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Comparative evaluation of four drugs on hematology and recovery time of canine monocytic Ehrlichiosis

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Abstract

Canine monocytic ehrlichiosis causes acute, sub-clinical, and chronic clinical complications with changes in hematological parameters: hemoglobin, hematocrit, and white blood cell count; doxycycline is suitable for treatment. The objective was to evaluate the effect of four drugs (doxycycline, minocycline, rifampicin, and imidocarb) on hematological parameters and the recovery of dogs with canine monocytic ehrlichiosis. Twenty-nine dogs were randomly distributed into 4 groups: minocycline at 10 mg/kg PO every 12 hours (G1), rifampicin at 10 mg/kg PO every 24 hours (G2), doxycycline at 10 mg/kg PO every 24 hours (G3), and imidocarb dipropionate at 6.6 mg/kg IM with 2-week intervals (G4). The effect of the drugs on hematological parameters and recovery on days 7, 14, 21, and 28 were tested by analysis of variance; means ± SD were compared with the Tukey test ($\alpha = 0.05$). The treatment of canine monocytic ehrlichiosis with three antibiotics (doxycycline, minocycline, and rifampicin) was more effective compared to imidocarb due to the rapid increase in platelets, leukocytes, and lymphocytes between 14 and 28 days. Based on hematological results, this study concludes that doxycycline, minocycline (semi-synthetic tetracyclines), and rifampicin were more suitable than imidocarb dipropionate for the treatment of canine monocytic ehrlichiosis. Therefore, in clinical practice, the administration of doxycycline and minocycline is recommended first, although rifampicin can be used in canine monocytic ehrlichiosis therapy as an optional alternative.

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Introduction

The intracellular gram-negative bacteria *Ehrlichia canis* is the primary etiological agent of canine monocytic ehrlichiosis (CME) (1,2). CME is potentially fatal in dogs and requires rapid and accurate diagnosis to initiate adequate therapy that leads to a favorable prognosis (3,4). CME is an endemic disease distributed globally in tropical and subtropical regions (5-8), on all continents except Australia (8). Brown hard ticks such as *Rhipicephalus sanguineus* transmit *E. canis* by biting. Under experimental conditions with blood transfusion or tick inoculation, it has an incubation period of 8 to 20 days. In the acute phase, the clinical signs show 2 to 4 weeks; the subclinical phase shows 40 to 120 days or years; and in the chronic phase, the classic clinical signs are manifested (4,5). Making a distinction between these phases is challenging in the clinical setting (3). Ehrlichiosis is a disease associated with leukopenia, thrombocytopenia, and anemia (9). Fever, depression, anorexia, generalized lymphadenopathy, splenomegaly, pale mucous membranes, bleeding tendency, and uveitis are typical clinical signs of spontaneous CME. Severely affected dogs may be persistently infested with ticks (3). In chronic phases, ulcerative stomatitis, necrotizing glossitis, swelling of the hind limbs or scrotum, and nervous symptoms such as seizures, ataxia, vestibular disorders, and neck pain are more common. Hemorrhagic diathesis can occur in both the acute and chronic phases of CME, with the chronic phase being

more frequent and severe, with petechiae on the skin and mucous membranes, hematuria, melena, and prolonged bleeding (3,8). The predominant hematological abnormalities are thrombocytopenia, anemia (mainly normocytic normochromic), and lymphopenia (10). Dogs with acute infections usually recover spontaneously, depending on their immune status, but in this phase, specific treatments are required to facilitate recovery and avoid clinical conditions or death. In CME's acute or subclinical phases, dogs with normal immune systems can clear the infection or reduce the bacterial load in tissues to levels inadequate for molecular detection by PCR amplification (11,12). However, sometimes, myelosuppression can develop without any warning symptoms, indicating an acute and subclinical stage (13). Furthermore, a percentage of dogs infected with *E. canis* may progress to a chronic phase characterized by severe bone marrow hypoplasia, severe peripheral pancytopenia, sepsis, and severe bleeding that predispose to death (13-15). In some areas where *E. canis* is found, such as Greece, Palestine, and Brazil, and occasionally in other regions, such as Turkey and Southeast Asia, CME may be the main cause of life-threatening pancytopenia in dogs (15-17). The seroprevalence of *E. canis* varies depending on the country; for example, in Paraguay, it is 10.41% (18). In Brazil, prevalence ranges between 22 and 76% (19,20). In regions of Peru, the prevalence of *E. canis* infection reaches between 22.6 and 59.4% (21-23).

