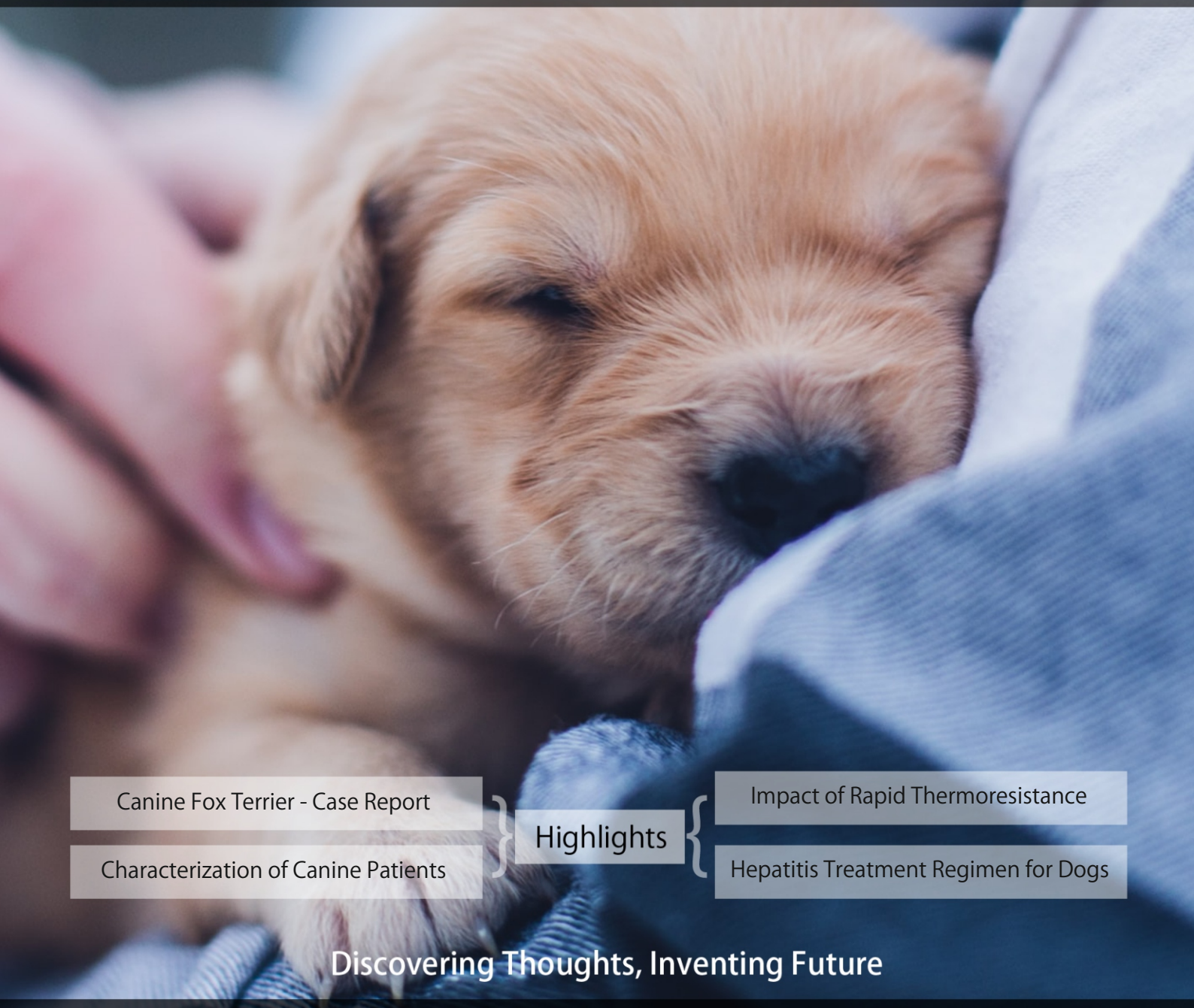
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Canine Fox Terrier - Case Report

Characterization of Canine Patients

Highlights

Impact of Rapid Thermoresistance

Hepatitis Treatment Regimen for Dogs

Discovering Thoughts, Inventing Future

VOLUME 24 ISSUE 1 VERSION 1.0

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Evaluating the Impact of Rapid Thermoresistance (RTT) on Membrane and Chromatin Features in Post-Thawed Bovine Semen

By Denise Martins Reis, Augusto Urzedo Pereira Queiroz, Chamberttan Souza Desidério, Amanda Pifano Neto Quintal, Marcelo Emílio Beletti, Bruno Augusto Nassif Travençolo, Guilherme Costa Venturini & André Belico de Vasconcelos

Universidade de Uberaba (UNIUBE)

Abstract- This work aims to evaluate the effects of rapid thermoresistance on the morphofunctional aspects of membranes using flow cytometry, as well as the quality of sperm chromatin using the Toluidine Blue technique, both before and after the thermoresistance test. Six sets of frozen semen from ten bulls were used. The samples were thawed in a water bath at 36°C and separated into two aliquots. The first aliquot, control group (C), was kept in a test tube previously heated to 36°C with subsequent evaluationsusingmicroscopy, cytometry, and chromatin analyses. The second aliquot, experimental group (E), underwent the rapid thermoresistance test (RTT) in a water bath preheated at 46°C for 30 minutes with subsequent evaluations usingmicroscopy, cytometry, and chromatin analyses. For statistical analysis, the paired T test, significance ($P<0.05$), was used.

Keywords: fertility, membranes, sperm cells, thermoresistance.

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Evaluating the Impact of Rapid Thermoresistance (RTT) on Membrane and Chromatin Features in Post-Thawed Bovine Semen

Denise Martins Reis [α], Augusto Urzedo Pereira Queiroz [σ], Chamberttan Souza Desidério [ρ], Amanda Pifano Neto Quintal [ω], Marcelo Emílio Beletti [¥], Bruno Augusto Nassif Travençolo [§], Guilherme Costa Venturini [χ] & André Belico de Vasconcelos [ν]

Abstract- This work aims to evaluate the effects of rapid thermoresistance on the morphofunctional aspects of membranes using flow cytometry, as well as the quality of sperm chromatin using the Toluidine Blue technique, both before and after the thermoresistance test.Six sets of frozen semen from ten bulls were used. The samples were thawed in a water bath at 36ºC and separated into two aliquots. The first aliquot, control group (C), was kept in a test tube previously heated to 36ºC with subsequent evaluationsusingmicroscopy, cytometry, and chromatin analyses. The second aliquot, experimental group (E), underwent the rapid thermoresistance test (RTT) in a water bath preheated at 46ºC for 30 minutes with subsequent evaluations usingmicroscopy, cytometry, and chromatin analyses. For statistical analysis, the paired T test, significance (P<0.05), was used. It was observed that from the results presented, semen samples after the resistance test showed a reduction of approximately 56% for motility and 36% for vigor (P< 0.05) when compared to samples before the test. In the flow cytometry evaluation, a significant difference was observed for all points analyzed (plasma membrane and acrosomal membrane). The results obtained with the Toluidine Blue technique, it can be observed that the RTT does not interfere with the chromatin structure, and this is probably due to the structure of mammal sperm DNA. It was concluded that the rapid thermoresistance test (RTT) alters the structures, mainly acrosomal structures, and has no interference in the chromatin compression structure, and should be considered, in association with other evaluation tests, a complementary parameter in the evaluation of bovine semen quality.

Keywords: fertility, membranes, sperm cells, thermoresistance.

I. Introduction

Artificial insemination with the use of genetically superior animals has been gaining prominence due to the excellent results in the commercialization of cryopreserved bovine semen. Thus, the concern with bull fertility is recurrent among rural producers, as well as scholars who dedicate themselves to sperm analysis to detect changes in sperm cells *(Alquézar-Baeta et al 2019)*.

The fertility potential of male cattle is determined through semen analysis in a set of evaluations. However, for this analysis to be more efficient, it is also necessary to evaluate the internal structure of the sperm in conjunction with the rapid thermoresistance test (RTT) *(Alves et al 2015)*. This can be used to evaluate the fertility of frozen semen, simulating a biological process. One of the possibilities to mimic this condition is to promote thermal stress, under controlled conditions, in a water bath, a situation close to the conditions to which it is exposed in the female genital tract *(Cunha et al 2012)*.

The potential study of RTT is to promote thermal stress in sperm, and in conjunction with flow cytometry and combined with the use of fluorophores, it allows evaluating the integrity of the plasma and acrosomal membrane, as well as the integrity of the DNA, after the induction of stress, providing pre- and post-heating parameters of post-thawed bull semen *(Arruda et al 2007)*. Also adding to the analysis, the Toluidine Blue (TB) technique has been widely used in the study of chromatin changes *(Mello 1982)*.

Therefore, this research aims to evaluate the effects of rapid thermoresistance on the morphofunctional aspects of plasma and acrosomal membranes, as well as on the chromatin quality of post-thawed bull semen, both before and after the rapid thermoresistance test.

II. Materials and Methods

a) Obtaining Bovine Semen Samples

Six batches of frozen semen from ten bulls were used, obtained from Sexing Technologies Repro I.C.M.G.A. Ltda. (Brindes) Use Authorization Protocol 135190833813983 08/11/2019 – CNPJ 23.694.902/0 001-70 – Indaiatuba – SP.Only samples containing at least 30% sperm with progressive motility were used in the study *(CBRA Manual, 2013)*.

b) Sperm Preparation

To carry out the experiment, the samples were thawed in a water bath at 36.8ºC *(CBRA Manual, 2013)* and separated into two aliquots. The first aliquot, control

Author α σ χ ν: Universidade de Uberaba (UNIUBE), Uberaba, MG, Brazil. e-mail: andre.vasconcelos@uniube.br
Author ρ: Universidade Federal do Triângulo Mineiro (UFTM), Uberaba, MG, Brazil.
Author ω: Faculdade Associadas de Uberaba (FAZU), Uberaba, MG, Brazil.
Author ¥ §: Universidade Federal de Uberlândia (UFU), Uberlândia, MG, Brazil.

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group (C), was kept in a test tube previously heated to 36.8°C and subsequently evaluated by microscopy, cytometry and chromatin analysis. The second aliquot, experimental group (E), was kept in a water bath at 46°C for 30 minutes to perform the rapid thermoresistance test (RTT) *(Arruda et al 2007, Cunha et al., 2012)*, followed by microscopy, cytometry and chromatin analyses. The experiment was carried out in triplicate and the evaluations were carried out by a single evaluator.

c) Sperm Evaluations

For the microscopic evaluations, a bright field microscope (Nikon eclipse e200) was used. 10 µL of the sample were added between the slide and coverslip, both before and after the thermoresistance test, and sperm motility and vigor were evaluated at 10x magnification, according to Brazilian College of Animal Reproduction *(Manual CBRA, 2013)*.

In the cytometric evaluations, a flow cytometer (FACSC alibur™) was used to detect the integrity parameters of the plasma (propidium iodide /1.5 mM) and acrosomal (Fitc-PNA/1.125 g/mL) membranes. A 560 nm short pass dichroic mirror,green fluorescence (FL1) was collected using a 515 meter545 nm bandpass filter. Red fluorescence (FL3) was collected through a 650 nm long-pass filter, after 640 nm filtering. long pass filter. The sheath/sample was placed on "low" andadjusted to a flow rate of 100 cells per second when analyzing a sample with a concentration of 1.25 x105 sperm/mL. Data acquisition from 15,000 cells was collected in list modeusing BD Cell Quest Pro software version 4.0 (Becton Dickinson®, San Jose, CA, USA)as described by *(Vasconcelos et al 2017).*

For chromatin analysis, the protocol as reported by Beletti and collaborators *(2005)* was followed. For this, the smear technique was performed on the samples taken at the two moments (before and after the thermoresistance test). They were fixed with absolute alcohol: acetic acid: 3:1, for one minute and afterwards immersed in 70% alcohol for three minutes. With this, four slides of each sample were made, which were later kept in 4N hydrochloric acid for 25 minutes, followed by washing in distilled water. After this procedure, the slides were kept at room temperature (dry) followed by the addition of three to four drops of Toluidine blue and covered with a coverslip. For the analysis, a microscope (Leica DM500) coupled to an image capture system (Leica ICC50) with a 100X oil-immersion objective in an optical microscope coupled to the camera was used, from which 30 to 50 images were obtained of each prepared slide.

The images obtained were light blue in color when they presented normal chromatin condensation, a variation from light blue to dark blue when the chromatin was moderately decondensed and a variation from dark blue to violet when the chromatin was highly decondensed.In view of this, to avoid the subjectivity of the chromatin alteration evaluations, sperm head segmentation was used, developed in MATILAB and executed with Octave software. The system performs the reading of the sperm according to the color present (light blue to magenta), making it possible to define the chromatin compaction index *(Beletti et al 2005).*

Statistics

For statistical analysis, the program GraphPad prism 6.0[5] was used. Fisher's and Student-Newman-Keuls tests were used to analyze progressive sperm motility, sperm with plasma and acrosomal membranes integrity and analyze chromatin expressed as means and standard deviation (SD) (three repetitions), test at 5% significance ($P<0.05$).

III. Results

The semen samples before the rapid thermoresistance test showed mean values of 37.5% for motility and 2.5 for vigor. These values were within the range established by the Brazilian College of Animal Reproduction, with references of 30% and 3 for motility and vigor, respectively. However, after the rapid thermoresistance test, a significant drop ($P<0.05$) in motility (16.5%) and vigor (1.6) was observed, with a reduction of approximately 56% and 36% for motility and vigor, respectively (Figure 1). According to Cunha et al. (2012), bovine semen after being subjected to rapid thermoresistance test (RTT) should show a decrease in straight, progressive sperm motility and sperm vigor.

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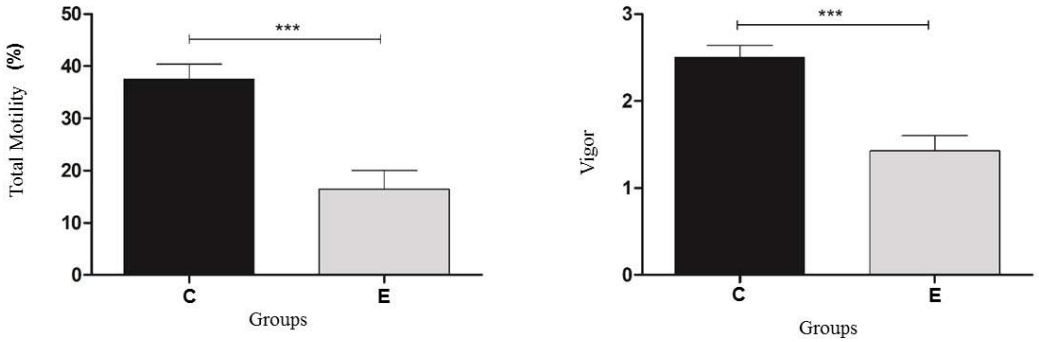


Figure 1: Result of the mean and standard deviation of the total motility and vigor of bovine sperm samples, after thawing (36°C) Control Group (C) and after the rapid thermoresistance test (46°C/30min), Experimental Group (E) Statistical significance ($P < 0.05$)

For the flow cytometry analysis, the correspondence of red, IP (FL3 fotodetector), green and Fitc-PNA (FL1 Fotodetector) fluorescence was observed. The sperm that fluoresced in red were classified as having non-intact plasma membranes and those that fluoresced in green had a reacted outer acrosomal membrane.

The results of thermoresistance test the Control (C) and experimental (E) Groups. Q1 (IP positive) percentage of sperm with reactive plasma membrane; Q2 (IP positive /PNA positive) percentage of sperm with reactive plasma and acrosomal membranes; Q3 (PNA positive) percentage of sperm with intact plasma and acrosomal membranes; Q4 (IP negative/PNA negative). The program Cell Quest was used for this analysis (Figure 2).

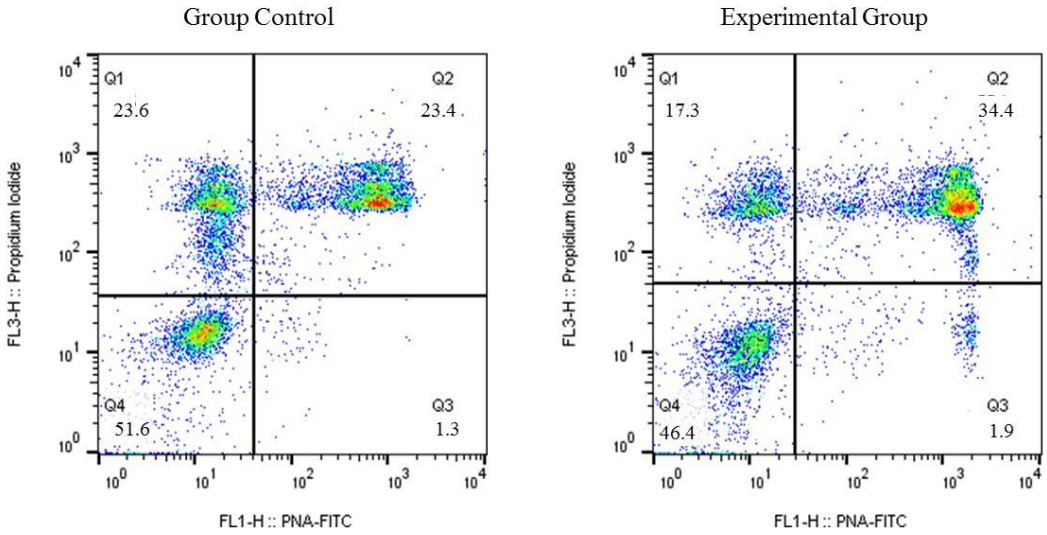


Figure 2: Graphic representation of spot distribution of bovine sperm marking, post-thawing, from Control and Experimental groups, marked with: Q1 - Propidium iodide (PI); Q2 - double marking of propidium iodide (IP) and Fic – PNA; Q3 – Fitc-PNA; Q4 without marking (viable sperm)

Before the thermoresistance test (Control Group), it was observed that the sperm were less reactive to the markings. Because of this condition, the values of the control group (pre-test) were higher than the cells from the experimental group (post-test) in regard to the plasma membrane Q1 (23.6% *vs* 17.3%) and the number of viable Q4 cells (51.6% *vs* 46.4%), respectively. For the Q2 double marking evaluation, higher percentages were observed for the experimental group (34.4%) when compared to the control group (23.4%); a result also observed in the Q3 group evaluations (1.4% *vs* 1.9%).In the flow cytometry analysis, a significant difference ($P<0.05$) was observed for all points analyzed for both the plasma membrane and the acrosomal membrane. (Figure 3).

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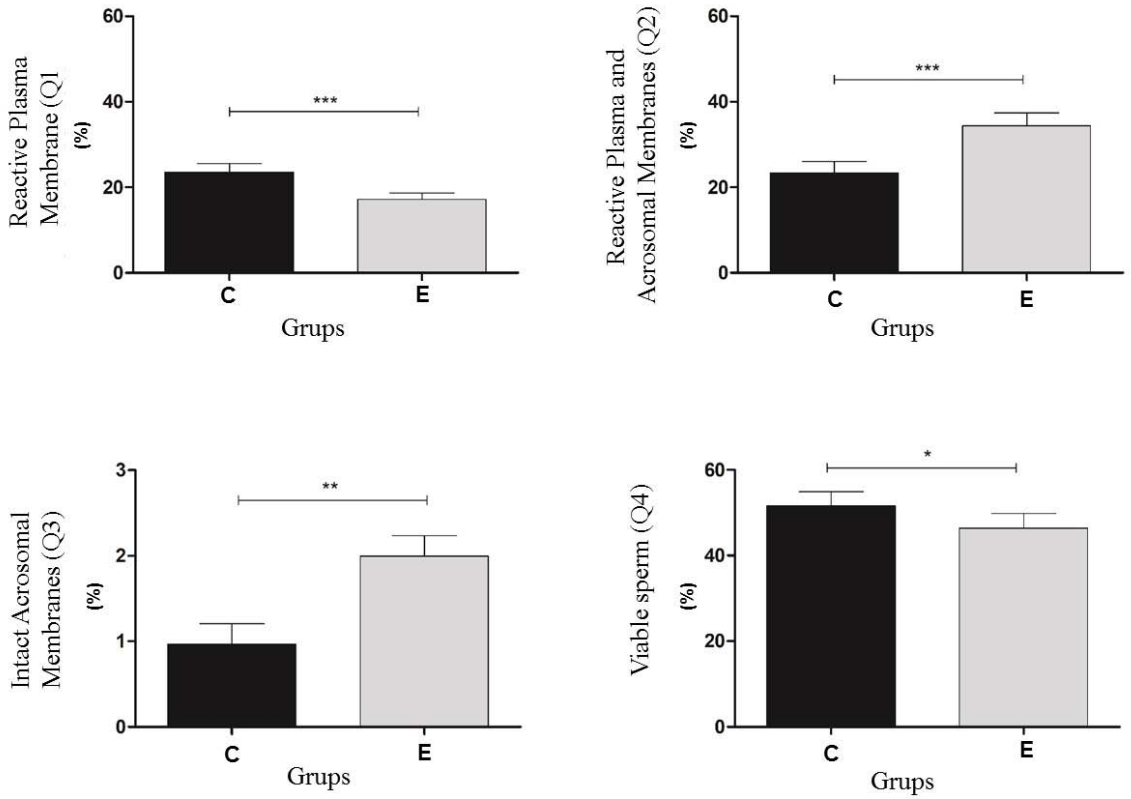


Figure 3: Experimental analysis of the flow cytometry tests of the Control (C) and experimental (E) Groups. Q1 (IP positive) percentage of sperm with reactive plasma membrane; Q2 (IP positive /PNA positive) percentage of sperm with reactive plasma and acrosomal membranes; Q3 (PNA positive) percentage of sperm with intact plasma and acrosomal membranes; Q4 (IP negative/PNA negative) percentage of sperm with reactive acrosomal membrane. Statistical significance ($P < 0.05$)

Sperm membranes perform numerous functions that are related to cellular metabolism and the maintenance of motility and training in sperm. Therefore, the loss of membrane integrity, as seen in figure 2 and figure 3, indicates a change in the maintenance of cellular homeostasis, directly observed in the decrease in sperm motility (figure 1)

From the decompaction and heterogeneity analyses, all sperm were averaged, both before and after the thermoresistance test. As shown in Figure 4, it was observed that there was no significant difference ($P > 0.05$) regarding the two evaluated points.

