

REVIEW ARTICLE

Efectividad de nitazoxanida, teclozán y fenbendazol en el tratamiento de giardiasis canina. Nitazoxanide, teclozan and fenbendazole effectiveness in treating canine giardiasis.

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Abstract

Background: Canine giardiasis is parasite illness of worldwide distribution that has been showing resistance phenomena to conventional treatment in tropical zones. Objective: to determine the effectiveness of nitazoxanida, teclozan and fenbendazole on the canine giardiasis treatment in dog kennel situation. Materials and methods: 45 canines selected, positive to the presence of *Giardia duodenalis* in a canine kennel in Caldas, Antioquia, Colombia. Canines were crossbred, of both sex and different ages. All were submitted to test carpological in four times: zero (initial), first (8 days), second (30 days), third (90 days) of application. Also were conducted in the same time hematological tests, with blood count, alanine amino transferees and creatinine, respectively. The selected patients for the controlled clinical trial were randomly selected and received three treatment protocols, Group A: nitazoxanida 10 mg/kg oral every 24 hours for three days; Group B: Teclozan 10 mg/kg oral every 24 hours for three days and, Group C: fenbendazole 50 mg/kg oral every 24 hours for three days. Results: all the medicines were effective in the canine giardiasis treatment at long and short terms. The fenbendazole and teclozan were the most effective at short term (60%), whereas, all were effective at long term, in 30 days (93.3%), and in 90 days (100%), respectively. None of the medicines shows alterations hematology, renal and hepatic in the study. Conclusion: All the evaluated medicines were effective for the canine giardiasis treatment in dog kennel situation and without secondary effects in the treated canines.

Key words: Diarrhea, Dog, Enteritis, *Giardia Duodenalis* and Zoonotic (Fuente: Mesh).

Abbreviations: MCV: Mean Corpuscular Volume; HGCM: Mean Corpuscular Hemoglobin; CHGCM: Mean Concentration of Corpuscular Hemoglobin

Resumen

Antecedents: la giardiasis canina es una enfermedad parasitaria de distribución mundial, que ha venido mostrando fenómeno de Resistencia al tratamiento convencional en zonas tropicales. Objetivo: determinar la Efectividad de nitazoxanida, teclozán y fenbendazole en el tratamiento de la giardiasis canina en situación de Pereira. Materials y methods: fueron seleccionados 45 canines positivos a la presencia de *Giardia duodenalis* en UN alergias canina, en Caldas, Antioquia, Colombia. Los canines eran mestizos, de ambos sexos y todas las edades. Todos fueron sometidos a prueba carpológica en cuatro tiempos, cero (inicial), Uno (8 días), dos (30 días) y tres (90 días) de aplicación. Edemas, fueron realizados en los mismos tiempos pruebas hematológicas, con hemograma, alanina amino transferas y creatinina, respectivamente. Los pacientes seleccionados para ensayo clínico controlado fueron seleccionados aleatoriamente, recibieron tres protocolos de tratamiento, Grupo A: nitazoxanida 10 mg/

kg oral cada 24 horas por tres días, Grupo B: teclozán 10 mg/kg oral cada 24 horas por tres días y Grupo C: fenbendazol 50 mg/kg oral cada 24 horas por tres días, respectivamente. Resultados: todos los Farmacos fueron efectivos en el tratamiento de la giardiasis canina a corto y largo plazo. El fenbendazole y teclozán fueron los más efectivos a corto plazo (60%), mientras que a largo plazo todos fueron efectivos, a los 30 (93.3%) y 90 días (100%), respectivamente. Ninguna de las pruebas presentó alteraciones hematológicas, renales y hepáticas en el estudio. Conclusion: todos los Farmacos evaluados fueron efectivos para el tratamiento de la giardiasis canina en situación de alergias y sin efectos secundarios en los canines tratados.

Introduction

Canine giardiasis is caused by *Giardia duodenalis* [1]; this protozoan affects the dog and other species, including man [2].

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It is a cosmopolitan parasite, causing malabsorption syndrome, with a gastroenteritis [2, 3, 1] because giardia has tropism by intestinal mucosa, produces ulcerative lesions, considered as a zoonotic disease of global importance [1], the prevalence is higher in areas of unhealthy conditions [3, 4]. Transmission is ore-fecal, humans and canines ingest the cysts [1, 4] and subsequently developing gastroenteritis, this route allows outbreaks between dogs and humans frequently, especially in rural areas and Canine shelters [5, 6].