Despite this, knowledge about the effectiveness of treatment against *E. canis* in dogs from Quillabamba, Cusco region is limited. The objective of the present study was to evaluate the effect of four drugs (doxycycline, minocycline, rifampicin, and imidocarb dipropionate) on hematological parameters and their recovery against CME during 28 days of follow-up.

Materials and methods

Ethical approve

All procedures were approved by the Ethics Committee of the National University of San Antonio Abad del Cusco (CBI–UNSACC) modified by - No. 079-2021-CU-UNSAAC, following Peruvian National Law No. 30407 (Animal Protection and Welfare Law).

Location, animals, and serological test

The study was conducted between August and November 2022 in Quillabamba City, Cusco region, Peru, located 1050 meters above sea level, characterized by a tropical climate with temperatures between 21 and 30.74°C. With a population of 3692, a random sample of 115 dogs with the typical clinical signs of fever, bleeding, pale gums, inflammation of superficial lymph nodes, petechiae, and uveitis was determined. They were naturally infected cross-breed pets, born in Quillabamba city, included of all ages as classified (24) in 9 puppies (0-6 months), 9 juveniles (6 months to 1 year), 71 young adults (1-2 years), 12 mature adults (2-6 years), 9 seniors (7-11 years), and 5 geriatrics (12 years or older). Twenty-nine (13 males and 16 females, 4 puppies, 2 juveniles, 17 young adults, 1 mature adult, 4 seniors, and 1 geriatric) of the 115 dogs tested positive for the rapid serological test (*Ehrlichia canis* Antibody Rapid Test Kit, S & C Biotech, China). The *E.canis* Ab Rapid test is based on sandwich lateral flow immunochromatographic assay. The components in this kit have been quality control tested. The procedure was applied following the guidelines of their respective manufacturers. Blood plasma was obtained by centrifugation (3000 rpm for at least 10 min at room temperature) in a centrifuge model 800D (Greetmed®, USA). Using the capillary dropper, place 10μL of the prepared specimen into the sample hole "S" of the test device. Then, 2 drops (approx. 80μL) of the assay buffer were immediately poured into the sample hole. Results were interpreted between 5 and 10 min.

Treatment design and hematology analysis

The seropositive dogs were randomly distributed into 4 groups. Each group was administered the drug, with informed consent given by the owners. The criteria used for selecting the specific dosages at 10 mg/kg PO for doxycycline (first-line treatment), minocycline, and rifampicin (second-line treatments) are supported by the current scientific literature (2,3,5,12) and for imidocarb dipropionate at 6.6 mg/kg, IM, 2 injections given 2 weeks apart (1,11). G1: minocycline at 10 mg/kg PO (Minocycline, Genfar, Colombia) every 12 h for 28 days, G2: rifampicin at 10 mg/kg PO (Rifampicin, Laboratorios Britania, Dominican Republic) every 24 h for 28 days, G3: doxycycline at 10 mg/kg PO (Doxitel 100, Veterline®, Peru) every 24 h for 28 days and G4: imidocarb dipropionate at 6.6 mg/kg IM (Imidox® 120, Agrovetmarket, Peru) with 2-weeks intervals for 28 days. Whole blood samples with EDTA (0.5 mL) from the dogs were sent for laboratory analysis (hematocrit, hemoglobin, and leukocyte, segmented neutrophil, lymphocyte, monocyte, eosinophil, and platelet counts) every 7 days, starting one week before the first treatment until day 28 post-treatment. Hematology was performed at Tecnolab Clinical Laboratory (Quillabamba, Peru) in an automated device 5-Part-Diff Auto Hematology Analyzer, model BH-5100 (URIT Medical Electronic Co., Ltd., China), following the procedures according to the instruction manual, under laboratory standard conditions of temperature and relative humidity, between 10 and 30°C, ≤ 85% RH, respectively. In addition, reference intervals (25,26) were used: 6.5-19 x 10^9/L (leukocytes), 3-11.5 x 10^9/L (segmented neutrophils), 1.2-5.2 x 10^9/L (lymphocytes), 0.2-1.3 x 10^9/L (monocytes), 0-1.2 x 10^9/L (eosinophils), 12.5-19 g/dL (hemoglobin), 35-54 % (hematocrit), 150-400 x 10^9/L (platelet count).

Statistical analysis

The effect of each drug (doxycycline, minocycline, rifampicin, and imidocarb dipropionate) and the follow-up time (7, 14, 21, and 28 days) on the hematological parameters were analyzed separately by one-way analysis of variance (without assuming equal variances). For multiple comparisons of the mean ± SD, the Tukey test ($\alpha = 0.05$) was used. Analyzes were performed using the software R version 4.2.3 (27).

