racial complementarity.

Overall, these results indicate that intermediate genetic combinations (50% OD - 50% D in G2; 75% OD - 25% D in G3) better optimize reproductive and productive performance by effectively exploiting the prolificacy genes of breed D and the growth abilities of breed OD. However, the fourth-generation G4 (37.5% OD - 62.5% D) performances could be further improved by initiating the second phase of the program, which focuses on multiplication and selection.

5. Conclusion

This study shows a positive impact of lambing order (parity) on the reproductive and productive performance of crossbred ewes (37.5% Ouled Djellal blood, 62.5% D'man blood). Multiparous ewes, particularly those that have reached their fourth lambing, demonstrate an increased ability to produce and rear lambs, highlighting the importance of managing lambing order (parity) in breeding programs. Furthermore, an overall analysis of results across all generations revealed that the best performance is achieved with intermediate proportions of D'man blood (particularly 50% in G2). All crossbred generations perform better than the purebred Ouled Djellal breed and worse than the purebred D'man breed. This crossbreeding pattern could be an appropriate strategy for increasing the productivity of local sheep flocks. Prospects for optimization are based on targeted selection and better control of genetic and non-genetic factors. These approaches would strengthen and stabilize performance, while promoting the dissemination of improved genotypes in Algerian breeding systems.

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1992 / 1996

Um relato sobre o CPGCV da UFPR

Antônio Felipe Paulino de Figueiredo Wouk

Em janeiro de 1985, retomei minhas atividades docentes no Curso de Medicina Veterinária da UFPR, após concluir o doutorado na Escola de Veterinária de Toulouse e no Instituto Nacional Politécnico de Toulouse, na França. Nos primeiros dias, fui convidado para uma reunião com os professores Metry Bacila e José Milton Andriguetto. Tive então a alegria de saber que estavam sendo realizadas as etapas necessárias à criação de um Mestrado em Ciências Veterinárias, abrangendo as grandes áreas de Patologia Animal, Produção Animal e Zootecnia de Produtos Aquáticos Renováveis. A partir desse momento, juntamente com outros colegas doutores, livres-docentes ou professores titulares, passamos a compor o grupo de primeiros docentes que, ao lado dos mestres Bacila e Andriguetto, deram forma ao projeto final submetido à CAPES. Tornamo-nos, assim, os primeiros orientadores, com linhas de pesquisa registradas. O curso teve início no ano seguinte, 1986, e permaneceu sob a coordenação do professor Bacila e vice-coordenação do professor Andriguetto até 1992.

Ao final desse período, o professor Bacila consultou a mim e à professora Clotilde de Lourdes Branco Germiniani sobre a possibilidade de darmos continuidade à gestão do curso – eu como coordenador e a professora Clotilde como vice-coordenadora. Para mim, foi uma honra inaudita suceder aos insígnies mestres Bacila e Andriguetto, ao lado da querida e brilhante professora Clotilde Germiniani. A secretaria da coordenação ocupava um espaço no primeiro andar do prédio central do Setor de Ciências Agrárias, ao lado da direção do setor. Desde 1986, a saudosa Tania Mara Schrank exercia, com absoluta competência, a secretaria da pós-graduação, permanecendo nessa função. Mais tarde, juntou-se a ela a não menos competente servidora Deleuse Cherobim.

Para melhor caracterizar a cultura do CPGCV em seus primeiros anos, cito o professor Metry Bacila em um pronunciamento feito ao final da nossa gestão, em 1996, por ocasião dos 10 anos do curso: “O CPGCV não nasceu de inopino, nem foi improvisado como resultado de um atrelamento a uma política de pós-graduação que se implantou no país e que chegou a criar um Conselho Nacional de Pós-Graduação, afortunadamente desativado tão logo foi percebido o exagero de medidas dessa ordem. Ao contrário, o nosso CPGCV foi resultado de um longo processo de amadurecimento, muito próprio do espírito paranaense, que se iniciou em 1939, quando o inolvidável mestre de todos nós, professor Dr. Marcos Augusto Enrietti, com toda a sabedoria e o descortino que eram a marca de sua inteligência e de seu ideal, fundou o Instituto de Biologia e Pesquisa Tecnológicas – IBPT – hoje Instituto de Tecnologia do Paraná – TECPAR.”

De fato, é apropriado associar a cultura do nosso curso à do TECPAR, uma vez que alguns dos professores fundadores, a começar pelo professor Bacila, têm sua origem científica nessa instituição, e o TECPAR seguiu colaborando com o curso nos seus primeiros anos, como veremos adiante.

Naquela época, os estudantes selecionados para o Mestrado possuíam bolsa e dedicavam-se integralmente ao curso – um diferencial que gerava pertencimento e uma vivência científica marcada pela intensa presença de discentes e docentes.

A cautela e o senso crítico dos fundadores do programa levaram a optar pela consolidação do Mestrado para, então, pensar no curso de Doutorado. A prioridade era a produção e a defesa de dissertações – então chamadas de teses – e as publicações delas decorrentes. Ao final dos primeiros dez anos, 66 teses haviam sido defendidas.

Ocorreram movimentos de internacionalização do CPGCV. Estabeleceu-se um convênio com a Universidade americana de Cornell, que enviou alguns de seus professores para ministrar cursos, seminários e conferências em nosso Programa de Pós-Graduação. Com a Universidade McGill, houve um intercâmbio que permitiu a alguns de nossos mestrandos realizar parte de seu trabalho de tese no Canadá; posteriormente, alguns deles retornaram àquele país para cumprir programas de Doutorado.

Mais adiante, sob minha coordenação, foi firmado um convênio CAPES-COFECUB – um acordo de cooperação internacional com universidades francesas, neste caso com as Escolas de Veterinária de Alfort, Toulouse e Lyon. Nessa ação internacional, houve intercâmbio de professores, e três mestrandos desenvolveram parte de seus experimentos nas escolas de veterinária de Alfort, Lyon e Toulouse.



Para proporcionar aos mestrandos uma visão ampla da profissão, da cultura geral e de suas responsabilidades sociais, o professor Bacila criou a disciplina de Seminários Gerais. As discussões versavam não apenas sobre temas técnicos, mas também culturais. Introduzir o humanismo e a filosofia no conhecimento técnico acadêmico fez uma grande diferença.

Foram promovidos neste período, os Simpósios de Ciências Médicas e Biológicas.

Os três primeiros simpósios homenagearam fatos importantes para a ciência paranaense e para o Curso de Pós-Graduação em Ciências Veterinárias.

O Simpósio aconteceu em 20 de novembro de 1991, homenageando o octogésimo aniversário do professor Dr. Heitor Segundo Guilherme Medina; foram apresentadas 47 comunicações, cujos resumos foram publicados sob o patrocínio do Instituto de Tecnologia do Paraná.

O II Simpósio realizou-se nos dias 29 e 30 de novembro de 1995, comemorando o cinquentenário da descoberta da *Leishmania enrietti*; também patrocinado pelo TECPAR, contou com 77 comunicações apresentadas e publicadas.

O III Simpósio ocorreu de 15 a 18 de outubro de 1996, ao final da nossa gestão, celebrando o décimo aniversário do CPGCV. Foram selecionadas 99 comunicações, e os resumos foram publicados no volume 1, número 1 dos *Archives of Veterinary Sciences*, periódico do curso então criado. A abertura desse III Simpósio aconteceu no Teatro da Reitoria, e as reuniões no Hospital Veterinário, no Setor de Ciências Agrárias da UFPR. Contamos com o apoio do Instituto de Tecnologia do Paraná, da Companhia de Cimento Portland Rio Branco e do CNPq.

O CPGCV entre 1992 e 1996 seguiu consolidando as bases de um programa de pós-graduação assentado no rigor acadêmico, na colaboração interinstitucional e na visão humanista da ciência. As sementes então plantadas desde a disciplina de Seminários Gerais até os convênios internacionais com Cornell, McGill e as escolas francesas – germinaram em iniciativas que ainda hoje distinguem o curso. Olhando para o futuro, o grande desafio será preservar esse espírito de integração entre excelência técnica, responsabilidade social e inserção global, ao mesmo tempo em que se ampliam as fronteiras do conhecimento em áreas emergentes, como a saúde única, a biotecnologia animal e a sustentabilidade da produção. Que as próximas gestões saibam honrar o legado de seu fundador, o insigne Mestre Metry Bacila, sem jamais perder a ousadia de inovar. Que o CPGCV siga formando pesquisadores críticos, éticos e comprometidos com os desafios do mundo contemporâneo. O futuro, construído com a mesma cautela crítica e senso de pertencimento que marcaram seus primeiros dez anos, será tão promissor quanto sólida for a memória que o ancora.



Archives of Veterinary Science



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Morphological characterization of embryonic and larval stages in the viviparous fish *Poecilia reticulata* (Peters, 1859)

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Abstract: Knowledge of embryonic development in viviparous fishes is essential for understanding reproductive strategies, population dynamics and applications in ornamental aquaculture. Despite the ecological and economic importance of the guppy *Poecilia reticulata*, detailed descriptions of its embryonic development remain scarce. This study aimed to describe the embryonic development stages of *Poecilia reticulata*. For this purpose, adult females (n = 19) were collected, subjected to thermonarcosis, preserved and subsequently dissected to remove eggs, embryos and larvae. Developmental stages were recorded in photographs and classified according to specialized literature. A total of 379 images were analyzed, revealing the simultaneous presence of multiple developmental stages in the same female, consistent with superfetation. Three major developmental phases were recognized: gastrulation, organogenesis and larval development. Approximately 6% of the embryos were classified as gastrulation stage, characterized by a diameter of about 0.5 mm and the initial formation of the optic primordia and notochord. Organogenesis was the predominant phase (93%), with embryos averaging 1.3 mm in length and 1.5 mm in diameter, showing differentiation of the embryonic axis, pigmentation and development of major body structures. Larval stage embryos represented 1% of the observations and exhibited complete tail uncoiling, developed eyes, body pigmentation and mean dimensions of 1.7 mm in length and 0.3 mm in diameter. The results provide a morphological framework for identifying developmental stages in *P. reticulata* and confirm the occurrence of superfetation. The data contribute to the understanding of reproductive biology in viviparous poeciliids and provide useful information for ecological and ornamental aquaculture studies.

Keywords: Poeciliidae; Guppy; Lebiste; Reproduction of viviparous; Fish ecology.

1. Introduction

Knowledge of embryonic and larval development in fishes constitutes an essential foundation for studies in ecology and reproductive biology, particularly for identifying early life stages and understanding reproductive strategies (Andrade and Yasui, 2003; Fuiman and Werner, 2009; Reynalte-Tataje et al., 2020). However, research in this field faces significant methodological obstacles, ranging from the complexity of morphological identification to the scarcity of comparative literature and appropriate identification keys (Oliveira et al., 2012). In viviparous species, such as members of the family Poeciliidae, describing these stages is even more challenging, because embryonic development occurs within the maternal reproductive tract, which hinders direct observation under natural conditions and generally requires dissection (Turner, 1937; Turner, 1940; Mousavi-Sabet, 2014). This methodological limitation hinders the understanding of crucial aspects of these organisms' life cycles.