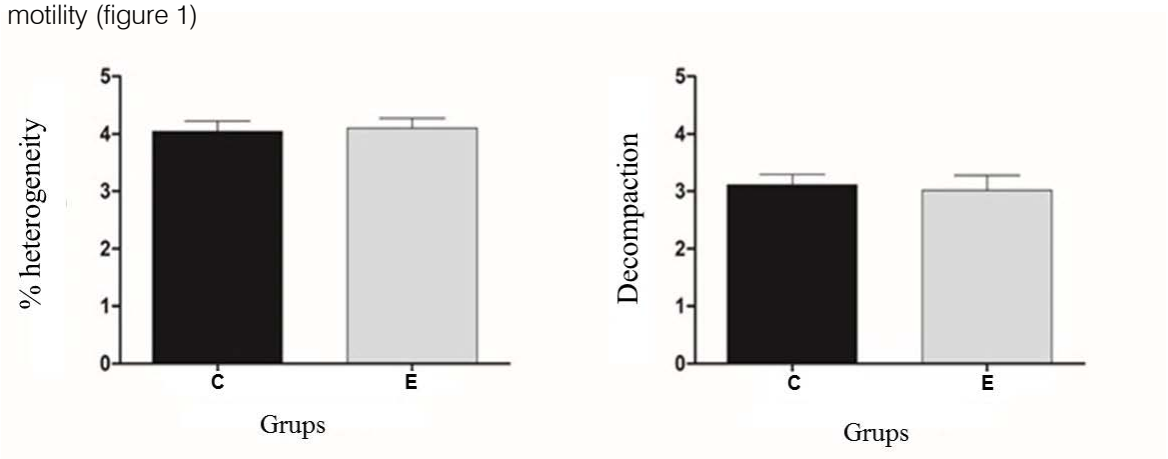


Figure 4: Experimental analysis of chromatin heterogeneity and decompaction of Control (C) and Experimental (E) Groups. No statistical difference ($P > 0.05$)

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The result may be related to the physiological aspect of nuclear differentiation that occurs during spermatogenesis, as in the condensation of sperm chromatin, somatic DNA histones are replaced by protamines (P1), compacted structures (toroid or donut).

IV. Discussion

During fertilization, sperm capacitation, acrosomal reaction and fusion with the ovum are events that require functional and intact plasma and acrosomal membranes. The cryogenics process injures the sperm, decreasing their viability. Aware of the extreme need for sperm to maintain good viability in order to obtain high fertility in a herd, the use of techniques in the field of reproduction biotechnology has intensified, being identified as extremely important tools for genetic improvement programs and efficiency in the production of animal products *(Coutinho et al 2010).*

The importance of applying additional techniques to evaluate semen batches before commercialization is evident, as it contributes to the quality control of the ejaculate, thus resulting in a better fertility rate and herd productivity *(Celeghini et al 2017).*

In this study, the effects of the rapid thermoresistance on the morphofunctional aspects of plasma and acrosomal membranes were evaluated through flow cytometry, as well as through chromatin quality, using the Toluidine Blue technique.

Semen samples taken before the rapid thermoresistance test presented values with greater significance in relation to those taken after the rapid thermoresistance test (RTT), possibly due to the time and temperature at which the sperm were kept. This may have led to a drop in sperm viability with changes in membrane structures, mainly the acrosomal structure. This aspect was observed in the flow cytometry analysis since the sperm were more reactive to the plasma and acrosomal membrane markings after the RTT.

The hypothesis for this result is established since, with the temperature increase, there is a structural change in the membrane's biochemical components, in addition to the promotion of an increase in the sperm's metabolic rate. It is known that the membrane is flexible, composed of lipids and proteins, and that it possesses self-sealing characteristics and ion channels defined by transmembrane proteins, which selectively act on solutes through active and passive transport *(Flesh & Gadella 2000).* Thus, the temperature increase can somehow act on ATP-dependent ion channels, such as the sodium/potassium ATPase pump and calcium ion channels, promoting instability in both membranes, and rather, greater reactivity to markers, Q2 quadrant. (PI Positive/PNA Positive).

Another point that corroborates this discussion is that in the experimental group (post-thermoresistance test), quadrant Q3 (PNA positive) showed an increase in the number of reactive sperm, which indicated a possible spontaneous capacitation induced by temperature.

Sperm capacitation is a physiological process that can be induced by several factors, such as variation in hydrogen potential and in intracellular ionic concentration. In addition, it is possible, given the results of the present study, that this sperm capacitation could also be related to the temperature used in the RTT. This is because the temperature can change the fluidity of the plasma membrane, which would promote an increase in metabolism and consequently greater motility, though for a very short period due to the availability of nutrients and oxygen in the medium *(Yanagimachi1994; Flesh F.M. & Gadella 2000).*

However, according to the results obtained with the Toluidine Blue technique, it can be observed that RTT does not interfere with the chromatin structure. This is probably due to the DNA structure of mammalian sperm, which have a basic unit of protamine toroid. Thus, after the interaction of these protamines with the sperm chromatin, it becomes an inert and highly stable structure, mainly due to the interaction of the protamine's amine residues with the phosphate groups of the DNA strands *(Hamilton et al 2016)*, which could promote greater DNA stability regarding temperature changes.

V. Conclusion

It can be concluded that the rapid thermoresistance test (RTT) alters the structures, mainly the acrosomal structure, and does not interfere with the structure of chromatin compaction, and should be considered, in association with other evaluations, as a complementary parameter in evaluating bovine semen quality.

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Characterization of Canine Patients with Heart Disease based on Age at the Pet`a Vet Veterinary Clinic in Guatemala City, Guatemala

By Estephan Mendez De Leon, Carlos de Leon & Renzo Ricardo Ayala
Universidad San Carlos de Guatemala

Abstract- In this study, the clinical records of canine patients seen at the Pet`a Vet veterinary clinic in Guatemala between 2018 and 2020 were analyzed with the aim of investigating heart disease in dogs in the region. From a total of 1215 records reviewed, 825 cases of heart disease were identified, with chronic mitral valve disease being the most frequent. A higher incidence was observed in males and in Schnauzer breed dogs. In addition, the majority of diagnosed patients were found to be 9 years of age or older. These results provide valuable information on the epidemiology of canine heart disease in Guatemala, which may be useful to improve its diagnosis and clinical management.

Keywords: *canine heart disease, chronic mitral valve disease, dogs, guatemala.*

GJMR-G Classification: *NLM: SF992.H4*

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Characterization of Canine Patients with Heart Disease based on Age at the Pet`a Vet Veterinary Clinic in Guatemala City, Guatemala

Caracterización de Pacientes Caninos con Cardiopatías en Función de la Edad en la Clínica Veterinaria Pet`a Vet de Ciudad de Guatemala

Estephan Mendez De Leon [α], Carlos de Leon [σ] & Renzo Ricardo Ayala [ρ]

Abstract- In this study, the clinical records of canine patients seen at the Pet`a Vet veterinary clinic in Guatemala between 2018 and 2020 were analyzed with the aim of investigating heart disease in dogs in the region. From a total of 1215 records reviewed, 825 cases of heart disease were identified, with chronic mitral valve disease being the most frequent. A higher incidence was observed in males and in Schnauzer breed dogs. In addition, the majority of diagnosed patients were found to be 9 years of age or older. These results provide valuable information on the epidemiology of canine heart disease in Guatemala, which may be useful to improve its diagnosis and clinical management.

Keywords: canine heart disease, chronic mitral valve disease, dogs, guatemala.

Resumen- En la clínica veterinaria Pet`a Vet, especializada en cardiología y neumología, situada en la ciudad de Guatemala, se llevó a cabo un análisis exhaustivo de las fichas clínicas de todos los pacientes atendidos entre 2018 y 2020, con el objetivo de recopilar información estadística sobre las enfermedades cardíacas en perros. Los datos obtenidos permitieron realizar una caracterización detallada de las cardiopatías en la población canina guatemalteca. De un total de 1215 fichas clínicas revisadas, se identificaron 825 casos de cardiopatías en perros, lo que representa el 99% de los pacientes evaluados. La enfermedad cardíaca más común fue la enfermedad crónica de la válvula mitral, diagnosticada en el 66% de los casos. Además, se observó que aproximadamente 7 de cada 10 pacientes con sospecha de enfermedad cardíaca fueron confirmados con algún tipo de cardiopatía. En cuanto al sexo de los pacientes, se encontró que los machos presentaron una mayor incidencia, representando el 53% de los casos, mientras que las hembras constituyeron el 47%. Sin embargo, la diferencia entre sexos fue mínima. La raza con mayor prevalencia de cardiopatías fue el Schnauzer. Esta tendencia podría relacionarse con la popularidad de esta raza en la región. Además, se observó que al menos 9 de cada 10 pacientes diagnosticados con alguna cardiopatía pertenecían a una raza específica. Se evidenció una mayor frecuencia de cardiopatías en perros de edad avanzada, siendo los grupos de 9 a 12 años y 13 años en adelante los más afectados. De hecho, aproximadamente 7 de cada 10 pacientes diagnosticados con cardiopatía tenían 9 años o más. Estos hallazgos proporcionan una visión significativa sobre la epidemiología de las cardiopatías en la población canina de Guatemala, lo que puede contribuir a una mejor comprensión y manejo de estas enfermedades en la práctica clínica veterinaria.

Palabras clave: cardiopatías caninas, enfermedad crónica de la válvula mitral, perros, Guatemala.

Abstract- At the veterinary clinic Pet`a Vet, specializing in cardiology and pneumology, located in Guatemala City, an exhaustive analysis of the clinical records of all patients seen between 2018 and 2020 was carried out, with the aim of compiling statistical information on heart disease in dogs. The data obtained allowed a detailed characterization of heart disease in the Guatemalan canine population. From a total of 1215 clinical records reviewed, 825 cases of heart disease in dogs were identified, representing 99% of the patients evaluated. The most common heart disease was chronic mitral valve disease, diagnosed in 66% of the cases. In addition, it was observed that approximately 7 out of 10 patients with suspected heart disease were confirmed to have some type of heart disease. Regarding the sex of the patients, it was found that males presented a higher incidence, representing 53% of the cases, while females constituted 47%. However, the difference between sexes was minimal. The breed with the highest prevalence of heart disease was the Schnauzer. This trend could be related to the popularity of this breed in the region. In addition, it was observed that at least 9 out of 10 patients diagnosed with heart disease belonged to a specific breed. There was evidence of a higher frequency of heart disease in older dogs, with the groups aged 9 to 12 years and 13 years and older being the most affected. In fact, approximately 7 out of 10 patients diagnosed with heart disease were 9 years of age or older. These findings provide significant insight into the epidemiology of heart disease in the canine population of Guatemala, which may contribute to a better understanding and management of these diseases in veterinary clinical practice.

Keywords: canine heart disease, chronic mitral valve disease, dogs, guatemala.

I. Introducción

La cardiología veterinaria en Guatemala y en Centroamérica se configura como una disciplina especializada dentro del ámbito de la Medicina Veterinaria, orientada al estudio exhaustivo del sistema

Author α σ ρ: Universidad San Carlos de Guatemala.
e-mail: mendezestephan@gmail.com

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cardiovascular en animales. Para hacerlo, se fundamenta en las premisas y conocimientos adquiridos en distintas áreas del saber. Al abordar específicamente las cardiopatías en poblaciones caninas, se torna esencial identificar las patologías más recurrentes, considerando variables como la raza, la edad y el sexo, aspectos de gran relevancia debido a sus implicaciones tanto en la medicina preventiva como en las enfermedades asociadas a procesos infecciosos o degenerativos que puedan vincularse con el entorno. Las condiciones ambientales y los agentes etiológicos, variables según la región, ejercen un papel determinante en la aparición de las patologías cardíacas en las poblaciones caninas de Guatemala, lo que subraya la necesidad prioritaria de discernir su contribución en este contexto.

Diversos estudios de caracterización de enfermedades cardiacas en poblaciones caninas, llevados a cabo en distintas latitudes, han concluido que los pacientes de edad avanzada (7 años o más) presentan una mayor susceptibilidad a desarrollar estas enfermedades. Dentro de este grupo, los pacientes seniles exhiben una mayor propensión o riesgo a padecer enfermedades adquiridas, mientras que los jóvenes son más propensos a enfermedades congénitas. Además, se observa una predisposición a ciertas cardiopatías según el sexo del animal. Este tipo de tipificaciones en poblaciones caninas proporcionan al médico veterinario clínico una guía para identificar una etiología altamente probable (Pereira et al., 2014).

Actualmente, no se dispone de información local que describa la frecuencia de las cardiopatías en las poblaciones caninas según su edad en Guatemala. La ausencia de datos específicos para este país impide determinar si existen factores predisponentes que inciden en la prevalencia de cardiopatías específicas en las poblaciones caninas de la región. Entre estos posibles factores predisponentes podrían mencionarse la influencia genética y enfermedades parasitarias como la enfermedad del gusano del corazón. En el presente estudio, se clasificaron a los pacientes caninos con cardiopatías utilizando la clasificación del Colegio Americano de Medicina Interna Veterinaria (ACVIM, por sus siglas en inglés), en función de la enfermedad diagnosticada. Ante la carencia de estudios locales especializados en cardiología veterinaria, surge la necesidad de llevar a cabo investigaciones descriptivas en este campo.

El estudio actual puede sentar las bases para investigaciones posteriores más amplias que busquen explicar las características idiosincráticas o autóctonas de las cardiopatías en Guatemala. Es crucial generar información local sobre estas patologías. Ejemplos de otras caracterizaciones incluyen enfermedades dermatológicas y óseas, así como casos sospechosos de Distemper, que constituyen puntos de partida para el desarrollo de perspectivas futuras en medicina veterinaria preventiva y clínica cotidiana. Los resultados y conclusiones de este estudio pueden servir como fundamento teórico para investigaciones posteriores en medicina veterinaria con especialización en cardiopatías en perros.

II. Materiales y Métodos

a) Materiales

Recurso humano

- Médicos Veterinarios
- Personal de apoyo en Clínica
- Estudiante de investigación

Recursos físicos

- Hospital veterinario
- Fichas clínicas
- Registros médicos
- Computadora
- Hojas
- Programas de cómputo y análisis de datos

b) Métodos

En el Hospital Pet`a Vet especializado en cardiología veterinaria en Guatemala, se llevó a cabo una recopilación exhaustiva de expedientes clínicos correspondientes al periodo comprendido entre 2018 y 2020. Esta recopilación se centró específicamente en pacientes caninos. Los datos obtenidos fueron posteriormente tabulados y analizados, tomando en consideración variables como la especie, el grupo etario, el sexo, la raza y el diagnóstico de cada paciente. Una vez completado el proceso de recopilación y tabulación, se realizó una minuciosa revisión comparativa con información proveniente de estudios previos y revisión bibliográfica.

Durante esta investigación retrospectiva, se recopiló información de tres años consecutivos (2018-2020), examinando los expedientes clínicos de pacientes caninos diagnosticados con cardiopatías y atendidos en el mencionado hospital especializado en cardiología veterinaria. Los pacientes fueron clasificados inicialmente por especie y posteriormente por grupos etarios. Una vez completada esta clasificación, se procedió al análisis detallado de los resultados y a su discusión en profundidad (Ver Cuadro No. 1).

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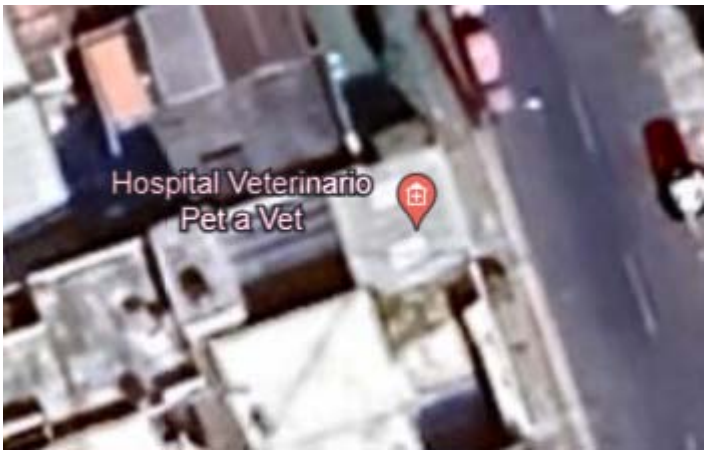
Cuadro No. 1: Pasos de la Metodología

c) Antecedentes del Hospital

El Hospital Veterinario Pet`a Vet cuenta con una trayectoria clínica de 15 años, destacándose por su especialización en Cardiología, Neumología y evaluación preanestésica veterinaria. Ofrece servicios de alta calidad destinados al cuidado y diagnóstico de los perros.

d) Ubicación

El presente estudio se llevó a cabo en el Hospital Veterinario Pet`a Vet, situado actualmente en la 2 calle B 13-44 zona 15, Tecún Uman.



e) Grupos Etarios

Para la recopilación de datos se agruparon las edades por grupos (Ver Tabla No. 1). Estos grupos etarios nos permitieron el análisis de las edades en las poblaciones caninas y así se pudo determinar el grupo etario que más frecuente manifestaba las enfermedades cardiacas, si en pacientes jóvenes o seniles. Algunos autores reportan en sus estudios que la mayor frecuencia de cardiopatías en edades avanzadas (Mucha, 2007) (Detweiler & Patterson, 1965) (Pereira et al., 2014).

Tabla No. 1: Modelo de grupos etarios

Raza o Grupo	Grupos Etarios: Edad en Años				
	0-1	1-4	5-8	9-12	13+

III. Resultados y Discusión

Se examinaron un total de 1215 pacientes caninos sospechosos de padecer alguna enfermedad cardíaca desde enero de 2018 hasta diciembre de 2020. De estos, el 68% (825 casos) presentaron hallazgos compatibles con cardiopatía, mientras que el 32% restante (390 casos) mostraron resultados normales. Dentro de este último grupo de pacientes normales, se incluyeron evaluaciones preanestésicas. Es importante destacar que, de cada 10 pacientes sospechosos de una patología cardíaca, al menos 7 pudieron ser confirmados (Ver Figura No. 1).

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En un estudio previo realizado por Calderón, se describe que solo un 2% (260 casos) de los pacientes caninos estudiados presentaban cardiopatía en comparación con la muestra general (Calderón et al., 2014).

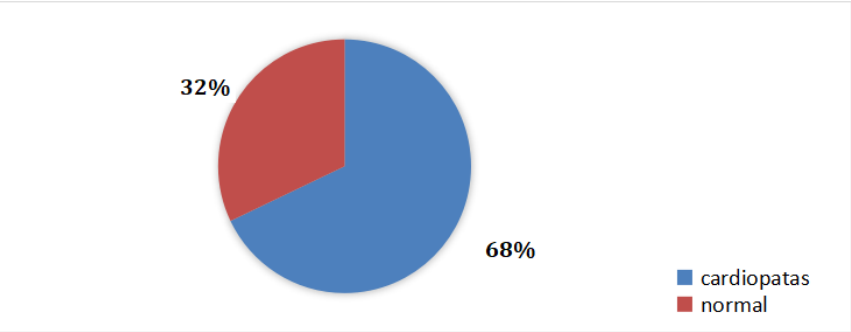


Figura No. 1: Cardiópatas vs normales. Elaboración propia, 2021

En el estudio se evidenció que el 99% de los casos de enfermedades cardíacas correspondían a perros, totalizando 816 casos. En términos generales, la mayoría de los pacientes caninos se situaban en el rango de edad de 9 a 12 años y mayores, tal como fue reportado previamente por Pereira (2014) en poblaciones caninas, lo cual se refleja en la Figura 2.

