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Research Article

Retrospective Analysis and Constraints in Epidemiological Monitoring of Canine Ehrlichiosis

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Abstract

Canine ehrlichiosis is a hemoparasitosis with significant impact on veterinary clinic and public health, caused by the obligate intracellular pathogen *Ehrlichia canis* and transmitted by the tick *Rhipicephalus sanguineus*. This study aimed to conduct a retrospective survey of cases with presumptive suspicion of ehrlichiosis treated at a private veterinary clinic in the municipality of Monte Alegre de Minas-MG, from January to December 2024, identifying the main risk factors, clinical signs, and laboratory abnormalities. The medical records of 197 dogs that presented suggestive signs and a history of exposure to the vector were analyzed. Of this total, 110 were males and 87 were females. Diagnosis was based on clinical evaluation – with main findings including apathy, vomiting, fever, anorexia, epistaxis and petechiae – and complete blood count. More than 90% of the animals evaluated presented leukocytosis, thrombocytopenia and anemia concomitantly. The therapeutic protocol established was based on the use of doxycycline associated with supportive therapy. The results revealed severe limitations in the surveillance and consolidation of local data**:** ethical commitments regarding the confidentiality of medical records and the absence of a unified database prevented the differentiation between new and old cases, making it impossible to determine broad population epidemiological indicators in the region. In addition, the risk of inaccurate diagnoses and false-positives resulting from the exclusive dependence of blood count in the local routine is discussed, given the lack of support from gold-standard tests, such as ELISA, CRP, or indirect immunofluorescence (IFA). It is concluded that the clinical suspicion of the disease is highly frequent in the city's routine, a scenario aggravated by the high density of stray dogs. There is an urgent need to raise awareness among owners, implement more specific diagnostic methods and structure an efficient veterinary data storage system to enable assertive prophylactic measures in the region.

Keywords: *Ehrlichia canis;* Retrospective Study; Thrombocytopenia; Laboratory Screening; Suspected Cases

Introduction

Hemoparasitosis is present throughout the Brazilian territory and is extremely important for veterinary medicine and public health in the country, as some have a zoonotic character. Among the main diseases transmitted by biological vectors in the routine of small animals, Canine Monocytic Ehrlichiosis (CME), Babesiosis and Hepatozoonosis stand out, caused by agents of the genera *Ehrlichia* sp., *Babesia* sp. and *Hepatozoon* sp. [4].

The prevailing tropical climate and the constant increase in temperature directly favor the biological development and proliferation of these parasites. Consequently, Brazil presents itself as a highly favorable geographical scenario for the laying and hatching of eggs of vectors, both of ehrlichiosis and canine babesiosis, with epidemiological studies reporting the possibility of reproduction and succession of generations of these ectoparasites up to four times during a period of one year. Additionally, in semi-arid regions, scientific evidence of a greater spread and endemicity of these pathologies is observed [2,35-37].

Contaminated ticks act as the main biological vectors responsible for the active transmission of several hemoparasitosis, and their variants present differences in relation to each specific infectious agent. Hematozoa and hemotropic bacteria act as obligate parasites inside the host's blood cells, triggering severe physiological dysfunctions and multisystem complications in the animals' bodies, such as hemolytic anemia, thrombocytopenia, renal failure and, in extreme clinical scenarios, can lead to the patient's death [3].

In Brazil, the first formal scientific description of ehrlichiosis occurred in 1973, in the city of Belo Horizonte, Minas Gerais, while babesiosis was reported as a pioneer much earlier, in 1918. In both diseases, pathogens are disseminated and inoculated through the blood meal of the tick *Rhipicephalus sanguineus* [12]. Ehrlichiosis is caused by a Gram-negative bacterium belonging to the Anaplasmataceae family, called *Ehrlichia canis*, which preferentially infects circulating leukocytes. Cell colonization triggers profound changes in the kinetics of platelet production and destruction, with thrombocytopenia being the most frequently reported hematological finding in affected dogs [27]. Such pathophysiological repercussions routinely manifest themselves in association with other peripheral cytopenias in the laboratory routine, such as anemia and leukopenia [6].