The family Poeciliidae, popularly known as guppies, lebistes, mosquitofish or guarus, comprises teleost fishes with approximately 274 species distributed among about 29 genera (Lucinda, 2003; Nelson et al., 2016; Fricke et al., 2019). Poeciliids exhibit a wide variety of adaptive reproductive strategies. These include internal fertilization, the capacity for clonal reproduction and developmental modes that encompass oviparity and viviparity. In addition, these fish exhibit different forms of embryonic nutrition, including lecithotrophy and matrotrophy. They may also undergo superfetation – where multiple gestations occur simultaneously at different developmental stages – as well as store sperm from their partners after copulation (Thibault and Schultz, 1978; Reznick, 2018; Facey et al., 2022). Males possess a distinctive morphological adaptation: the anterior rays of the anal fin are modified into a copulatory organ known as the gonopodium – formed by the third, fourth or fifth anal-fin ray – which is essential for the transfer of spermatophores (Evans and Meisner, 2019).

The family exhibits pronounced sexual dimorphism. In general, males are significantly smaller (approximately 4.0 cm) and display vibrant coloration and complex patterns, which play a decisive role in sexual selection (Houde, 1997). Females, in contrast, may reach up to 6.5 cm in length and exhibit more cryptic coloration and a more robust body, which contributes to protection against predators (Lucinda, 2003; Andrade et al., 2005; Shoji et al., 2006).

Poecilia reticulata is a Neotropical species native to Central America and northern South America. Omnivorous and highly adaptable, it has historically been introduced into various regions of the world, including Brazil, as part of biological control programs targeting disease-vector mosquitoes, due to its efficiency in consuming larvae (Lucinda, 2003; Deacon, 2023; El-Sabaawi et al., 2016). At the same time, its hardiness, the striking coloration of males and high fecundity have consolidated its position as one of the most popular species in the ornamental fish trade (Endler, 1980; Deacon, 2023; Patel et al., 2023).

Despite its ecological and economic relevance, knowledge of its early ontogenetic development remains fragmented. Most of the literature focuses on aspects of sexual selection, behavior or ecology (e.g., Endler, 1980; Magurran, 2005), whereas detailed,

standardized descriptions of embryonic and larval stages are scarce. This gap hinders comparative studies of life histories and the ecological impacts of their populations, which are often invasive.

Therefore, this study aims to describe the embryonic and larval development stages of *P. reticulata*. This research seeks to fill a gap in basic knowledge of this species' reproductive biology by providing a morphological reference framework that may support future studies in areas such as ecology, fisheries management, ornamental aquaculture and investigations into the reproductive plasticity of poeciliids.

2. Materials and Methods

Adult females of *P. reticulata* (n = 19) were collected from the Santa Fé River, in the municipality of Palotina, Paraná State, southern Brazil (24°17'65" S; 53°50'53.72" W). The sampled stretch is characterized by lentic conditions, predominantly sandy substrate, an average depth of approximately 50 cm and well-preserved riparian vegetation. Specimens were captured along the river margins using hand sieves with a 5 mm mesh size.

In the field, females presenting visibly distended abdomens, indicative of advanced reproductive condition, were selected for analysis. The specimens were transported alive to the Laboratory of Ecology, Fisheries and Ichthyology (LEPI), Federal University of Paraná, Palotina Campus, in aerated containers. All procedures were conducted in accordance with animal welfare guidelines and approved by the Ethics Committee on Animal Use of the Federal University of Paraná (CEUA/UFPR; Protocol No. 42/2015).

A total of 19 adult females were analyzed. Body mass (g) and total length (cm) were recorded before fixation. Females presented a mean body mass of 0.24 ± 0.11 g and a mean total length of 2.88 ± 0.39 cm. Subsequently, specimens were euthanized by thermanarcosis, following Freire and Gonçalves (2013), and fixed in Davidson's solution, following Pérez-Robles et al. (2016). After fixation, females were dissected under a stereomicroscope and all embryos present in the ovarian cavity were carefully removed. The embryos were preserved in 70% ethanol for subsequent analysis. Embryonic developmental stages were identified based on external morphological characteristics, following the criteria proposed by Sanches et al. (2001).

Images were obtained using an AVerMedia image capture system (AVerVision CP155) coupled to a stereomicroscope (Leica® DM1000). A total of 379 images from embryos and larvae were recorded and analyzed. For each developmental stage, morphological characteristics such as body shape, eye development, pigmentation pattern, notochord formation, yolk sac presence and tail position were evaluated. Whenever possible, embryo length and diameter were measured from digital images.

Data were analyzed descriptively. Female body mass and total length are presented as mean $\pm$ standard deviation. The frequency of each developmental stage was expressed as a percentage of the total number of embryos analyzed. Morphological descriptions were used to characterize and compare the observed developmental stages.

3. Results and Discussion

Females of *P. reticulata* possess a single gonad, formed by the anastomosis of the two primordial ovaries, constituting a pouch of germinative tissue that contains oogonia and oocytes (Betito, 2006). Development begins with the proliferation of oogonia through successive mitotic divisions, followed by the onset of the first meiotic division, which is arrested at prophase I (Wootton and Smith, 2014).

In studies involving already-fertilized females, as in the present study, direct observation of the initial stages (oogonia and primary oocytes) is hindered, leaving only advanced-stage oocytes visible. These post-copulatory oocytes are characterized by a significant accumulation of yolk, a typical adaptation of viviparous development that ensures embryonic nutrition (Campos, 1999; Reznick et al., 2002).

The difficulty of sampling females in the pre-copulatory phase is intrinsically linked to the species' reproductive singularity. Males of *P. reticulata* use a modified gonopodium to transfer spermatophores (spermatocysts). During copulation, this gonopodium may detach and remain attached to the female urogenital opening (Evans et al., 2011). This mechanism ensures successive fertilization of multiple batches of oocytes over several reproductive cycles without the need for additional copulations (Campos, 1999; Constantz, 1989). This strategy of internal fertilization and sperm storage represents an adaptive advantage in variable environments (Magurran, 2005).

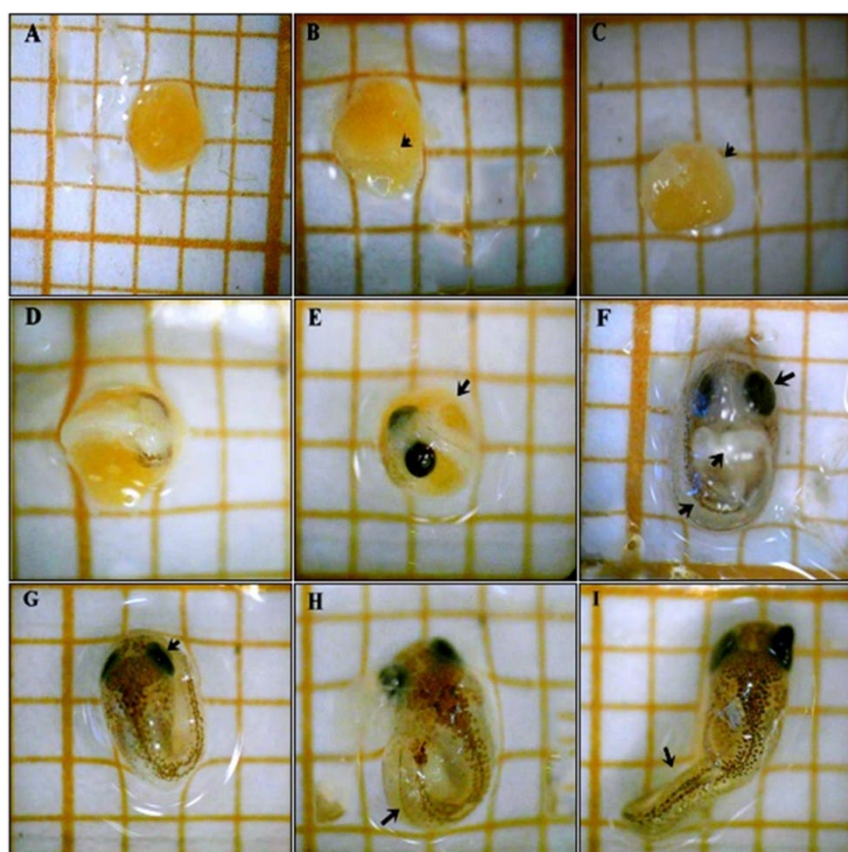


Figure 1 – Embryonic and larval development of *Poecilia reticulata*. A-C: Early stages of gastrulation – A) oocytes approximately 0.5 mm in diameter; B) optic primordia (arrows); C) indications of notochord formation (arrows). D-F: Organogenesis stages. Embryos approximately 1.5 mm in diameter and 1.3 mm in length. D) Development of the notochord (whitish structure) and the onset of eye formation, still with little pigmentation. E) Lateral view – tail coiled around the embryo's body. F) Ventral view – tail coiling, eye position, remnants of the yolk sac, and the onset of body pigmentation. G-I: Larval development. G) Embryo at the larval stage showing well-developed and pigmented eyes, with the tail beginning to uncoil. H) Advancement of tail uncoiling and definition of the pigmentation patterns previously described. I) Complete uncoiling of the tail. Structures indicated by arrows.

Among the 379 images recorded and analyzed, the simultaneous presence of different embryonic stages within the same female was observed, indicating superfetation. The presence of multiple embryonic developmental stages in a single female confirms superfetation in *P. reticulata*, a pattern described for Poeciliidae (Turner, 1937; Thibault and Schultz, 1978; Aranzábal et al., 2006). This condition is typical of Poeciliidae, in which the same gonad may simultaneously exhibit multiple embryonic stages, functioning both as germinative tissue and as an incubator (Aranzábal et al., 2006; Betito, 2006). This reproductive strategy allows greater adaptive success in unstable environments by ensuring continuous offspring release. Of the total embryos analyzed, approximately 6% were at the gastrulation stage (Figure 1 A-C), 93% at organogenesis (Figure 1 D-F) and 1% at the larval stage (Figure 1 G-I). During gastrulation, embryos exhibited approximately 0.5 mm in diameter, bright yellow coloration and early indications of optic primordia and notochord formation (Figure 1 C). Gastrulation represents the final phase of cell division and may show early signs of eye formation (Sanches et al., 2001). During organogenesis, embryos reached approximately 1.3 mm in length and 1.5 mm in diameter, with the formation of the embryonic axis and differentiation of the cephalic and caudal regions (cephalo-caudal elongation). The notochord became well defined, eyes were pigmented, the yolk sac was reduced, the first somites were observed and the onset of body pigmentation occurred (Figure 1 D-F; Sanches et al., 2001; Gilbert, 2002; Faustino et al., 2011). The predominance of the organogenesis phase suggests it is the longest stage in the species' embryonic cycle. The larval stage was characterized by complete tail uncoiling, full eye development and the presence of dark pigmentation distributed throughout the body (Figure 1 G-I). Individuals averaged 1.7 mm in length and 0.3 mm in diameter. According to the literature on other species, this stage corresponds to the point at which the larva, with the tail completely free, is ready to hatch. The predominant coloration is grayish, with melanotic pigments dispersed over the body surface, gills and a functional mouth (Sousa and Severi, 2000; Sanches et al., 2001; Andrade et al., 2014).