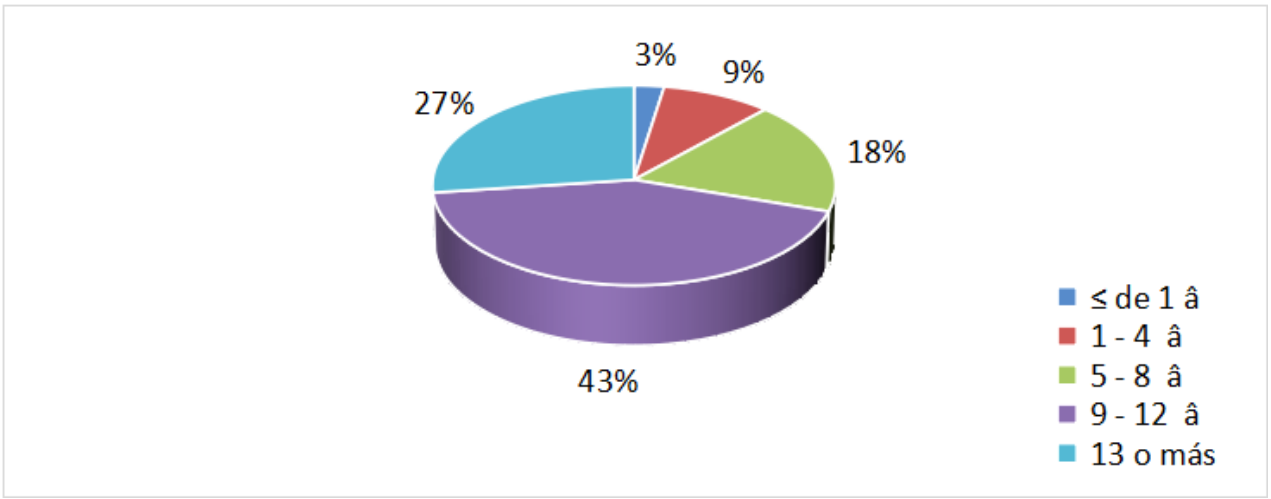


Figura No. 2: Grupo etario de pacientes enfermos en general. Elaboración propia, 2021

En caninos menores de un año, según lo descrito por Patterson (1968), el 86% de los casos (18 ejemplares) presentan una raza definida, mientras que solo el 14% (3 casos) carece de definición racial. Por otro lado, en caninos de 9 a 12 años, tal como lo han señalado Detweiler y Patterson (1965), se observa un incremento en la prevalencia de cardiopatías caninas en consonancia con la edad. Este patrón ha sido corroborado por Calderón, Dávila y Gavidia en (2014), así como por Pereira (2014), quienes encontraron una casuística similar en sus estudios. Específicamente, los grupos de edad entre 9 y 12 años, y aquellos mayores de 12 años, exhibieron una mayor propensión a desarrollar enfermedades cardíacas en comparación con otros grupos etarios.

La Figura No. 3 muestra un aumento progresivo en la frecuencia de cardiopatías en caninos a medida que aumenta la edad.

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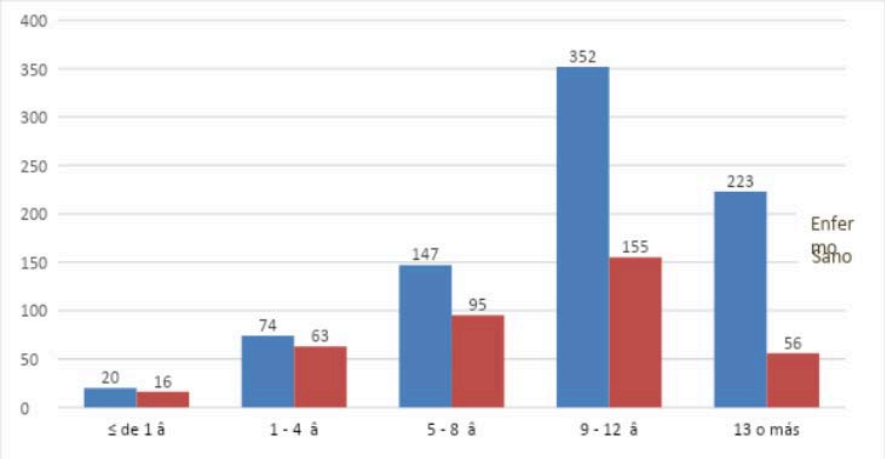


Figura No. 3: *Grupo etario de pacientes caninos sanos vs enfermos*

En la población canina examinada, se identificaron ciertas tendencias relevantes en relación con las cardiopatías. Entre las razas estudiadas, el Schnauzer y el French Poodle mostraron la mayor incidencia de estas enfermedades, destacando el Schnauzer como la raza más afectada. Además, se observó que los machos presentaban una mayor predisposición a desarrollar cardiopatías en comparación con las hembras.

El análisis por grupos etarios reveló que los perros de 9 a 12 años tenían la frecuencia más alta de casos, seguidos por aquellos de 13 años o más, lo que sugiere un aumento en la prevalencia de cardiopatías con la edad. Las enfermedades más comunes fueron la enfermedad valvular mitral, seguida de la hipertensión pulmonar postcapilar asociada a valvulopatías y la cardiomiopatía fenotipo dilatada.

En cuanto a las cardiopatías congénitas, se encontró que representaban el 2.42% de los casos en perros, una cifra menor en comparación con el 11% observado en gatos. Basándose en datos de diferentes países, se sugiere realizar consultas cardiológicas para perros a partir de los 7 años de edad.

Se recomienda incrementar el número de casos clínicos examinados, especialmente en la población felina. Además, es esencial evitar la reproducción de razas con antecedentes de cardiopatías congénitas y hereditarias en perros. También se destaca la importancia de descartar la presencia de enfermedad renal crónica en perros con enfermedad crónica de la válvula mitral, según lo señalado por Martinelli (2016).

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Evolution of Degenerative Myxomatous Mitral Valve Disease in Canine Fox Terrier - Case Report

By Keren Souza, Maryanne Santos & Carlos Carreli

Abstract- Degenerative mitral valve disease (DMVD) consists of progressive degeneration of the mitral valve that can occur either in isolation or in association with the tricuspid valve, leading to incomplete coaptation of its leaflets and subsequent valve regurgitation. It's the most common cardiac condition in small animals, accounting for about 75% of cases of heart disease in dogs, mainly affecting elderly small-breed dogs. Breeds with a genetic predisposition, such as Cavalier King Charles Spaniels and Bull Terriers, tend to develop the disease early. It can be classified into four stages: A, B, C, and D, with B having subdivisions (B1 and B2). Animals in stages A, B1, and B2 are asymptomatic, showing clinical signs only from stage C onwards. The report details the case of a 13-year-old Fox Terrier diagnosed at stage B2 of the disease, progressing to stage C within two years—a cardiologist conducted annual monitoring.

Keywords: *degenerative myxomatous mitral valve disease. pimobendan. pulmonary edema.*

GJMR-G Classification: *NLMC Code:14.280.484, SF991*



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Evolution of Degenerative Myxomatous Mitral Valve Disease in Canine Fox Terrier - Case Report

Keren Souza [α], Maryanne Santos [σ] & Carlos Carreli [ρ]

Simple Summary- This work aims to report the case of an animal referred to a cardiologist in 2021, being diagnosed with Myxomatous Mitral Valve Disease in stage B2, and demonstrating the evolution of the disease to date.

Abstract- Degenerative mitral valve disease (DMVD) consists of progressive degeneration of the mitral valve that can occur either in isolation or in association with the tricuspid valve, leading to incomplete coaptation of its leaflets and subsequent valve regurgitation. It's the most common cardiac condition in small animals, accounting for about 75% of cases of heart disease in dogs, mainly affecting elderly small-breed dogs. Breeds with a genetic predisposition, such as Cavalier King Charles Spaniels and Bull Terriers, tend to develop the disease early. It can be classified into four stages: A, B, C, and D, with B having subdivisions (B1 and B2). Animals in stages A, B1, and B2 are asymptomatic, showing clinical signs only from stage C onwards. The report details the case of a 13-year-old Fox Terrier diagnosed at stage B2 of the disease, progressing to stage C within two years—a cardiologist conducted annual monitoring.

Keywords: degenerative myxomatous mitral valve disease. pimobendan. pulmonary edema.

I. Introduction

Degenerative myxomatous mitral valve disease (DMVM) is the most common heart disease in small animals, accounting for around 75% of cases of heart disease in dogs [9]. The prevalence of MVD increases markedly with age in small dogs, with up to 85% showing evidence of valve damage by 13 years of age [3].

According to Petrus Gimenes and Mantovani (2019), the proper functioning of the mitral valve is based on the structural and functional performance of six components: the posterior wall of the left atrium, the valve ring, the valve leaflets or cusps, the tendon chordae, the papillary muscles of the left ventricle and the left ventricular wall, each of these components plays an independent and synergistic role, contributing to complex functions that maintain valve competence. Any structural changes in the elements of the mitral apparatus affect valve mechanics, compromising its efficiency.

The disease involves a gradual myxomatous degeneration over time with the disorganization of collagen bundles and a reduction in their content, excess production of glycosaminoglycan that results in a change in the valve structure leading to poor coaptation of the leaflets. Poor coaptation of the leaflets allows regurgitation of the mitral valve, resulting in the murmur characteristic of the disease [7].

According to the latest consensus published by ACVIM in 2019, an echocardiogram (ECHO) and chest x-ray in the absence of ECHO are recommended to diagnose the disease. However, caution should be taken due to the marked variation in thoracic conformation and racial differences in the vertebral cardiac scales. The disease can also be recognized during a screening or routine examination by auscultating a typical heart murmur when there is regurgitation of the mitral valve.

The main symptoms that the animal presents when it reaches the advanced stage of the disease are Exercise intolerance, dry cough, decreased appetite, difficulty breathing, and syncope, which are findings associated with a worse prognosis in affected dogs [6].

According to the classification system for treating dogs with DMVM, published by Atkins et al. 2019, described below (Table 1) [9], there are four primary stages of the disease.

Table 1: Four preliminary stages of degenerative myxomatous mitral valve disease

Stages	Description
Stage A	Identifies patients at high risk of developing heart disease but who do not yet have identifiable structural changes in the heart.
Stage B	Identifies patients with structural heart disease but who have never developed clinical manifestations of heart failure. This stage is divided into:
	B1: Asymptomatic patients who do not present radiographic or echocardiographic evidence of cardiac remodeling in response to valve disease.

Author α σ: Graduating in Veterinary Medicine – Una – Pouso Alegre/MG – Brazil. e-mail: kerencristina90@hotmail.com
Author ρ: Internship supervisor, Veterinary Cardiologist - Pouso Alegre/MG – Brazil.

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	B2: Asymptomatic patients who present hemodynamically significant mitral valve regurgitation, evidenced by radiographic and echocardiographic findings of left heart enlargement.
Stage C	Patients with previous or current clinical symptoms of heart failure associated with structural changes in the heart.
Stage D	Patients with end-stage heart disease with signs of heart failure who are refractory to mainstay therapy require particular or advanced treatment strategies to remain comfortable regardless of the presence of the disease.

Source: Petrus; Gimenes; Mantovani - Treatise on cardiology of dogs and cats

This work aims to report the case of an animal referred to a cardiologist in 2021, diagnosed with Myxomatous Mitral Valve Disease in stage B2, and demonstrate the evolution of the disease to date.

II. Case Report

A Fox Terrier dog male, 13 years old, neutered, weighing 12 kg, was treated at the Veterinary Clinic Animal Medical Center (AMC) in Pouso Alegre - MG, Brazil, and was sent for cardiological evaluation. He consulted a general practitioner due to complaints of intense tremors, where a chest x-ray was requested. During this examination, an increase in the cardiac silhouette was observed in a topography corresponding to the left atrium, resulting in the patient being referred to a specialist.

In the anamnesis, the owners reported that the animal never had coughs, syncope, or convulsions, just tremors since it was a puppy. On physical examination, a grade 3/6 murmur was heard in the mitral focus, normal heart, and respiratory rates, systemic arterial hypertension (180mmHg), and normal-colored mucous membranes.

An echocardiogram (ECHO) (Figure 1) showed a thickened mitral valve and enlarged heart chambers. At first was prescribed Pimobendan PO at a dose of 0.25 mg/kg BID, continuous use, and requested to return in 30 days.

Upon return, the owners reported that the animal was more active and was not quickly tired. His pressure had already decreased from 180 mmHg to 160 mmHg, and he continued using pimobendan.

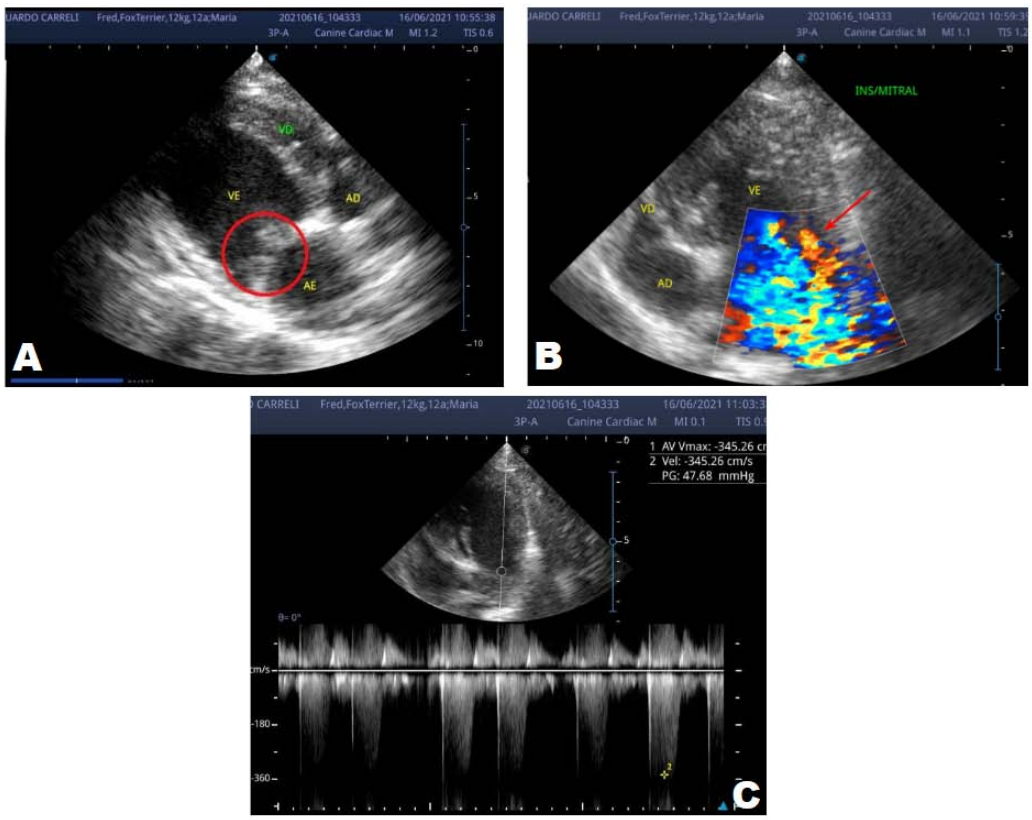


Figure 1: Echocardiogram 2021: Thickened/degenerated mitral valve (A); Observed in Doppler mode, significant mitral valve insufficiency (B); Hemodynamic assessment - Mitral regurgitation gradient: 3.45 m/s / 47.68 mmHg; enlargement of left cardiac chambers; left ventricular diastolic dimension above normal limits was observed

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One year later, the animal returned for a new evaluation, repeating the ECHO (Figure 2), which showed the progression of the increase in the left atrium. The electrocardiogram (ECG) (Figure 3) shows baseline sinus arrhythmia with the presence of a premature ventricular complex (Figure 4). Owners report drowsiness, hyporexia, and syncope. However, they did not present tiredness or cough. Blood pressure was 80 mmHg.

Due to the arrhythmia, Sotalol was prescribed at a dose of 25 mg/kg PO, BID for continuous use, and Omega 3 PO at a dose of 600 mg/kg SID became necessary, and pimobendan continued.

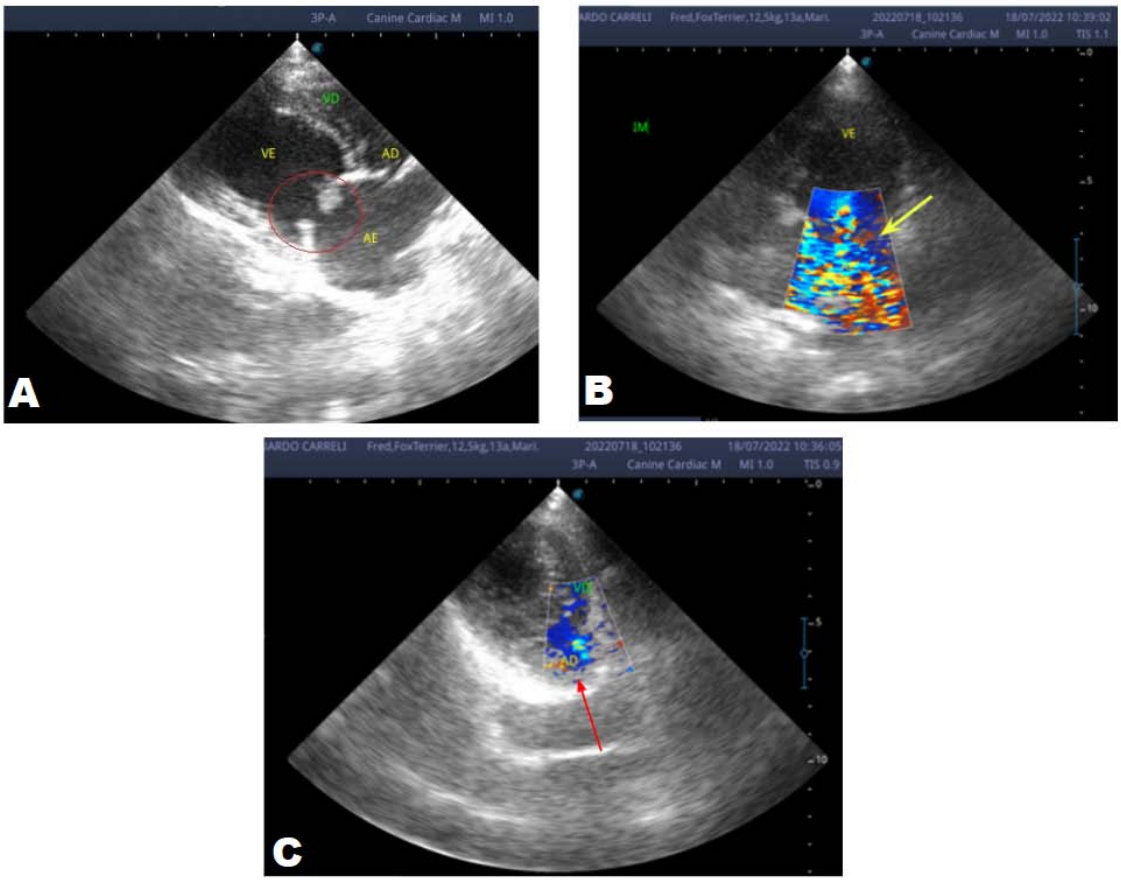


Figure 2: Echocardiogram (2022): observed increase in the left atrium and ventricle; thickened/degenerated mitral valve (A); observed in a Doppler study, turbulent systolic flow within the left atrium, characterizing significant mitral valve insufficiency (B); systolic turbulent flow within the right atrium, representing mild tricuspid valve insufficiency (C); hemodynamic assessment - maximum velocity gradient mitral regurgitation: 3.51 m/s /49.22 mmHg; observed left ventricular diastolic dimension above normal limits with normal systolic function parameters, characterizing systolic dysfunction; preserved diastolic function

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Figure 3: Electrocardiogram (2022)

Table 2: Electrocardiographic report (2022)

Observed parameters	Observed parameters	Observed parameters
QRS axis: 67.07 °	QT Interval: 210 ms	Duration of T: 54 ms
P axis: 58.01 °	PR Interval: 108 ms	QRS duration: 72 ms
Amplitude of S: -0.06 mV	R amplitude: 2.26 mV	Minimum HR: 49 bpm
PR Segment: 52 ms	P amplitude: 0.22 mV	Average HR: 95 bpm
ST segment: 84 ms	T amplitude: -0.74 mV	Maximum HR: 297 bpm
Duration of P: 56 ms		

Comments

Baseline sinus arrhythmia.

The QRS axis is within normal limits for the species. Episodes of the premature ventricular complex were observed.

An increase in the P wave and QRS complex duration was observed, suggesting atrial and left ventricular overload.

Ventricular repolarization disorder was observed due to the rise in the amplitude of the T wave (> 25% of the R wave), which is compatible with electrolyte changes and myocardial hypoxia.