The clinical course of the disease is classically divided into three distinct phases: acute, subclinical, and chronic. The acute phase manifests itself soon after the post-inoculation incubation period, characterized by active proliferation of the agent in the mononucleated cells of crucial target organs such as lymph nodes, liver and spleen; Thus, infected cells circulate freely in the bloodstream and reach multiple organs, making the initial clinical signs vague and nonspecific. Then, the subclinical phase is established, in which there are usually no evident symptoms, although the animal presents antibody titers and subtle hematological changes. Finally, the chronic phase manifests predominantly in immunosuppressed animals, a stage in which symptoms can remain for long periods or take on complex aspects of autoimmune diseases, resembling the pathogenesis of lupus [34].

Routine diagnostic conduct, in the absence of confirmatory molecular or serological tests, should be based on the association between thorough clinical screening and the execution of complementary laboratory tests. Clinical diagnosis is based on the identification of the signs presented by the patient, while laboratory diagnosis is consolidated through blood counts, biochemical tests and urinalysis. In routine hematological examination, the marked presence of thrombocytopenia, normocytic and normochromic anemia, eosinopenia, lymphopenia, and nuclear deviation of neutrophils to the left becomes noticeable, and this set of hematological findings is considered the main presumptive and suggestive marker of *E. canis* infection [20].

Babesiosis, in turn, is caused by an intraerythrocyte protozoan called *Babesia canis*. By biting the vector, the parasite settles in the definitive host and gains the surrounding bloodstream, where it reproduces, invading and destroying the erythrocytes. Another form of transmission of great clinical importance, although less common in routine, occurs via blood transfusion [39]. According to the precepts of Nelson and Couto [31], the confirmatory diagnosis of babesiosis requires direct visual demonstration of the presence of protozoa inside the erythrocytes. Blood smears are the most commonly used and economically viable diagnostic methods in veterinary medical routine; in these cases, priority is given to peripheral blood collection, which is subsequently submitted to hematological stains based on the Romanowsky principle, such as Giemsa, Wright, Rosenfeld or Diff-Quick.

Another hemoparasitosis of worldwide distribution caused by the same brown tick is anaplasmosis. Canine thrombocytic anaplasmosis is a pathogenesis caused by a Gram-negative bacterium, which belongs taxonomically to the order Rickettsiales, family Anaplasmataceae and genus *Anaplasma* [5,17,19,23]. Clinical cases of this cyclic thrombocytopenia have increased exponentially globally, with new occurrences reported in countries on different continents, including the United States, Greece, Taiwan, Spain, Southern China, Australia, Thailand, and Venezuela. In the national scenario, anaplasmosis is also one of the main causes of thrombocytopenia in dogs in several Brazilian regions, and the occurrence of simultaneous co-infections with *E. canis* and *B. vogeli* has been described, since the tick *R. sanguineus* acts efficiently as the same biological vector for the three species portrayed [13]. This disease is pathophysiologically characterized by parasitemia of clinical thrombocytes, progressing clinically to thrombocytopenia and generalized lymphadenopathy [16]. Several serological tests are described in the literature for the detection of agents of the Anaplasmataceae family, highlighting the complement fixation technique, indirect hemagglutination, Indirect Immunofluorescence Assay (IFA), Enzyme-linked Immunosorbent Assay (ELISA), Dot-blot ELISA and Western blot [27].

Canine hepatozoonosis is also part of this complex of tick-borne pathologies. The protozoan that causes the disease belongs to the genus *Hepatozoon*, which has a specific biological tropism for neutrophils and monocytes of intermediate hosts [8]. According to Baneth [7], the parasite has a wide geographical distribution worldwide, with a higher prevalence of *H. canis* in regions of Africa, Asia, Southern Europe, and North and South America, while *H. americanum* is endemically concentrated in the United States. In contemporary times, both species of *Hepatozoon* cause distinct clinical changes in dogs. The first (*H. canis*) mainly affects the hemolymphoid organs, while *H. americanum* severely affects the musculoskeletal system [7,8,40]. The resulting clinical signs are not pathognomonic and vary according to the severity of the infection. According to Ivanov and Tsachev [25], the main symptoms noticed in the medical routine include anemia, edema and fever peaks intermittently.

Regarding prevention and control measures, the continuous prophylactic use of ticks plays a crucial role, as it interrupts the biological life cycle of the tick. Spraying should be carried out using long-lasting active ingredients both in the animals and in the environment [32]. Based on the need to understand the distribution of clinical care related to this diagnostic suspicion and the resulting impacts on the canine population cared for locally, it was essential to structure the present study.