Results

Pharmacological treatment with four drugs on the hematology and recovery time of canine monocytic ehrlichiosis

The point prevalence of CME was 25.2%. A greater proportion of young adult dogs were affected 15%, but there was no distinction between males 11.3% and females 13.9%. Fever, epistaxis, bleeding and/or pale gums, swollen superficial lymph nodes, petechiae in the abdominal region, scrotal edema in males, and uveitis were the clinical signs compatible with CME, regardless of the phase of the disease and the hematological abnormalities.

Tables 1, 2, 3, 4 and 5 showed low hematocrit and hemoglobin values (anemia) due to *E. canis* infections. Hematological abnormalities were evident on days 0 and 7 for such conditions as leukopenia, lymphocytopenia, monocytopenia, thrombocytopenia, and a significant increase in polymorphonuclear cells such as neutrophilic leukocytosis and eosinophilic leukocytosis. In this context, pharmacological treatment with doxycycline, minocycline, rifampicin, and imidocarb dipropionate allowed an increase in hemoglobin and hematocrit, platelets, leukocytes, and lymphocytes, at the same time, decreased segmented neutrophils and eosinophils (Figures 1-6).

Table 1: Effect of doxycycline on changes in hematological parameters during the study follow-up period

Hematological parameters	Day 00	Day 07	Day 14	Day 21	Day 28
Hemoglobin (g/dL)	6.03 ± 1.53e	10.3 ± 1.58d	13.2 ± 1.14c	15.4 ± 0.82b	18.1 ± 0.40a
Hematocrit (%)	16.6 ± 3.64b	27.3 ± 3.25b	36.6 ± 1.99ab	43.2 ± 2.48ab	70.0 ± 56.6a
Platelet count ($x10^9$/L)	106 ± 19.2d	148 ± 10.5c	176 ± 22.5c	248 ± 37.6b	335 ± 21.9a
Leukocytes ($x10^9$/L)	2.83 ± 1.00e	6.00 ± 0.75d	9.31 ± 1.59c	13.5 ± 1.42b	17.5 ± 0.91a
Segmented neutrophils ($x10^9$/L)	14.8 ± 1.00a	11.6 ± 0.52b	9.93 ± 0.69c	7.27 ± 0.94d	4.67 ± 0.93e
Lymphocytes ($x10^9$/L)	0.13 ± 0.35d	1.05 ± 0.61c	1.73 ± 0.60c	2.63 ± 0.47b	3.79 ± 0.44a
Monocytes ($x10^9$/L)	0.00 ± 0.00d	0.31 ± 0.16c	0.79 ± 0.14b	1.03 ± 0.07a	1.13 ± 0.14a
Eosinophils ($x10^9$/L)	2.35 ± 0.51a	1.83 ± 0.15ab	1.34 ± 0.22bc	0.99 ± 0.42c	0.25 ± 0.46d

Different letters in the same row indicate mean differences (Tukey, $\alpha = 0.05$).

Table 2: Effect of imidocarb dipropionate on changes in hematological parameters during the study follow-up period

Hematological parameters	Day 00	Day 07	Day 14	Day 21	Day 28
Hemoglobin (g/dL)	9.07 ± 1.62e	6.71 ± 0.03d	13.3 ± 0.82c	15.2 ± 0.16b	17.0 ± 1.36a
Hematocrit (%)	25.0 ± 3.51c	36.9 ± 0.18b	37.7 ± 1.90b	43.2 ± 0.25a	43.6 ± 1.42a
Platelet count ($x10^9$/L)	132 ± 16.4c	131 ± 1.83c	186 ± 15.9b	188 ± 0.14b	306 ± 11.7a
Leukocytes ($x10^9$/L)	3.77 ± 1.55d	3.81 ± 0.04d	8.07 ± 1.12c	9.81 ± 0.15b	14.3 ± 0.80a
Segmented neutrophils ($x10^9$/L)	14.0 ± 0.87a	13.0 ± 0.03b	9.02 ± 0.34c	6.73 ± 0.12d	4.48 ± 0.48e
Lymphocytes ($x10^9$/L)	0.00 ± 0.00e	1.58 ± 0.03d	3.01 ± 0.43c	3.76 ± 0.03b	4.26 ± 0.17a
Monocytes ($x10^9$/L)	0.00 ± 0.00d	1.11 ± 0.02a	0.79 ± 0.14b	0.43 ± 0.02c	1.22 ± 0.02a
Eosinophils ($x10^9$/L)	2.13 ± 0.48b	2.72 ± 0.02a	0.91 ± 0.41c	0.14 ± 0.01d	0.14 ± 0.38d

Different letters in the same row indicate mean differences (Tukey, $\alpha = 0.05$).