Conclusions: Baseline sinus arrhythmia with the presence of premature ventricular complex

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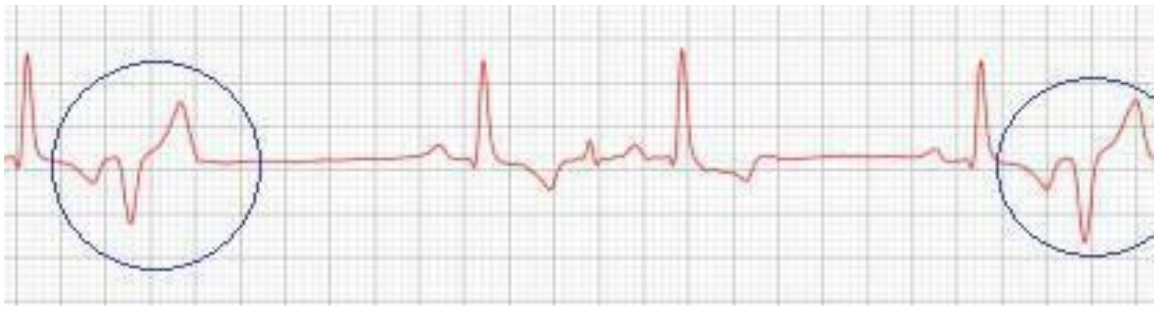


Figure 4: Electrocardiographic tracing (2022): showing episodes of premature ventricular complex (circles)

At the beginning of 2023, the patient returned to repeat the cardiological evaluation. During the anamnesis, the Guardian reported that the patient did not present episodes of syncope and remained stable during this period, continuing the previously adopted treatment.

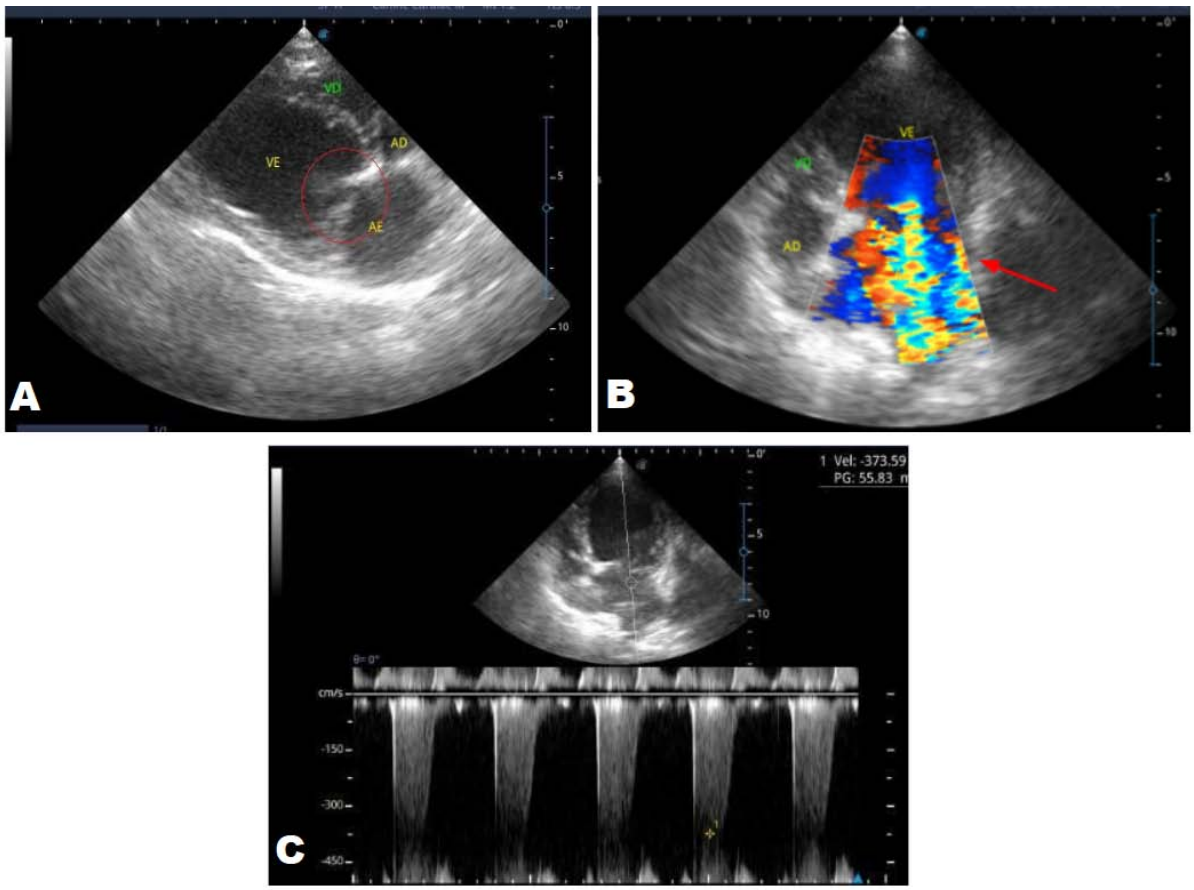


Figure 5: Echocardiogram (January/2023): observed increase in the left atrium and ventricle; thickened/degenerated/prolapsed mitral valve (A); observed in Doppler study, systolic turbulent flow within the left atrium characterizing severe mitral valve insufficiency (B). Hemodynamic assessment - Maximum velocity gradient mitral regurgitation: 3.74 m/s / 55.83 mmHg: Observed left ventricular diastolic dimension above standard limits, with normal systolic function parameters, characterizing systolic dysfunction; preserved diastolic function

After eight months, the patient returned with a worsening condition, repeated ECHO (Figure 6) and ECG (Figure 7), where diastolic dysfunction and worsening of the arrhythmia were observed. Amiodarone 8.7 mg/kg orally was prescribed, replacing Sotalol, one tablet BID, and Furosemide (Lasix) 1.7 mg/kg orally was prescribed every 12 hours. Pimobendan was continued. After a week, a reassessment was carried out, and the ECG demonstrated stability in the arrhythmia (Figure 8).

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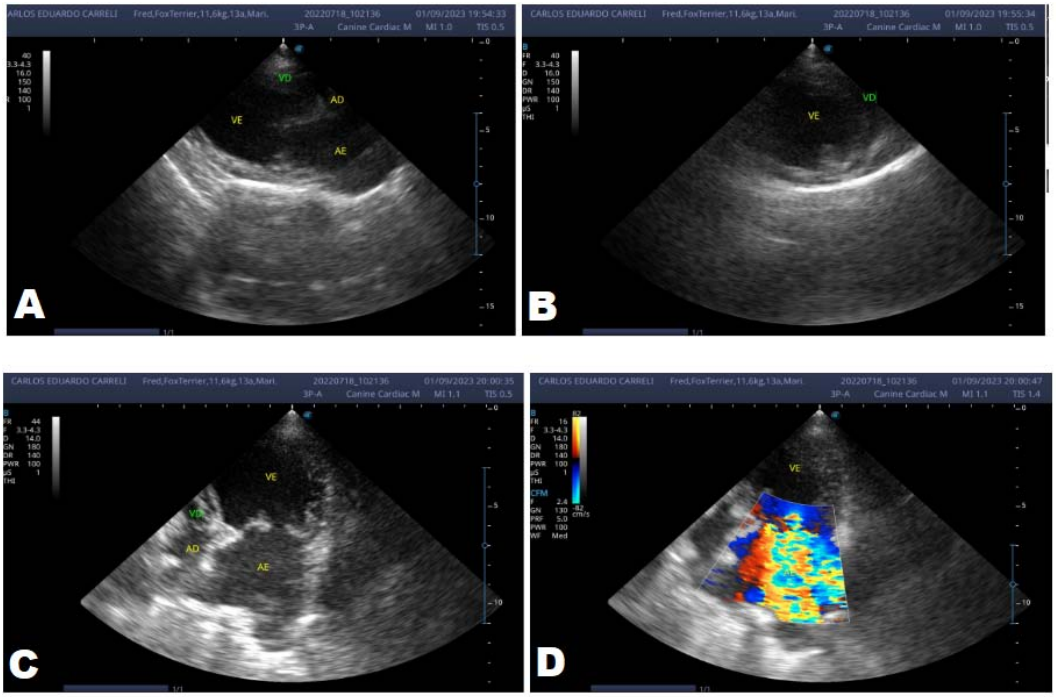


Figure 6: Echocardiogram (September/2023): significant enlargement of the left heart chambers observed; observed in a Doppler study, systolic turbulent flow within the left atrium characterizing significant mitral valve insufficiency; Observed left ventricular diastolic dimension above standard limits, with normal systolic function parameters, indicating systolic dysfunction; A left ventricular filling pattern of the E>A wave type and an increased E/A ratio were observed, characterizing diastolic dysfunction (restrictive or pseudo-restrictive pattern)



Figure 7: Electrocardiogram (September/2023)

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Table 3: Electrocardiographic report (September/2023)

Observed parameters	Observed parameters	Observed parameters
Minimum HR: 71 bpm	T duration: 68 ms	S amplitude:: -0.01 mV
Average HR: 125 bpm	QT interval: 196 ms	T amplitude: -0.32 mV
Maximum HR: 400 bpm	ST segment: 56 ms	P axis: 83.89 º
P duration:: 48 ms	P amplitude: 0.29 mV	QRS axis: 81.32 º
QRS duration:: 72 ms	R amplitude: 1.69 mV	PR Interval: 104 ms

Comments:

Sustained ventricular bigeminy with clusters of ventricular trigeminy and sinus arrhythmia.

Duration and amplitude of the P wave and QRS complex with values within normal limits for the patient's species, size, and age.

Normal electric axis.

Normal T wave.

Presence of polymorphic premature ventricular extrasystoles, isolated in pairs, in triplets, and at times organized into ventricular bigeminy and trigeminy.

Nothing else is worth noting during the 4 minutes and 41 seconds of monitoring.

Conclusions: Sustained ventricular bigeminy with clusters of ventricular trigeminy and sinus arrhythmia.



Figure 8: Reevaluation of the electrocardiogram (September/2023)

III. Discussion

In the reported, he was asymptomatic, and the echocardiographic examination showed enlargement of the left heart chambers, thickened and degenerated mitral valve (Figure 1), left ventricular diastolic dimension above normal limits. , with normal systolic function parameters, characterizing systolic dysfunction. The thickening and degeneration of the mitral valve indicates its insufficiency, and the other changes represent diastolic dysfunction.

Diagnosed with degenerative myxomatous mitral valve disease, treatment with pimobendan began. According to Boswood *et. al*. (2016), its mechanism of action includes a positive inotropic combination and balanced vasodilation caused by calcium sensitization and phosphodiesterase inhibition, as a result, the effects of pimobendan may consist of increased cardiac output (CO), myocardial contractility and decreased preload and afterload.

In 2022, the animal returned for annual exams one year after the diagnosis. The ECHO showed an increase in the left atrium and ventricle, thickened/degenerated mitral valve (Figure 2), preserved diastolic function, and low probability of pulmonary hypertension; the ECG detected an increase in the duration of the P wave and the QRS complex (Figure 3), suggestive of atrial and left ventricular

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overload, baseline sinus arrhythmia with the presence of a premature ventricular complex.

In most cases, cardiac arrhythmias are not clinically relevant unless they are associated with heart disease or cause an extreme change in heart rate, being very slow, fast, or irregular. [10]. When choosing a medication for treatment, one must consider some things, such as clinical status, associated cardiac or systemic comorbidities, and drug association [13]. Class III antiarrhythmics block potassium channels, preventing a new action potential from occurring before complete repolarization, causing a prolongation of this potential and the refractory period [5].

Sotalol was prescribed to control the arrhythmia presented by the animal with DMVM. According to Treseder et al. (2019), Sotalol is an antiarrhythmic agent commonly used in human and veterinary medicine to control ventricular and supraventricular arrhythmias. Its antiarrhythmic properties are well established and are attributed mainly to the blockade of potassium channels with concomitant non-selective β-adrenergic blockade.

In 2023, the animal returned and repeated only the ECHO (Figure 5), where no significant changes were observed during this period, except for a structural change in the mitral valve, identifying a prolapse and increase in the left ventricular diastolic dimension, with systolic function parameters, characterizing systolic dysfunction; diastolic function remained preserved.

Petrus, Gimenes, and Mantovani (2019, p. 156) report that mitral valve prolapse is a common complication that generally occurs with the progression of MVD. As MVD progresses, mitral valve regurgitant flow increases, promoting volume overload in the left atrium, with an increase in filling pressures in the left heart chambers. There is also progressive cardiac remodeling, characterized by eccentric hypertrophy, initially in the left atrium and, later, in the left ventricle. Systolic dysfunction occurs in the advanced stages of MVD, which culminates in hemodynamic changes and progressive diastolic dysfunction of the left ventricle, affecting general cardiac performance and determining the appearance of congestive heart failure syndrome.

In the same year, eight months later, the patient returned with a worsening condition, presenting pulmonary edema. The animal's condition progressed to stage C with these changes, requiring repeat echocardiographic and electrocardiographic examinations.

The ECHO (Figure 6) showed an increase in the cardiac chambers and diastolic dysfunction compared to the previous exam. The ECG (Figure 7) revealed polymorphic premature ventricular extrasystoles. Due to this change, Sotalol was replaced by amiodarone.

Amiodarone is also a class III antiarrhythmic, being the most indicated in these cases as it is considered broad spectrum and can be used in supraventricular and ventricular arrhythmias [5]. However, its use should be reserved for cases where the animal no longer responds to sotalol treatment due to its various side effects [10]. Its most common adverse effects are liver changes, causing an increase in the concentration of gastrointestinal and enzymes, such as vomiting, diarrhea, and anorexia [11]. Usually, these effects are dose-dependent and reversible if their supply is interrupted at the beginning of clinical manifestations [10].

For pulmonary edema, furosemide (Lasix) was prescribed. Furosemide is a loop diuretic widely used in veterinary clinics. The Food and Drug Administration (FDA) has approved furosemide to treat conditions with volume overload and edema secondary to exacerbation of congestive heart failure, liver failure, or renal failure, including nephrotic syndrome [4]. Its mechanism of action is to inhibit the tubular reabsorption of sodium and chloride in the proximal and distal tubules and in the thick ascending loop of Henle, inhibiting the sodium chloride cotransport system, resulting in excessive excretion of water along with sodium, chloride, magnesium, and calcium [4].

One week later, the patient returned for a reevaluation of the ECG (Figure 8), demonstrating stability in the arrhythmia.

In 2023, there were some complications regarding the patient's treatment. The owner reported that he was no longer able to administer the medications as recommended, providing pimobendan every 24 hours or at a longer interval. Regarding other medications, it was said that the animal accepted it more quickly but could not administer it at the correct time, which may justify the worsening of the condition in a short period. The animal is currently stable and continues to receive monitoring.

IV. Conclusion

Myxomatous mitral valve degeneration is a progressive disease responsible for the most significant number of heart diseases in small animals. We observed that the time for the disease to progress from stage B2 to C was two years, with correct monitoring and treatment reducing its progression over a while. Medications were essential in this case to keep the animal's condition stable.

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Effectiveness of the use of DAFS-25 in the Standard Hepatitis Treatment Regimen for Dogs

By Poberezhets E. P. & Rodionova T. N.
Astrakhan Tatishchev State University

Abstract- The article provides a study on the effectiveness of the drug DAFS-25 in the standard hepatitis treatment regimen for dogs. The study was conducted in a veterinary clinic «Alabai», Astrakhan, based on the treatment of dogs of the Caucasian wolfhound breed from Kangly kennel. Key indicators of liver status in dogs were taken in the study: alkaline phosphatase (ALP), ALT, AST, ALB (albumin), (TB) total bilirubin, (TP) total protein; in the process of treatment, based on the results of the biochemical rapid analyzer Seamaty 120VP, animals were divided into three groups (one control and two experimental groups) by analyzing their state. Fifteen dogs aged 3-6 were reviewed in the study, the average weight of animals was 65 kg. Biochemical analysis was carried out at the beginning of the study, on the twentieth day and after the end of therapy. The standard treatment regimen was used in the control group and the drug DAFS-25 was added into the standard treatment regimen of experimental groups at a dose of 1.6 mg/kg (104 mg/head) in the second group and 4.8 mg/kg (312 mg/head) in the third group.

Keywords: drug, DAFS-25, hepatitis, caucasian wolfhound, treatment regimen.

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Effectiveness of the use of DAFS-25 in the Standard Hepatitis Treatment Regimen for Dogs

Эффективность Применения Препарата Дафс-25 В Стандартной Схеме Лечения Гепатитов У Собак

Poberezhets E. P. [α] & Rodionova T. N. [σ]

Реферат- **Вданной статье предоставляется исследованиепо изучению эффективности применения препарата ДАФС-25 в стандартной схеме лечения гепатитов у собак.Исследование проводилось в ветеринарной клинике «Алабай» г. Астрахань на основе лечения собак породы кавказский волкодав питомника «Канглы». При исследовании были взяты основные показатели состояния печени у собак: щелочная фосфатаза (ALP), ALT, AST,ALB (альбумины), (TB) общий билирубин, (TP) общий белок,впроцессе лечения по результатам биохимического экспресс анализатора Seamaty 120VP, анализируя состояние животных, их распределил их на три группы – однаконтрольная и две опытные. В изучении было обследовано 15 собак, от 3 до 6лет, средний вес животных 65 кг. Биохимический анализпроводили в начале исследования, на двадцатый день и после окончания терапии. В контрольной группе применялась стандартная схема лечения, а в опытных в стандартную схему лечения был добавлен препарат ДАФС-25в дозах 1,6 мг/кг (104 мг/гол) - вторая группа и 4,8 мг/кг (312 мг/гол) - третья группа. Летальных исходов в период исследования не было. Для исследования был взят препарат ДАФС-25 так как он обладает выраженными антиоксидантными свойствами, благоприятно влияет на организм животных от негативных факторов внешней среды, восполняет недостачу селена в организме животных. Благоприятно влияет на состав крови, улучшает обменные процессы всего организма. В результате лечения общее состояниеживотных которые получали терапию пришло к физиологической норме, биохимические показатели взятие в конце терапии нормализовались, что свидетельствовало о полном выздоровлении собак. Исследования показали, эффективность применения препарата в второй и третьей опытных группах, что позволяет предложить данный препарат ДАФС-25 как альтернативу в лечении заболеваний печении и гепатитов другимгепатопротекторным препаратам.**

Ключевые слова: препарат, ДАФС -25, гепатит, кавказский волкодав, схема лечения.

Author α: graduate student of FSBEI HE Astrakhan Tatishchev State University (ORCID 0009-0001-1575-5743).
e-mail: Petrovnaalena86@gmail.com
Author σ: professor, Doctor of Biological Sciences, FSBEI HE Saratov State Vavilov Agrarian University (ORCID-0000-0003-2444-5269)

Abstract- The article provides a study on the effectiveness of the drug DAFS-25 in the standard hepatitis treatment regimen for dogs. The study was conducted in a veterinary clinic «Alabai», Astrakhan, based on the treatment of dogs of the Caucasian wolfhound breed from Kangly kennel. Key indicators of liver status in dogs were taken in the study: alkaline phosphatase (ALP), ALT, AST, ALB (albumin), (TB) total bilirubin, (TP) total protein; in the process of treatment, based on the results of the biochemical rapid analyzer Seamaty 120VP, animals were divided into three groups (one control and two experimental groups) by analyzing their state. Fifteen dogs aged 3-6 were reviewed in the study, the average weight of animals was 65 kg. Biochemical analysis was carried out at the beginning of the study, on the twentieth day and after the end of therapy. The standard treatment regimen was used in the control group and the drug DAFS-25 was added into the standard treatment regimen of experimental groups at a dose of 1.6 mg/kg (104 mg/head) in the second group and 4.8 mg/kg (312 mg/head) in the third group. There were no lethal outcomes during the study period. The drug DAFS-25 was taken for the research, as it has strong antioxidant properties, positively affects the animals' body from negative environmental factors, fills selenium deficiency in the body of animals. It positively affects the composition of the blood, improves metabolic processes of the whole organism. As a result of treatment, the general condition of the animals who received therapy came to the physiological norm, biochemical parameters taken at the end of therapy returned to normal, which indicated a complete recovery of the dogs. Studies have shown the efficacy of the drug in the second and third experimental groups, which allows to suggest the drug DAFS-25 as an alternative to other hepatoprotective drugs in the treatment of liver diseases and hepatitis.

Keywords: drug, DAFS-25, hepatitis, caucasian wolfhound, treatment regimen.

I. Введение/Introduction

В последнее время в ветеринарной практике у собак встречаются очень часто болезни печени. Одно из самых распространенных заболеваний печени является гепатит. Гепатит –это воспалительные заболевания печени, в результате чего орган не выполняет свои основные функции (вывод токсинов из организма, обмен веществ, усвоение витаминов и микроэлементов), и это приводит к ослаблению организма, нарушению работы других органов и систем, интоксикации. Причины возникновения гепатитов у собак

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различен. Алиментарный гепатит является одним из часто встречающихся заболеваний в ветеринарной практике. Возникновение заболевания напрямую зависит от нерационального питания, которое приводит к нарушению функционального состояния печени, обмена веществ и в целом на организм животного. Процент смертельных исходов составляет 30-40%.[1] В результате разнообразия патоморфологических изменений при гепатите у собак как правило отмечают пат изменения в желчном пузыре и печени, а так же желтушность слизистых[1].

I. Introduction

ecently in veterinary practice, liver diseases are common in dogs. Hepatitis is one of the most common liver diseases. Hepatitis is an inflammatory liver disease as a result of which the organ does not perform its basic functions (removal of toxins from the body, metabolism, assimilation of vitamins and trace elements) and this leads to a general weakening of the body, violations in the work of other organs and systems, intoxication. The causes of hepatitis in dogs are different. Nutritional hepatitis is one of the most common diseases in veterinary practice. The occurrence of the disease directly depends on malnutrition, which leads to a violation of the functional state of liver, metabolism and generally the whole organism of an animal. The percentage of deaths is 30-40%. As a result of the diversity of pathomorphological changes with hepatitis in dogs pathological changes in the gall bladder and liver, as well as yellowing of mucous membranes are usually noted.