4. Conclusion

This study characterized the embryonic and larval developmental stages of *P. reticulata*, confirming superfetation and revealing morphological patterns consistent with those reported for other teleosts. These findings enhance the understanding of the species' reproductive biology and provide a basis for comparative and ecological studies, as well as for applications in ornamental aquaculture management.

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Murray E. Fowler: A consolidação da Medicina de Animais Selvagens na UFPR

Pedro Ribas Werner

Em 1985, durante a gestão do Professor Metry Bacila na coordenação do Programa de Pós-Graduação em Ciências Veterinárias da Universidade Federal do Paraná, foi concebido o I Curso de Medicina e Cirurgia de Animais Selvagens. Coordenada pelo Professor Pedro Ribas Werner, a iniciativa trouxe a Curitiba o Professor Murray E. Fowler, da University of California, Davis, reconhecido internacionalmente como um dos pioneiros da Medicina de Animais Selvagens e de Zoológico. O curso representou um divisor de águas para a especialidade no Brasil, influenciando a formação de profissionais e a consolidação de uma área que, à época, ainda dava seus primeiros passos no meio acadêmico nacional.

A realização daquele curso constituiu uma decisão ousada e visionária. Em meados da década de 1980, a Medicina de Animais Selvagens ainda ocupava espaço restrito nas escolas veterinárias brasileiras, e a vinda de um especialista de reconhecimento mundial exigiu capacidade de articulação institucional, determinação e, sobretudo, visão. A proposta inicialmente voltada aos alunos regularmente matriculados no Programa despertou grande interesse e foi posteriormente aberta a médicos-veterinários de diferentes regiões do país, reunindo participantes provenientes do Amazonas ao Rio Grande do Sul.

A presença do Professor Fowler em Curitiba ultrapassou os limites de um evento acadêmico. Seu vasto conhecimento, sua experiência profissional e seu entusiasmo inspiraram uma geração de médicos-veterinários que viria a construir as bases da Medicina de Animais Selvagens no Paraná e no Brasil. Entre eles, destacam-se nomes como o do Professor José Ricardo Pachaly, posteriormente responsável por importantes avanços na especialidade e idealizador do Ambulatório de Animais Selvagens da UFPR, o primeiro vinculado a uma universidade brasileira. Anos mais tarde, o Professor Rogério Ribas Lange deu continuidade a esse legado, assumindo papel de destaque na área e contribuindo significativamente para o fortalecimento do ensino, da pesquisa e da assistência em Medicina de Animais Selvagens na Universidade Federal do Paraná, mantendo e ampliando o trabalho iniciado por seu antecessor.

Além das atividades didáticas, a visita de Fowler proporcionou oportunidades singulares de intercâmbio científico e humano. Durante sua permanência no Brasil, realizou expedições de campo ao lado do Professor Pedro Ribas Werner, incluindo uma memorável viagem ao Pantanal Mato-Grossense.

Os desdobramentos daquela visita histórica estenderam-se muito além do curso realizado em 1985. A criação de grupos de estudo, o fortalecimento da atuação do Hospital Veterinário da UFPR na área de animais selvagens, a formação de recursos humanos especializados e o estabelecimento de uma sólida tradição acadêmica podem ser associados, direta ou indiretamente, à semente plantada por Murray Fowler em sua primeira passagem pela instituição.

Em reconhecimento à extraordinária contribuição de Murray E. Fowler, a então Associação Paranaense de Medicina de Animais Selvagens – Grupo Fowler instituiu a Comenda Fowler, destinada a homenagear pessoas e instituições com atuação destacada em prol da Medicina de Animais Selvagens e da conservação da fauna brasileira.

Ao revisitar essa trajetória, torna-se evidente que a história do Programa de Pós-Graduação em Ciências Veterinárias da Universidade Federal do Paraná também se constrói a partir dos encontros que transformam pessoas e instituições. A iniciativa coordenada pelo Professor Pedro Ribas Werner representa um exemplo da importância da visão na construção das instituições acadêmicas. Ao reconhecer oportunidades, reunir pessoas e transformar ideias em ações concretas, contribuiu para que o PPGCV ampliasse seus horizontes e deixasse uma marca duradoura na história da Medicina Veterinária brasileira.

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Manufatura aditiva aplicada na fabricação de dispositivos assistivos para animais

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Resumo: A Manufatura Aditiva (MA) evoluiu da prototipagem para a manufatura de produtos finais complexos e de alto valor agregado. Diversos setores têm incorporado a MA em seus processos de desenvolvimento e fabricação, com destaque para a área da saúde que tem empregado amplamente na produção de instrumentos cirúrgicos, modelos anatômicos, próteses, órteses e implantes. No entanto, a sua aplicação na medicina veterinária ainda é recente e pouco explorada. Animais com deformações congênitas ou traumáticas frequentemente não utilizam dispositivos ortopédicos devida escassez de evidências científicas sobre sua eficácia, além do alto custo e da complexidade de sua fabricação. Neste contexto, o objetivo deste trabalho foi desenvolver soluções assistivas personalizadas para dois animais com necessidades específicas, utilizando tecnologias de projeto e de fabricação de baixo custo. O primeiro caso envolveu uma cadela sem raça definida que sofreu amputação total do membro pélvico esquerdo. A região amputada foi digitalizada para a obtenção da anatomia e para o posterior desenvolvimento e fabricação de uma prótese articulada utilizando MA. O segundo caso envolveu um marreco com úlcera de córnea em um dos olhos, que necessitava de proteção contra traumas e de instilação de colírios para tratamento. Para este animal, foi realizada, no projeto e na fabricação, por MA, uma digitalização indireta e a fabricação de um capacete de proteção para o olho direito. Em ambos os casos, foi possível demonstrar a versatilidade da MA na criação de dispositivos personalizados que promovem a qualidade de vida e viabilizam tratamentos para diferentes espécies animais.

Palavras-chaves: Manufatura aditiva, impressão 3D, medicina veterinária, prótese, saúde veterinária.

1. Introdução

Por mais de um século, os produtos industriais se limitavam às capacidades de fabricação dos processos tradicionais de usinagem, conformação, moldagem, entre outros. A manufatura aditiva (AM, do inglês *Additive Manufacturing*) muda fundamentalmente isso e viabiliza a criação de objetos sob demanda, complexos, *free-forms* e com diversas características antes impensadas (Fischer et al., 2004). A AM é a maior novidade na área da manufatura nas últimas três décadas (Thompson et al., 2016; Sossou et al., 2018). Seu diferencial é o método de fabricação por adição sucessiva de camadas de material, com base em informações de um modelo 3D computacional (Volpato, 2017).

Atualmente, a norma ISO/ASTM 52900:2021 classifica a AM em sete categorias, diferenciadas pela forma como os materiais são processados. Enquanto algumas categorias e tecnologias ainda são restritas a grandes corporações e centros de pesquisa, outras, devido à sua simplicidade operacional, ao custo acessível e à ampla disponibilidade de material, são mais difundidas. Destacam-se, entre essas, a fabricação por extrusão de material em forma de filamento fundido (FFF). Esse processo utiliza materiais poliméricos em forma de filamentos, que são aquecidos e extrudados por meio de um bico metálico.

Diversos setores têm empregado a MA na prototipagem e no desenvolvimento de produtos, na fabricação de ferramentais e também na produção final. Na fabricação de produtos finais, destaca-se a área da saúde, que demanda produtos e soluções alinhados às potencialidades da MA, como elevado grau de personalização, concepção rápida e fabricação de geometrias complexas, entre outras. As principais aplicações da MA na área da saúde incluem a fabricação de tecidos e órgãos (Frankowski et al. 2023), a elaboração de modelos anatômicos (Santos et al. 2025), o desenvolvimento de próteses e implantes personalizados (Mendoza-DeCal et al., 2023), soluções farmacêuticas, especialmente em formas de administração de medicamentos (Dimitrov et al., 2023, etc). O emprego da MA na área da saúde ainda é recente, mas Culmone, Smit e Breedveld (2019) apontam que, apesar dos desafios ainda existentes em uma área tão nova, a MA representa uma grande oportunidade para melhorar o acesso à assistência médica em países em desenvolvimento, além de ser um avanço significativo rumo a uma medicina específica para o paciente. Kumar et al. (2021) indicam um crescimento expressivo de mais de 18 vezes nas publicações científicas que envolvem o emprego da MA na área médica entre 2011 e 2020.

Na medicina veterinária, as aplicações da MA são ainda mais recentes e há poucos relatos na literatura. Mesmo as órteses e próteses convencionais utilizadas na medicina veterinária ainda apresentam diversas limitações, como a adaptação às necessidades específicas de cada animal e o elevado custo dos dispositivos (Arauz et al., 2021). Lee (2021) menciona que essas dificuldades são exacerbadas pela escassez de experiência profissional na área, pela limitada disponibilidade de dispositivos personalizados e pela falta de resultados científicos consistentes sobre a eficácia dos tratamentos em pacientes veterinários. A aplicação da MA, embora

promissora, enfrenta obstáculos na pesquisa e no desenvolvimento de soluções específicas para a medicina veterinária e demanda mais estudos e avanços tecnológicos (Chopra, 2023).

Outra dificuldade observada na fabricação de órteses e próteses por MA é na etapa de modelagem do produto, que exige considerar aspectos de DFAM (*Design for Additive Manufacturing*) e de ergonomia para garantir a eficiência do produto. DFAM é um conjunto de regras e boas práticas que considera fatores como a tecnologia de MA a ser empregada, a geometria da peça, os materiais e o custo durante a modelagem, de modo a resultar em peças geometricamente otimizadas (Fischer et al., 2004). Por outro lado, a ergonomia na modelagem dos produtos deve considerar a interação do produto com o animal, visando garantir a eficiência, melhorar o uso, a adaptação e o bem-estar do animal. Wang (2024) explorou a aplicação da ergonomia no projeto de próteses caninas fabricadas por MA para melhorar a mobilidade de animais de estimação com deficiência e reduzir lesões secundárias decorrentes de próteses mal projetadas. Os projetos tradicionais de membros protéticos geralmente carecem de adaptabilidade, mas a ergonomia pode melhorar significativamente o conforto e a funcionalidade das próteses por meio de design personalizado e tecnologia eletrônica.

Diante disso, este trabalho tem o objetivo de contribuir para o avanço no desenvolvimento de soluções assistivas na medicina veterinária, desenvolvendo soluções personalizadas para dois animais, de espécies distintas, com necessidades especiais, utilizando DFAM e ergonomia na modelagem, tecnologias modernas de projeto, digitalização 3D e fabricação por MA de baixo custo.

2. Desenvolvimento

Com o objetivo de desenvolver soluções assistivas para dois animais com necessidades especiais, foram empregados conceitos de DFAM e de ergonomia para projetos personalizados, de rápida concepção e focados na fabricação de baixo custo por MA. Na sequência, os dois casos são descritos, cada um com suas peculiaridades.

2.1. Caso 1 – Prótese articulada

O primeiro projeto foi desenvolvido para uma cadela sem raça definida que sofreu amputação total do membro pélvico esquerdo. A cadela se adaptou a locomover-se sem uma das patas, de modo a compensar a falta do membro; porém, essa adaptação causa maior distribuição de peso sobre a pata traseira direita, que fica sobrecarregada, além de torcioniar a coluna vertebral do animal, o que acarreta frequentes lesões.