Table 3: Effect of minocycline on changes in hematological parameters during the study follow-up period

Hematological parameters	Day 00	Day 07	Day 14	Day 21	Day 28
Hemoglobin (g/dL)	6.93 ± 1.21e	12.0 ± 0.58d	14.3 ± 0.69c	16.2 ± 0.81b	17.9 ± 0.94a
Hematocrit (%)	19.1 ± 3.18d	30.1 ± 3.67c	38.2 ± 2.87b	44.0 ± 3.55a	48.4 ± 3.00a
Platelet count ($x10^9$/L)	109 ± 10.2e	158 ± 7.59d	236 ± 14.6c	289 ± 27.1b	342 ± 14.7a
Leukocytes ($x10^9$/L)	3.90 ± 0.96e	6.90 ± 1.12d	10.6 ± 1.50c	14.5 ± 1.08b	16.7 ± 0.87a
Segmented neutrophils ($x10^9$/L)	14.4 ± 0.80a	11.3 ± 0.34b	8.95 ± 0.43c	6.51 ± 1.12d	4.28 ± 0.24e
Lymphocytes ($x10^9$/L)	0.00 ± 0.00e	1.30 ± 0.24d	2.45 ± 0.38c	3.22 ± 0.33b	4.15 ± 0.28a
Monocytes ($x10^9$/L)	0.36 ± 0.94a	0.57 ± 0.66a	0.84 ± 0.29a	0.89 ± 0.40a	0.99 ± 0.44a
Eosinophils ($x10^9$/L)	2.21 ± 1.19a	1.89 ± 1.05a	1.06 ± 0.42ab	0.36 ± 0.52b	0.14 ± 0.38b

Different letters in the same row indicate mean differences (Tukey, $\alpha = 0.05$).

Table 4: Effect of rifampicin on changes in hematological parameters during the study follow-up period.

Hematological parameters	Day 00	Day 07	Day 14	Day 21	Day 28
Hemoglobin (g/dL)	6.54 ± 0.57e	11.1 ± 0.35d	13.5 ± 0.43c	15.2 ± 0.48b	17.7 ± 0.46a
Hematocrit (%)	20.4 ± 0.83e	29.1 ± 2.04d	36.7 ± 1.47c	41.9 ± 1.15b	45.9 ± 2.23a
Platelet count (x10^9/L)	100 ± 3.27e	148 ± 2.23d	170 ± 7.98c	257 ± 8.97b	325 ± 13.7a
Leukocytes (x10^9/L)	3.26 ± 0.43e	5.40 ± 0.38d	7.93 ± 0.44c	12.4 ± 0.73b	16.7 ± 0.91a
Segmented neutrophils (x10^9/L)	14.2 ± 0.90a	11.3 ± 0.33b	8.68 ± 0.33c	6.30 ± 0.86d	4.42 ± 0.38e
Lymphocytes (x10^9/L)	0.00 ± 0.00e	2.00 ± 0.62d	2.89 ± 0.41c	3.56 ± 0.30b	4.39 ± 0.32a
Monocytes (x10^9/L)	0.00 ± 0.00d	0.41 ± 0.13c	0.81 ± 0.11b	1.06 ± 0.10a	1.14 ± 0.10a
Eosinophils (x10^9/L)	2.17 ± 0.11a	1.80 ± 0.38a	1.14 ± 0.30b	0.17 ± 0.45c	0.00 ± 0.00c

Different letters in the same row indicate mean differences (Tukey, α = 0.05).

Table 5: Effect of pharmacological treatment against canine monocytic ehrlichiosis on changes in hematological parameters at 14 and 28 days of follow-up.