The general objective of this study was to collect retrospective data on suspected cases of ehrlichiosis in the city of Monte Alegre de Minas, located in the state of Minas Gerais, in order to evaluate the frequency and distribution of this suspected diagnosis in the local clinical routine. It is, therefore, relevant to carry out this survey of cases in order to estimate the occurrence of clinical suspicions, the associated symptomatology and the problems resulting directly from the disease. This approach seeks to provide scientific support so that the veterinary community can act more effectively in clinical management, early diagnosis and preventive prophylaxis, aiming to mitigate the spread and increase of these infections, in addition to contributing to the improvement of the well-being and quality of life of animals.

Methodology

The present study is characterized as a retrospective, descriptive study with a quantitative approach, based on the collection of data from clinical and laboratory records. The research was carried out from the digital and physical archive of a private veterinary clinic located in the municipality of Monte Alegre de Minas, Triângulo Mineiro region, state of Minas Gerais. The period delimited for sampling comprised the services carried out between January 1 and December 31, 2024.

The study population was composed of dogs of different breeds, sexes and ages that were treated at the aforementioned institution with initial clinical suspicion of hemoparasitosis or a history of direct exposure to the vector *Rhipicephalus sanguineus*. The eligibility criteria for sample inclusion were the complete completion of the clinical records and the concomitant completion of the serial hematological profile at the time of the primary consultation. Medical records that were incomplete, erased, or that made it impossible to tabulate the variables of interest were excluded from the survey. At the end of the screening, a total sample of 197 dogs was obtained.

Data collection consisted of the systematic extraction of information from the medical records, mapping epidemiological and clinical variables, such as the sex of the animal (male or

female), the main complaints reported by the owners (apathy, vomiting, fever, anorexia) and presumptive clinical signs observed in the general physical examination (epistaxis and petechiae). In the laboratory setting, the absolute parameters of the complete blood count routinely processed in the clinic were collected, focusing on global leukocyte count (evaluation of leukocytosis or leukopenia), platelet count (investigation of thrombocytopenia) and measurement of cell volume/hematocrit (determination of anemia). The pathophysiological interpretation of such hematological alterations and the criteria for the classification of cytopenias were based on Kaneko's precepts of clinical biochemistry and hematology [26].

Because it was data from a private institution, the study followed strict ethical criteria of confidentiality. The absolute confidentiality of personal information and the identity of the tutors and animals was guaranteed, and the data was analyzed in an anonymized and aggregated way. Due to the absence of a unified return tracking system or confirmatory molecular biology and serology tests (such as PCR or ELISA) in the standard routine of the locality, the data were tabulated assuming the bias of presumptive diagnosis based on clinical-laboratory findings.

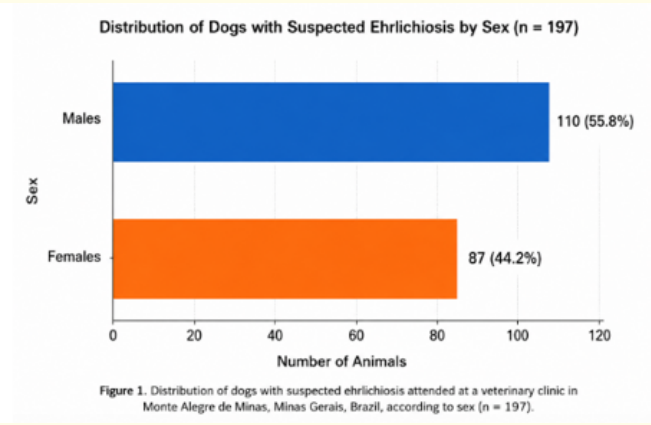
The raw data obtained were organized and tabulated in electronic spreadsheets. Statistical analysis was limited to descriptive epidemiology, using the calculation of absolute frequencies (n) and percentages (%) to characterize the profile of the sample treated at the clinic. In view of the structural limitations of storage and the impossibility of distinguishing chronic reconsultations from new diagnoses, the results were discussed from the perspective of a local frequency survey, emphasizing the methodological impossibility of calculating the real incidence or prevalence rates of ehrlichiosis for the entire municipality.