II. Материалы и Методы Исследования/Materials and Methods of Research

Исследования проводились с апреля по май 2023 года, в рамках ветеринарной клиники «Алабай» и питомника кавказских волкодавов «Канглы» в г. Астрахань.В результате работы было сформировано три группы по пять собак в каждой группе, в возрасте от 3 до 6 лет, весом 65кг. Всего в работе было обследовано 15 собак.Распределение больных животных по группам имело случайный характер. Группы были выделены на основании схем лечения. Первая (контрольная)группа животных получали лечение по стандартной схеме, которая включала препараты 5%раствор глюкозы 8мл/кг, гептрал 400мг на гол, мильгамма 2мл на гол ,0,9% растворNaCl 10мг/кг. Вторая(опытная)группа так же получала стандартное лечение, но в схему был добавленселеноорганический препарат ДАФС-25 в дозе 1,6 мг/кг (104мг/гол). Третья группа (опытная) гдеживотные получали лечение по той же схеме, что и вторая с добавлением селеноорганическогопрепарата ДАФС-25 в дозе 4,8 мг/кг (312 мг/гол).

III. Результаты Исследований/Research Results

На момент исследования у собак отмечались четко выраженные клинические признаки гепатита, температура тела повышалась до 40 градусов, при осмотре было выявлено бледность слизистых оболочек ротовой полости, десен и склеры глаз, у некоторых животных отмечалась незначительная желтушность, желудочно-кишечный тракт нарушен (понос, а у некоторых животных кал содержал непереваренную пищу), снижение аппетита, угнетение общего состояния собак, периодическая рвота. При пальпации отмечалась болезненность в области печени. При УЗИ диагностике увеличение печени, края неровные, повышение эхогености.[5]
Для оценки влияния препарата ДАФС-25 производили забор крови в начале исследования, на седьмой день и в конце опытанатощак. Биохимический анализ крови проводился на экспресс анализаторе Seamaty 120VP. Для наглядности и подтверждения диагноза были взяты наиболее информативные биохимические показатели крови: щелочная фосфатаза (ALP), ALT,AST, общий билирубин (TB) , альбумины (ALB), общий белок (TP).[4].

В начале исследования после забора крови полученные показатели были усреднены и представлены в таблице1

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Таблица 1: Биохимические показатели крови собак в начале исследования

Обозначения	Показатели	Норма	1 группа (контрольная группа)	2 группа опытная (ДАФС-25 в дозе 104 мг/гол)	3 группа опытная (ДАФС-25 в дозе 312 мг/гол)
ALB	Альбумины ,г/л	22-39	17±1,20*	19±1,50*	20,6±1,51*
TB	Общий билирубин, мкмоль/л	0,9-10,6	11,7±0,23*	13,4±0,25*	11,5±0,23*
TP	Общий белок, г/л	50-100	43±2,11*	46±2,08*	47±2,09*
ALP	Щелочная фосфатаза, ед/л	10,6-100,7	111,8±3,22*	115,2±3,25	110,3±3,20*
ALT	АЛТ, ед/л	8,2-57,3	67±2,24*	65,3±2,22*	68,8±2,24*
AST	АСТ, ед/л	8,9-48,5	55,3±2,12*	57,9±2,13*	58,2±2,13*

Примечание: * $p<0,05$ относительно физиологической нормы*

При исследовании крови собак больных гепатитом в начале лечения отмечается понижение альбуминов у контрольной группы на 29,41% по отношению физиологической нормы, в опытных (второй и третьей) на 15,79%-6,80%, снижение общего белка у контрольной первой группы на 16,28%, а второй и третьей опытных групп на 8,70% -6,38% от нормы. Повышение показателей АСТ и АЛТ, АСТ на 14,02% в контрольной группе, а у опытных 19,38-19,59%, АЛТ повысился на 16,93% в первой группе, второй и третьей на 13,96%-18,67%относительно физиологической нормы крови. Показатель щелочной фосфатазы крови был увеличен у первой контрольной на 11,02%, у опытных 14,20% - 9,53% по отношению нормы. Уровень общего билирубина у всех собак был повышен относительно физиологической нормы на 10,38% первая группа и на 22,64% -8,49%.

Повторное исследование крови собак больных гепатитом проводилось на двадцатый день лечения у всех групп

Таблица 2: Биохимические показатели крови собак на двадцатый день исследования

Обозначения	Показатели	1группа (контрольная)	2группа опытная (ДАФС-25в дозе 104 мг/гол)	3группа опытная (ДАФС-25 в дозе 312 мг/гол)
ALB	Альбумины, г/л	18,5±1,21	20,8±1,23*	21,7±1,24*
TB	Общий билирубин, мкмоль/л	10,9±0,11	10,7±0,10*	10,6±0,10*
TP	Общий белок, г/л	45±2,08	47±2,09*	49±2,11*
ALP	Щелочная фосфатаза, ед/л	101,3±3,21	65,3±2,22*	63,7±2,23*
ALT	АЛТ, ед/л	58,2±2,13	52,6±2,12*	51,8±2,10*
AST	АСТ, ед/л	52,4±2,10	50,7±2,08*	49,2±2,02*

*Примечание: * $p<0,05$ относительно результатов анализов первой контрольной группы*

При взятии биохимических анализов крови на двадцатый день после начала лечения было отмечено, что показатели были значительно понижены в второй и третьей группах относительно первой. Щелочная фосфатаза была ниже на 59,03%-55,85%, показатель АЛТ был снижен на 12,36%-10,65%, АСТ понизился 5,69%-2,56%, общий билирубин так же был снижен на 2,83%-1,87%. Отмечено повышение альбуминов у второй и третьей группах на 12,43%- 17,30% по отношению к контрольной первой группе. Общий белок так же повысился 4,44%-8,89% по отношению к первой группе.

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Таблица 3: Биохимические показатели крови собак по окончанию исследования

Обозначения	Показатели	1 группа (контрольная)	2группа опытная (ДАФС-25 в дозе 104 мг/гол)	3группа опытная (ДАФС-25 в дозе 312 мг/гол)
ALB	Альбумины, г/л	23±1,52	25±1,53*	27±1,55*
TB	Общий билирубин, мкмоль/л	8,7±0,21	5,4±0,18*	3,5±0,20*
TP	Общий белок, г/л	51±2,15	58±2,17*	63±2,22*
ALP	Щелочная фосфатаза, ед/л	23,3±1,23	22,5±1,08*	18,4±1,20*
ALT	АЛТ, ед/л	37,8±1.43	35,5±1,40*	33,8±1,42
AST	АСТ, ед/л	24,8±1,22	21,5±1,12*	19,3±1,13*

Примечание: $p<0,05$ относительно результатов анализов первой контрольной группы*

При повторном взятии крови на биохимические показатели после лечения наблюдались достоверныеизменения в крови.Так щелочная фосфатаза у всех опытных животных снизилась до 18,5 г/л – третьей группы, второй 22,5 ед/л против 23,3 ед/л контрольной группы. Отмеченоснижение ферментов печени у собак второй и третьей групп АЛТдо 33,8 ед/л -35,5ед/л против37,8 ед/лконтрольной группы, АСТ в опытных группах снизилась до 21,5 ед/л во второй группе и до 19,3 ед/л в третьей опытной группе против 24,8 ед/л относительно первой группы. Отмечено снижение общего билирубина у собак опытных групп до 3,5 – 5,4мкмоль/л против 8,7 мкмоль/л контрольной группы. Повысились альбумины до 27 г/л – 25 г/л по отношению к 23 г/л,общий белок до 63 г/л – 58 г/л по отношению к 51 г\л первой контрольной группе.

Курс лечения у собак на момент исследования составил 45 дней. В результате чего на момент окончания лечения у собак экспериментальных групп (контрольной группы) и (в схемах включающих стандартное лечение и ДАФС-25 в дозах 1,6мг/кг (104 мг/гол) и 4,8мг/кг(312 мг/гол)) улучшилось общее состояние животных, аппетит нормализовался, отсутствовала рвота, масса тела пришла в норму, температура тела отсутствовала, что свидетельствовало об активизации обменных процессов в печени. Возобновилась работа желудочно-кишечного тракта, видимые слизистые ротовой полости и склеры глаз были розового цвета. В результате пальпации отсутствовала болезненность в области печени.

IV. Выводы/Conclusions

Прииссследовании использование различных схемлечения показало, что стандартные схемы лечения с добавлением препарата ДАФС-25 обладают более эффективным влиянием на печень. Препарат ДАФС-25 обладает сильными антиоксидантными свойствами и выраженным гепатопротекторным действием при гепатите у собак, о чем свидетельствуют данные исследованийбиохимических показателей и общего состояния животный на момент исследования.

Effectiveness of the use of DAFS-25 in the standard hepatitis treatment regimen for dogs.

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Effect of the Intravaginal Inoculation of Phytobiotics in a Murine Experimental Model

By Antonella Marchesi, Jessica Alejandra Silva, Priscilla Romina De Gregorio & María Elena Fátima Nader-Macías

Abstract- Lactobacilli play a fundamental role in maintaining health and preventing infections of the female urogenital tract. Complementary use of vegetal extracts selected for their ethnopharmacological characteristics and conventional uses for the different conditions in the human body as an adequate alternative therapy for the restoration of the vaginal microbiome has emerged. Compatibility of phytoextracts with lactobacilli for the design of phytobiotic formulas was determined previously. Safety of selected combinations by the intravaginal (i.va.) administration in a murine model was evaluated to determine if some type of adverse effect was produced in the host. Lactobacillus gasseri CRL1320, Limosilactobacillus reuteri CRL1324, Ligilactobacillus salivarius CRL1328 and Lacticaseibacillus rhamnosus CRL1332 combined with Hamamelis-virginiana, Amaranthus-muricatus and Smilax-áspera, were inoculated individually or combined in mice vagina (7 daily doses).

Keywords: *beneficial lactobacilli, vegetal-extracts, phytobiotics, safety, vaginal murine, adverse effect.*

GJMR-G Classification: *LCC: QP251*



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Effect of the Intravaginal Inoculation of Phytobiotics in a Murine Experimental Model

Antonella Marchesi [α], Jessica Alejandra Silva [σ], Priscilla Romina De Gregorio [ρ] & María Elena Fátima Nader-Macías [ω]

Abstract- Lactobacilli play a fundamental role in maintaining health and preventing infections of the female urogenital tract. Complementary use of vegetal extracts selected for their ethnopharmacological characteristics and conventional uses for the different conditions in the human body as an adequate alternative therapy for the restoration of the vaginal microbiome has emerged. Compatibility of phytoextracts with lactobacilli for the design of phytobiotic formulas was determined previously. Safety of selected combinations by the intravaginal (i.va.) administration in a murine model was evaluated to determine if some type of adverse effect was produced in the host. *Lactobacillus gasseri* CRL1320, *Limosilactobacillus reuteri* CRL1324, *Ligilactobacillus salivarius* CRL1328 and *Lacticaseibacillus rhamnosus* CRL1332 combined with *Hamamelis-virginiana*, *Amaranthus-muricatus* and *Smilax-áspera*, were inoculated individually or combined in mice vagina (7 daily doses). Vaginal washes were taken for microbiological (cultivable lactobacilli) and cytological (May Grünwald-Giemsa technique) evaluations, and vagina for histological (by Hematoxylin-Eosin) and ultrastructural (by electronic microscopy) analyses. Results obtained demonstrated that the i.va. phytobiotics administration did not produce adverse effects in the murine vaginal tract, by absence of inflammatory response. There were no modifications of the vaginal tract at the structural and ultrastructure level, suggesting the safety of phytobiotic formulas. Results obtained in this stage are original because information of viability and safety of natural extracts with strains of beneficial lactobacilli in *in vivo* trials are limited, and will allow progress in the design of beneficial formulas of reproductive age women.

Keywords: beneficial lactobacilli, vegetal-extracts, phytobiotics, safety, vaginal murine, adverse effect.

I. Introduction

Lactobacilli play a fundamental role in the urogenital tract by maintaining health or preventing infections through different mechanisms of action, demonstrated by a wide diversity of publications (Lazarenko et al., 2012; Karlsson et al., 2012; Wagner and Johnson et al., 2012; De Gregorio et al., 2014, 2015, 2016, 2019; Nader and Juárez-Tomas 2015; Nader Macías et al., 2021; Mashatan et al., 2023; Szczerbiec et al., 2024; Gupta et al., 2024). Plants extracts are applied to treat different pathologies in human and animals, which is one of the reasons to support their selection, supported by their ethnopharmacological characteristics to be used in urogenital tract infections (UGTI) prevention or treatment (Argentine Pharmacopoeia; Palmeira-de-Oliveira et al., 2015; Flower et al., 2016; Montorsi et al., 2016; Aziz y col., 2017: Moreno et al., 2018; Marchesi et al., 2020). Different products were applied for UGTI treatment, most of them derived from natural products, given the requirement of new therapies to prevent and treat chronic infections (Palmeira-de-Oliveira et al., 2013). In recent years, the frequent use of antimicrobials (such as antibiotics, antimicotics, and antivirals) is constantly questioned due to the appearance of resistant strains (Falagas et al., 2006; Flores-Mireles et al., 2015; Karam et al., 2019) which support the search for alternatives therapies or strategies to prevent or treat female urogenital infections, and to restore the microbiota of vaginal tract.

"*Probiotics*" are defined as "*live microorganisms, when administered in adequate amounts, evidence a beneficial physiological effect on the consumer*" (Hill et al., 2014). "*Pharmabiotics*" are "*living or dead microorganisms and their microbial constituents and metabolites that can beneficially interact with the host*" (Shanahan et al., 2009). "*Phytobiotic*" formulas refer to the "*combination of plant extracts with probiotic microorganisms to maintain or prevent health*" (Nader-Macías and Juárez-Tomás et al., 2015). "*Phytoextracts*" or "*Vegetal extracts*" are the substances obtained by maceration process of plants in 40% alcohol for medicinal use, approved by pharmacopoeias (Argentine Pharmacopoeia Method). Despite the fact that lactobacilli are generally recognized as safe (Generally Regarded as Safe, GRAS) by international organizations (FAO/WHO, 2012), and plant extracts which different applications in humans are described in pharmacopoeias (Argentine and European Pharmacopoeias) there is an imperative requirement to determine their safety and innocuity. It is of high importance to demonstrate that there is not production of adverse effects in animal models, before advancing in the evaluation of i.va. formulations in human clinical trials (Falagas et al., 2007; Nader-Macías et al., 2008;

Author α σ: Antonella Marchesi and Jessica Alejandra Silva have contributed at the same level in performing the experimental protocols.
Author ρ: Centro de Referencia para Lactobacilos-Consejo Nacional de Investigaciones Científicas y Técnicas de Argentina (CERELA-CONICET). Chacabuco 145. San Miguel de Tucumán. 4000. Argentina.
Author ω: Prof. Dra. María Elena Fátima Nader-Macías CERELA-CONICET Chacabuco 145. 4000 San Miguel de Tucumán. Argentina. e-mails: fnader@cerela.org.ar, fatynader@gmail.com

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2021; Alfaro et al., 2013; Silva et al., 2023). Selection of strains and extracts was also performed to further design phytobiotic formulas aimed to treat, prevent or maintain the human vaginal health (Mishra et al., 2018).

Safety of vaginal lactobacilli was determined previously, supported by requirements established for the design of probiotic formulas for human beings. Intraurethral and i.va. administration of vaginal lactobacilli was applied in a murine experimental model evidencing their persistence in the urogenital tract, absence of adverse effects, protection against *Staphylococcus aureus*, *Streptococcus agalactiae*, *Escherichia coli* and *Candida albicans* challenges, and immune system modulation (Silva-Ruiz et al., 2004; Zárate et al., 2007; De Gregorio et al., 2012; 2015; 2016; 2019; Leccese-Terraf et al., 2015). Experimental animal model in BALB/c mice was used for its small size, easy manipulation and short reproductive cycle. Safety of lactobacilli was also demonstrated in phase I trial in healthy women (De Gregorio et al., 2020). Our research group demonstrated that the i.va. administration of functional nanofibers was safe in murine models, producing a viable lactobacilli increase and promoting their permanence in the murine vaginal tract (Silva et al., 2023).

The physiological characteristics of the murine vaginal tract are different from those of women, mainly in the neutral vaginal pH, the low numbers of lactobacilli in the autochthonous microbiota and the length and characteristics of the sexual cycle (Patras et al., 2013). McLean et al. (2012) described a simple and non-invasive protocol to determine the estrous cycle stage of female mouse without altering its reproductive cycle, similar to the one used in this work. Murine models success described by numerous scientists to evaluate the vaginal tract is useful to predict the expected behaviour in human beings (Silva-Ruiz et al., 2001; Zárate et al., 2009; Muench et al., 2009; Spurbeck and Arvidson 2011; Joo et al., 2011; Joo et al., 2012; Patras et al., 2013; De Gregorio et al., 2014; 2015; 2016; 2019).

Phytobiotics were formulated previously in our research group with 30 different beneficial vaginal lactobacilli (BVL) strains combined with phytoextracts (selected by their application and characteristics related with vaginal health improvement) to produce a synergic or complementary pharmacological effect (Marchesi et al., 2020). However, no safety assays were published referred to formulas designed with vegetal extracts and probiotic bacteria combined, administered by the vaginal way. Thus, the aim of this work was to evaluate the safety of phytobiotic formulas designed with beneficial lactobacilli and vegetal extracts combined, in order to define the permanence of the strains, and if some type of adverse reactions was evidenced in the murine experimental model.

II. Results and Discussion

a) Quantification of Viable Lactobacilli in Vaginal Washing

The results obtained when evaluating the number of viable lactobacilli from vaginal washing (v.w.) of mice inoculated with the different phytobiotics did not show significant differences ($p>0.05$) referred to mice inoculated only with BVL (control), indicating that a stimulatory or inhibitory effect by the phytoextracts on the colonization capability of lactobacilli was not produced (Fig.1). The comparison of the i.va. inoculation of the four BVL strains, showed higher viable cell numbers of *L. gasseri* CRL1320 (10^6 CFU/ml v.w.), while *L. reuteri* CRL1324 and *L. salivarius* CRL1328 were in a lower value (10^{2-3} CFU/ml). In control mice, viable lactobacilli were not detected (in MRS-pH-5.5, selective medium) suggesting that the vaginal isolated lactobacilli came from the exogenous administration. Mice receiving phytobiotics-*L. rhamnosus* CRL1332showed similar values than *L. gasseri* CRL1320. *L. salivarius* CRL1328+phytocompounds mice produced a higher number of viable lactobacilli, but not significant compared with the strain without vegetal extracts. At the end, *L. salivarius* and *L. rhamnosus* demonstrated a higher colonization in mouse vagina when administered in phytobiotic formulas. These results were different to those obtained from *in vitro* assays (Marchesi et al., 2020) where some vegetal-extracts showed a stimulatory or inhibitory effect on BVL. Differences between *in vitro* and *in vivo* assays were also observed in BVL interaction with *St. agalactiae* (De Gregorio et al., 2014). These differences support the requirement to apply different criteria for probiotic selection, including both *in vitro* an *in vivo* safety assays (Nader-Macias and Juárez, 2015; Nader-Macias et al., 2021). Viable lactobacilli quantification in v.w. were similar to those obtained previously when the optimal i.va. dose of *L. reuteri* CRL1324 was determined (De Gregorio et al., 2015).