A primeira etapa do projeto envolveu a digitalização direta para obtenção da anatomia do animal. O animal foi colocado em uma posição confortável, em pé, com apoio da tutora, para que permanecesse parado enquanto uma digitalização de 360° era realizada. A digitalização foi realizada por meio do aplicativo *Kiri Engine*, instalado em um smartphone modelo iPhone 14, versão iOS 18.1.1, resultando em uma superfície poligonal exportada na extensão STL (do inglês *Stereolithography*).

A superfície digitalizada foi editada no programa gratuito Meshmixer (Autodesk, EUA), no qual foi projetada uma interface para a fixação da prótese à anatomia da região pélvica do animal (Fig. 1).

A interface segue a anatomia do animal e possui aberturas circulares para reduzir o peso, o contato e permitir a ventilação. A ausência de coto torna a fixação da prótese mais difícil, e a solução adotada foi adicionar uma ancoragem na perna direita existente, ambas ligadas ao peitoral, para auxiliar na fixação da prótese/cão. Com base na digitalização do animal, foi projetada uma prótese articulada, modelada a partir da anatomia da região amputada, utilizando um sistema CAD 3D (do inglês *Computer-Aided Design*), empregando o DFAM, de modo a facilitar a fabricação por MA. A prótese desenvolvida conta com uma articulação que permite ao cão sentar ou deitar sem a retirada da própria prótese, proporcionando uma melhor adaptação às necessidades cotidianas do animal. As partes foram montadas com elementos de fixação, e a articulação foi projetada com parafuso na função de eixo e mola de torção para o retorno à posição vertical, assim que o cão volta a se erguer.

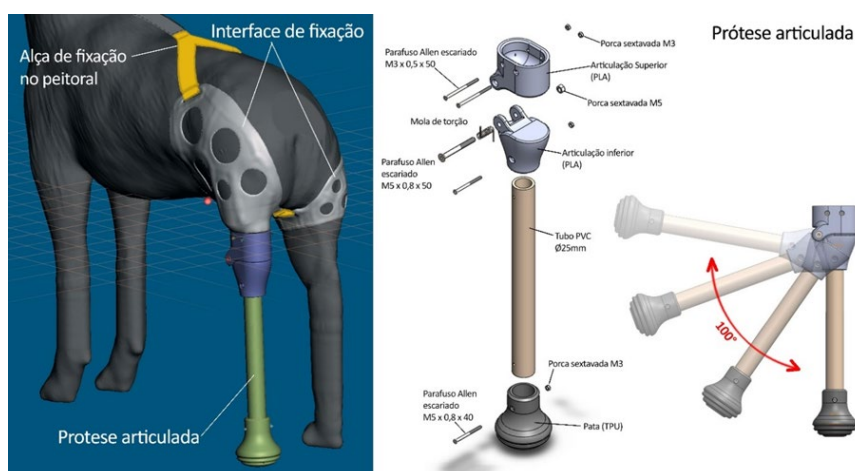


Figura 1 – Projeto da prótese articulada com a interface de fixação na anatomia digitalizada do animal.

Para a prototipação da maioria das peças, foi utilizado o processo de MA com o princípio FFF. A interface de fixação e a pata foram fabricadas com filamento flexível de TPU (termoplástico de poliuretano) para melhorar a adaptação e o conforto das peças ao corpo do cão; as demais peças foram fabricadas com filamento de PETG (polietileno tereftalato glicol) para aumentar a resistência mecânica do componente. O PETG é um polímero amplamente disponível, de baixo custo e de fácil processamento. Foi utilizada, na impressão 3D, uma máquina CR10 V2 da Creality, com volume de impressão de 300 x 300 x 400 mm (CxLxA), que possui mesa aquecida e estrutura aberta. O planejamento do processo de impressão foi realizado no programa *Open Source Ultimaker Cura 5.0*. Os principais parâmetros de impressão utilizados estão apresentados na Tabela 1. A haste principal da prótese foi fabricada com tubo de PVC de 25 mm de diâmetro. Na montagem da prótese, utilizou-se uma mola de torção para a articulação do joelho, além de elementos de fixação, como parafusos e porcas. Para a fixação da prótese ao peitoral, foram costuradas alças com velcro ajustáveis. Após a prototipagem das peças e montagem da prótese, ela foi testada no animal em um atendimento intermediado pela tutora do animal, pela veterinária do projeto e pela equipe de desenvolvimento.

Parâmetro	Valores	
	TPU	PETG
Altura da camada	0,2 mm	0,2 mm
Temperatura de impressão	230°C	232°C
Temperatura da 1ª camada	235°C	237°C
Temperatura da mesa de impressão	60°C	80°C
Veloc. de extrusão na 1ª camada	10 mm/s	20 mm/s
Veloc. de extrusão geral	20 mm/s	40 mm/s
Contornos sólidos na parede	4	4
Camadas sólidas inferior	4	4
Camadas sólidas superior	4	4
% de preenchimento interno	20%	20%
Geometria de preenchimento	Linear cruzada	Linear cruzada
Distância de retração	1mm	5 mm
Velocidade de retração	10 mm/s	50 mm/s

Tabela 1 – Principais parâmetros do processo de fabricação por MA em TPU e em PLA.

2.2. Caso 2 – Capacete de proteção

O segundo caso trata-se de um projeto de dispositivo para um marreco com úlcera de córnea no olho direito, cuja necessidade era a proteção do olho contra traumas, bem como impedir que a ave raspasse o olho em superfícies ásperas durante crises de dor ou prurido. Em virtude deste problema, o animal já havia perdido a visão do olho esquerdo. O dispositivo também precisava permitir a instilação regular de colírios para tratamento.

Para este animal, a digitalização direta por escaneamento foi descartada devido à dificuldade em mantê-lo estático até o final do processo. A anestesia também foi descartada por haver riscos à vida do animal. Para contornar este problema, foram tiradas fotos e realizadas medições da cabeça do marreco. Essas informações foram utilizadas para esculpir, em massa, plastilina, uma réplica da cabeça, região de interesse. Essa réplica foi digitalizada para o desenvolvimento do projeto e para a fabricação, por MA, de um capacete de proteção para o olho direito (Figura 2a).

A escultura da cabeça da ave foi digitalizada em um escâner de base giratória da *Matter and Form* (Canadá). A precisão da digitalização deste equipamento é de $\pm 0,1$ mm, e o volume de escaneamento é de 25 cm (altura) x 18 cm (diâmetro). Um arquivo na extensão STL foi gerado e exportado para o programa Meshmixer, para edição da superfície resultante. No próprio programa, foi projetado um capacete para proteção do olho direito, com os seguintes requisitos: leveza, ergonomia, conforto, facilidade de montagem e de fabricação por MA.

Na figura 2b, pode-se ver o projeto do capacete modelado sobre a superfície digitalizada. Para facilitar a montagem e a fixação, foi criada uma abertura na parte de trás, com furos para fixar com cordão ou fita. Áreas vazadas foram criadas para aumentar o conforto, reduzindo material, peso e área de contato.

Na região do olho doente, foi projetada uma calota de proteção com furos circunferenciais para garantir a visibilidade e um furo central para a administração de remédios. O capacete foi fabricado em TPU flexível para que a prótese se acomodasse melhor à cabeça, e os parâmetros de impressão foram os mesmos utilizados no projeto anterior e estão listados na Tabela 1.

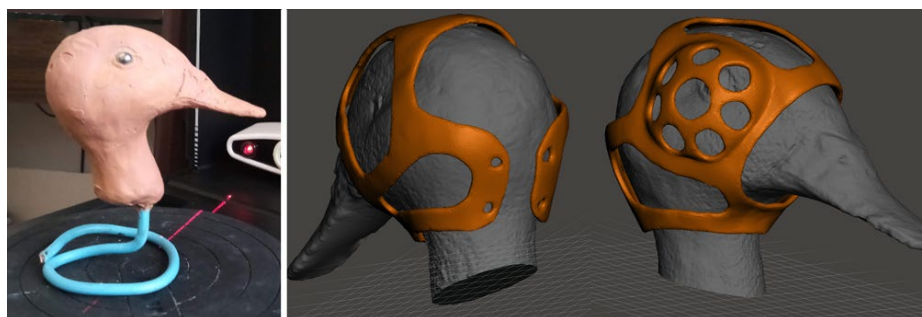


Figura 2 – Em (a), a digitalização 3D da escultura; em (b), o projeto do capacete sobre a superfície digitalizada.

2.3. Testes nos animais e avaliação

Ambos os dispositivos foram inicialmente montados nos animais, com a presença dos tutores e das médicas veterinárias do projeto, que orientaram os ajustes. Após um mês de uso, foi solicitado aos tutores o preenchimento de uma ficha de avaliação da satisfação com o dispositivo assistivo desenvolvido. Essa ficha foi adaptada da ficha de avaliação proposta por Demers, Weiss e Ska (2002), utilizada na avaliação de pessoas usuárias de soluções de Tecnologia Assistiva. O questionário contém doze perguntas sobre o grau de satisfação com o dispositivo utilizado. As respostas são dadas em uma escala de 1 a 5, em que: 1 - Insatisfeito, 2 - Pouco satisfeito, 3 - Mais ou menos satisfeito, 4 - Bastante satisfeito e 5 - Totalmente satisfeito. No final, essas notas são computadas e resultam em uma pontuação máxima. Além disso, foi solicitado aos tutores que, ao final, informassem os três itens de maior satisfação. Foram considerados aprovados os dispositivos que obtiverem nota final igual ou superior a quatro.

Avaliação da satisfação do cuidador/veterinário com o dispositivo assistivo instalado no animal com deficiência.

- O objetivo do questionário abaixo é avaliar o grau de satisfação com o dispositivo tecnológico assistivo e os serviços relacionados que um animal com deficiência está usando.

Dispositivo instalado: _____
Data da avaliação: _____
Qual espécie / raça do animal: _____
Idade do animal: _____
Qual a deficiência e causa: _____
Quanto tempo está usando o dispositivo: _____
Em média quantas horas diárias o animal usa o dispositivo? _____
O animal fez fisioterapia: _____
Observações extras: _____

• Para cada um dos itens, avalie sua satisfação usando a seguinte escala de 1 a 5:

1	2	3	4	5
Insatisfeito	Pouco satisfeito	Mais ou menos satisfeito	Bastante satisfeito	Totalmente satisfeito

- Por favor não deixe nenhuma pergunta sem resposta.
- Assinale entre as 12 perguntas as 3 mais importantes pra você.
- Obrigado por dedicar seu tempo e participar desta pesquisa. Esta pesquisa é totalmente anônima.

Nº	Qual é o seu grau de satisfação com o dispositivo assistivo:	Nota	3 mais import.
1	As dimensões (tamanho, altura, comprimento, largura)		
2	O peso		
3	A facilidade de ajustar (fixar, afivelar)		
4	A estabilidade e a segurança		
5	A durabilidade (acabamento, revestimento, rigidez, resistência, etc)		
6	A facilidade de uso		
7	O conforto		
8	A eficácia (o quanto o recurso atende às necessidades)		
9	O processo desenvolvimento e entrega (procedimentos, tempo de espera)		
10	Reparos e a assistência técnica (manutenção) prestados		
11	A qualidade dos serviços prestados (inform., atenção) recebida para o uso		
12	Os serviços de acompanhamento (serviços de suporte contínuos) recebidos		

Figura 3 – Ficha de registro do animal e de avaliação da satisfação com o produto assistivo.