The signs are due to gastroenteritis [1], for the damage of the villi of the intestine, which leads to malabsorption syndrome [5, 7, 8]. The diagnosis is made by direct smear and flotation with zinc sulfate [9], in addition to other molecular and serological techniques [10], among which the Snap® test stands out (IDEXX ©, laboratories, USA) [11] and ELISA and immunofluorescence [12]. The treatment is with fenbendazole [11] or febantel [13], but due to the constant reinfection and resistance phenomena, these have lost effectiveness; There are other pharmacological protocols for this problem, among which are metronidazole [1, 11, 21], secnidazole, imidazole and albendazole [14-17]. This is the reason for the search for other alternatives such as Nitazoxanide and teclozan widely described in humans for the treatment of resistant giardiasis [7, 18].

Materials and Methods

Study type

A controlled and randomized clinical trial, conducted between January and July 2016.

Population

88 canines were selected from a canine shelter in the municipality of Caldas, Antioquia, Colombia (6°5 '19 "N, 75° 38' 10" W), the breed used was mixed, both males and females of various ages and fed with Colombian commercial concentrate, of which 45 were positive for canine giardiasis by direct smear and flotation with zinc sulphate.

Ethical aspects

The owner of the canines signed informed consent where he accepted the conditions of the study; in addition the recommendations for animal research were followed according to Law 84 of 1989, chapter 3 and 6, Law 576 of 2000, chapter 6 and Law 1774 of 2016 of the congress National of Colombia.

Inclusion protocol

The positive canines selected were of sexes, all ages and any race and no history of renal or hepatic involvement.

Experimental protocol

There were 45 separate specimens in three groups of 15, with diagnosis of *G. duodenalis*, measured by cysts according to qualitative scale in crosses, being 0 (null), 1 (scarce), 2 (moderate), 3 (abundant). The faces were evaluated according to variables: fat, leukocytes, vegetable fiber and starch in the same qualitative scale. Complete blood count, creatinine

measurement and alanine amino transferases (ALT) were performed. Group A received Nitazoxanide 10 mg / kg orally every 24 h, group B fenbendazole 50 mg / kg orally every 24 h and group C teclozan 10 mg / kg orally every 24 h, all for three days.

Protocol evaluation

Coprinological analyzes were performed in reference laboratory Medellin, Colombia, in three times, 8, 30 and 90 days respectively, the carpological variables were the same as at the beginning. Blood counts were performed at the same time, taking blood from the peripheral vein; blood was collected in EDTA tube and refrigerated at 2°C, sent to the laboratory. The samples were processed in Abacus Junior Vet® device and were evaluated: hematocrit (Hct), hemoglobin (Hgb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (HGBM), mean concentration of corpuscular hemoglobin (CHGBM), erythrocytes, leukocytes, neutrophils, eosinophils, lymphocytes, monocytes, bands, platelets and plasma proteins. In addition, ALT and creatinine tests were performed in the same time, ALT and creatinine was performed with blood without anticoagulant, processed in Bio systems® A15 equipment at the same time. Pharmacological efficiency evaluation was carried out at times one, two and three, as well as the number needed to treat (NNT), following the formula below.

$$\text{Efficacy} = \frac{\text{number of animals without giardiasis}}{\text{total number of animals}} \times 100$$

$$\text{NNT} = 100 / (\% \text{ patients without giardia's new drug}) - (\% \text{ patients without guardians is with standard}).$$
 Statistical protocol Data were tabulated in Microsoft® Excel® and analyzed Stat graphics Centurion XV®, with a level of significance of 5%. ANOVA, Turkey HSD Test and Chi2 were performed.

Results

Population 88 dogs were evaluated, of which 45 were positive, of which 20 (44.4%) were males and 25 (55.6%) females, age was not taken into account for the study and race, because they are animals of feral origin, mestizos and age not determinable. For fenbendazole 7 females and 8 males, nitazoxanida 7 females and 8 males and for teclozan 4 males and 11 females were evaluated. No significant statistical difference was found between the presence of *G. duodenalis* and sex, since all were positive. Experimental protocol No It was determined that there is a relationship between *G. duodenalis* and the variables steatorrhea, starch and plant fiber in feces ($p \geq 0.05$). In the evaluation of each treatment group, it was found that for fenbendazole, at time 0 and 2, only one canine (6.6%) presented steatorrhea, while at time 1 and 3, no dog was present, for nitazoxanida only (6.6%) was maintained with steatorrhea during times 0, 1 and 2, while at time 3 did not show fat in stools and teclozán only one canine (6.6%) showed fat in feces at time 0, in the rest of days there was no presence of steatorrhea, no difference between groups was found. The starch showed that for fenbendazole 53.3% of