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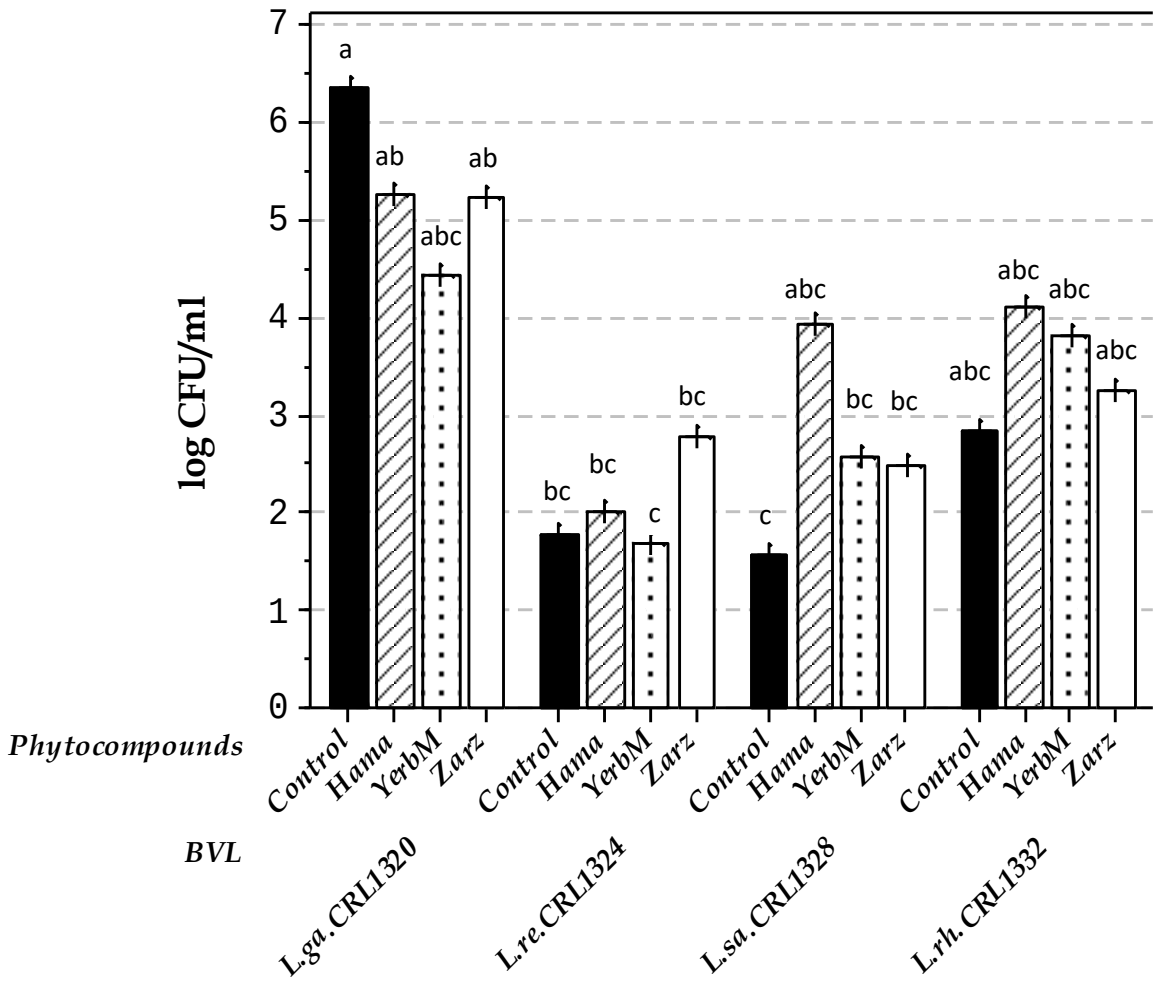


Fig.1: Quantification of viable BVL in murine v.w. of mice inoculated with lactobacillivegetal extracts: Control (black-bar: ■), *Hama:Hamamelis* (oblique-bar: ▨), *YerbM:Yerba meona* (dot-bar: ⬚) and *Zarz*: *Zarzaparilla* (white-bar: □). The results represent the log CFU/ml mean values of *L. gasseri* CRL1320, *L. reuteri* CRL1324, *L. salivarius* CRL1328, and *L. rhamnosus* CRL1332 ± standard error. Significant differences in the number of each BVL strain and their combination with extracts are indicated by different letters ($p < 0.05$).

b) Vaginal Cytology

Intravaginal administration of phytobiotic formulas did not produce adverse effect or inflammatory response in the murine experimental model. May Grünwald-Giemsa vaginal smears from phytobiotics, BVL or vegetal extracts inoculated mice obtained, by optical microscopy are included in Fig.2.A. Cytological evaluation evidenced absence of adverse effect at this level. All samples showed similar patterns to control mice, indicating pseudo-estrous state induced by hormonal inoculation, characterized by the presence of keratinized epithelial cells (irregular shape of scales) and absence of nucleated epithelial cells and leukocytes (which are indicative of an inflammatory or adverse response). Safety of BVL in the urogenital tract was also demonstrated previously. Silva de Ruiz et al. (2003) administered intraurethrally *L. fermentum* CRL1508 in a murine model with no adverse effect or significant changes in the organs (kidney, ureter, bladder or urethra) at the structural and ultra-structural level, indicating its safety. De Gregorio et al. (2012) evidenced that the administration of five different human BVL to BALB/c-mice for 4 163 days did not produce adverse effects in cytological and immunological assays. Zarate et 164 al. (2009) also showed the protection against *S. aureus* challenge by the i.va. 165 administration of human BVL strains with no adverse effects. Recently, Silva et al. (2023) demonstrated the absence inflammatory response at the cytological level after the i.va. administration of nanofibers with *L. rhamnosus* CRL1332 immobilized in murine model.

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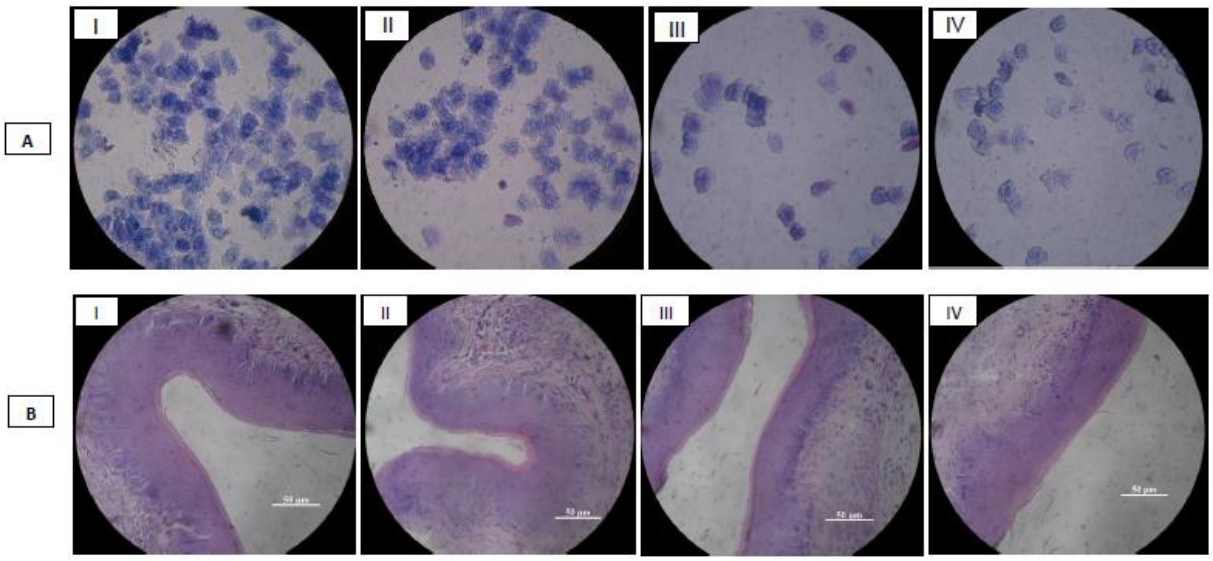


Fig. 2: Vaginal Cytology (A) and Histology (B) images of BALB/c mice after 171 intravaginal administration with different phytobiotics.A-I: *L. rhamnosus* 172 CRL1332+*Hamamelis;*A-II: *Hamamelis;* A-III: *L. reuteri* CRL1324+*Hamamelis;*A173 IV: Control. B-I: *L. gasseri* CRL1320+*Hamamelis*; B-II: *Hamamelis*; B-III: *L. gasseri* 174 CRL1320; B-IV: Control.

c) Vaginal Histology

Microscopy evaluation is useful to facilitate diagnosis, as some authors claim. Donders 178 et al. (2019) recommend the use of microscopy for an exact observation of vaginal 179 epithelium condition, and helps to define different therapeutic alternatives. Histological 180 structure of vaginal tract of mice i.va. inoculated with phytobiotics, phytocompounds or 181 lactobacilli showed normal lamina propia characteristics, multilayer epithelium, 182 keratinized epithelial cells, indicating the pseudo-estrous state (Fig. 2. B). The observed 183 pattern was similar to control mice (Fig. 2. B-IV), with absence of inflammatory response 184 in murine tissue.

Zarzaparilla has antifungal, antiseptic and diuretic uses; *Hamamelis* has antiviral, antiseptic and anti-inflammatory activities, while *Yerba meona* evidenced diuretic, antitumor, drastic purging, warts, herpes, depurative, and other applications, supporting their selections to evaluate the beneficial properties when used in combination with BVL (Argentine-Pharmacopoeia; European-Pharmacopoeia; Theisen et al., 2014; Qi et al., 2017; Marchesi et al., 2020). Different scientists published the effect of plant derivatives administered orally or intravaginally in women suggesting a variety of effects and mechanisms of action. Moraes et al. (2012) demonstrated the efficacy and safety of *Mentha crispa* as a suitable alternative in the therapy of *Trichomonas vaginalis* infections. Satthakarn et al. (2015) administered *Houttuynia cordata* aqueous-extract in women urogenital tract determining an increase of cells participating in vaginal immune response. Espino et al. (2019) showed the complementary antifungal effect when administering *L. plantarum* cream+two extracts in candidiasis treatment in *in vitro* protocols. No publications were detected referred to protocols of i.va. administration of phytobiotics in a murine experimental models. Recently, Miranda and Nader-Macías (2024) showed that the i.va. administration of probiotic and phytobiotic formulations to pregnant female cows at pre and postpartum increased significantly the number of lactic acid bacteria with no adverse local and systemic effects in cows.

d) Ultrastructure of Murine Vagina

Ultrastructure of murine vagina inoculated with phytobiotics was characterized by a keratinized epithelium with anucleated polystratified cells, supported on the lamina propia with anucleated cells. Microphotographs of the vaginal tract of lactobacilli and phytocompounds i.va. inoculated mice did not evidence modifications, being similar in experimental group (*L. salivarius* CRL1328+*Hamamelis)* (Fig.3.A,B) and control mice (Fig. 3. G,H). Absence of inflammatory response, ultrastructure maintenance, and no other cells participating in inflammation indicate no adverse effect. Normal polystratified murine vaginal epithelium was evidenced. Mice inoculated with phytobiotics and lactobacilli showed bacteria or bacilli close or in contact with the epithelial cells surface (black arrows). These bacteria were not detected in mice i.va. inoculated only with phytoextracts or control animals. Therefore, it could be suggested that the bacteria evidenced in vagina of mice are those i.va. inoculated for 7-days, with no adverse effects. As control mice did not show bacteria, lactobacilli permanence is supported by their exogenous administration to mice.

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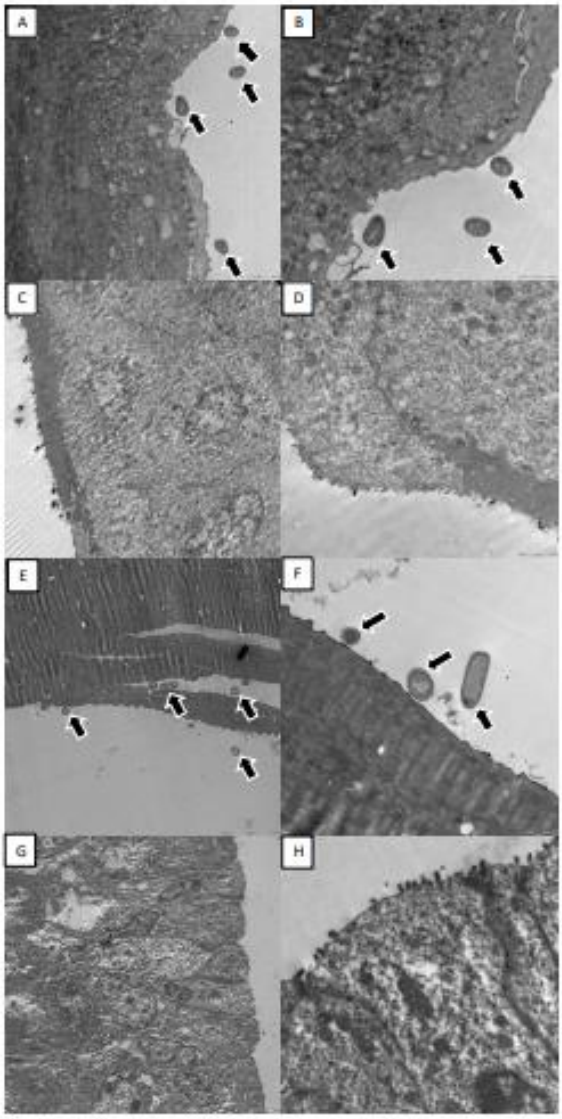


Fig. 3: Ultrastructure of mouse vagina i.va. inoculated with phytobiotics, lactobacilli or phytocompounds analysed by transmission electron microscopy. A:*L. salivarius* CRL1328+*Hamamelis* (2500X); **B:** *L. salivarius* CRL1328+*Hamamelis* (4000X); **C:** *Hamamelis* (1200X); **D:** *Hamamelis* (4000X); **E:** *L. salivarius* CRL1328 (1200X); **F:** *L. salivarius* CRL1328 (4000X); **G:** Peptone-water Control Mice Group (800X); **H:** peptone-water Control Mice Group (4000X). Bacteria or bacilli close or in contact with the epithelial vaginal cells are indicated with black arrows.

III. Materials and Methods

Microorganisms: Four BVL strains previously characterized and genetically identified were used in this work:*Lactobacillus gasseri* CRL1320, *L. reuteri* CRL1324, *L. salivarius* CRL1328 and *L. rhamnosus* CRL1332 (Marchesi et al., 2020) (Table 1). The strains were freezed-stored in milk yeast-extract (10% skim-milk, 0.5% yeast-extract, 1% glucose), and they were inoculated, and subcultured 3 times in MRS broth (De-ManRogosa and Sharpe) (Biokar-Diagnostics-Beauvais-France) at 37°C for 13-14 h before their use. For the i.va. administration in mice, the strains were centrifuged at 3000g for 10 min (Presvac-Argentina), and resuspended in 50μl agarose-peptone [1% meatpeptone, 1.5% agar] or combined with the vegetal extracts selected (Table 2).

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Table 1: Beneficial Vaginal Lactobacilli (BVL) Properties

BVL Strains	Beneficial Properties
L. gasseri CRL1320	H_2O_2 and lactic acid production, high hydrophobicity, pathogens inhibition, biofilm formation
L. reuteri CRL1324	H_2O_2 and lactic acid production, high hydrophobicity, biofilm formation, pathogens inhibition, colonization, of BALB/c vaginal tract mice, adhesion to fibrinogen and mucin
L. salivarius CRL1328	bacteriocin production, pathogens inhibition
L. rhamnosus CRL1332	H_2O_2 production, high hydrophobicity, biofilm, formation, high resistance to lyophilization, pathogens inhibition, colonization of BALB/c mice vaginal tract, adhesion to fibrinogen and mucin

Table 2: Vegetal Extracts and uses

Scientific name	Popular name	Uses	Pharmacopoeia
Hamamelis viriginiana	*Hamamelis*	Astringent, antiseptic, antiinflammatory, antiviral, venotonic	Argentine 8th edition
Amaranthus muricatus	*Yerba-meona*	Diuretic, antitumor, drastic purging, warts, herpes, depurative	Argentine 6th edition
Smilax aspera	*Zarzaparrilla*	Diuretic renal depurative, antibacterial, antifungal, antiseptic	European

Phytocompounds: "*Hamamelis-virginiana*" (*Hamamelis),* "*Amaranthus-muricatus*" *(Yerba meona)* and "*Smilax-áspera" (Zarzaparilla)* were selected for their ethnopharmacological properties and compatibility against different BVL, summarized in Table 2 (Marchesi et al., 2020). From dry extracts of *Hamamelis* (leaves), *Yerbameona* (leaves-stem), and *Zarzaparilla* (bark+branches+fruits), previously obtained by maceration (according to the Argentine Pharmacopoeia) and dried, 1 mg of extract was weighed with 1 ml of alcohol 40%, taking only 20 *µ*l of each plant extract (1mg/ml in 40%-alcohol) were mixed with 30*µ*l agarose-peptone, 50*µ*l i.va. administered to mice.

Microorganisms+Phytocompounds: the pellet from 3rd BVL subculture was mixed with 20µl vegetal-extract+30µl agarose-peptone for mouse i.va. administration. The protocol of administration in the murine model is indicated in Figure 4.

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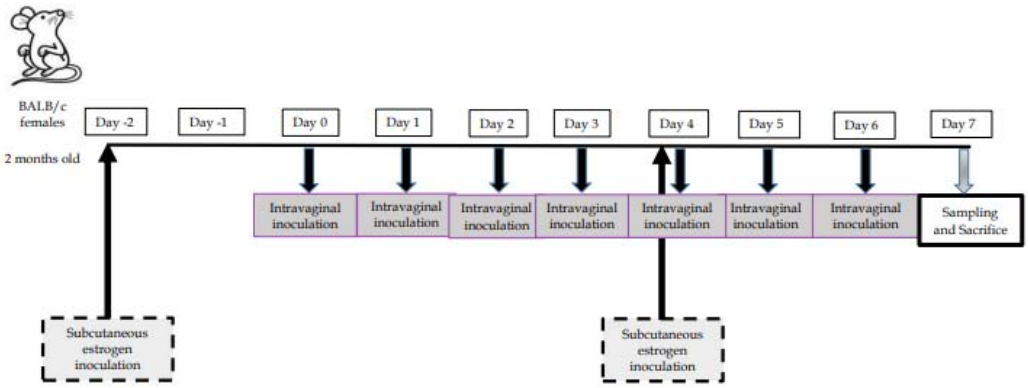


Fig. 4: Protocol of Administration of Phytobiotics, Lactobacilli and Phytocompounds by I.Va. Route to Estrogenized-Adult BALB/C Female Mice

a) Female BALB/C Mice as Experimental Model

Female BALB/c mice, 45-days-old, 20-25 average-weight, were provided and maintained in CERELA nursery, keeping constant environmental conditions, fed *ad libitum* with conventional balanced diet. Pseudostrous status was induced with β-estradiol-valerate subcutaneous administered on days "-2" and "3", according to previously set-up protocols, to avoid the variations from the estrous cycle state, and to promote lactobacilli permanence (De Gregorio et al., 2012). Hormone was prepared from a stock solution (2 mg/ml, Sigma-Life-Sciences, Switzerland) resuspended and diluted in sesame-oil (Sigma-Life-Sciences, Mexico) at 0.2 mg/ml. Then, 0.1 ml was injected subcutaneously (0.02 mg). The experimental protocol, hormone administration and sampling days are shown in Figure 4. 100 mice were used, divided in 20 groups, assigning 5 mice randomly to each group, as follows:

1) *Phytobiotic Groups (12):* The following combinations of probiotic BVL+phyto extracts were i.va administered for 7 days: *L. gasseri* CRL1320+ *Hamamelis*, *L. gasseri* CRL1320+ *Zarzaparrilla*, *L. gasseri* CRL1320+*Yerba-meona*, *L. reuteri* CRL1324+*Hamamelis,L. reuteri* CRL1324+ *Zarzaparrilla*, *L. reuteri* CRL1324+ *Yerbameona*, *L. salivarius* CRL1328+*Hamamelis, L. salivarius* CRL1328+*Zarzaparrilla, L. salivarius* CRL1328+ *Yerba-meona*, *L. rhamnosus* CRL1332+ *Hamamelis, L. rhamnosus* CRL1332+*Zarzaparrilla,* and *L. rhamnosus* CRL1332+*Yerba-meona*.
2) *Vegetal Extracts Groups (3):* mice were i.va. administered for 7 days with: *Hamamelis-virginiana* (*Hamamelis*), *Amaranthus-muricatus* (*Yerba-meona*) and *Smilaxáspera* (*Zarzaparrilla*).
3) *BVL Strains Groups (4):Lactobacillus gasseri* CRL1320, *Limosilactobacillus reuteri* CRL1324, *Ligilactobacillus salivarius* CRL1328 or *Lacticaseibacillus rhamnosus* CRL1332 were individually i.va. administered during 7 days, at 10^7-10^8 CFU each dose:
4) *Control Group (1):* 20μl saline+30μl 1%-agarose-peptone.

Two independent assays were carried out. CERELA Institutional Committee for the Care and Use of Laboratory Animals approved the experimental protocol CRL-BIOT-LMP2010/1A.

b) Mice Sampling and Analytical Procedures

Murine vagina was washed in sterile conditions with 50μl phosphate-buffered-saline (PBS: 8.1mM Na_2HPO_4, 1.5mM KH_2PO_4, 140mM NaCl, pH 7.2) 7 times and v.w. of each animal pooled. Vaginal washes were used for different assays:

1) *Quantification of microorganisms:* serial dilutions were prepared and inoculated in selective MRS agar (pH 5.5) to quantify viable BVL after 48 h incubation at 37°C. Number of microorganisms was expressed as log CFU/ml v.w.
2) *Cytological studies:* 10μl aliquots were spread on slides, fixed and stained with MayGrünwald-Giemsa technique to assess whether inoculation with phytobiotics produced any type of adverse effect at cytological level. Preparations were observed under light microscope (40x, Axio-Scope-A1, Carl-Zeiss) (McLean et al., 2012).
3) *Histological studies:* mice were sacrificed by cervical dislocation at the 7th day and dissected to extract vagina, which was transferred to the appropriate solvents for subsequent process for histological and electronic microscope observation, as follows:
   i. *Histological evaluation (light microscopy):* vaginal tissues were fixed in 4% (v/v) formaldehyde at 4°C, embedded in paraffin by applying routine laboratory methods. Organs were processed according to Silva de Ruiz et al. (2003) using Carl Zeiss Microscope (40x).
   ii. *Ultrastructural evaluation (transmission electron microscopy):* mouse vagina samples were placed in Karnovsky's fixative (2.66% paraformaldehyde,

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0.1M sodiumphosphate-buffer, pH 7.4, 1.66% glutaraldehyde) for 1 week. The technique applied was detailed previously (Zampini et al., 2020). Samples were processed and observed at the Zeiss Libra 120 electron microscope (Carl Zeiss, Oberkochen, Germany) of Integral Center for Electronic Microscopy in Tucumán (CIME-CONICET).

c) *Statistics Analysis*

Analysis of variance (ANOVA) using a general linear model was applied to define the main effects of experimental groups on the number of viable lactobacilli. Significant differences (p-value$<$0.05) between mean values were determined by Tukey's test, using MINITAB statistical software (version-16 for Windows).