Results and Discussions

Currently, the genus in question encompasses five distinct species duly validated: *E. canis*, *E. chaffeensis*, *E. ewingii*, *E. muris* and In the national scenario, however, the documented isolations in canines are restricted only to *E. canis* [28]. Among the varieties described, a group of three species has the ability to infect humans: *E. chaffeensis*, *E. canis* and *e. ewingii*. This zoonotic potential linked to *E. canis*, associated with the scarce clinical records reported in human medicine, raises an alert in the sphere of public health, especially due to the close coexistence of dogs with the population and with the biological vector [9].

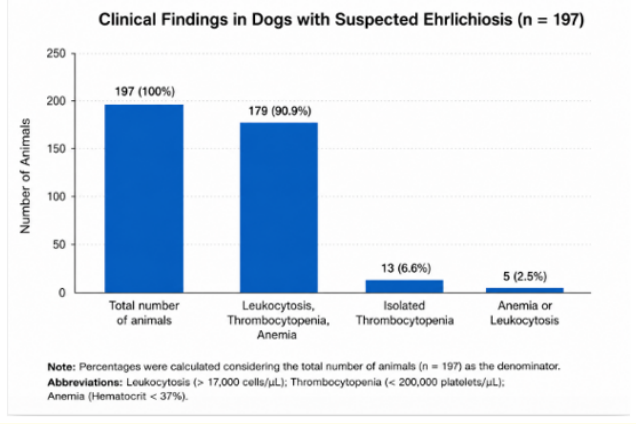
The occurrence of infections in dogs by *E. canis* is largely linked to the geographic distribution of the vector, which is the tick *Rhipicephalus sanguineus*. This tick is found predominantly in tropical and temperate areas. It is characterized by being monotropic, that is, it feeds on the same species in all its stages of development and requires three hosts, since its biological cycle depends on a different host for each stage of the blood meal. In addition, there may be accidental hosts, such as humans, that are not inserted in the natural chain of the tick. This demonstrates that *R. sanguineus* is a cosmopolitan tick, capable of adopting several strategies to ensure its survival and the maintenance of the species when necessary [30].

According to Foley [21], transmission occurs exclusively horizontally, that is, the tick acquires the infection and passes it on to the next hosts after the change of its biological stage, passing from larva to nymph and, later, to the adult stage. There is no vertical transmission. The female tick does not contaminate her eggs with *E. canis*, as this bacterium does not replicate in the ovaries of engorged females. The main risk factor for E*. canis infection* in Brazil is the direct contact of dogs with *R. sanguineus* [1,10,11,14]. The result of the present retrospective survey showed a total of 197 dogs with presumptive suspicion of canine ehrlichiosis, screened based on hematological alterations and compatible clinical manifestations**.** Of these animals, 110 were males and 87 females, of various ages and different breeds, represented as shown in graph 1.



Graph 1: Sample profile according to the sex of the canines evaluated in the retrospective survey.

After the detailed physical examination, all dogs in the research underwent hematological analysis, used as a complementary hematological evaluation resource. In the evaluation of blood counts, the most prevalent clinical abnormalities were thrombocytopenia, anemia and leukocytosis, following the pattern shown in Graph 2.



Graph 2: Prevalence of leukocytosis, thrombocytopenia and anemia in dogs with suspected ehrlichiosis.

Graph 2 shows that more than 90% of the sampled population showed simultaneous signs of leukocytosis, thrombocytopenia, and anemia in the blood count. Corroborating the findings described by Brandão., *et al*. thrombocytopenia stands out as the most common hematological finding in routine diagnostic screening [27]. Through the information obtained, it was also possible to observe that the hematological alterations are consistent with Balwani's descriptions, which lists anemia, thrombocytopenia and variations in white blood cell count as the main markers in affected animals [6]. The diagnoses were also based on clinical signs presented by the animals, the main signs reported were listed in table 1.

Key Clinical Signs Observed in Dogs with Suspected Ehrlichiosis	
Clinical Sign	**Description**
Apathy	Reduction or absence of normal activity; animal appears quiet, disinterested or unresponsive to stimuli.
Vomiting	Forceful expulsion of stomach contents through the mouth.
Fever	Elevated body temperature above the normal reference range (> 39.2 °C).
Anorexia	Partial or complete loss of appetite or refusal to eat.
Epistaxis	Nasal bleeding caused by rupture of blood vessels in the nasal mucosa.