Follow-up on day 14					
Hematological parameters	Doxycycline (n=8)	Imidocarb (n=7)	Minocycline (n=7)	Rifampicin (n=7)	Pr(>F)
Hemoglobin (g/dL)	13.2 ± 1.14	13.3 ± 0.82	14.3 ± 0.69	13.5 ± 0.43	0.093
Hematocrit (%)	36.6 ± 1.99	37.7 ± 1.89	38.2 ± 2.87	36.7 ± 1.47	0.391
Platelet count (x10^9/L)	176 ± 22.5b	186 ± 15.9b	236 ± 14.6a	170 ± 7.98b	<0.001
Leukocytes (x10^9/L)	9.31 ± 1.59ab	8.07 ± 1.12b	10.6 ± 1.50a	7.93 ± 0.44b	0.002
neutrophils (x10^9/L)	9.93 ± 0.69a	9.02 ± 0.34b	8.95 ± 0.43b	8.68 ± 0.33b	<0.001
Lymphocytes (x10^9/L)	1.73 ± 0.60b	3.01 ± 0.43a	2.45 ± 0.38a	2.89 ± 0.41a	<0.001
Monocytes (x10^9/L)	0.79 ± 0.14	0.79 ± 0.14	0.84 ± 0.29	0.81 ± 0.11	0.925
Eosinophils (x10^9/L)	1.34 ± 0.22	0.91 ± 0.41	1.06 ± 0.42	1.14 ± 0.30	0.142
Follow-up on day 28					
Hemoglobin (g/dL)	18.1 ± 0.40	17.0 ± 1.36	17.9 ± 0.94	17.7 ± 0.46	0.134
Hematocrit (%)	70.0 ± 56.6	43.6 ± 1.42	48.4 ± 3.00	45.9 ± 2.23	0.306
Platelet count (x10^9/L)	335 ± 21.9a	306 ± 11.7b	342 ± 14.7a	325 ± 13.7ab	0.002
Leukocytes (x10^9/L)	17.5 ± 0.91a	14.3 ± 0.80b	16.7 ± 0.87a	16.7 ± 0.91a	<0.001
neutrophils (x10^9/L)	4.67 ± 0.93	4.48 ± 0.48	4.28 ± 0.24	4.42 ± 0.38	0.640
Lymphocytes (x10^9/L)	3.79 ± 0.44a	4.26 ± 0.17b	4.15 ± 0.28ab	4.39 ± 0.32b	0.008
Monocytes (x10^9/L)	1.13 ± 0.14	1.22 ± 0.02	0.99 ± 0.44	1.14 ± 0.10	0.335
Eosinophils (x10^9/L)	0.25 ± 0.46	0.14 ± 0.38	0.14 ± 0.38	0.00 ± 0.00	0.617

Different letters in the same row indicate mean differences (Tukey, α = 0.05).

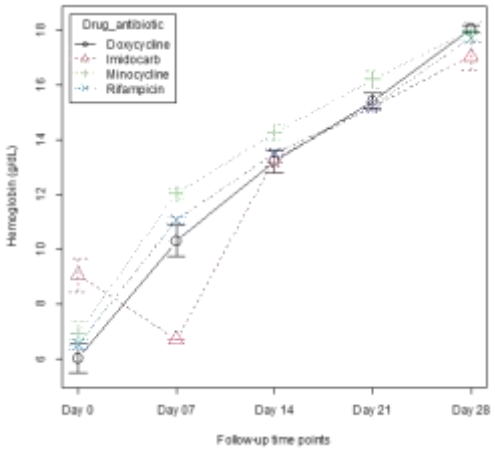

Figure 1: Mean and standard error for the effect of drugs on changes in hemoglobin during the follow-up period.

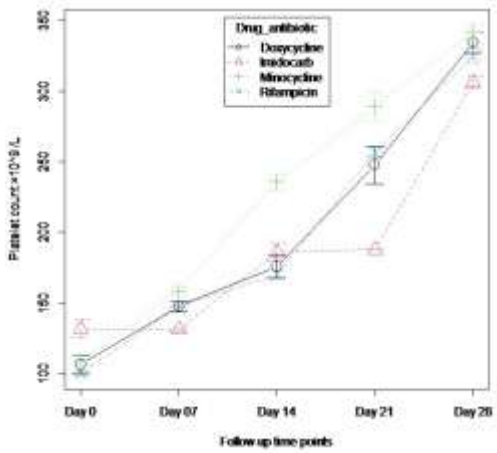

Figure 2: Mean and standard error for the effect of drugs on changes in platelet count during the follow-up period.

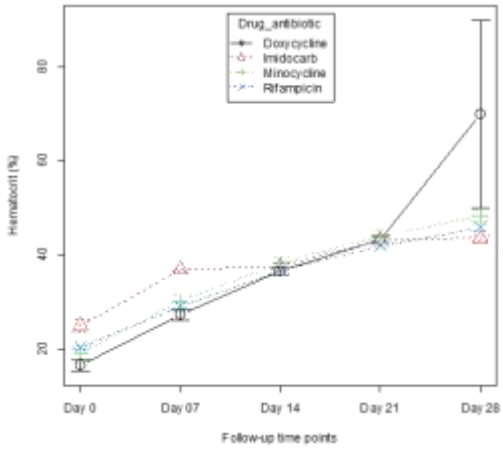

Figure 3: Mean and standard error for the effect of drugs on changes in hematocrit during the follow-up period.