the dogs had presence of starch on day 0, which continued at 8 d and increased to 66.6% at 30 d and 90 days 67.39% was found respectively, Nitazoxanide showed that 53.3 % had starch in time 0, then only 46.6% had starch in times 1, 2 and 3, while teclozán started with 86.6% in time 0, then the values were 60% time 1, 20% time 2 and 25% in the time 3 respectively. The plant fiber all treatments initiated with 80% in time 0 and 1, in time 2 it was 66.6% and for time 3 it was increased to 82.6%. The presence of cysts of *G. duodenalis*, was found that 100% of dogs were positive at day 0, the values for each treatment can be seen in (Table 1).The effectiveness of fenbendazole in time 1 is 60%, in time two of 93.3% and in time three 100%, respectively. In addition, significant statistical difference was found between time 0 and times 1, 2 and 3 ($p \leq 0.05$), effectiveness of Nitazoxanide at time 1 is 46.7%, time at time 2 is 93.3% and time 100% at time 3

Between the times 0, 1, 2 and 3 significant statistical difference was found ($p \leq 0.05$), while the effectiveness of teclozan in time 1 is 60%, for time 2 is 93.3% and 100% time three, found difference between treatment times see (Table 1). Regarding the values found in the calculation of NNT, for each treatment in the three times these can be observed in the following times see (Table 2).Hematology showed on day zero values within of the standard for dogs (Table 3). No statistical difference could be found between the hematological values over time. The creatinine showed that at time 0 the value was 1.10 ± 0.30 mg / ld. (0.5-1.5 mg / ld.), normal for canines, and there was no difference between treatments and times (Table 4).

The ALT showed a value of 55.77 ± 17.6 IU / L (21-102 IU / L) on day 0, normal for dogs, and found no statistical difference between treatments and times (Table 4).

Cyst <i>G. duodenalis</i> (Crosses) Fenbendazole Nitazoxanide Teclozan												
	T0	%	T1	%	T2	%	T3	%	T0	%	T1	%
	T2	%	T3	%	T0	%	T1	%	T2	%	T3	%
0	0a	0	9b	60	14c	93.3	15c	100	0a	0	7b	46.6
	14c	93.3	15c	100	0a	0	9b	60	14c	93.3	15c	100
1	0a	0	3b	20	1c	6.6	0	0	0a	0	4b	26.6
	1c	6.6	0	0	5a	33.3	4b	26.6	1c	6.6	0	0
2	6a	40	1b	10	0c	0	0	0	6a	40	4b	26.6
	0c	0	0	0	6a	40	2b	13.3	0c	0	0	0
3	9a	60	1b	10	0c	0	0	0	9a	60	0b	0
	0c	0	0	0	4a	26.6	0b	0	0c	0	0	0
Total	15	100	15	100	15	100	15	100	15	100	15	100
	15	100	15	100	15	100	15	100	15	100	15	100

Super index with different letters a, b, c, indicate significant statistical difference ($p \leq 0.05$).

Table 1: Presence values of *G duodenalis* according to treatment.

Nitazoxanide			Teclozan		
T1	T2	T3	T1	T2	T3
7.4	0	0	0	0	0

Table 2: NNT values for nitazoxanide and teclozan.

Variable	Unit	Average	SD	Reference*
Erythrocyte	Millón/ μ L	7.59	0.97	5.5-8.5
Hematocrit	%	47.93	6.61	12-18
Hemoglobin	gr/dL	16.21	2.20	37-55
VCM	ft	63.30	2.60	60-70
HBCM	pg	21.29	0.99	22-27
CMHB	ft	33.61	0.86	-
White blood cell	Mil/ μ L	14.4	0.38	6-17
Neutrophil	Mil/ μ L	8.73	0.28	3.3-10
Eosinophilic	Mil/ μ L	1.01	0.67	0.1-1.5
Lymphocytes	Mil/ μ L	5.79	0.90	1-4.5
Monocytes	Mil/ μ L	0.05	0.1	0.1-0.7
Platelets	Miles/ μ L	319	0.95	200-500
Plasma protein	gr/dL	7.36	0.96	5.5-7.5

*Canine hematology reference (Meyer y Harvey 2004).

Table 3: Average values $\pm$ DS, of the hematological variables in T0.

Analite/unit	Fenbendazole				Nitazoxanide				Teclozan			
Tiemp	0	1	2	3	0	1	2	3	0	1	2	3
ALT UI/L	39.45	43.38	43.38	44.89	67.33	43.63	43.63	46.25	59.38	47.54	47.54	41.48
Creatinine mg/dL	1.12	1.08	1.08	1.02	1.07	1.05	1.05	1.02	1.12	0.88	0.88	1.04

Table 4: Average values of ALT and creatinine for treatments and times.

