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# Efficacy of doxycycline versus doxycycline-metronidazole and cellular drainer® in the acute clinical phase in dogs with ehrlichia

*Efficacy of doxycycline against doxycycline-metrodinazole and cell drainer® in the acute clinical phase in dogs with ehrlichia*

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**Palabras claves:**
Garrapatas
Erliquia
Ehrlichiosis canina
Inmunocromatografía

**Resumen**

**Introducción.** La Ehrlichiosis canina se define como una enfermedad hemoparasitaria causada por bacterias Gram negativas. Estas bacterias se dirigen a las células diana de los leucocitos, pertenecientes tanto al grupo de los granulocitos como a los agranulocitos. La enfermedad se transmite a los perros a través de garrapatas. En cuanto a la sintomatología que presentan los pacientes afectados por esta bacteria, se clasifica en leves, agudos y crónicos. **Objetivo.** Esté estudio tiene como objetivo el comparar el efecto de la *doxiclina* frente a *doxiclina* + *metrodinazol* + drenador celular en fase clínica agudas en perros con *erliquia* que asistieron a la Clínica Veterinaria JM. **Metodología.** Para el desarrollo de este artículo científico, se plantea el método de investigación experimental de Métodos transversales y técnicas de observación de leucogramas, signos clínicos de cada canino de la clínica para valores cualitativos, los instrumentos de investigación serán de forma comparativa y el universo de estudio corresponderá a 20 perros para el tratamiento 1 y 20 perros con tratamiento 2, se utilizaron estadística descriptiva para el tratamiento de los datos. **Resultados.** Se logró determinar que con el tratamiento 2 hubo 16 mascotas que obtuvieron resultados satisfactorios mientras que con el tratamiento 1 solo 10 mascotas obtuvieron buenos resultados. **Conclusión.** Tras la recopilación de datos, se considera válida dicha hipótesis, ya que los resultados estadísticos evidenciaron la mayor eficacia del tratamiento T2 en comparación con el tratamiento T1. **Área de la ciencia:** Medicina veterinaria, enfermedades infecciosas, medicina interna etc.

**Keywords:**
ticks
Erlichia
canine ehrlichiosis
Immunochromatography

**Abstract**

**Introduction**. Canine Ehrlichiosis is defined as a hemoparasitic disease caused by Gram-negative bacteria. These bacteria target the target cells of leukocytes, belonging to both the granulocyte and agranulocyte groups. The disease is transmitted to dogs through ticks. Regarding the symptoms presented by patients affected by these bacteria, they are classified as mild, acute, and chronic. Aim.

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This study aims to compare the effect of doxycycline versus doxycycline + metrodinazole + cell drainer in the acute clinical phase in dogs with erlichia that attended the JM Veterinary Clinic. Methodology. For the development of this scientific article, the experimental research method of cross-sectional methods and observation techniques of leukograms, clinical signs of each canine in the clinic is proposed for qualitative values, the research instruments will be comparative and the study universe It will correspond to 20 dogs for treatment 1 and 20 dogs with treatment 2, descriptive statistics were used to process the data. Results. It was determined that with treatment 2 there were 16 pets that obtained satisfactory results while with treatment 1 only 10 pets obtained satisfactory results. Conclusion. After data collection, this hypothesis is considered valid since the statistical results showed the greater effectiveness of treatment T2 compared to treatment T1. Science area: Veterinary medicine, infectious diseases, internal medicine.

## Introduction

Ehrlichia canis was initially identified as Rickettsia canis. During the Vietnam War, this disease was responsible for the loss of hundreds of military canines. It is classified as a bacterial infection and is quite common in veterinary tests.

According toPerez-Trallero & Vicente(2010), Ehrlichia canis is transmitted by ticks via a transstadial route, ruling out the transovarial route. This indicates that the infection originates during the feeding of ticks on an infected host.Perez-Trallero & Vicente(2010), classifies the disease into three phases: acute, subclinical and chronic.

Treatment for this disease involves protocols with doxycycline during the acute phases, which are less than 3 weeks. However, starting in 2022 and in line with the lack of demonstrated efficacy, the recommendation was adjusted. According to the consensus of the Infectious Diseases Study Group of the American College of Veterinary Internal Medicine (ACVIM), the use of doxycycline for 28 days is suggested, with a dose of 10 mg/kg administered orally (Monsalve et al., 2017).