3. Resultados e discussões

A digitalização direta da cadela (caso 1) por meio do aplicativo *Kiri Engine* (Shenzhen, China), instalado em um iPhone 14, gerou uma malha poligonal incompleta e com muitas falhas, conforme pode ser visto na Fig. 4. Foi necessária a correção extensa da malha e a modelagem complementar no programa Meshmixer (www.meshmixer.org/), usando como referência fotos e medidas do animal. O uso de um escâner específico para a digitalização de superfícies orgânicas poderia gerar um melhor resultado e reduzir o processo de modelagem. Somente com a superfície completamente reconstruída foi possível projetar a interface de fixação. Apesar da necessidade de uma grande reconstrução da superfície digitalizada, o resultado final da interface se ajustou bem ao animal, conforme pode ser visto na Fig. 5.

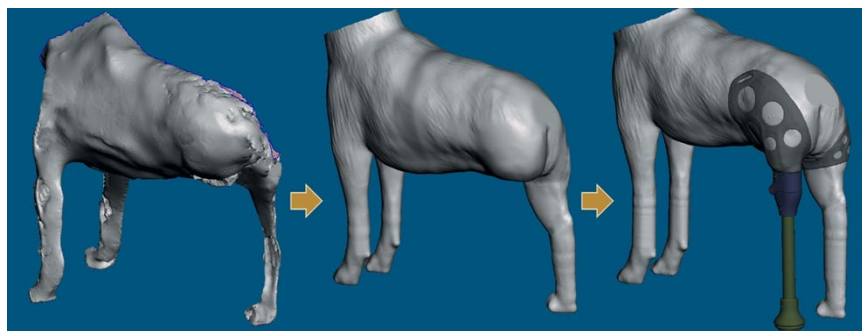


Figura 4 – Resultado da digitalização e da edição da superfície anatômica para o projeto da interface de fixação.



Figura 5 – Prótese fabricada por MA: (a) detalhe da articulação, (b) montagem e fixação e (c) treinamento de uso da prótese.

A digitalização indireta da cabeça do marreco, no caso 2, por meio do escaneamento da réplica em massa de modelar, foi uma estratégia bem-sucedida, pois gerou uma superfície poligonal de boa qualidade, sem aberturas na malha e com reprodução dos detalhes necessários. A alta qualidade da digitalização garantiu um projeto de capacete que se adequou perfeitamente ao animal, protegendo a região de interesse. O sucesso dessa estratégia dependeu da habilidade na modelagem manual da réplica, seguindo as medições do animal.

A fabricação por meio da MA por FFF, em ambos os casos, permitiu a obtenção de produtos com geometrias complexas, em material flexível, de baixo custo e com concepção rápida. A fabricação da prótese do caso 1 (cadela) levou quarenta e duas horas de impressão e custou R\$ 80,00 com filamentos, sendo trinta e cinco horas de impressão com TPU (450g, R\$ 67,00) e sete horas de impressão com PETG (106g, R\$ 13,00).

No caso 2, do marreco, a fabricação do capacete levou duas horas e consumiu em torno de 20 g de material, a um custo aproximado de R\$ 3,00.

No caso 1, após a fabricação e a montagem da prótese, esta foi montada no animal. A cadela, extremamente dócil e calma, deixou que a prótese fosse instalada facilmente. Em um primeiro momento, ela não entendeu o que estava acontecendo, mantendo o peso distribuído entre suas três patas. O primeiro passo para a adaptação da prótese foi forçar, gentilmente, a cadela a apoiar parte do seu peso sobre a prótese. Isso foi alcançado ao erguer levemente a pata traseira direita do chão. A cadela rapidamente entendeu que poderia apoiar-se na prótese. Em seguida, a cadela foi incentivada a começar a caminhar. No início a cadela tendeu a andar como habitualmente, em três patas, mas com a utilização de um tecido passando por baixo do abdômen para que a posição normal de caminhar de um cão com quatro patas fosse alcançada e dificultando assim o apoio apenas em três patas, a cadela começou a utilizar a prótese como a quarta pata para caminhar, ver Fig. 5c. Em seguida, a cadela foi incentivada a se sentar e em seguida a deitar para a avaliação da funcionalidade da articulação. A articulação funcionou como previsto e a cadela conseguiu sentar e deitar com a prótese facilmente. O processo de adaptação depende fortemente do interesse e da dedicação dos tutores, que precisam dedicar tempo e paciência para que a cadela se acostume à prótese e crie a confiança necessária para caminhar com ela.

A colocação do capacete no marreco do Caso 2 foi realizada de maneira simples, sem dificuldades e com fácil ajuste (Fig. 6). Inicialmente, o animal demonstrou leve desconforto, mas se adaptou rapidamente, uma vez que a órtese foi projetada com tamanho adequado e material leve. O capacete facilitou a instilação dos colírios para o tratamento da úlcera de córnea, contribuindo efetivamente para a recuperação. Durante o uso, o marreco tentou esfregar o olho afetado com os pés, mas a órtese desempenhou seu papel protetor, evitando danos adicionais à área afetada.



Figura 6 – Prótese fabricada por MA: (a) detalhe da articulação, (b) montagem e fixação e (c) treinamento de uso da prótese.

Os conceitos de DFAM e de cuidados ergonômicos no projeto se mostraram eficazes nas soluções desenvolvidas. Apesar da geometria complexa, os produtos foram facilmente fabricados por MA com pouca ou nenhuma necessidade de estruturas de suporte. Os produtos se adaptaram perfeitamente à anatomia dos animais e não causaram nenhum desconforto visível. Wang (2024) menciona que, no futuro, a ergonomia tem um vasto potencial de aplicação no design de produtos para animais. Com o avanço da tecnologia, as técnicas ergonômicas permitirão uma simulação mais precisa da biomecânica natural dos animais, fornecendo próteses e órteses que se alinham melhor às suas estruturas fisiológicas, aumentando, assim, o conforto e a mobilidade. Além disso, o desenvolvimento de sistemas de monitoramento inteligentes permitirá uma análise de dados em tempo real mais eficaz durante o processo de recuperação de animais de estimação com próteses, ajudando a otimizar ainda mais o design e os ajustes necessários.

3.1. Resultado da avaliação de satisfação com o produto desenvolvido

Ambos os dispositivos foram inicialmente montados nos animais, com a presença dos tutores e das médicas veterinárias do projeto.

A avaliação de satisfação dos tutores com os dispositivos desenvolvidos foi realizada por meio do questionário adaptado, ilustrado na Figura 3. Os tutores atribuíram notas de 1 a 5 e realçaram os três itens de maior importância; ver célula colorida de cinza na Tabela 2. No caso 1, os tutores ficaram satisfeitos com o peso, a facilidade de ajuste e as dimensões da prótese. Porém, apontam necessidades de melhoria no ajuste, na estabilidade e na segurança.

No caso 2, os tutores ficaram bastante satisfeitos com a eficácia, a facilidade de uso e de ajuste, e totalmente satisfeitos com os demais itens. No geral, a avaliação médica foi de 4,45, o que indica que se enquadra como bastante satisfeito com o produto desenvolvido.

Qual é o seu grau de satisfação com:	Caso 1	Caso 2	Média
As dimensões (tamanho, altura, comprimento, largura)	4	5	4,5
O peso	5	5	5
A facilidade de ajustar (fixar, afivelar)	5	4	4,5
A estabilidade e a segurança	3	5	4
A durabilidade (acabamento, revestimento, rigidez, resistência, etc)	4	5	4,5
A facilidade de uso	4	4	4
O conforto	3	5	4
A eficácia (o quanto o recurso atende às necessidades)	3	4	3,5
O processo desenvolvimento e entrega (procedimentos, tempo de espera)	4	5	4,5
Reparos e a assistência técnica (manutenção) prestados	5	5	5
A qualidade dos serviços prestados (inform., atenção) recebida para o uso	5	5	5
Os serviços de acompanhamento (serviços de suporte contínuos) recebidos	5	5	5
Avaliação média de cada dispositivo	4,17	4,75	4,45

Tabela 2 – Avaliação de satisfação dos responsáveis, utilizando os dispositivos desenvolvidos.

4. Conclusão

Com o objetivo de desenvolver soluções assistivas personalizadas para dois animais, observou-se que o emprego de tecnologias de projeto modernas, digitalização 3D e fabricação de baixo custo por MA permite produzir soluções que melhoram a condição de vida dos animais. No caso 1 da cadela amputada, a MA mostrou-se eficiente na confecção de uma prótese personalizada, leve, ergonômica, funcional e de baixo custo. A prótese produzida mostrou-se eficiente não apenas na funcionalidade, mas também possibilitou um bom ajuste no corpo do animal, permitindo que ele passasse a caminhar como um cão de quatro patas. São necessárias algumas melhorias para aumentar o conforto, a estabilidade e a segurança do dispositivo. No caso 2 do marreco com

úlceras no olho direito, a MA, usando um material flexível, permitiu a fabricação de um capacete com geometria complexa, leve, ergonômico e que cumpriu a função de proteção, de garantir a visão e de administrar medicação quando necessária.

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Marcelo Beltrão Molento

As pesquisas lideradas pelo **Dr. Marcelo Beltrão Molento**, Professor Titular do Departamento de Medicina Veterinária da UFPR, têm importância estratégica e direta para a saúde animal, a sociedade e a sustentabilidade ambiental do Brasil. Suas contribuições têm como objetivo apresentar modelos de sustentabilidade dos recursos para a manutenção da saúde animal, incluindo o combate à resistência aos medicamentos.

O Dr. Molento é pioneiro no estudo da resistência genética de parasitos aos antiparasitários convencionais na criação de bovinos, ovinos e equinos, bem como das características epigenéticas da tolerância dos hospedeiros. Suas pesquisas evidenciam falhas nos tratamentos e calculam as perdas para a sociedade e para os animais. O prof. trabalha com métodos alternativos para reduzir o uso excessivo de produtos químicos, estudando as características específicas de cada propriedade, aplicando o método FAMACHA. Em 2004, ele apresentou o Sistema Integrado de Controle Parasitário (SICOPA), que é aplicado no Brasil e em vários países latino-americanos.



Food and Agriculture Organization of the United Nations

Suas pesquisas focam em terapias limpas para o manejo de vermes e carrapatos, utilizando fitoterapia (extratos de plantas), fungos nematófagos, biotecnologia e metais bioativos. Essas tecnologias de baixa toxicidade reduzem a presença de resíduos químicos nos animais e no ambiente brasileiros. As pesquisas do prof. têm sido levadas ao público-alvo sob a forma de publicações técnicas, livros e cursos de formação para centenas de pessoas, em parceria com várias entidades no Brasil.

