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Evaluation Of The Antiparasitological Effect Of Seeds Of *Cucurbita ficifolia* In Stray Dogs.

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ABSTRACT

The dog (*Canis lupus familiaris*) being a sniffer animal and being in direct contact with the ground, is very prone to parasite attacks. Parasites are living beings that spend part or all of their lives inside or outside a host, with endoparasites being the ones that most affect canids. So, the objective of this research was: To evaluate the deworming effect of *Cucurbita ficifolia* seeds in stray canines, for which a simple randomized complete block design was applied, distributed in three treatments: T1 (10 g of antiparasitic/kg of live dog weight during 2 consecutive days); T2 (20 g of antiparasitic/kg of live dog weight during 2 days) and T3 (30 g of antiparasitic/kg of live dog weight during 2 days) in all three treatments a repetition of the dose was performed on day 20 and a coproparasitological analysis at day 23. After the application of the natural antiparasitic and coproparasitological analysis, the incidence of *Ancylostoma* reduced from 1.4 to 0.8 forms/field, of the *Toxocara canis* nematode, reduced from 0.5 forms/field to 0.3, in *Capillaria* reduced from 0.1 to 0.0 forms/field. Concluding, that, in the dogs subjected to the dewormer based on calabaza seeds, the parasitic load decreases.

Keywords: Anti-parasitological, effect, seeds, *Cucurbita ficifolia*

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INTRODUCTION

The dog is a pet whose scientific name is *Canis lupus familiaris*, is a carnivorous mammal of the family of canids, which is a subspecies of the wolf (*Canis lupus*). He has a very developed ear and nose, the latter being his main sensory organ, his average life is around 15 years [1]. They have a digestive system that processes the food they eat; this process starts in the mouth and ends in the year with the elimination of unused food [2].

The dog being a sniffer animal and being in direct contact with the ground, is very prone to parasite attacks. Parasites are living beings that spend part or all of their lives in the interior or exterior of another living being, called host, from which it is nourished, causing it many times serious injuries, thus, the parasites are subdivided into: **Ectoparasites** (external location of the host, such as lice, bedbugs, fleas, ticks); **Endoparasites** (internal location of the host, such as protozoa, cestodes, nematodes) and **Visceral** (develop in internal organs such as fasciola, which develops in the liver) [3, 1].

The nematodes, are nemathelminths worms of the super phylum Ecdysozoa, these animals have a digestive system with the shape of a straight duct, that occupies the whole extension of the body, in general, they usually live in the aquatic environment, although they also inhabit the surface, among the more than twenty-five thousand species detected, there are nematodes of autonomous existence and other parasites of humans, plants and animals [4].

The factors that influence the pathological manifestation of certain parasites in dogs are: young animals with low nutritional levels, high temperatures and humidity adding to poor hygienic conditions [5].

Ancylostoma caninum. - It is a parasite whose length oscillate between 12 mm and 15mm, its eggs measure 65 μ m and its walls are thin, the larvae hatch in 24 to 72 hours in warm and humid soils [6]. After the excretion of the eggs in the feces, the larvae develop inside and hatch in 2 to 9 days, completing their development to infective larvae L-III [7].

Capillaria. - Life cycles are not known in detail, but they are supposed to have an indirect cycle with earthworms as an intermediate host. Pets are infected by consuming worms or other secondary hosts (eg birds). Thus, the infective larvae that migrate through various routes to their favorite organs are released where they complete their development to adults [8].

Toxocara canis. - They are cylindrical worms with pointed ends with 3 lips in their mouth, light pink-nacreous, they measure between 7 and 15 cm, the eggs measure 80 μ m and the larvae 0.4 mm long x 0.02 mm wide, given the right conditions, eggs can survive for 2 to 4 years [9].

Dipylidium caninum. - They are flat worms of simpler bilateral symmetry, has three well defined germ layers, in addition, two or more types of tissues can form organs [10]. Its main vector are fleas, once inside the flea, the parasite develops to its next phase, the oncosphere, which penetrates the intestinal wall and develops over time to the cysticercoid state, as soon as it reaches the interior of the in the intestine, the cysticercoid will anchor with the scolex to enter the interior of the intestinal wall [11].

The calabazo (*Cucurbita ficifolia*) is a cucurbitacea native to America, whose family contains about 760 species of tropical and sub-tropical distribution, are characterized by vines, climbers or crawlers of rapid growth [12].