Doxycycline is a synthetic amphoteric compound of the tetracycline group that degrades in markedly alkaline or acidic media. Like other tetracyclines, it is a bacteriostatic drug with a broad spectrum of action, including microorganisms of the order Rickettsiales, Mycoplasmas and Chlamidiales (Monsalve et al., 2017).

Tasaycoet al. (2016), report a comparative study of treatments with tetracycline hydrochloride and synthetic tetracycline (doxycycline), in dogs positive for Ehrlichia canis, 3 groups of 10 canines were formed, being T0 the name of the control group, in addition the group that was treated with oral Doxycycline was named T1, and T2 the group that was administered treatment with injectable Tetracycline hydrochloride, the treatment period was planned at 21 days. After the established time, the test was performed with the Anigen Kit again, where the result was that 73.3% remained positive for Ehrlichia canis, so it is considered that it was due to the short treatment time, which is considered insufficient (21 days), also the absence of important differences between the treatments was observed (T1 and T2); In addition, it was found that the positive canines mostly live indoors, their owners' socioeconomic level is seen as medium to low, their respective health statuses were considered as good; it was also seen that they were not applied external deworming, with the above it is concluded that, in the present study, the two treatments gave similar results, with the presence of an effectiveness of 13.3%, during their application.

The incidence of gastric mucosal alterations in working dogs previously diagnosed with canine ehrlichiosis, which underwent treatment with doxycycline in Medellin, Colombia, regarding the evolution of canine ehrlichiosis, in a population of 15 of 19 patients detected as positive from a total population of 55; the aspects and symptoms evaluated were gagging, reflux, vomiting and diarrhea; in a period of 28 days, for which the stages during and after treatment with oral doxycycline were considered. In addition, a descriptive study was carried out, where the most representative breed is Rottweiler with 57.8%, with an average age of 57 months, 63.15% of the patients analyzed were males; In addition to a percentage of 79% of patients who tested positive in serology, there was no presence of reflux in any patient during the respective process of the evaluation month, considering that 3 of the patients, representing 20%, had an episode of diarrhea and retching; 2 of them, representing 13%, had vomiting. Thanks to this study, which provides important information on the possible physical changes that patients could present when undergoing prolonged treatment with oral doxycycline, however, there is a need for more descriptive and prospective studies to know with greater certainty the effects.

McCown et al. (2016) consider that dogs that have been infected by vector-borne diseases such as mosquitoes and ticks become suitable sources for zoonotic diseases.

It is expected that by evaluating the effect of doxycycline versus doxycycline + metronidazole and cellular drainer in the acute clinical phase in dogs with ehrlichia at the

JM veterinary clinic in the city of Babahoyo, the efficacy of the combination of doxycycline with metronidazole and the cellular drainer versus doxycycline in the treatment of canines that tested positive for Ehrlichia will be established.

*Erlichia*

Canine monocytic ehrlichiosis (CME), also known as hemorrhagic pyrexia, canine tropical pancytopenia, bleeding disorder, is a tick-borne disease caused by the Ehrlichia bacteria, is immunosuppressive, chronic evolutionary course, generally caused by Gram-negative intracellular rickettsiae, belonging to the genera Erhlichia spp and Anaplasma, which have a varied conformation (coccoid - ellipsoidal) with a thickness of 0.5 mm, fixed inside the platelets and leukocytes, with a morula appearance due to intracytoplasmic inclusions; for these microorganisms an arthropod (ticks) is necessary as a vector for transmission and a mammal as a reservoir, resulting in ticks of the genera Ixodes spp. and Rhipicephalus spp., the most common; They can also be transmitted through blood transfusions from an infected mammal to another with susceptible conditions or through fomites (Moncayo, 2022).

Pathogenesis of ehrlichia

Canine infection occurs when infected ticks feed, thus their salivary secretions come into contact with the blood and contaminate the feeding site. Tick saliva contains a wide variety of anti-inflammatory, anticoagulant and immunoregulatory molecules, which facilitate the transmission and acquisition of the pathogen.