Por meio de estudos com modelos epidemiológicos e do uso da biologia molecular, suas pesquisas também investigam a expansão geográfica de doenças parasitárias de importância na interação humano-animal, como a *Fasciola hepatica*. Este e outros trabalhos conferiram ao prof. Molento mais de uma dezena de prêmios em pesquisa, reforçando seu destaque mundial. O Dr. Molento atua diretamente como consultor especialista para a Organização das Nações Unidas para a Alimentação e a Agricultura (FAO/ONU), a Organização Mundial da Saúde (OMS) e a Organização Mundial de Saúde Animal (OMSA), o que posiciona o Brasil na vanguarda científica global do setor agroveterinário.

Recentemente, o prof. Marcelo Molento passou a ocupar a cadeira número 18 da Academia Paranaense de Medicina Veterinária, ACAPAMEVE, fato bastante relevante para o curso de Medicina Veterinária, para o Programa de Pós-graduação e para toda a UFPR.



Archives of Veterinary Science



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Dedicação!



Revisão sistemática integrativa das principais neoplasias do tecido neural em cães

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Resumo: As neoplasias do tecido neural são pouco relatadas na espécie canina e incluem diferentes tipos e subtipos histológicos, como o meningioma, astrocitoma, oligodendroglioma, tumor do plexo coroide e ependimoma, sendo este último um dos mais raros. Em geral, esses tumores afetam animais de meia-idade, sem predisposição sexual comprovada. Em relação às raças, há ampla variabilidade; entretanto, parece haver maior acometimento na raça Boxer, bem como entre os cães sem raça definida. A finalidade deste estudo foi realizar uma revisão sistemática integrativa das principais neoplasias do tecido neural em cães. Foi realizada uma busca sobre as principais neoplasias do tecido neural em cães, por meio de periódicos nacionais e internacionais já publicados, nos bancos de dados PubMed, Scielo, BVS e Google Acadêmico. O meningioma foi o tipo histológico mais relatado, com 535 casos; seguido pelo glioma, com 199 casos, divididos em astrocitomas e oligodendrogliomas; pelo tumor de plexo coroide, com 120 casos; e pelo ependimoma, com 19 casos. Não houve predisposição sexual evidente. A idade média observada foi de 8 anos, embora tenha havido uma ligeira variação entre os diagnósticos. No que diz respeito às raças, os cães braquicefálicos e os animais sem raça definida, além dos Boxers, foram os mais descritos. Por meio do presente estudo, foi possível reafirmar a prevalência dos meningiomas, seguidos pelos gliomas, bem como a superioridade na incidência da raça Boxer. Além disso, podem afetar uma ampla faixa etária, dos animais de meia-idade aos senis. Contudo, demonstra-se também a existência de contradições em relação a outras descrições, o que torna necessária a continuidade das pesquisas.

Palavras-chave: glioma; meningioma; sistema nervoso; tumores.

1. Introdução

Os tumores do tecido neural podem causar impacto significativo no bem-estar dos cães, com uma variedade de sinais clínicos que afetam a qualidade de vida e podem exigir intervenções terapêuticas complexas (Horta et al., 2013). Esse impacto é potencializado pelas dificuldades atualmente presentes no diagnóstico, no tratamento e na prevenção na neuro-oncologia veterinária. Os tumores são responsáveis por uma parcela significativa das neuropatias em cães, sendo as neoplasias primárias do tecido neural entre as causas mais frequentes de sinais clínicos neurológicos em animais senis (Platt and Olby, 2013).

As neoplasias intracranianas representam até 2% das neoplasias diagnosticadas em cães (Miller e Rossmeisl, 2019). Entre elas, as mais frequentes são meningiomas, gliomas e neoplasias do plexo coroide. O meningioma tem origem em células das meninges; já o glioma tem origem em células da glia e pode ser classificado em astrocitoma ou oligodendroglioma, quando sua origem está nos astrócitos ou nos oligodendrócitos, respectivamente. As neoplasias do plexo coroide surgem das células produtoras de líquido, enquanto os ependimomas têm origem nas células ependimárias (Dewey e Da Costa, 2016).

A avaliação neurológica geralmente é dificultada devido à baixa especificidade dos sinais clínicos, principalmente em animais de idade avançada, que, em sua maioria, apresentam outras enfermidades concomitantes. A manifestação clínica observada, em geral, não depende do tipo tumoral, mas sim da sua neurolocalização. Apesar das diversas apresentações, alguns sinais clínicos são mais frequentemente observados, como crises epiléticas, andar em círculos, déficits nas reações posturais e alterações comportamentais (Daleck and Nardi, 2016).

A pesquisa contínua é crucial para aprimorar a compreensão das neoplasias do tecido neural, o que pode levar ao desenvolvimento de novas estratégias terapêuticas e ao aperfeiçoamento dos resultados clínicos (Higgins et al., 2017). Nesse contexto, o objetivo deste trabalho foi realizar um levantamento retrospectivo das principais neoplasias do tecido neural em cães, avaliando a distribuição geral dessas neoplasias quanto à localização. Além disso, foi avaliado o perfil epidemiológico associado a cada tipo neoplásico, buscando padrões de acometimento por raça, sexo e idade.

2. Materiais e Métodos

Foi realizado um estudo sistemático, integrativo e retrospectivo, com revisão da literatura, sobre as principais neoplasias do tecido neural em cães. O levantamento bibliográfico e a coleta de informações foram realizados nas bases de dados PubMed, SciELO, Google Acadêmico e na Biblioteca Virtual em Saúde (BVS), utilizando descritores em dupla linguagem, em português e em inglês (Tabela 1).

Termos de Busca em Português	Termos de Busca em Inglês
Neoplasias Primárias do Sistema Nervoso em Cães	<i>Primary Neoplasms of the Nervous System in Dogs</i>
Neoplasias Primárias Intracranianas em Cães	<i>Primary Intracranial Neoplasms in Dogs</i>
Tumores Cerebrais Primários em Cães	<i>Primary Brain Tumors in Dogs</i>

<https://doi.org/10.5380/avs.v31i2.102964>

1

Meningioma Canino	<i>Canine Meningioma</i>
Glioma Canino	<i>Canine Glioma</i>
Astrocitoma Canino	<i>Canine Astrocytoma</i>
Oligodendroglioma Canino	<i>Canine Oligodendroglioma</i>
Tumor de Plexo Coróide Canino	<i>Canine Choroid Plexus Tumor</i>
Ependimoma Canino	<i>Canine Ependymoma</i>

Tabela 1 – Termos de busca utilizados nos idiomas português e inglês, com o intuito de ampliar o alcance dos trabalhos.

Após a busca inicial, procedeu-se à seleção dos trabalhos, com a eliminação dos sem aprovação ética, dos não originais e dos que apresentaram duplicidade. Após isso, iniciou-se o processo de filtragem dos trabalhos restantes.

A filtragem dos trabalhos se deu em três etapas: inicialmente, a seleção por análise do título; em seguida, a seleção por meio da leitura do resumo (*abstract*); e, por fim, a filtragem após a leitura integral dos trabalhos.

Para a classificação dos tipos/subtipos histológicos, adotou-se o seguinte padrão: o meningioma foi classificado em meningotelial, transicional, anaplásico, psamomatoso, fibroblástico, papilar, microcístico, granular, atípico, vacuolar e mixoide. O glioma foi classificado como astrocitoma e oligodendroglioma; o primeiro, como anaplásico, gemistocístico, fibrilar, protoplasmático, pilocítico e atípico; já o segundo, como anaplásico. O ependimoma foi classificado como papilar, tancítico e anaplásico. O tumor do plexo coróide foi classificado como papiloma, carcinoma ou atípico (Higgins et al., 2017).

Também foram analisadas as características de população, sexo, idade e raça. As informações obtidas foram incluídas conforme constavam nos trabalhos originalmente consultados. Casos em que determinada informação não constava foram classificados como não informados (NI). Posteriormente, por meio das etapas de filtragem, foi possível selecionar trabalhos acadêmicos condizentes com a proposta apresentada, que foram utilizados na análise final dos dados.

3. Resultados

Foram acessados 3.553 artigos publicados entre 1964 e 2023, que foram posteriormente submetidos às etapas de filtragem, resultando em 78 artigos no total (Figura 1). Dentre os trabalhos incluídos por autor/as (Figura 2) na pesquisa, foram contabilizados 873 casos de neoplasias do tecido neural em cães, classificados quanto ao tipo histológico (Tabela 2 e Figuras 3 e 4) e analisados separadamente quanto ao perfil epidemiológico dos animais acometidos. A variável racial foi avaliada para cada tipo histológico e para a casuística total do trabalho (Tabela 3 e Figura 5).

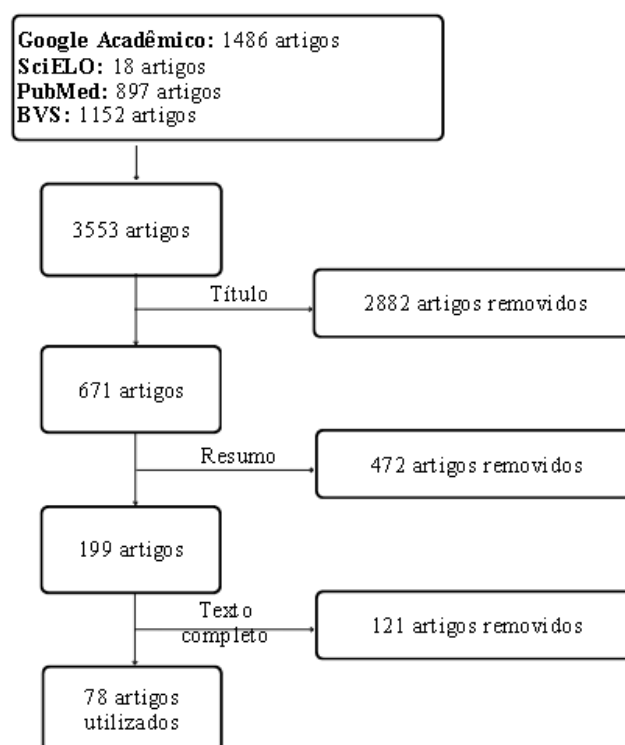


Figura 1 – Fluxograma da pesquisa e suas respectivas filtrações, com base na leitura do título, do resumo e do texto completo.

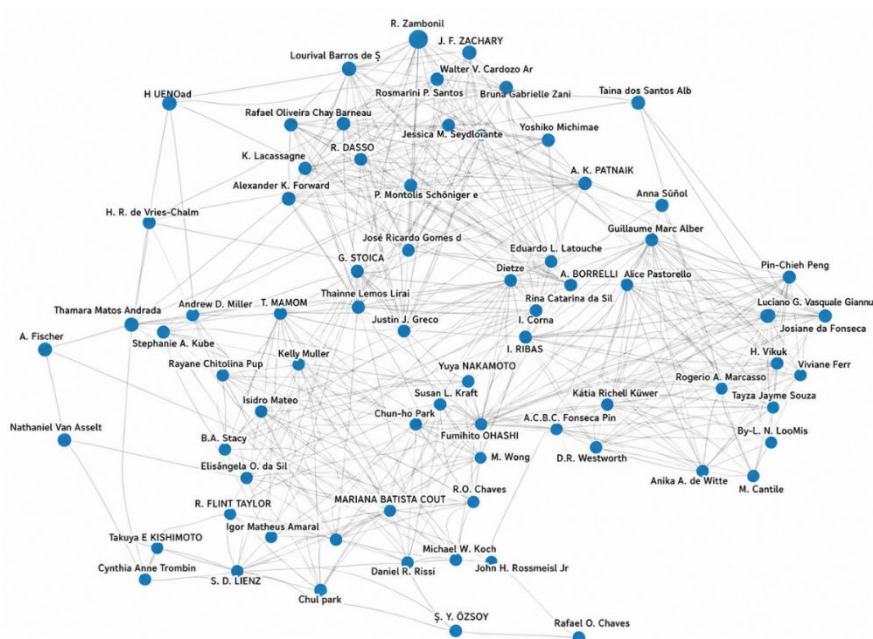


Figura 2 – Mapa em rede ilustrando a correlação entre os autores e os trabalhos incluídos sobre neoplasias em cães.