Nutritionally, calabaza contains up to 24.5% protein, fatty acids, minerals, amino acids contain leucine, tyrosine, peporesin, vitamin B, provitamin B, provitamin A and phosphorus, essentials, cucurbitite and cucuric acid. They have anti-inflammatory, emollient and antiparasitic properties [13]. So, the pulp has been used to treat urinary inflammations, kidney failure, hemorrhoids, dyspepsia, enteritis, dysentery, constipation, heart disease, insomnia, diabetes, the tegument of its seeds have been considered as antiparasitic especially as anthelmintic not irritant and non-toxic [14].

Considering the previously described, as objective we have proposed, to evaluate the deworming effect of calabazo seeds (*Cucurbita ficifolia*) in stray dogs of the shelter 2 "O" of the City of Guaranda (Ecuador).

MATERIALS AND METHODS

Demographic description

The present investigation was carried out in the shelter called 2 "O", located in the city of Guaranda (Ecuador), with the following construction characteristics: four mesh facilities and a small enclosure where the individual kennels of 8 m each are located, 12 dogs remain in each enclosure. So, fecal samples were collected from 36 dogs of both sexes with an average age of 3.5 years of different races (Mestizo= 16, Castellano= 6, Fresh poodle= 5, Salchicha= 3, Schauzer= 2, Labrador= 1, Gran Danés= 1, Golden Retriever= 1 y Shitzu= 1).

Faecal samples duly identified were transported within a time period of no more than 45 min to the Huellitas of the city San Miguel de Bolívar veterinary laboratory for the respective coproparasitological analysis.

The following methods were used: Observation method (a clinical examination of each dog was carried out); **Experimental method** (degree of infestation by parasites, and the decrease of the same post natural treatment) and **Exploratory Method** (direct examination of the dogs in the shelter and copro-parasitological analysis of fecal material in the laboratory for the identification of parasites before and after the natural treatment). Also, records of the medical records were taken. The collected data was analyzed by the ordinal, nominal and interval scales.

Preparation of the natural dewormer.

Seeds of calabazo (*Cucurbita ficifolia*) were collected aseptically in an approximate amount of 2 kilograms. Then processed according the follow figure.

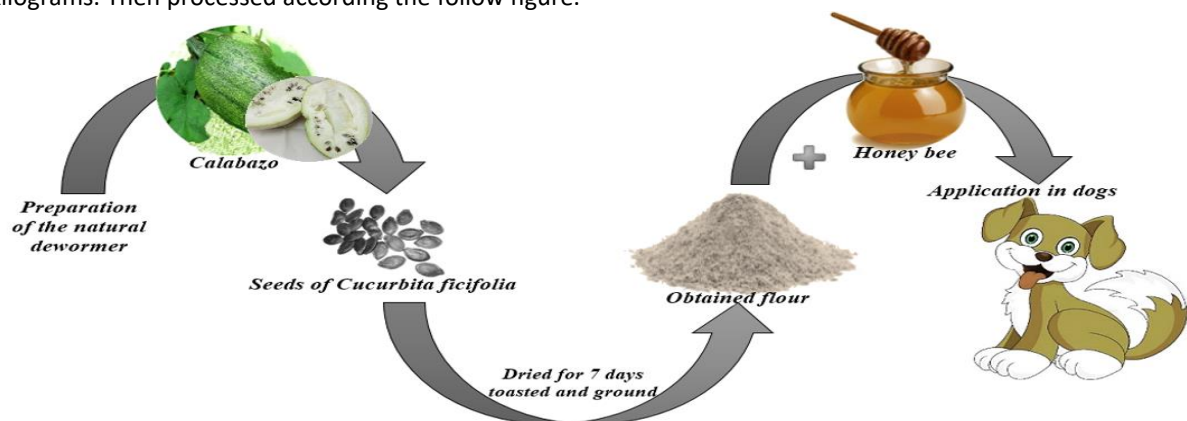


Figure 1. Preparation process of the natural dewormer

Previously to the application in the dogs the flour was heavy in gr

Once obtained the results of the coproparasitological analysis of the 36 canines (22 females and 14 males), three groups of 12 dogs were made in each one (conformed of different ages, races, weight, condition and body). To this end, a simple randomized complete block design (RCBD) was applied, distributing it in three treatments, in addition, for the administration of the deworming preparation, a syrup with honey and purified water was elaborated.