Table 1: Clinical manifestations and symptoms reported in the screening of the animals evaluated.

In view of the absence of pathognomonic clinical manifestations and the adoption of the blood count as a basic screening tool in the evaluated sample, it is imperative to consider the broad spectrum of differential diagnoses**.** In view of the absence of confirmatory molecular or serological tests in the routine of the clinical center evaluated, as well as the lack of longitudinal follow-up of the subsequent clinical development of the patients, the limitation in establishing the prevalence or actual epidemiological incidence of the infection in the municipality is highlighted**.** The data obtained strictly reflect the frequency of care for suspected cases in the private sample studied**.**

The therapeutic protocol was based on the use of doxycycline (5mg/kg PO every 12 hours or 10mg/kg PO every 24 hours for at least 28 days). The treatment of choice established in the literature for canine ehrlichiosis involves the use of antimicrobials associated with supportive therapy. This complementary approach includes the reestablishment of hydroelectrolyte and energy balance, the administration of corticosteroids, blood transfusions when indicated, as well as immunomodulators and stimulants of hematopoiesis [30]. The evaluation of the therapeutic response was carried out by monitoring the remission of clinical signs, characterized by the return of appetite, behavioral reestablishment and progressive improvement of the hematological condition [24].

The prognosis tends to be favorable for dogs that manifest the clinical picture compatible with the acute phase and variable to reserved for those with suspected chronic evolution. Clinical manifestations such as fever, petechiae, vomiting, diarrhea, epistaxis and thrombocytopenia constantly resolve within a few days after the start of early therapy [3].

The present study focused on a descriptive survey focused on the frequency of suspected cases seen in the clinical routine. The data collected were tabulated in absolute values, with no chronological distinction between acute or relapsed infections. The available information was restricted to gender variables and the total number of individuals with clinical-laboratory suspicion, in order to safeguard the privacy and confidentiality of the patients' medical records in accordance with local ethical precepts. In view of these technical and sampling limitations, it was not feasible to measure incidence rates or infer comprehensive population epidemiological data.

In view of these circumstances, the relevance of standardization and adequate storage of clinical data is understood, since the

scarcity of detailed records limits a more assertive analysis of the true distribution of hemoparasitosis. Another relevant factor in the propagation dynamics is the population density of stray dogs, which, by remaining exposed to the vector without prophylactic control, act significantly in the maintenance and dissemination of the pathogen in the environment.

Considering that many veterinary centers regionally use the hematological profile as the main tool for presumptive screening, there are challenges in differentiating with other pathologies that course with similar cytopenias, which reinforces the importance of interpreting the data from this survey as estimates of clinical suspicion in the locality of Monte Alegre de Minas**.**

Thus, the importance of enabling diagnostic tests with greater accuracy in the local veterinary clinical routine becomes notorious. Enzyme-linked immunosorbent assays (ELISA), Polymerase Chain Reaction (PCR) and Indirect Immunofluorescence Assay (IFA) stand out in the literature for conferring high sensitivity and specificity for the detection of *Ehrlichia* spp. at different stages of infection [22]. Direct hematological diagnosis, performed by means of microscopy of peripheral blood smears stained by Giemsa, also remains useful in the routine due to the possibility of direct visualization of bacterial morulas inside leukocytes [22,34].

Therefore, the need for the development of new scientific studies aimed at the molecular and serological characterization of *Ehrlichia* spp. in the region of Monte Alegre de Minas is emphasized. Such data will allow the consolidation of more efficient prophylactic measures, assisting in assertive clinical management, prevention and correct understanding of the real impacts resulting from the disease in the municipality, aiming at the well-being and quality of life of the canine population.

Conclusion

A retrospective study in Monte Alegre de Minas outlined the profile of dogs with suspected ehrlichiosis, evidencing diagnostic limitations due to the absence of confirmatory methods (PCR/ELISA) and failures in data storage. The dependence on the blood count, with its clinical and hematological non-specificity, generates a risk of inaccurate diagnoses and false positives in the clinical routine. It is concluded that there is a need to adopt confirmatory methods and structure medical records, recommending future studies with laboratory validation for a more robust epidemiological panorama.

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