Tipo histológico	N
Meningioma	535
Glioma	199
Tumor de Plexo Coróide	120
Ependimoma	19

Tabela 2 – Tipos histológicos das neoplasias no SNC, descritos em valores absolutos.

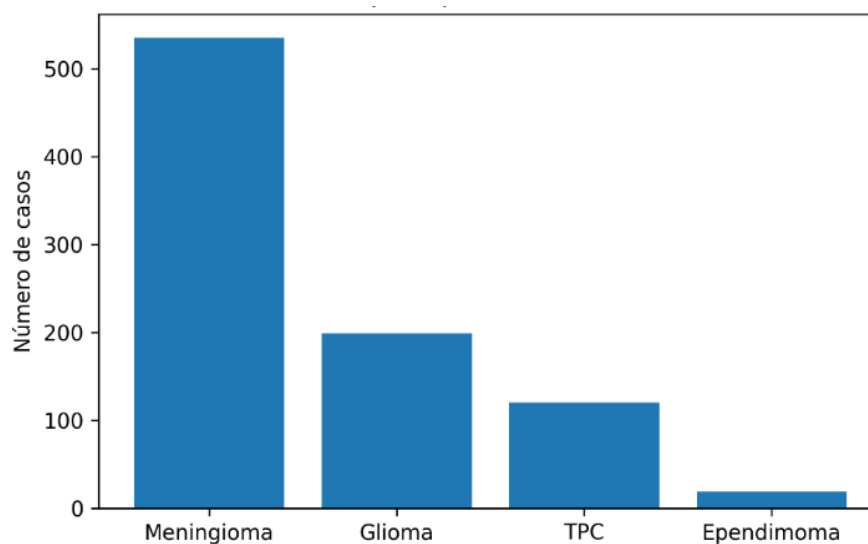


Figura 3 – Distribuição dos principais tipos histológicos de neoplasias do tecido neural em cães e o número de casos descritos nos estudos incluídos nessa revisão.

O meningioma foi a neoplasia mais frequentemente observada (Figura 4), correspondendo a 61,3% dos casos avaliados, seguido pelos gliomas, que incluem astrocitomas e oligodendrogliomas, com 22,8%; em terceiro, os tumores do plexo coroide, com 13,7%; e, por fim, os ependimomas, com 2,3% dos casos, valores que corroboram as descrições da literatura.

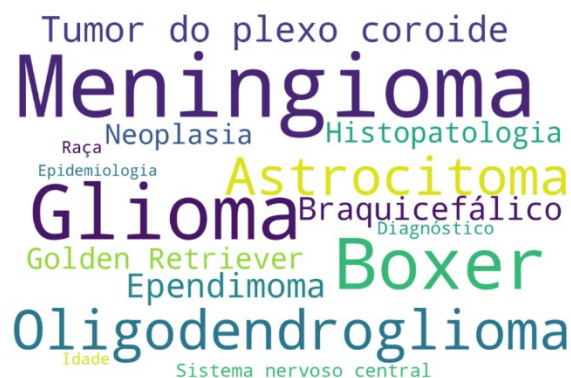


Figura 4 – Nuvem de palavras utilizadas nos 78 artigos incluídos nesta revisão sistemática integrativa. Os termos incluem títulos e subtítulos, resumos e palavras-chave presentes nos trabalhos. O tamanho das palavras indica sua frequência nos textos; as cores dividem a categoria dos termos.

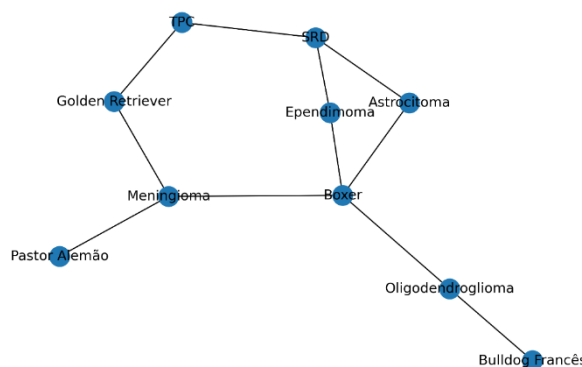


Figura 5 – Rede de associação entre os principais tumores neurais em cães e as raças caninas mais acometidas. De modo que, quanto mais próxima a raça do tipo histológico, mais frequentemente essa raça é correlacionada com o neoplasma.

Observa-se forte associação entre meningiomas e cães das raças Boxer, Pastor Alemão e Golden Retriever. Os oligodendrogliomas apresentaram maior incidência em cães da raça Bulldog Francês, enquanto os tumores do plexo coróide acometeram mais cães da raça Golden Retriever. Ademais, os ependimomas e astrocitomas afetaram mais frequentemente cães sem raça definida (SRD) e cães da raça Boxer.

Raças	Meningioma	Astrocitoma	Oligodendroglioma	Ependimoma	T.P.C.
Alsatian		1			
Boxer	64	18	16	5	
Bulldog Francês			25		
Bulldog Inglês		2	1		
Bulldog			2		
Beagle				1	1
Bloodhound				1	
Boston Terrier	2		5		
Boerboel					1
Chihuahua	7				
Cane Corso					1
Cocker Spaniel					2
Dachshund	13				
Dálmata	2	1			
Fox terrier					1
Golden	42	6	5		16

Husky	4				1
Labrador	26				1
Lhasa Apso		1			
Maltês				1	1
Mongrel				1	
Poodle	17			1	
Pitbull	3		1		
Pinscher		1			
Pequinês				1	
Pointer				1	
Pastor Alemão	48	3	1		
Rottweiler	6				1
Shnauzer	7				
Shih tzu		1			
Sharpei					2
Staffordshire					1
Setter Inglês					
SRD		20	11	6	5
Veadeiro Pampeano				1	
Weimaraner					1
Yorkshire			3		
NI	173	53	20	0	84

Tabela 3 – Relação de raças observadas em todas as neoplasias, em valores absolutos.

3.1. Meningioma

Os trabalhos sobre meningioma somaram 535 casos, dos quais 233/535 machos e 235/535 fêmeas, com 67/535 casos não informados, tornando-se a neoplasia mais relatada nesta pesquisa. No que se refere à idade média observada, os meningiomas foram predominantes em cães com idade média igual ou superior a 8 anos, sendo raros em animais mais jovens. Em relação às raças, destacaram-se os cães sem raça definida (SRD), com 121/535 dos casos, seguidos por Boxer (64/535), Pastor Alemão (48/535) e Golden Retriever (42/535). Ainda assim, 173/535 dos casos foram de raças não informadas. As demais raças encontradas nesta pesquisa foram descritas na Tabela 3. Os subtipos histológicos dos meningiomas foram descritos na Tabela 4, na qual se observou predominância do subtipo meningotelial.

Subtipo histológico	N
Meningotelial	65
Transicional	59
Anaplásico	44
Psamomatoso	25
Fibroblástico	12
Papilar	10
Microcístico	7
Granular	4
Atípico	3
Vacuolar	1
Mixóide	1
NI	304

Tabela 4 – Subtipos histológicos do meningioma, descritos em valores absolutos.

3.2. Glioma

Os gliomas foram o 2º tipo neoplásico de maior incidência, classificados em astrocitomas e oligodendrogliomas, ambos de grande relevância entre as células da glia do sistema nervoso central.

3.2.1. Astrocitoma

Os casos de astrocitoma somaram 107, sendo a neoplasia mais frequente entre os gliomas nesta pesquisa. Quanto ao sexo, foram descritos 34/107 machos, 36/107 fêmeas e 37/107 casos sem informação. No que diz respeito à idade média, os astrocitomas foram predominantes em cães com idade média igual ou superior a 6 anos, com acometimento considerável em animais mais jovens. Em

relação às raças, destacaram-se os cães sem raça definida (SRD), com 20/107 dos casos, seguidos pelas raças Boxer (18/107), Golden Retriever (06/107) e Pastor Alemão (03/107). As demais raças foram descritas na Tabela 3. Os subtipos histológicos do astrocitoma de maior prevalência foram, respectivamente, anaplásico (9), gemistocístico (7), fibrilar (5), protoplasmático (3), pilocítico (2), atípico (1) e não informados (80).

3.2.2. Oligodendroglioma

Os casos de oligodendroglioma somaram 92, o que os torna a segunda neoplasia entre os gliomas mais relatados neste trabalho. Destes, 33/92 eram machos, 22/92 eram fêmeas e 37/92 casos não foram informados. Quanto à idade média, os oligodendrogliomas foram frequentemente relatados em cães com idade média igual ou superior a 8 anos, assim como os meningiomas, com acometimento raro em animais mais jovens. Em relação às raças, evidenciou-se a raça bulldog francês como a mais predominante, com 25/92 dos casos, seguida pelas raças boxer (16/92), golden retriever (05/92) e boston terrier (05/92). As demais raças foram descritas na Tabela 3. Os subtipos histológicos do oligodendroglioma observados foram anaplásicos (31) e não informados (58).

3.3. Tumor de plexo coroide

Os casos de tumor do plexo coroide (TPC) somaram 120, o que o torna a 3ª neoplasia mais frequentemente relatada nesta pesquisa. Quanto à diferenciação sexual, 52/120 eram machos e 46/120 eram fêmeas, com 22/120 casos não informados. Quanto à idade média, os tumores do plexo coroide foram predominantes em cães com idade média igual ou superior a 7/8 anos, com acometimento raro em animais jovens. Em relação às raças, destacaram-se os cães da raça Golden Retriever, com 16/120 casos, seguidos pelos cães sem raça definida (SRD), com 5/120 casos, e pelos Sharpei, com 2/120 casos. Os casos de raça não informada somaram 84/120. As demais raças encontradas foram descritas na Tabela 3. Os subtipos histológicos do tumor de plexo coroide encontrados foram, respectivamente, papiloma (32), carcinoma (6), atípico (5), não informado (77).

3.4. Ependimoma

Os casos de ependimoma somaram 19, o que o torna a 5ª neoplasia mais frequentemente relatada nesta pesquisa. Destes, 10/19 (52,63%) eram machos e 9/19 (47,36%) fêmeas. A idade média dos ependimomas foi de 6 a 7 anos. Em relação às raças, destacam-se os cães sem raça definida (SRD), com 06/19 (31,57%) dos casos, seguidos pelas raças Boxer, com 05/19 (26,31%), e Bloodhound, com 01/19 (5,26%). As demais raças foram descritas na Tabela 3. Os subtipos histológicos do ependimoma encontrados foram, respectivamente, papilares (6), anaplásicos (3), tancíticos (1) e não informados (9).