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Diphylla ecaudata Spix, 1823 in the Caatinga-Cerrado Ecotone: From Feeding Plasticity to Rabies Propagation in Northeastern Brazil

By Marcelo Cardoso da Silva Ventura, Randyson da Silva Pinheiro, Marcos Vinicius Costa Santos, Beatriz da Silva Borges, Rômulo Oliveira Barros, Michael Anderson Teneu Costa, Mayky Carvalho de Oliveira, Elba Regina Sampaio de Lemos & Marco Aurélio Pereira Horta

Resume- In the Brazilian Northeast, the state of Piauí exhibits gaps in bat species diversity, with limited knowledge about their ecology and zoonotic disease transmission. This study updated the distribution of *Diphylla ecaudata* in the region, exploring feeding behaviors and recording the presence of the rabies virus. Fieldwork in Picos, Pedro II, and Milton Brandão resulted in the capture of nine bats, following ethical protocols. Previous records from 2004 to 2008 were also noted. Research showed these bats' adaptability to various environments, including the Peruvian biome, and their diet flexibility beyond birds, their typical prey.

Keywords: *Brazil, ecotone, bats, piauí, one health.*

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Diphylla ecaudata Spix, 1823 in the Caatinga-Cerrado Ecotone: From Feeding Plasticity to Rabies Propagation in Northeastern Brazil

Marcelo Cardoso da Silva Ventura [α], Randyson da Silva Pinheiro [σ], Marcos Vinicius Costa Santos [ρ], Beatriz da Silva Borges [ω], Rômulo Oliveira Barros [¥], Michael Anderson Teneu Costa [§], Mayky Carvalho de Oliveira [χ], Elba Regina Sampaio de Lemos [ν] & Marco Aurélio Pereira Horta [Θ]

Resume- In the Brazilian Northeast, the state of Piauí exhibits gaps in bat species diversity, with limited knowledge about their ecology and zoonotic disease transmission. This study updated the distribution of *Diphylla ecaudata* in the region, exploring feeding behaviors and recording the presence of the rabies virus. Fieldwork in Picos, Pedro II, and Milton Brandão resulted in the capture of nine bats, following ethical protocols. Previous records from 2004 to 2008 were also noted. Research showed these bats' adaptability to various environments, including the Peruvian biome, and their diet flexibility beyond birds, their typical prey. Significantly, a rabies virus variant (AgV3), commonly found in *Desmodus rotundus*, and traces of human blood in *Diphylla ecaudata* feces were detected, raising public health concerns. These findings highlight potential risks for zoonotic disease transmission to humans, underscoring the need for ongoing monitoring in wildlife-human interactions.The broader relevance of this study lies in its implications for zoonotic disease surveillance across diverse biomes, not just in Piauí. Bats are key reservoirs of pathogens, and their ability to adapt to different ecosystems suggests a wider risk for emerging zoonoses. This work emphasizes the importance of integrating ecological and health monitoring under the One Health approach, providing insights that can inform future research and guide public health and conservation strategies aimed at mitigating the spread of zoonotic diseases globally.

Keywords: Brazil, ecotone, bats, piauí, one health.

Author α: Federal Institute of Piauí, Teresina-CEP 64000-040, Brazil. Molecular Biology and Epidemiology Laboratory of the Federal Institute of Education of Piauí, Teresina-CEP 64000-040, Brazil.
e-mail: marceloventura@ifpi.edu.br
orcid: 0000-0003-2169-362X
Author σ: Molecular Biology and Epidemiology Laboratory of the Federal Institute of Education of Piauí, Teresina-CEP 64000-040, Brazil.
e-mail: randysonpinheiro22@gmail.com
orcid: 0000-0001-9140-9079
Author ρ: Molecular Biology and Epidemiology Laboratory of the Federal Institute of Education of Piauí, Teresina-CEP 64000-040, Brazil. e-mail: viniciusmarcos1212@gmail.com
orcid: 0009-0003-1916-9658
Author ω: Molecular Biology and Epidemiology Laboratory of the Federal Institute of Education of Piauí, Teresina-CEP 64000-040, Brazil.
e-mail: beatrizborg37@gmail.com
orcid: 0000-0002-6385-6012
Author ¥: Federal Institute of Piauí, Teresina-CEP 64000-040, Brazil.
e-mail: romulobarros@ifpi.edu.br
orcid: 0000-0002-0000-033X
Author §: University Federal of Piauí – CEP64.049-550-Programa de Pós-Graduação em Tecnologias Aplicadas a Animais de Interesse Regional (PPGTAIR). e-mail: michaelcostta2801@outlook.com
orcid: 0000-0001-9595-3792
Author χ: Federal Institute of Piauí, Teresina-CEP 64000-040, Brazil.
e-mail: mayky.c.oliveira@gmail.com
orcid: 0000-0003-3255-9717
Author ν: Laboratório de Hantaviroses e Rickettsioses, Oswaldo Cruz Institute, Fiocruz, Rio de Janeiro 21040-360, Brazil.
e-mail: erslemos@gmail.com
orcid: 0000-0003-3761-0200
Author Θ: Biosafety Level 3 Facility, Oswaldo Cruz Institute, Fiocruz, Rio de Janeiro 21040-900, Brazil. e-mail: marco.horta@fiocruz.br
orcid: 0000-0003-4117-5379

I. Introduction

Knowledge about the chiropteran fauna in the state of Piauí is characterized by substantial gaps, despite significant advancements in the field of study in the Brazilian Northeast over the past two decades. Understanding the distribution of bats in the state is crucial for species conservation and habitat maintenance. In particular, focusing on the subfamily Desmodontinae, which includes only three monotypic genera (*Desmodus*, *Diphylla*, and *Diaemus*), highlights their important role in maintaining rabies in natural environments, an infection that affects the nervous system of mammals, including humans (Barreto et al. 2019).

Among hematophagous bats, the genus *Diphylla* stands out as a monotypic taxon, characterized by the absence of callosities on their anterior appendicular limbs. The epithet "hairy-legged bat" refers to the rudimentary femoral membrane covered in hair, which is a vestige of a lateral uropatagium (Vizotto and Taddei, 1973). The species *Diphylla ecaudata* (Spix, 1823), which means "tailless hairy leaf," ranks second in geographical distribution and capture frequency among blood-feeding bats, following Desmodus rotundus.

According to Uieda (1982), *Diphylla ecaudata* specializes in feeding on birds. however, Ruschi (1951) notes that in the absence of their usual prey, they may also attack pigs, cattle, and horses. This specialized diet makes hematophagous bats significant players in the spread of diseases such as rabies, a viral encephalitis with nearly 100% lethality in both animals and humans worldwide (Streicker and Allgeier, 2016).

The association between *D. ecaudata* and the rabies virus is noteworthy, as these viruses primarily

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affect mammals, even though these bats mostly prey on birds. This interaction can be explained by their shared habitats, particularly in caves with *Desmodus rotundus*, the primary reservoirs of the rabies virus variant AgV3, facilitating the exchange of viral particles (Albas et al. 2011). Since caves are also inhabited by non-hematophagous bats, positive findings for rabies extend to these species as well.

Updating the numbers for Brazil, there are records of seven variants of the *Lyssavirus* genus. Among these are variant 2 (AgV2), found in *Phyllostomus discolor* (Albas et al. 2011), and variants 4 and 6 (AgV4 and AgV6), found in the insectivorous bats *Tadarida brasiliensis* and *Lasiurus cinereus*, respectively (Brasil, 2023).

Regarding distribution, *D. ecaudata* has been recorded throughout much of the national territory, being present in all regions, with notable occurrences in the Southeast and Northeast of Brazil (Rocha et al., 2014). In the state of Piauí, Castilho et al. (2010) made the first record of *D. ecaudata* in the municipalities of São Miguel do Tapuio and Buriti dos Montes based on field research conducted in 2004. It was not until 2008 that Gregorin, Carmignotto, and Percequillo described its occurrence in the Serra das Confusões National Park (PNSC) in Gilbués, Piauí.

Understanding the distribution and ecology of *Diphylla ecaudata* not only addresses the gaps in knowledge about this species but also enhances our understanding of the broader chiropteran fauna in Piauí. This study aimed to update the geographical distribution of *Diphylla ecaudata* in the Brazilian Northeast, associating it with new foraging approaches and records of rabies virus occurrence in the region for this species. By highlighting these findings, the research contributes to ongoing conservation efforts and public health policies in the region, which aim to mitigate the risks associated with zoonotic diseases and promote biodiversity preservation.

II. Metodology

a) Research Area

Located between 2° 44' 49" and 10° 55' 05" latitude South and 40° 22' 12" and 45° 59' 42" longitude West, the state of Piauí is bordered by the states of Ceará and Pernambuco to the east, Bahia to the south and southeast, Tocantins to the southwest, with the Parnaíba River marking the border with Maranhão to the west, and the Atlantic Ocean to the north (Lima et al., 2020). It occupies 251,755.481 km², which is 16.16% of the 1,558,000 km² of the Northeast region of Brazil. Piauí is the third-largest state in the Northeast, smaller only than Bahia and Maranhão, and the tenth-largest state in Brazil, accounting for 2.9% of the national territory (IBGE, 2022).

Piauí contains a wide variety of ecosystems, including stretches of the Cerrado and Caatinga biomes, which are known for their biodiversity and varying climatic conditions (Santos-filho*et al.*, 2018). These biomes, along with a significant number of cave systems and fragmented forest patches, provide ideal habitats for bat species (Pereira, 2022). The state's mosaic of natural environments, particularly cave habitats, supports both hematophagous and insectivorous bat populations. These bats play critical roles in ecosystem services such as pollination and seed dispersal but are also key reservoirs for zoonotic diseases, including rabies (Stoner-Duncan *et al.,* 2014).

The choice of Piauí for this study was motivated by these unique ecological features and its role as a transition zone between the Cerrado and Caatinga biomes. This makes the region a hotspot for studying bat ecology and disease transmission. The presence of both preserved and disturbed habitats, including agricultural areas, increases the chances of interactions between bats and humans or livestock, which is relevant for understanding the dynamics of rabies transmission.

b) Sampling

i. Primary Data

Primary distribution data were obtained through passive sampling. Mist nets measuring three meters in height by nine and twelve meters in length were used, set up in strategic areas based on factors favorable for the flight routes of these animals and the presence of their usual prey. The traps were positioned at two collection points at 6 pm and dismantled at midnight of the following day, totaling a sampling effort of 4,968 m²h in the municipality of Picos, 864 m²h in the municipality of Pedro II, and 162 m²h in the municipality of Milton Brandão (Straube and Bianconi, 2002).

The animals were immediately removed from the mist nets upon capture and placed in pre-numbered cloth bags. The identification of the specimens was based on specialized literature cited in the work of Reis et al. (2017). The study was submitted to and approved by the System of Authorization and Information on Biodiversity (SISBio) under number 69264-7, and the Ethics Committee on Animal Use under number 002/CEUAIFPI/2021, with registration number AF78746 in the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SISGEN). The specimens were designated for the Natural History Collection of UFPI (CHNUFPI) in the municipality of Floriano-PI.

ii. Secondary Data

Data were obtained through a review of specialized bibliography and digital platforms such as the *Global Biodiversity Information Facility* (GBIF), including references characterizing the biodiversity of

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chiropterans in the state of Piauí and the occurrence of *D. ecaudata* in the regional context.

Vegetation characteristics, geographic boundary information, territorial distances, and biomes for each collection site were extracted from research conducted on the website of the Brazilian Institute of Geography and Statistics (IBGE). This information was processed using QGIS software version 3.34.1 for map plotting (Figure 3).

III. Results and Discussion

The geographical distribution of bats of the species *D. ecaudata* is quite broad, with faunal individuals found throughout much of the American continent (Scheffer et al., 2015). The update of occurrence records in this study was achieved through field captures and secondary data, which indicated 13 specimens of *D. ecaudata* collected and identified in the state of Piauí up to the production of this study. These specimens originated from research focused on surveying diversity and/or studying zoonoses associated with chiropterans in Piauí. Figure 1 shows a photograph of a male specimen of *Diphylla ecaudata* captured in the northern region of the state, in the municipality of Pedro II.



Source: Authors' archive

Figure 1: Photograph of a faunal specimen of *Diphylla ecaudata*, collected in the municipality of Pedro II, Piauí

Note from Table 1 that in the village of Sertão de Dentro located in Milton Brandão – PI, five specimens were collected, and these captures were prompted by a request from a local resident who reported several chicken deaths likely caused by bats. Subsequently, we set up a mist net measuring 9 x 3 meters near the chicken roost. In Figure 2 (resting place of the chickens), we noted the height above ground level and marked it at 50 cm.

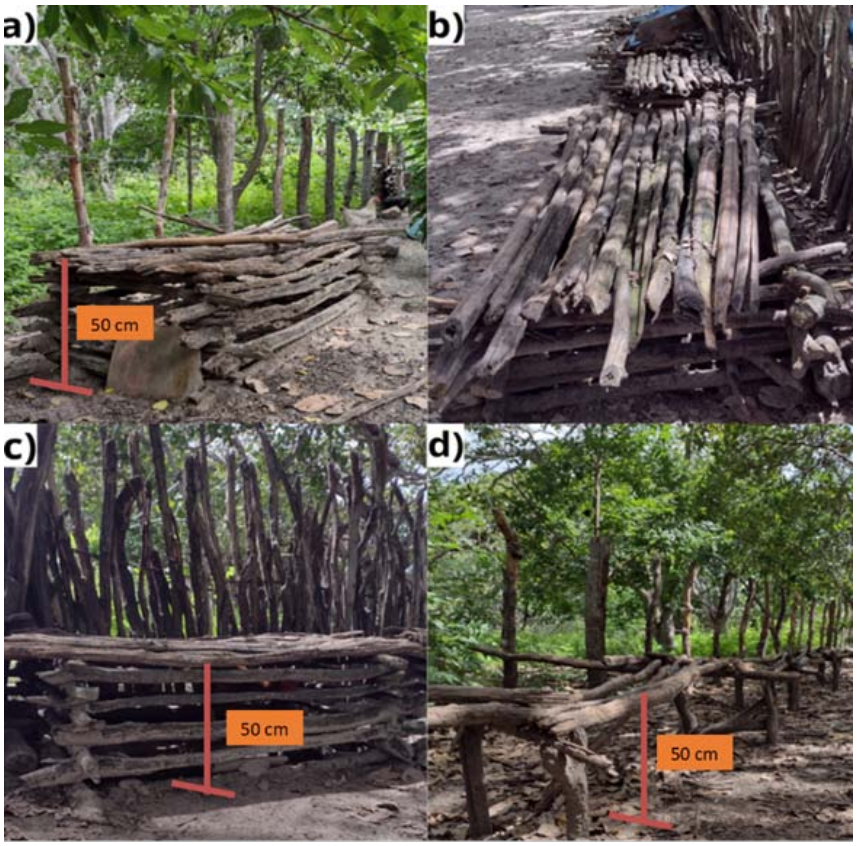
Table 1. Compilation of Information on Captures of *D. ecaudata* in the State of Piauí, Northeast Brazil (2004-2023)

Location	Source	Coordinates	Quantity
Pedro II (Serra dos Matões)	Own work	S 4°24'2,3" W 41°26'44,6"	2
Milton Brandão (Sertão de dentro)	Own work	S 4°47'03,2" W 041°28'54,2"	5
Picos	Own work	S 7°02'18,0" W 41°27'4,6"	2
Guaribas (PNSC)	Gregorin *et al.* (2008)	S 09°13'12" W 43°29'52"	1
São Miguel do Tapuio and Buriti dos Montes*	Castilho *et al.* (2010)	Not provided	3

**The record of the three animals occurred in 2004, however, the publication dates back to 2010, and we were unable to discriminate the quantity of animals for the mentioned municipalities.*

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Uieda (1982), in his experimental work with specimens of *D. ecaudata*, reports these animals' preference for preying on birds within a height range of 2 to 4 meters above the ground. However, observations made by our team throughout the night in a net set up near the birds' resting place show a behavioral change, as predation occurred closer to the ground, at around 0.5 meters height, thereby expanding the spatial spectrum for predation (Figure 2). This demonstrates the flexibility in predation habits among these animals, indicating adaptations in foraging behavior.



Source: Authors' archive

a) Chicken coop with dimensions of 1.5 meters in length x 0.5 meters in width and 0.5 meters in height from the ground at its highest point; b) Top view of the chicken coop; c) Front view of the chicken coop; d) Chicken roost.

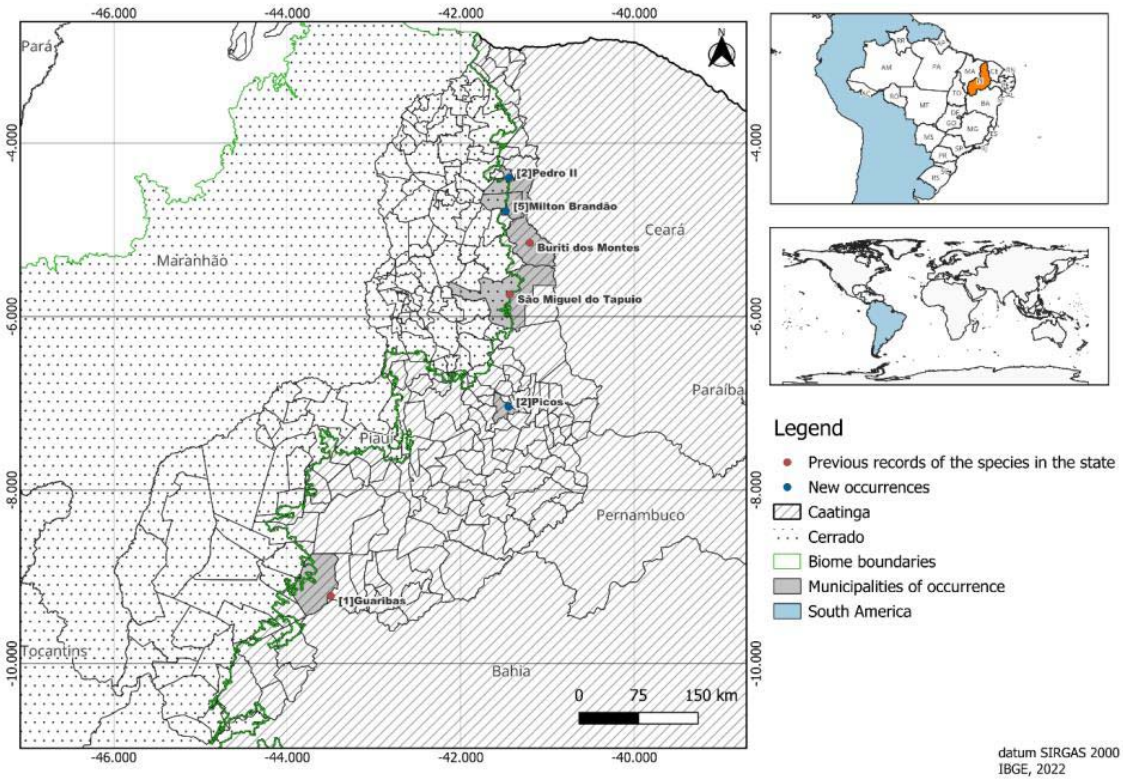
Figure 2: Images of Chicken Roosts in the Backyard of a Local Resident in the Village of Sertão de Dentro, in the Municipality of Milton Brandão-PI, recorded in 2023

Figure 3 presents the map of Piauí with the occurrence of faunal individuals of the genus *Dyphylla* recorded to date. The expansion of the taxon's geographical distribution is evident from records made in PNSC (Serra das Confusões National Park) in Gilbués-PI, in 2008 by Gregorin and colleagues, and in 2010 by Castilho and colleagues in the municipalities of São Miguel do Tapuio and Buriti dos Montes, in the northern part of the state of Piauí. The update on the quantity of *D. ecaudata* was conducted by our research team.

The Parna Serra das Confusões (PNSC) is located between coordinates 9° 27' to 9° 31' S and 43° 05' to 43° 56' W, encompassing the municipalities of Canto do Buriti, Tamboril do Piauí, Jurema, Alvorada do Gurguéia, Bom Jesus, Guaribas, and Cristino Castro. The PNSC has a legal area of 823,843.08 hectares (or 8,238.43 km^2), which is approximately equivalent to 763,000 soccer fields (Brazil, 2017).