*Treatment of Ehrlichia*

“To detect the disease, the “Anigen Rapid E. canis Ab” test kit is used, which is a chromatographic immunoassay for the qualitative detection of Ehrlichia canis antibodies in canine serum, plasma or whole blood (Anigen Rapid Test Kit for E. canis Ab)” (Tasaycoet al., 2016).

Of the tetracyclines, doxycycline is considered the antibiotic of choice to be used for rickettsial infections (Gutiérrez et al., 2016).

The treatment of choice is doxycycline (5 mg/kg/12 h or 10 mg/kg/24 h) for 4 weeks, generally, clinical improvement occurs within a few days, as an option it can be combined with imidocarb although in some cases it is not effective, quinolones are less effective than doxycycline according to some published studies and are not considered first choice, in cases of neurological involvement or immune-mediated factors, steroid corticosteroids are used at 1-2 mg/kg/24h (Madrigal, 2016).

*Doxycycline*

According to Monsalve et al. (2017), doxycycline is a bacteriostatic antimicrobial belonging to the synthetic tetracycline family that has been used orally in small species for more than 4 decades, due to its high capacity to dissolve or mix with lipid solutions compared to other groups of tetracyclines, it contains a greater volume of distribution that allows it a better implantation in the tissues, considering its high percentage of adherence to plasma proteins, it is seen as an antibiotic that is used in the treatment of various canine infections, including those caused by Ehrlichia canis, anaplasma, respiratory problems, stomatitis, pyorrhea, gingivitis and infections in the genitourinary tract, for which a regimen for a period of 28 days is recommended.

*Doxycycline use*

ForPerez-Trallero & Vicente(2010),Doxycycline does not alter the bacterial flora as much as short-acting tetracyclines; it is more effective than tetracycline in treating canine ehrlichiosis, as it penetrates the cells adequately. As it is not eliminated via the kidneys, it can be recommended for patients with renal failure; it is important to note that it irritates the stomach, so it should be administered with food.

*Benefits of doxycycline*

It is a bacteriostatic antibiotic since it inhibits the protein synthesis of sensitive microorganisms, it has a broad spectrum of antimicrobial action, acting on Gram-positive and Gram-negative aerobic and anaerobic bacteria, chlamydia, rickettsia, spirochetes, mycoplasmas and some protozoa. Doxycycline, compared to other tetracyclines, is more lipophilic, resulting in greater tissue penetration, greater intracellular penetration, larger volumes of distribution and better antimicrobial properties (Cruz, 2023).

Dose

The oral dose of doxycycline that is frequently administered to dogs is 10 mg per kilogram of the dog. Another possibility is to administer 5 mg per kilogram of the dog every 12 hours. The dose will be adjusted by the veterinarian in each particular case (Navas, 2019).

*Doxycycline Side Effects*

According to González (2021), doxycycline negatively influences the growth and bone development of animals. In addition, tetracyclines, if administered in the last 2-3 weeks of gestation to the mother or during the first weeks of life in puppies, can discolor the teeth. In this regard, it should be noted that doxycycline seems to cause side effects than other tetracyclines, because it binds less to calcium.

*Elimination Route*

For Monsalve et al. (2017), doxycycline, unlike other tetracyclines, is eliminated by mechanisms other than the renal route. Some studies have reported concentrations of doxycycline in bile close to 50 µg/ml (25 times higher than the plasma concentrations obtained). Likewise, the elimination of doxycycline via the urinary route is 20%, where 75% of doxycycline passes through the blood into the intestinal lumen by means of passive diffusion, and another 5% from the bile to be subsequently excreted in the feces, it irritates the stomach, so it should be administered with food.

*Metronidazole*

Metronidazole is a synthetic antibacterial and antiparasitic drug belonging to the nitroimidazole class that has been used in clinical practice for more than 35 years. Its original indication was for the treatment of infections caused by Trichomonas vaginalis, but over time its scope of action has been expanded and it is currently used to treat a variety of infections caused by different types of organisms. Metronidazole was originally approved for human use in 1963 by the US Food and Drug Administration (FDA) and is available in oral, parenteral, vaginal and topical formulations (Montes, 2019).Bandesky & Menéndez (2001) consider that MTZ is a CYP2C9 inhibitor and can therefore block the metabolism of substrates of this isoenzyme such as tolbutamide, S-warfarin, phenytoin, ibuprofen and flurbiprofen.