Discussion

The 45 positive specimens to *G. duodenalis*, for the shelter represent a prevalence of 51.17%, being high for Colombia. In a study of 122 stool samples in Bogotá, the prevalence is 0.81% [19], other authors in Envigado describe 13.9% [20] and in shelters in Medellín it is 6.8% and 10.3% for shelters in eastern Antioquia [9]. The high prevalence can be due to re infection [21] or linked to street origin [19, 22] and the infection is linked to many factors including geographical area, general state of the host and population habits [20]. For the breed and sex, it was found that 100% of the specimens were mestizos and therefore there is no relationship between race and giardiasis, similar findings described by other authors in the medium where 84.6% of the giardiasis occurs in mestizo animals [19-21], but it is related to the fact that it is the most frequent population in shelters and not that it is more susceptible. The proportions between males (44.4%) and females (55.6%) are very homogeneous, which indicates that the disease has no relationship with sex [1]. This corresponds to previous studies where there was no difference between the presence of giardiasis, sex and age [9, 19, 20]. However, it has been described that Young animals are more predisposed to giardiasis than adults, which could not be determined in the present work [23]. In the macroscopic carpological analysis, the appearance of faces was considered an indicator of giardiasis, in previous studies *G. duodenalis* is related to pasty, soft and diarrheic stools [24-26], in the present study, no relationship was found between the consistency of feces and the presence of canine giardiasis, similar to what [27], where there is the same probability of giardiasis in pasty stools as in formed stools [27]. In the microscopic aspect, the present study could not find a relationship between the presence of *G. duodenalis* and the sign of steatorrhea [1], a condition frequently described by several authors [28, 29, 46]. On the other hand no malabsorption syndrome problems were found, since the presence of stool starch was not statistically significant in relation to the presence of the parasite, as described by [46], which can be giardiasis pictures Chronic, where there is an epizootic equilibrium of the parasite [31], in addition the absence of intestinal signs of inflammation such as leukocytes, mucus and blood, may be indicating that this same endemic balance is present, already described in humans from cosmopolitan cities [6]. All the patients were positive for the presence of *G. duodenalis*, there being no difference between the three treatment groups, which is evident is that after 8 days of starting fenbendazole treatment an efficiency of 60% was found, which agrees with the descriptions of several authors [17, 32, 33], in which it is indicated that fenbendazole is effective for the treatment of canine giardiasis [11, 34, 13], for patients undergoing Nitazoxanide at the same time, 46.6% efficiency was found after 8 days of treatment, which differs

from the findings found by other authors [35-37], disagree for the descriptions made for children with giardiasis, in which the efficiency was 80% [36]. While for teclozán showed efficiency of 60% in the same period of time, which corresponds to the descriptions made for children and women in gestation [38]. In the evaluation 30 days after the treatment was found that for fenbendazole had an efficiency of 93.3%, for Nitazoxanide the efficiency values were also 93.3% and for teclozan also 93.3% which corresponds to the descriptions made in canine and human patients respectively [11, 18, 32].

For the evaluation at 90 days it was found that the values of *G. duodenalis* cysts for fenbendazole were 0, for Nitazoxanide 0 and teclozan of 0, which indicates that all the treatments showed to be efficient (100%) in time. Regarding the evaluation of NNT, it was determined that for Nitazoxanide it is required to treat 7 patients so that they do not present the giardiasis symptoms, while with tell ozone does not need to treat any, being highly effective after 8 days, in terms of NNT at 30 days no patient is required to treat and the same was obtained after 90 days, both Nitazoxanide and teclozán being effective in the treatment of canine giardiasis at 30 and 90 days. In the hematological evaluation of day zero it was found that the values were within the averages for the canine species, it was not possible to determine statistical difference between treatments, which is indicative not only that The presence of giardiasis is not related to the presence of changes in the haemoleucogram, as described by some authors [9, 20, 21], it could also be determined that the use of the drugs described does not cause changes in hematology, similar to how their therapeutic safety in humans has been described [39, 40, 45], in addition it was not possible to determine a relationship between the presence of giardiasis and changes in eosinophil's, a frequent phenomenon in parasitoids. In terms of renal safety, creatinine values were measured, which were normal and within normal for the canine species, which indicates that the three drugs are safe to be delivered to canines and do not produce changes in creatinine during 90 days of evaluation, this renal safety has been described for humans and other animal species [41-44].

Hepatic safety was also evaluated finding normal values for canines, which indicates that these drugs are not very hepatotoxic for the canine species and are safe in the administration, as they do not induce changes in hepatic transaminase ALT.

Conclusion

Phoendazole and teclozan were found to be more effective at short term (8 d), while Nitazoxanide turned out to be effective in the long term (30 d). All the drugs in this study were effective in the treatment of canine giardiasis.

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Conflict of Interests

In this study, no sponsorships were received by commercial agencies; it is a personal idea in search of pharmacological development.

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