The city of Picos – PI, characterized by typical Caatinga vegetation, is located 330 km from PNSC and 249 km from the Cerrado-Caatinga enclave that includes the municipalities of Pedro II - PI and Milton Brandão - PI, further north in the state, with a distance of 35 kmbetween them. Close to these two municipalities are São Miguel do Tapuio and Buriti dos Montes, located in an interbiome area, with a distance of 54 km between them.

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Source: Authors

Figure 3: Map showing the occurrence of *D. ecaudata* in the state of Piauí, delineated by municipality and biomes, spanning from 2008 to 2023

Given its geographical position, the Cerrado and Caatinga biomes dominate the state of Piauí, with a significant presence of a Cerrado-Caatinga enclave, identified as a green line on the map (Figure 3), which can be characterized as a typical transition zone according to the latest update by IBGE (2019). It is highlighted that the updates for *Diphylla ecaudata* recorded in this study were conducted in areas delimited by the following domains: Caatinga (Picos - PI) and the Cerrado-Caatinga enclave (Pedro II - PI, Milton Brandão - PI, São Miguel do Tapuio-PI, Buriti dos Montes-PI, and PNSC - PI) (IBGE, 2024).

Studying the vegetation of different biomes, especially in transition areas, leads to a classification of domains that is still subject to change. In this regard, considering the municipalities studied, all exhibit characteristics of Savanna-Woodland, with highly branchedbranching generally provided with thorns. However, Guaribas - PI and Milton Brandão - PI also have patches of Deciduous Mountain Seasonal Forest and Shrub Savanna-Woodland, respectively, which designate these two biomes as dominant in the state of Piauí. It is also noted that the focus of occurrences was in zones with a semi-arid tropical climate, with an average temperature above 18°C and a maximum of 39°C (Brazil, 2024).

Understanding the ecosystem types allows for a more informed analysis of the biology of this bat group. In this context, the Caatinga, confined to northeastern Brazil, is the least studied biome, with only about 2% of its entire territory protected by conservation units. Unsustainable use of its resources has led to extensive environmental alteration and degradation, resulting in rapid loss of species diversity, elimination of unique species, key ecological processes, and desertification (Leal; Tabarelli; Silva, 2003).

The hairy-legged vampire bat is often associated with tropical zones in its distribution, being classified as the most specialized among hematophagous bats in feeding behavior, both in natural environments and in captivity (Uieda, 1992; Uieda, 1994). Conversely, Ito, Bernard, and Torres (2016) highlight the flexibility in the feeding habits of these bats due to the scarcity of usual prey, with regular records of feeding on human blood based on molecular analysis of *D. ecaudata* feces.

The report of the AgV3 variant in *D. ecaudata* by Castilho et al. (2010) in northern Piauí adds important information to be considered by competent health authorities. It indicates evidence of rabies virus circulation in a group of animals that are poorly sampled in the region, within an area recognized for underreporting or even lack of information. This complicates the establishment of an epidemiological framework regarding rabies transmission cycles,

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particularly focusing on the sylvatic aerial cycle for this disease.

IV. Final Considerations

The alert has been given! The information presented in this study brings new information regarding the behavior of *D. ecaudata* bats preying on birds close to the ground, different from what was experimentally portrayed, when *D. ecaudata* was observed preferring to prey on birds perched in higher roosts. In addition, it shows that these bats can attack different prey, such as mammals, including humans in search of maintaining their blood-eating diet.

The data point to the need for systematic, continuous and cooperative surveillance between researchers and bodies responsible for health and environmental surveillance agencies in order to converge proposals for improving and optimizing monitoring for this animal group with the aim of maintaining harmony and rapid response in cases of positivity of pathogens disseminated by this faunal group.

We understand that only joint efforts can act effectively to understand the epidemiological cycles provided by bats and their harbored microbiota. And may this essay serve as a guiding focus for further research in similar ecotonal areas and other biomes where this species of bat occurs. Establishing a working network based on the research model proposed here can be a positive factor in uncovering knowledge gaps about the spatial distribution of this taxon and keeping us connected regarding its biology amid current and future climate changes.

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Although low-quality images are sufficient for review purposes, print publication requires high-quality images to prevent the final product being blurred or fuzzy. Submit (possibly by e-mail) EPS (line art) or TIFF (halftone/ photographs) files only. MS PowerPoint and Word Graphics are unsuitable for printed pictures. Avoid using pixel-oriented software. Scans (TIFF only) should have a resolution of at least 350 dpi (halftone) or 700 to 1100 dpi (line drawings). Please give the data for figures in black and white or submit a Color Work Agreement form. EPS files must be saved with fonts embedded (and with a TIFF preview, if possible).

For scanned images, the scanning resolution at final image size ought to be as follows to ensure good reproduction: line art: >650 dpi; halftones (including gel photographs): >350 dpi; figures containing both halftone and line images: >650 dpi.

Color charges: Authors are advised to pay the full cost for the reproduction of their color artwork. Hence, please note that if there is color artwork in your manuscript when it is accepted for publication, we would require you to complete and return a Color Work Agreement form before your paper can be published. Also, you can email your editor to remove the color fee after acceptance of the paper.

Tips for writing a good quality Medical Research Paper

1. Choosing the topic: In most cases, the topic is selected by the interests of the author, but it can also be suggested by the guides. You can have several topics, and then judge which you are most comfortable with. This may be done by asking several questions of yourself, like "Will I be able to carry out a search in this area? Will I find all necessary resources to accomplish the search? Will I be able to find all information in this field area?" If the answer to this type of question is "yes," then you ought to choose that topic. In most cases, you may have to conduct surveys and visit several places. Also, you might have to do a lot of work to find all the rises and falls of the various data on that subject. Sometimes, detailed information plays a vital role, instead of short information. Evaluators are human: The first thing to remember is that evaluators are also human beings. They are not only meant for rejecting a paper. They are here to evaluate your paper. So present your best aspect.

2. Think like evaluators: If you are in confusion or getting demotivated because your paper may not be accepted by the evaluators, then think, and try to evaluate your paper like an evaluator. Try to understand what an evaluator wants in your research paper, and you will automatically have your answer. Make blueprints of paper: The outline is the plan or framework that will help you to arrange your thoughts. It will make your paper logical. But remember that all points of your outline must be related to the topic you have chosen.

3. Ask your guides: If you are having any difficulty with your research, then do not hesitate to share your difficulty with your guide (if you have one). They will surely help you out and resolve your doubts. If you can't clarify what exactly you require for your work, then ask your supervisor to help you with an alternative. He or she might also provide you with a list of essential readings.

4. Use of computer is recommended: As you are doing research in the field of medical research then this point is quite obvious. Use right software: Always use good quality software packages. If you are not capable of judging good software, then you can lose the quality of your paper unknowingly. There are various programs available to help you which you can get through the internet.

5. Use the internet for help: An excellent start for your paper is using Google. It is a wondrous search engine, where you can have your doubts resolved. You may also read some answers for the frequent question of how to write your research paper or find a model research paper. You can download books from the internet. If you have all the required books, place importance on reading, selecting, and analyzing the specified information. Then sketch out your research paper. Use big pictures: You may use encyclopedias like Wikipedia to get pictures with the best resolution. At Global Journals, you should strictly follow here.



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6. Bookmarks are useful: When you read any book or magazine, you generally use bookmarks, right? It is a good habit which helps to not lose your continuity. You should always use bookmarks while searching on the internet also, which will make your search easier.

7. Revise what you wrote: When you write anything, always read it, summarize it, and then finalize it.

8. Make every effort: Make every effort to mention what you are going to write in your paper. That means always have a good start. Try to mention everything in the introduction—what is the need for a particular research paper. Polish your work with good writing skills and always give an evaluator what he wants. Make backups: When you are going to do any important thing like making a research paper, you should always have backup copies of it either on your computer or on paper. This protects you from losing any portion of your important data.

9. Produce good diagrams of your own: Always try to include good charts or diagrams in your paper to improve quality. Using several unnecessary diagrams will degrade the quality of your paper by creating a hodgepodge. So always try to include diagrams which were made by you to improve the readability of your paper. Use of direct quotes: When you do research relevant to literature, history, or current affairs, then use of quotes becomes essential, but if the study is relevant to science, use of quotes is not preferable.

10. Use proper verb tense: Use proper verb tenses in your paper. Use past tense to present those events that have happened. Use present tense to indicate events that are going on. Use future tense to indicate events that will happen in the future. Use of wrong tenses will confuse the evaluator. Avoid sentences that are incomplete.

11. Pick a good study spot: Always try to pick a spot for your research which is quiet. Not every spot is good for studying.

12. Know what you know: Always try to know what you know by making objectives, otherwise you will be confused and unable to achieve your target.

13. Use good grammar: Always use good grammar and words that will have a positive impact on the evaluator; use of good vocabulary does not mean using tough words which the evaluator has to find in a dictionary. Do not fragment sentences. Eliminate one-word sentences. Do not ever use a big word when a smaller one would suffice.

Verbs have to be in agreement with their subjects. In a research paper, do not start sentences with conjunctions or finish them with prepositions. When writing formally, it is advisable to never split an infinitive because someone will (wrongly) complain. Avoid clichés like a disease. Always shun irritating alliteration. Use language which is simple and straightforward. Put together a neat summary.

14. Arrangement of information: Each section of the main body should start with an opening sentence, and there should be a changeover at the end of the section. Give only valid and powerful arguments for your topic. You may also maintain your arguments with records.

15. Never start at the last minute: Always allow enough time for research work. Leaving everything to the last minute will degrade your paper and spoil your work.

16. Multitasking in research is not good: Doing several things at the same time is a bad habit in the case of research activity. Research is an area where everything has a particular time slot. Divide your research work into parts, and do a particular part in a particular time slot.

17. Never copy others' work: Never copy others' work and give it your name because if the evaluator has seen it anywhere, you will be in trouble. Take proper rest and food: No matter how many hours you spend on your research activity, if you are not taking care of your health, then all your efforts will have been in vain. For quality research, take proper rest and food.

18. Go to seminars: Attend seminars if the topic is relevant to your research area. Utilize all your resources.

19. Refresh your mind after intervals: Try to give your mind a rest by listening to soft music or sleeping in intervals. This will also improve your memory. Acquire colleagues: Always try to acquire colleagues. No matter how sharp you are, if you acquire colleagues, they can give you ideas which will be helpful to your research.

20. Think technically: Always think technically. If anything happens, search for its reasons, benefits, and demerits. Think and then print: When you go to print your paper, check that tables are not split, headings are not detached from their descriptions, and page sequence is maintained.

21. Adding unnecessary information: Do not add unnecessary information like "I have used MS Excel to draw graphs." Irrelevant and inappropriate material is superfluous. Foreign terminology and phrases are not apropos. One should never take a broad view. Analogy is like feathers on a snake. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Never oversimplify: When adding material to your research paper, never go for oversimplification; this will definitely irritate the evaluator. Be specific. Never use rhythmic redundancies. Contractions shouldn't be used in a research paper. Comparisons are as terrible as clichés. Give up ampersands, abbreviations, and so on. Remove commas that are not necessary. Parenthetical words should be between brackets or commas. Understatement is always the best way to put forward earth-shaking thoughts. Give a detailed literary review.

22. Report concluded results: Use concluded results. From raw data, filter the results, and then conclude your studies based on measurements and observations taken. An appropriate number of decimal places should be used. Parenthetical remarks are prohibited here. Proofread carefully at the final stage. At the end, give an outline to your arguments. Spot perspectives of further study of the subject. Justify your conclusion at the bottom sufficiently, which will probably include examples.

23. Upon conclusion: Once you have concluded your research, the next most important step is to present your findings. Presentation is extremely important as it is the definite medium though which your research is going to be in print for the rest of the crowd. Care should be taken to categorize your thoughts well and present them in a logical and neat manner. A good quality research paper format is essential because it serves to highlight your research paper and bring to light all necessary aspects of your research.

Informal Guidelines of Research Paper Writing

Key points to remember:

- Submit all work in its final form.
- Write your paper in the form which is presented in the guidelines using the template.
- Please note the criteria peer reviewers will use for grading the final paper.

Final points:

One purpose of organizing a research paper is to let people interpret your efforts selectively. The journal requires the following sections, submitted in the order listed, with each section starting on a new page:

The introduction: This will be compiled from reference matter and reflect the design processes or outline of basis that directed you to make a study. As you carry out the process of study, the method and process section will be constructed like that. The results segment will show related statistics in nearly sequential order and direct reviewers to similar intellectual paths throughout the data that you gathered to carry out your study.

The discussion section:

This will provide understanding of the data and projections as to the implications of the results. The use of good quality references throughout the paper will give the effort trustworthiness by representing an alertness to prior workings.

Writing a research paper is not an easy job, no matter how trouble-free the actual research or concept. Practice, excellent preparation, and controlled record-keeping are the only means to make straightforward progression.

General style:

Specific editorial column necessities for compliance of a manuscript will always take over from directions in these general guidelines.

To make a paper clear: Adhere to recommended page limits.



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Mistakes to avoid:

- Insertion of a title at the foot of a page with subsequent text on the next page.
- Separating a table, chart, or figure—confine each to a single page.
- Submitting a manuscript with pages out of sequence.
- In every section of your document, use standard writing style, including articles ("a" and "the").
- Keep paying attention to the topic of the paper.
- Use paragraphs to split each significant point (excluding the abstract).
- Align the primary line of each section.
- Present your points in sound order.
- Use present tense to report well-accepted matters.
- Use past tense to describe specific results.
- Do not use familiar wording; don't address the reviewer directly. Don't use slang or superlatives.
- Avoid use of extra pictures—include only those figures essential to presenting results.

Title page:

Choose a revealing title. It should be short and include the name(s) and address(es) of all authors. It should not have acronyms or abbreviations or exceed two printed lines.

Abstract: This summary should be two hundred words or less. It should clearly and briefly explain the key findings reported in the manuscript and must have precise statistics. It should not have acronyms or abbreviations. It should be logical in itself. Do not cite references at this point.

An abstract is a brief, distinct paragraph summary of finished work or work in development. In a minute or less, a reviewer can be taught the foundation behind the study, common approaches to the problem, relevant results, and significant conclusions or new questions.

Write your summary when your paper is completed because how can you write the summary of anything which is not yet written? Wealth of terminology is very essential in abstract. Use comprehensive sentences, and do not sacrifice readability for brevity; you can maintain it succinctly by phrasing sentences so that they provide more than a lone rationale. The author can at this moment go straight to shortening the outcome. Sum up the study with the subsequent elements in any summary. Try to limit the initial two items to no more than one line each.

Reason for writing the article—theory, overall issue, purpose.

- Fundamental goal.
- To-the-point depiction of the research.
- Consequences, including definite statistics—if the consequences are quantitative in nature, account for this; results of any numerical analysis should be reported. Significant conclusions or questions that emerge from the research.

Approach:

- Single section and succinct.
- An outline of the job done is always written in past tense.
- Concentrate on shortening results—limit background information to a verdict or two.
- Exact spelling, clarity of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else.

Introduction:

The introduction should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable of comprehending and calculating the purpose of your study without having to refer to other works. The basis for the study should be offered. Give the most important references, but avoid making a comprehensive appraisal of the topic. Describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will give no attention to your results. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here.

The following approach can create a valuable beginning:

- Explain the value (significance) of the study.
- Defend the model—why did you employ this particular system or method? What is its compensation? Remark upon its appropriateness from an abstract point of view as well as pointing out sensible reasons for using it.
- Present a justification. State your particular theory(-ies) or aim(s), and describe the logic that led you to choose them.
- Briefly explain the study's tentative purpose and how it meets the declared objectives.

Approach:

Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done. Sort out your thoughts; manufacture one key point for every section. If you make the four points listed above, you will need at least four paragraphs. Present surrounding information only when it is necessary to support a situation. The reviewer does not desire to read everything you know about a topic. Shape the theory specifically—do not take a broad view.

As always, give awareness to spelling, simplicity, and correctness of sentences and phrases.

Procedures (methods and materials):

This part is supposed to be the easiest to carve if you have good skills. A soundly written procedures segment allows a capable scientist to replicate your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order, but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt to give the least amount of information that would permit another capable scientist to replicate your outcome, but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section.

When a technique is used that has been well-described in another section, mention the specific item describing the way, but draw the basic principle while stating the situation. The purpose is to show all particular resources and broad procedures so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step-by-step report of the whole thing you did, nor is a methods section a set of orders.

Materials:

Materials may be reported in part of a section or else they may be recognized along with your measures.

Methods:

- Report the method and not the particulars of each process that engaged the same methodology.
- Describe the method entirely.
- To be succinct, present methods under headings dedicated to specific dealings or groups of measures.
- Simplify—detail how procedures were completed, not how they were performed on a particular day.
- If well-known procedures were used, account for the procedure by name, possibly with a reference, and that's all.

Approach:

It is embarrassing to use vigorous voice when documenting methods without using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result, when writing up the methods, most authors use third person passive voice.

Use standard style in this and every other part of the paper—avoid familiar lists, and use full sentences.

What to keep away from:

- Resources and methods are not a set of information.
- Skip all descriptive information and surroundings—save it for the argument.
- Leave out information that is immaterial to a third party.



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Results:

The principle of a results segment is to present and demonstrate your conclusion. Create this part as entirely objective details of the outcome, and save all understanding for the discussion.

The page length of this segment is set by the sum and types of data to be reported. Use statistics and tables, if suitable, to present consequences most efficiently.

You must clearly differentiate material which would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matters should not be submitted at all except if requested by the instructor.

Content:

- Sum up your conclusions in text and demonstrate them, if suitable, with figures and tables.
- In the manuscript, explain each of your consequences, and point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation of an exacting study.
- Explain results of control experiments and give remarks that are not accessible in a prescribed figure or table, if appropriate.
- Examine your data, then prepare the analyzed (transformed) data in the form of a figure (graph), table, or manuscript.

What to stay away from:

- Do not discuss or infer your outcome, report surrounding information, or try to explain anything.
- Do not include raw data or intermediate calculations in a research manuscript.
- Do not present similar data more than once.
- A manuscript should complement any figures or tables, not duplicate information.
- Never confuse figures with tables—there is a difference.

Approach:

As always, use past tense when you submit your results, and put the whole thing in a reasonable order.

Put figures and tables, appropriately numbered, in order at the end of the report.

If you desire, you may place your figures and tables properly within the text of your results section.

Figures and tables:

If you put figures and tables at the end of some details, make certain that they are visibly distinguished from any attached appendix materials, such as raw facts. Whatever the position, each table must be titled, numbered one after the other, and include a heading. All figures and tables must be divided from the text.

Discussion:

The discussion is expected to be the trickiest segment to write. A lot of papers submitted to the journal are discarded based on problems with the discussion. There is no rule for how long an argument should be.

Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implications of the study. The purpose here is to offer an understanding of your results and support all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of results should be fully described.

Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact, you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved the prospect, and let it drop at that. Make a decision as to whether each premise is supported or discarded or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."

Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work.

- You may propose future guidelines, such as how an experiment might be personalized to accomplish a new idea.
- Give details of all of your remarks as much as possible, focusing on mechanisms.
- Make a decision as to whether the tentative design sufficiently addressed the theory and whether or not it was correctly restricted. Try to present substitute explanations if they are sensible alternatives.
- One piece of research will not counter an overall question, so maintain the large picture in mind. Where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

When you refer to information, differentiate data generated by your own studies from other available information. Present work done by specific persons (including you) in past tense.

Describe generally acknowledged facts and main beliefs in present tense.

The Administration Rules

Administration Rules to Be Strictly Followed before Submitting Your Research Paper to Global Journals Inc.

Please read the following rules and regulations carefully before submitting your research paper to Global Journals Inc. to avoid rejection.

Segment draft and final research paper: You have to strictly follow the template of a research paper, failing which your paper may get rejected. You are expected to write each part of the paper wholly on your own. The peer reviewers need to identify your own perspective of the concepts in your own terms. Please do not extract straight from any other source, and do not rephrase someone else's analysis. Do not allow anyone else to proofread your manuscript.

Written material: You may discuss this with your guides and key sources. Do not copy anyone else's paper, even if this is only imitation, otherwise it will be rejected on the grounds of plagiarism, which is illegal. Various methods to avoid plagiarism are strictly applied by us to every paper, and, if found guilty, you may be blacklisted, which could affect your career adversely. To guard yourself and others from possible illegal use, please do not permit anyone to use or even read your paper and file.

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Please note that following table is only a Grading of "Paper Compilation" and not on "Performed/Stated Research" whose grading solely depends on Individual Assigned Peer Reviewer and Editorial Board Member. These can be available only on request and after decision of Paper. This report will be the property of Global Journals.

Topics	Grades		
	A-B	**C-D**	**E-F**
Abstract	Clear and concise with appropriate content, Correct format. 200 words or below	Unclear summary and no specific data, Incorrect form Above 200 words	No specific data with ambiguous information Above 250 words
Introduction	Containing all background details with clear goal and appropriate details, flow specification, no grammar and spelling mistake, well organized sentence and paragraph, reference cited	Unclear and confusing data, appropriate format, grammar and spelling errors with unorganized matter	Out of place depth and content, hazy format
Methods and Procedures	Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads	Difficult to comprehend with embarrassed text, too much explanation but completed	Incorrect and unorganized structure with hazy meaning
Result	Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake	Complete and embarrassed text, difficult to comprehend	Irregular format with wrong facts and figures
Discussion	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

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