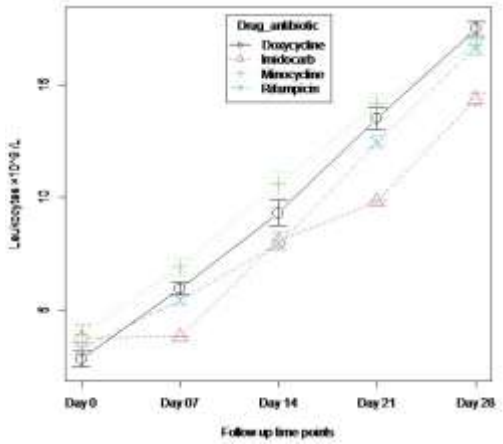

Figure 4: Mean and standard error for the effect of drugs on changes in leukocyte count during the follow-up period.

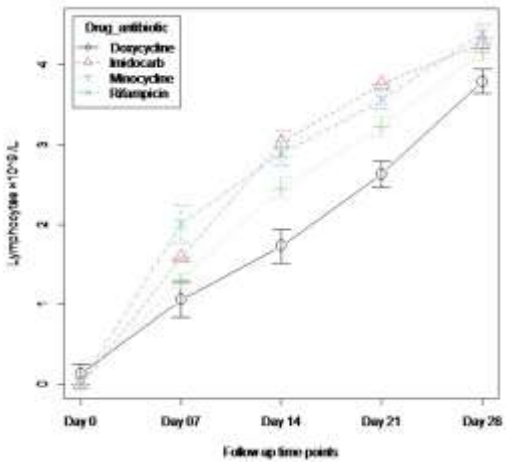

Figure 5: Mean and standard error for the effect of drugs on changes in lymphocyte count during the follow-up period.

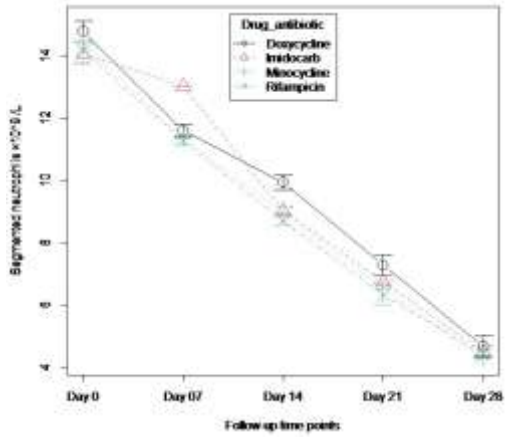

Figure 6: Mean and standard error for the effect of drugs on changes in segmented neutrophil count during the follow-up period.

Discussion

The point prevalence 25.2% observed in this study was similar to the 22.6% (21) in Chiclayo, Peru. Our result indicated that sex did not influence the point prevalence of CME. However, the disease was common in young adult dogs. Likewise, a similar pattern was reported in dogs from Huánuco, Peru (22). Dogs of this age or older, compared to puppies, would be more exposed to ticks because the owners take them out for walks in parks.

Conversely, age, race, and season are key risk factors associated with (21). The disease occurs in almost equal proportions of dogs of all ages and sexes (10). Most cases of CME occur during the warmest season in the northern hemisphere (May - September), corresponding to the greatest seasonal activity of the brown dog tick, consistent with our study carried out between August and September in cross-breed dogs. Furthermore, cross-breed dogs were more resistant to CME, so the differences in susceptibility can be attributed to the differential capacity of cellular and/or humoral immune responses (5,10).

In our study, canines with *E. canis* infection had the most obvious clinical signs of fever, bleeding, pale gums, inflammation of superficial lymph nodes, petechiae in the abdominal region, scrotal edema in males, and uveitis. These signs are compatible with the clinical signs described by other authors (4,5,9,28), who noted fever, lethargy, loss of appetite, weight loss, mucosal hemorrhages, uveitis, paleness, and edema. Sometimes, in chronic cases, dogs present cachexia and neurological signs (8). The bleeding tendency due to *E. canis* may be related to thrombocytopenia and platelet dysfunction associated with the disease. The severe anemia, severe leukopenia, pancytopenia, and bleeding tendency (especially epistaxis) in German Shepherds are important indicators of lower survival rates in cases of CME (10). Therefore, measuring and interpreting

hematological parameters can help clinicians provide a more accurate prognosis for dogs suffering from the disease (2,4,10).