*Immunochromatography*

The immunochromatography test is based on the principle of antigen-antibody reaction. When an antigen is present in a sample, it binds to specific antibodies that are immobilized on the test strip. The antibodies are conjugated to gold nanoparticles that produce a visible signal when they bind to the antigen. The test strip also contains a control line that confirms the validity of the test. The control line contains antibodies that bind to the gold nanoparticles, producing a visible signal indicating that the test is working properly. The gold nanoparticles play a key role in the test by amplifying the signal and making it visible to the naked eye.

*Common Ehrlichia Treatments*

Treatment of Erlichia in canines involves a variety of options, including antibiotics, supportive care, and blood transfusions. Doxycycline is the most commonly used antibiotic for the treatment of Erlichia, which is given for several weeks. Supportive care, such as fluid therapy and pain control, are also essential to manage the symptoms of Erlichia. In severe cases, blood transfusions may also be necessary. While these treatments are generally effective, they can have side effects such as vomiting, diarrhea,

and liver damage. Additionally, some strains of Erlichia have developed resistance to antibiotics, making treatment difficult.

The fact that they acquire greater liposolubility allows them to largely insert themselves into cells, which favors a longer persistence of these components against Ehrlichia, an obligate intracellular bacteria. Although doxycycline is also available intravenously, the distribution of these components is done orally. A new treatment was established based on doxycycline (10 mg/kg/24), imidocarb dipropionate (5 mg/kg) and the use of glucocorticoid (prednisolone and dexamethasone) at doses of 1-2 mg, repeating the administration of these last two drugs after 15 days (Madrigal, 2016).

- New treatment for Ehrlichia
- Metronidazole 10 mg/kg/24 hours/15 days
- Doxycycline 5mg/kg/12 hours for 4 weeks minimum
- Cellular drainer: take twice a day.

**Methodology**

This research is experimental in nature, carried outThrough observation, research and the use of leukograms were performed on young dogs positive for Ehrlichia canis and two treatments will be administered, doxycycline and doxycycline and doxycycline + metronidazole + cell drainer to have a new prescription for ehrlichia and in that way see the effectiveness in the recovery time in group 1 and group 2.

The research was carried out in adult dogs aged 15 - 22 months that were positive for Ehrlichia in the acute phase. Two types of treatment for Ehrlichia were used at the JM Veterinary Clinic in the city of Babahoyo in the province of Los Ríos. A sample of 40 dogs was used, which were distributed into two groups: the first group of 20 dogs was given doxycycline at a dose of 10 mg/kg/24 h. The second group was given doxycycline orally at 10 mg/kg/24 hours + metronidazole at 10 mg/kg/24 hours + cellular drainer at 3 cc/24 hours. The effectiveness of the treatments was evaluated, as well as the treatment that had the greatest recovery in biometry and appetite.and patient prognosis. For data analysis, the Chi-square test was used with the 2 different treatment groups, using statistical programs (SPSS).

**Results**

As shown in Table 1, concerning high monocytes, where in comparison of treatment T1 versus T2, there was a significant difference due to the p value obtained, which is less than 0.05. That treatment 2 does influence whether monocytes are high or normal

**Table 1**

*Contingency, absolute frequency, high monocytes*

| T2 Treatment | T1 Treatment | NO | YEAH | Total |
|---|---|---|---|---|
| No | No | 0 | 4 | 4 |
| Yeah | No | 6 | 0 | 6 |
| Yeah | Yeah | 10 | 0 | 10 |
| Total | Total | 16 | 4 | 20 |

| Statistical | Worth | gl | p |
|---|---|---|---|
| Pearson Chi Square | 20,00 | 2 | < 0.0001 |
| Chi Square MV-G2 | 20.02 | 2 | < 0.0001 |
| Cramer Contingent Coef. | 0.71 | | |
| Pearson Contingent Coef. | 0.71 | | |

As observed in Table 2, concerning high leukocytes, where in comparison of T1 versus T2 treatment, there is a significant difference, due to the p value obtained, which is less than 0.05.