4. Discussão

Os tumores primários representam a terceira maior casuística neurológica em cães, correspondendo a cerca de 5,9% de todas as doenças neurológicas (Chaves et al., 2018; Frade et al., 2018). As neoplasias mais observadas neste estudo foram meningiomas, gliomas (astrocitomas e oligodendrogliomas), tumores do plexo coroide e ependimomas.

O meningioma foi o histótipo mais presente, representando 61,3% dos casos, em concordância com relatos de que o meningioma é a neoplasia de maior prevalência no tecido neural de cães (Daleck and Nardi, 2016). A idade média encontrada foi de 8 anos, aproximando-se dos valores observados na literatura, que indicam uma média entre 7 e 8 anos, com maior ocorrência em idades iguais ou superiores a 5 anos (Babicsak et al., 2011; Motta et al., 2012; Santos et al., 2012). Contudo, há dados que indicam uma idade média de 10 anos nos casos de meningioma (Marcapasso et al., 2015). Em relação ao sexo, não se observou predisposição, em concordância com a literatura (Bagley, 2005; Foster et al., 1988; Motta et al., 2012; Montoliu et al., 2006; Patnaik et al., 1986).

No que diz respeito às raças, o Boxer se sobressaiu, em consonância com descrições que evidenciam a predominância dessa raça nesse tipo histológico (Andrews, 1973; Snyder et al., 2006; Sturges et al., 2008). Em seguida, foram observadas as raças Pastor Alemão (8,97%) e Golden Retriever (7,85%), ambas incluídas no relato de predisposição racial a meningiomas (Carvalho et al., 2016). Além disso, cães sem raça definida (SRD) obtiveram valores consideráveis (22,61%) nesta pesquisa e também foram mencionados em outros estudos (Chaves et al., 2018; Song et al., 2013). Os animais apresentados são, em sua maioria, cães de grande porte e dolicocefálicos, corroborando relatos semelhantes (Silva et al., 2014).

Os meningiomas são descritos em diversos subtipos histológicos. Isso ocorre devido à origem embriológica e às células do tecido nervoso (Marcapasso et al., 2015). No presente trabalho, foram identificados 11 subtipos, sendo o meningotelial e o transicional os mais frequentes; ambos somaram 23,16% do total de casos. Dados semelhantes já foram descritos (Higgins et al., 2017; Marcapasso et al., 2015; Montoliu et al., 2006).

Os gliomas foram a segunda neoplasia mais comum na pesquisa. Entre eles, o astrocitoma demonstrou-se o mais frequente, representando 12,3% dos casos totais, corroborando relatos de que a neoplasia glial mais frequente seria o astrocitoma (Maxie, 2007); contudo, os valores encontrados entram em conflito com alguns relatados na literatura, que retratam o oligodendroglioma como o mais comum em cães (Snyder et al., 2006; Truve et al., 2016). A idade média encontrada para esse tumor foi de 6 anos, em consonância com descrições que informam a idade média de astrocitomas em cães acima de 5 anos (Sembritzki et al., 2002; Storts, 1995; Summers et al., 1995; Zaki and Hurvitz, 1976); todavia, os resultados deste trabalho divergem de dados que relatam uma idade média de 8-9 anos (Bwangomoi, 1968; Moulton, 1961). Não há predileção sexual aparente (Bagley et al., 1999; Snyder et al., 2006; Summers et al., 1995; Lecouter, 2001), conforme observado na pesquisa.

Quanto às raças, há uma predisposição descrita em animais braquicefálicos para o astrocitoma canino (Bwangomoi, 1968; Higgins et al., 2017; O'Brien and Coates, 2010), o que reforça os dados desta pesquisa, com o Boxer (16,82%) como a raça mais comum pesquisada, em consonância com outros trabalhos (Sembritzki et al., 2002; Storts, 1995; Summers et al., 1995; Zaki and

Hurvitz, 1976). Neste estudo, observaram-se seis subtipos histológicos, com ênfase nos subtipos anaplásico (8,41%) e geminocístico (6,54%), conforme as descrições anteriores (Burger e Scheithauer, 1994; Lopes e Vandenberg, 2002).

Os oligodendrogliomas foram a segunda neoplasia mais frequente no grupo dos gliomas, menos frequentes do que os astrocitomas (Innes and Saunders, 1962; Moulton, 1961). Dados da literatura indicam que os oligodendrogliomas representam até 14% de todas as neoplasias intracranianas primárias em cães (Snyder et al., 2006). No presente trabalho, os oligodendrogliomas representaram 10,4% de todos os casos, o que os coloca atrás dos astrocitomas. A idade média encontrada foi de 8 anos, corroborando relatos de acometimento dos oligodendrócitos em cães com idade igual ou superior a 8 anos (Snyder et al., 2006; Triolo et al., 1994), sendo frequentemente observado em animais entre 5 e 11 anos (Luginbuhl et al., 1968). Considerando o fator sexual, observou-se maior acometimento em machos (35,86%), embora não haja predisposição sexual descrita (Bagley et al., 1999; Snyder et al., 2006; Summers et al., 1995; Lecouter et al., 2001).

No que se refere as raças, os oligodendrogliomas possuem uma predisposição observada em animais braquicefálicos, principalmente nas raças Boxers, Boston Terrier e Bulldog Inglês (Luginbuhl, 1963; Smit, 1961), o trabalho em vigência entra de acordo com as duas primeiras raças descritas, contudo, os dados desta pesquisa mostram que o Bulldog Francês se sobressai, dos 92 casos de oligodendroglioma apresentados, 25/92 (27,17%) foram desta raça, tendo em vista que o Bulldog Inglês obteve somente 1 caso relatado nesta pesquisa. Do mesmo modo que as demais neoplasias, os oligodendrogliomas também apresentam subtipos histológicos; contudo, apenas 2 foram identificados, sendo o anaplásico (33,69%) o mais comum (Koestner et al., 1999).

O tumor de plexo coróide é uma neoplasia incomum que afeta o epitélio do plexo coróide, localizado nos ventrículos, somando 7-10% de todas as neoplasias do tecido neural em cães (Dalton et al., 2019; Meuten, 2017), o que afirma os dados apresentados neste trabalho, onde o tumor de plexo coróide foi a terceira neoplasia do tecido neural em cães mais predominante, reunindo 120/873 (13,7%) dos casos descritos, atrás apenas do meningioma. A idade média identificada foi de 7/8 anos, ficando um pouco acima da média mencionada, onde descrevem uma média de 6 anos (Meuten, 2017; Summers et al., 1995), contudo, a média apresentada segue de acordo com outros trabalhos, pois, pode haver uma variação de 1-13 anos de idade (Cantile et al., 2002; Dalton et al., 2019; Hammer et al., 1990; Lipsitz et al., 1999; Miller et al., 2019; Patnaik et al., 1980; Ribas et al., 1989; Snyder et al., 2006; Wilson et al., 1989; Westworth et al., 2008), além disso, outros estudos sugerem uma variação entre 5-7 anos de idade (Nashold et al., 1961).

Em relação ao sexo, 52/120 (43,33%) eram machos e 46/120 (38,33%) foram fêmeas, concordando com relatos que descrevem uma certa predisposição ao macho sobre as fêmeas (Baba and Catoi, 2007; Moore et al., 1996), já outros autores reconhecem a predisposição sexual aos machos como verdadeira (Luginbuhl et al., 1968; Zaki and Nafe, 1980). Contudo, os estudos acerca das neoplasias do tecido neural, em sua grande maioria, não evidenciam predisposições sexuais; diante dos resultados, é possível que haja uma equalização dessa opinião (Thankey et al., 2006; Westworth et al., 2008). No que se refere às raças, não há predisposição racial confirmada para tumores do plexo coróide; no entanto, a raça Golden Retriever é a mais afetada (Westworth et al., 2008). Ademais, há relatos de que raças de grande porte podem ser as mais afetadas (Song et al., 2013). Conforme informado neste trabalho, esta raça destacou-se, representando 16/120 (13,33%) dos animais acometidos.

Entre os subtipos histológicos dos tumores do plexo coróide, os principais são: o carcinoma do plexo coróide, encontrado em 62-64% dos casos, seguido pelo papiloma do plexo coróide, em 36% (Dalton et al., 2019; Westworth et al., 2008). Entretanto, os dados apresentados diferem dos de alguns autores, pois, no presente estudo, o subtipo de papiloma do plexo coróide foi o mais frequentemente observado (25,83%), em comparação com o carcinoma (5%).

Os ependimomas em cães são tumores considerados incomuns a raros (Vandeveldt et al., 2012), podendo representar cerca de 3% dos tumores do tecido neural (Higgins et al., 2017). Seguindo essa linha, essa neoplasia foi a menos observada neste trabalho, totalizando 19/873 (2,3%) dos casos. A idade média encontrada, de 6/7 anos, reafirma as descrições de que a idade de acometimento dos ependimomas varia entre 6 e 12 anos (Higgins et al., 2017; Silva et al., 2014). Quanto ao sexo, não há predisposição específica em cães (Maxie & Youssef, 2007; Santos et al., 2012). No presente estudo, dos casos de ependimoma encontrados, 10/19 foram machos (52,63%) e 9/19 foram fêmeas (47,36%). No entanto, assim como os astrocitomas, apresentam alterações semelhantes às descritas por outros autores.

Dentre os animais acometidos, os que mais se destacam são os cães sem raça definida (SRD), principalmente os braquicefálicos, seguidos pela raça Boxer (Higgins et al., 2017; Silva et al., 2014). Bem como aqui exposto, os cães SRD foram os mais atingidos, representando 06/19 (31,57%), e, em seguida, a raça Boxer, com 05/19 (26,31%), o que confirma os dados informados pelos demais pesquisadores. O tumor das células ependimais também é classificado em subtipos histológicos; foram identificados 3 subtipos para essa neoplasia, em destaque o papilar (31,57%) e o anaplásico (15,78%), sendo este último considerado raro (Koestner and Higgins, 2002; Miller et al., 2019); outro subtipo não especificado foi o ependimoma benigno (Chaffee, 1977; de Vries-Chalmershoynk et al., 1988; Wright, 1985).

5. Conclusão

As neoplasias do tecido neural em cães são variadas e podem ou não apresentar predisposição racial e/ou sexual. Algumas podem acometer cães braquicefálicos; já outras, cães de grande ou pequeno porte. Podem afetar uma ampla gama de idades, desde animais muito jovens até animais senis; porém, a média seria de cães de meia-idade, com mais de 6 anos. Por meio deste estudo retrospectivo, foi possível reafirmar a predominância dos meningiomas, com destaque para seus subtipos meningotelial e transicional, quando comparados aos demais tumores, seguidos pelos gliomas – astrocitomas e oligodendrogliomas –, bem como a superioridade e a frequência da raça Boxer entre os animais afetados. Evidencia-se que cada neoplasia apresenta particularidades, desde a frequência de acometimento até variações histológicas, o que expõe contradições e similaridades em relação a outras descrições.

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