Due to the constant bleeding, anemia persists, in addition to a reduction in platelets and hematocrit that could be related to dehydration in dogs suffering from CME. In general, pancytopenia could be due to the obligatory intracellular tropism of *E. canis* in mononuclear cells (lymphocytes and monocytes) and macrophages (2,4,9). The administration of antibiotics such as doxycycline, minocycline, and rifampicin in therapeutic doses for 28 days showed the progressive reestablishment of hematological abnormalities such as blood cell count from day 7 to day 28 and hemoglobin and platelets also increased, due to the antibiotic action of tetracyclines on *E. canis*. The effects of minocycline and doxycycline are similar, providing evidence that minocycline could be an alternative treatment for non-acute *E. canis* infections when doxycycline is unavailable on the market (29).

Given that the acute, subclinical, or chronic phase of CME may affect the efficacy of doxycycline (30), accompanying therapy with rifampicin was suggested to effectively eliminate chronic persistent *E. canis* infections. The improved hematological abnormalities demonstrated that both doxycycline and rifampicin effectively alleviated the signs of the disease, and using rifampicin would be an alternative option (31). Doxycycline has immunomodulatory and anti-inflammatory properties associated with the proliferation function of leukocytes in the blood; due to these properties, it is the drug's first choice for the treatment of CME (5), specifically, the administration orally for 28 days is recommended (1).

With the 4 drugs, hematocrit increased moderately ≥ 40% up to 21 days, but with doxycycline, it reached between 45 and 70 % at 28 days. Doxycycline increases the count of platelets, erythrocytes, and the concentration of hemoglobin in dogs affected with *E. canis* (5). Likewise, the monocytes and platelets increase in CME due to treatment with doxycycline (32,33). In dogs treated with minocycline, leukocytes significantly increased compared to those treated with imidocarb dipropionate at 14 days ($p < 0.05$, Figure 4). These values were slightly higher than the 6.5 x 10^9/L (25). At 28 days, treatment with 3 antibiotics significantly increased lymphocytes between 16.7 and 17.5 x 10^9/L (Table 5), consistent with the reference values (25,26). However, there was no increase in white blood cells ($\times 10^3/\mu L$) in dogs with CME during the follow-up periods of 1, 2, and 6 months (5).

Doxycycline was less effective than the other drugs in increasing lymphocytes. Segmented neutrophils tended to decrease from 14 x 10^9 with the administration of 4 drugs and were restored between 9 and 4 x 10^9/L in 14 and 28 days, consistent with reference values (25,26). On the other hand, the restoration of cellular components with imidocarb dipropionate was slower than with tetracyclines and rifampicin due to the slowly normalized platelets (33). Treatment with rifampicin accelerated hematological abnormalities recovery (mainly platelets count), although it is not consistent in eliminating acute *E. canis* infection (12).

In this regard, platelet counts returned to normal more slowly in dogs treated with imidocarb dipropionate than those receiving doxycycline and combining both treatments (1,34). Therefore, doxycycline was more effective for treating CME than imidocarb dipropionate (1,35). At doses of 6.6 mg/kg, IM, 2 injections 2 weeks apart, imidocarb dipropionate did not eliminate experimental *E. canis* infection in dogs (11,33). Imidocarb dipropionate should be used cautiously as a potential treatment when combined with doxycycline (2,33,35). No adverse effects were observed with the doses applied during and after the pharmacological treatment. In clinical practice, doxycycline and imidocarb dipropionate are often used in combination, especially in severe cases of CME. However, the last one, as an antiparasitic, is mainly prescribed for dogs and puppies coinfected with *Babesia* spp. (2,34). On the other hand, the One Health approach to tick-borne diseases calls for physicians and veterinarians to unify their efforts in managing these diseases. The epidemiological control and prevention of these diseases is often difficult because it requires disrupting a complex transmission chain involving vertebrate hosts and ticks, which interact in a constantly changing environment (36).