**Table 2**

*Contingency absolute frequency, high leukocytes T1*

| T2 Treatment | T1 Treatment | NO | YEAH | Total |
|---|---|---|---|---|
| No | No | 4 | 0 | 4 |

**Table 2**

*Contingency absolute frequency, high leukocytes T1 (continued)*

| T2 Treatment | T1 Treatment | NO | YEAH | Total |
|---|---|---|---|---|
| Yeah | No | 6 | 0 | 6 |
| Yeah | Yeah | 0 | 10 | 10 |
| Total | Total | 16 | 10 | 20 |

| Statistical | Worth | gl | p |
|---|---|---|---|
| Pearson Chi Square | 20,00 | 2 | < 0.0001 |
| Chi Square MV-G2 | 27.73 | 2 | < 0.0001 |
| Cramer Contingent Coef. | 0.71 | | |
| Pearson Contingent Coef. | 0.71 | | |

As shown in Table 3, concerning appetite, where the results of the comparison of treatment T1 versus T2 also obtained a p value less than 0.05, which represents considerable differences between both treatments.

ISSN: 2600-5859
Vol. 7 No. 1.2, pp. 77 – 93, March 2024

**Table 3**

*Contingency absolute frequency, appetite T1*

| T2 Treatment | T1 Treatment | NO | YEAH | Total |
|---|---|---|---|---|
| No | No | 2 | 2 | 4 |
| Yeah | No | 6 | 0 | 6 |
| Yeah | Yeah | 1 | 9 | 10 |
| Total | Total | 9 | 11 | 20 |
| | | | | |
| Statistical | Worth | gl | p | |
| Pearson Chi Square | 12,32 | 2 | < 0.0021 | |
| Chi Square MV-G2 | 15.48 | 2 | < 0.0004 | |
| Cramer Contingent Coef. | 0.56 | | | |
| Pearson Contingent Coef. | 0.62 | | | |

Table 4 / Figure 1 / Figure 2 show the data obtained from the blood count of the population at the start of the treatments, for the respective assessment of effectiveness at the end of the proposed dosage.

**Table 4**

*Day 1 start of treatments*

| | Treatment 1 | | | Treatment 2 | | |
|---|---|---|---|---|---|---|
| | HIGH | Half | Low | High | Half | Low |
| Leukocytes | 7 | 6 | 7 | 7 | 6 | 7 |
| lymphocytes | 9 | 5 | 6 | 9 | 5 | 6 |
| monocytes | 3 | 6 | 11 | 5 | 4 | 11 |
| platelets | 4 | 7 | 9 | 4 | 6 | 10 |
| red blood cells | 5 | 4 | 11 | 3 | 6 | 11 |
| appetite | 6 | 7 | 7 | 6 | 7 | 7 |

ISSN: 2600-5859
Vol. 7 No. 1.2, pp. 77 – 93, March 2024

**Figure 1**

*Day 1 T1 treatment*

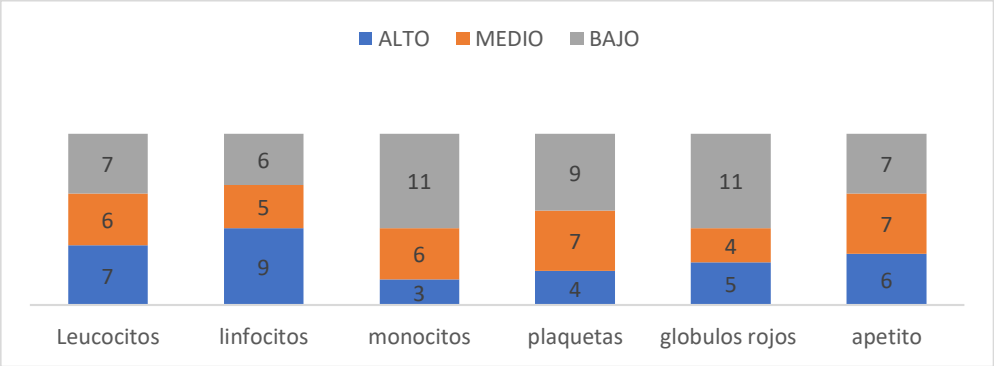
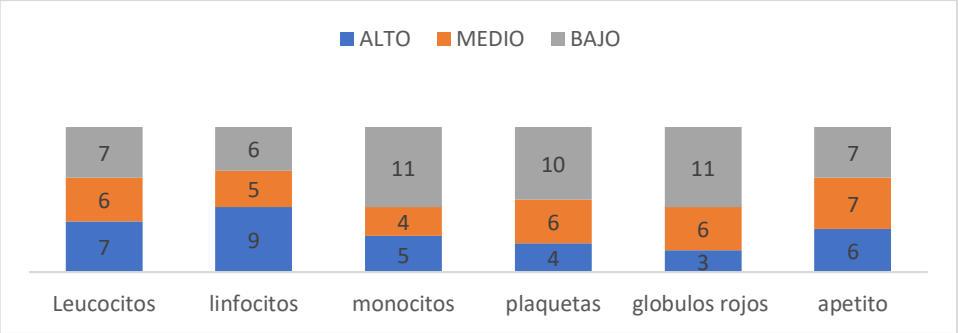



**Figure 2**

*Day 1 T2 treatment*




As observed in Table 5 / Figure 3 / Figure 4, corresponding to the results of the blood tests performed on the test population, after the application of the proposed treatment, it is noted that 10 treatments in T1 respond within 15 days and 16 treatments respond in T2 at the end of the same time.

**Table 5**

*Day 15 end of treatments*

| | Day 15 | | | | | |
|---|---|---|---|---|---|---|
| | Treatment 1 | | | Treatment 2 | | |
| | High | Half | Low | High | Half | Low |
| leukocytes | 7 | 6 | 7 | 1 | 15 | 4 |
| lymphocytes | 9 | 5 | 6 | 4 | 12 | 4 |

ISSN: 2600-5859
Vol. 7 No. 1.2, pp. 77 – 93, March 2024

| | | | | | | |
|---|---|---|---|---|---|---|
| monocytes | 13 | 5 | 2 | 1 | 18 | 1 |
| platelets | 10 | 7 | 3 | 3 | 16 | 1 |
| red blood cells | 12 | 6 | 2 | 1 | 18 | 1 |
| appetite | 9 | 7 | 4 | 2 | 17 | 1 |

**Figure 3**

*Day 15 T1 treatment*

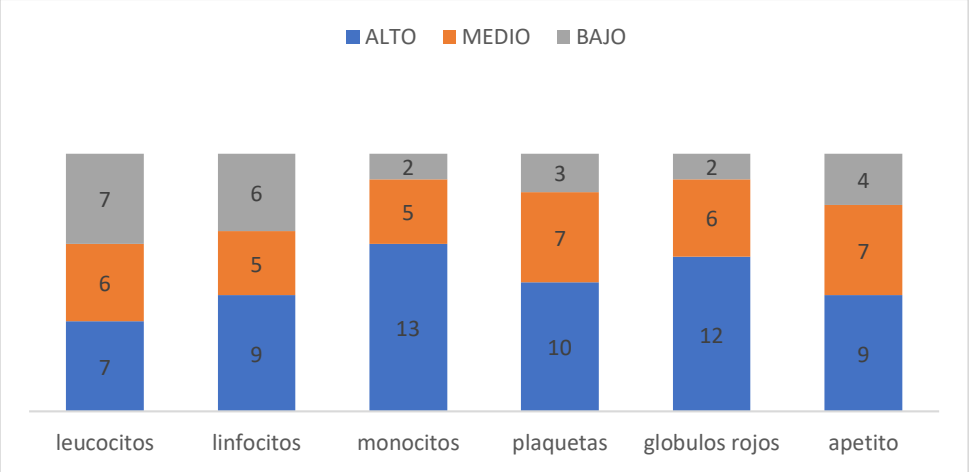
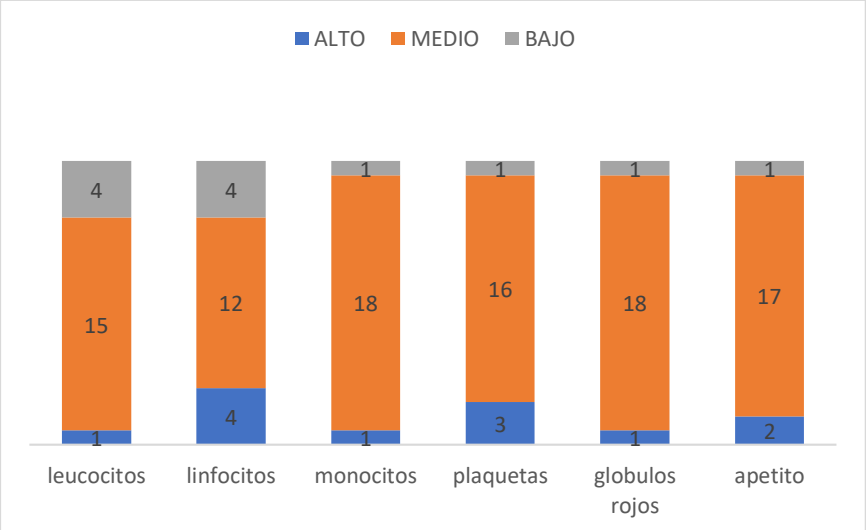