As described previously, the *E.canis* Ab test is based on sandwich lateral flow immunochromatographic assay (32,33). Furthermore, the kit used in this study has a high sensitivity of 99.8% and specificity > 98%, similar to 96.2% (sensitivity), 97.7% (specificity), and 97% (accuracy) reported for the rMAP2 ELISA test by comparing it to the indirect fluorescent-antibody assay gold standard (37). Nonetheless, the *E.canis* Ab test is limited to use only for *in vitro* veterinary diagnosis. It must be emphasized that serodiagnosis assays do not distinguish between current infection and prior exposure. All results should be considered, along with other clinical information available from the veterinarian. It is suggested that further confirmative methods such as PCR, microscopy, or cytology (blood smear evaluation) be applied when a positive result is observed (11-13,20,29-38). Therefore, it is recommended that the blood smear should be performed at the beginning of the examination, after two weeks, and at the end of treatment (11,12,38).

Due to the limitations of the serology, a complementary diagnosis by hematology was performed; additionally, the information on the clinical signs and the anamnesis allowed us to substantiate the clinical diagnosis. Diagnosis of CME must be made in light of anamnesis, clinical signs, and laboratory test results. Platelet counts and serology are good screening laboratory tests; however, PCR techniques and sequencing are the definitive confirmatory tests for *E. canis* infection (38).

Conclusion

Canine monocytic ehrlichiosis causes negative alterations in blood cellular components. Regardless of the effect of each drug, from day 7 to 28 days after treatment, hematological abnormalities were restored with a progressive increase in hemoglobin, hematocrit, platelets, leukocytes, lymphocytes, and a marked decrease in eosinophils and neutrophils. Treatment of canine monocytic ehrlichiosis with three antibiotics (doxycycline, minocycline, and rifampicin) was more effective than imidocarb dipropionate. Therefore, in clinical practice, the administration of doxycycline and minocycline is recommended first, although rifampicin can be used in canine monocytic ehrlichiosis therapy as an optional alternative.

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Conflict of interest

The authors declare no conflicts of interest regarding this manuscript's publication and/or funding.

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تقييم مقارن لأربعة أدوية على أمراض الدم ومدة التعافي من داء إيرليخية وحيدات النوى في الكلاب

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الخلاصة

يسبب داء إيرليخ أحادي الخلية في مضاعفات سريرية حادة وشبه سريرية ومزمنة مع تغيرات في المعايير الدموية: الهيموكلوبين والهيماتوكريت وعدد خلايا الدم البيضاء. الدوكسيسيكلين مناسب للعلاج. كان الهدف هو تقييم تأثير أربعة أدوية (دوكسيسيكلين، مينوسيكلين، ريفامبيسين، وإيميدوكرب) على المعايير الدموية وتعافي الكلاب المصابة بداء إيرليخ أحادي الخلية. تم توزيع تسعة وعشرين كلبا" بشكل عشوائي إلى ٤ مجموعات: مينوسيكلين بمقدار ١٠ ملغم/ كغم عن طريق الفم كل ١٢ ساعة (G1)، ريفامبيسين بمقدار ١٠ ملغم/ كغم عن طريق الفم كل ٢٤ ساعة (G2)، دوكسيسيكلين بمقدار ١٠ ملغم/ كغم عن طريق الفم كل ٢٤ ساعة (G3)، وإيميدوكارب ديبروبيونات بمقدار ٦,٦ ملغم/ كغم عن طريق الحقن العضلي بفواصل أسبوعين (G4). تم اختبار تأثير الأدوية على المعايير الدموية والتعافي في الأيام ٧ و ١٤ و ٢١ و ٢٨ عن طريق تحليل التباين. تمت مقارنة المتوسطات ± الانحراف القياسي باختبار Tukey ($\alpha = 0.05$). كان علاج داء إيرليخ أحادي الخلية في الكلاب بثلاثة مضادات حيوية (دوكسيسيكلين ومينوسيكلين وريفامبيسين) أكثر فعالية مقارنة بالإيميدوكارب بسبب الزيادة السريعة في الصفائح الدموية والبيضاء والخلايا الليمفاوية بين ١٤ و ٢٨ يوما. بناء على نتائج أمراض الدم، خلصت هذه الدراسة إلى أن الدوكسيسيكلين والمينوسيكلين (التتراسيكلين شبه الاصطناعية) والريفامبيسين كانت أكثر ملاءمة من إيميدوكارب ديبروبيونات لعلاج داء إيرليخ أحادي الخلية في الكلاب. لذلك، في التطبيقات السريرية، يوصى بإعطاء الدوكسيسيكلين والمينوسيكلين أولا، على الرغم من أنه يمكن استخدام الريفامبيسين في علاج داء إيرليخ أحادي الخلية في الكلاب كبديل اختياري.

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