**Figure 4**

*Day 15 T2 treatment*




As can be seen in cross-table 6, it can be determined that with treatment two there were 16 pets that obtained good results while with treatment one only 10 pets obtained good results.

Conciencia Digital
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www.concienciadigital.org

**Table 6**

*Crusade day 15 treatments*

| | | Treatment 1 | Treatment 2 | |
|---|---|---|---|---|
| Day 15 | It didn't work | 10 | 4 | 14 |
| | It worked | 10 | 16 | 26 |
| Total | | 20 | 20 | 40 |

As seen in Table 7, the analysis of the corresponding chi square testAt the significance value of 0.047 and this in turn is <0.05, the null hypothesis is rejected and the alternate hypothesis is accepted, which indicates that the combined treatment of doxycycline + metronidazole + cell drainer will be more effective than treatment with doxycycline alone in the clinical phase in dogs with ehrlichia.

It should be noted that when the significance of the Chi-square test is > 0.05, the null hypothesis is accepted and therefore the alternative hypothesis is rejected. Otherwise, if the significance value of the Chi-square test is < 0.05, the null hypothesis is rejected and the alternative hypothesis is accepted.

**Table 7**

*Chi-square tests*

| | Worth | df | Asymptotic significance (bilateral) | Exact significance (bilateral) | Exact significance (unilateral) |
|---|---|---|---|---|---|
| Pearson Chi-square | 3,956[to] | 1 | 0.047 | | |
| Continuity correction[b] | 2,747 | 1 | 0.097 | | |
| Likelihood ratio | 4,054 | 1 | 0.044 | | |
| Fisher's exact test | | | | 0.096 | 0.048 |
| N of valid cases | 40 | | | | |

## Discussion

There is statistically significant evidence supporting the rejection of the null hypothesis (H0), which stated that the combined treatment of doxycycline + metronidazole + cell drainer would not be more effective than treatment with doxycycline alone in the clinical

phase of dogs with Ehrlichia. This is attributed to the performance of Treatment T2 and its remarkable effectiveness compared to Treatment T1.

No statistically significant evidence was found to reject the null hypothesis (H0) that stated the lack of relationship between the sex of the dog and the performance of Treatment T2. In other words, the data collected do not offer sufficient support to conclude that there is a significant connection between these two variables.

This absence of evidence suggests that there is no significant disparity between the observed and expected frequencies, indicating similar efficacy of Treatment T2 under the null hypothesis of independence.

Initially, it was formulated as an alternative hypothesis that the combined treatment of doxycycline + metronidazole + cellular drainer would be more effective than exclusive treatment with doxycycline in the clinical phase of dogs with ehrlichia, treated at the JM Veterinary Center in the city of Babahoyo. This hypothesis, after obtaining data supporting the superiority of the T2 treatment over T1, is considered valid, evidenced by the 10-16 ratio in the effective results of the applied treatments.

## Conclusions

- At the beginning of the study, it was hypothesized that the combination of doxycycline + metronidazole + cell drainer would be more effective than treatment with doxycycline alone during the clinical phase in dogs with ehrlichia treated at the JM Veterinary Center in the city of Babahoyo. After data collection, this hypothesis is considered valid, since the statistical results showed the greater efficacy of the T2 treatment compared to the T1 treatment.

## Conflict of interest

Authors must declare whether or not there is a conflict of interest in relation to the submitted article.

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