Treatment 1. The dose administered in the first treatment of the natural antiparasitic was 10 gr / kg of live weight, that is to say 28 g in general average for each canine, this amount was given in 2 consecutive days and on day 20 the dose was repeated, finally, a laxative 5 mL of magnesium sulfate was administered. On the 23rd of the treatment the faecal samples were collected, the coproparasitological examination was of flotation by Faust, to check in this way the efficacy of the product.

Treatment 2. The dose administered in the second treatment of the natural antiparasitic was 20 gr / kg of live weight, that is to say 53 g in general average for each canine, this quantity was divided in four portions and delivered in 2 consecutive days and per day 20 the dose was repeated, finally administered a laxative, 5 mL of

magnesium sulfate. On day 23 of the treatment, fecal samples were collected, coproparasitic examination according to treatment 1.

Treatment 3. The dose administered in the third treatment of the natural antiparasitic was 30 g / kg of live weight, that is to say 63.3 g in general average this amount was divided in four portions to be supplied in 2 consecutive days and to the 20 day repeated the dose, finally the laxative was administered. On day 23, coproparasitic analysis of fecal samples was performed.

Statistical analysis

The study variables were subjected to an Analysis of variance (ADEVA) and separation of means through Duncan's multiple range at 5% probability.

RESULTS AND DISCUSSION

Weight of the animals under study

Table 1. Duncan test at 5% to compare the means of treatments in the variable body weight

Weight in Kg of the dogs		
Treatments	Means	Ranks
T1	11.47	A
T2	10.77	A
T3	8.63	A
X= 10.29 Kg (NS)		
CV=38.93%		

In general average of canines subjected to research for all treatments was 10.9 Kg of weight per animal, its coefficient of variation suggests that the group under study was heterogeneous within the treatments, it should be noted that it is a variable that does not was under the control of the researcher, this being stray dogs and subject to overcrowding.

According to the Duncan test at 5% there was no significant statistical difference, however, numerically there was a slight increase in the weight of treatment one (T1). The body conditions of each animal vary due to some factors such as race, physiological state, climate factors, among others, so the prevalence of intestinal helminths can act favorably to suitable corporal conditions, as this favors the animal inactivating the organism to react before any parasitic infestation or disease [15].

Coproparasitological analysis

After the coproparasitological analysis of flotation by Faust, the presence of parasites in the stool samples of the canids was evidenced in the first instance (Figure 2)

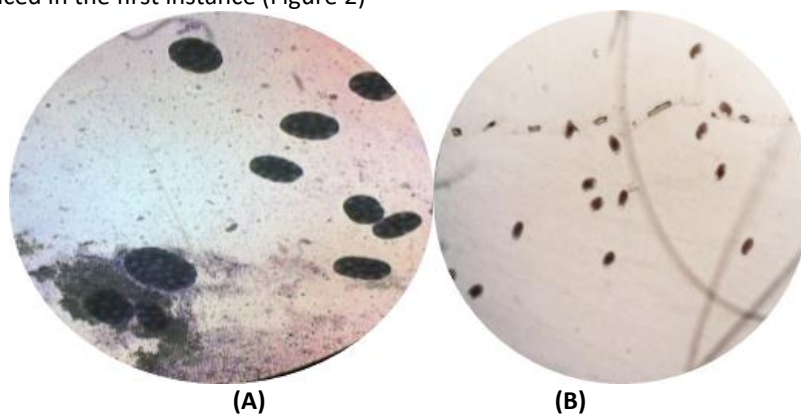


Figure 2. Parasite eggs identified by microscopy, A: *Ancylostoma caninum*; B: *Capillaria*

Dosage of natural dewormer administered

Table 2. Duncan's test at 5% of the treatment means in the variable dose of natural dewormer.

Dosage of dewormer (gr)		
Treatments	Means	Ranks
T3	63.3	A
T2	53.1	A
T1	28.6	B
X= 48.31 gr (*)		
CV=38.30%		

A natural dewormer was administered according to the dose applied (table 2), with an average of 48.31 gr. When performing the Duncan test at 5%, in its averages two ranges of significance were determined; registering as the highest dose of dewormer applied during the trial and located in the first rank (A), to the group of dogs belonging to the treatment (T3); not so that the lowest dose applied was in the group of T1, which was located in the last range.

In an investigation carried out in Guatemala by **Granados** [16], for the evaluation of the deworming effect of a natural product based on seeds of cucurbit pepo, flower of the dead, he mentions that with the application of the deworming preparation in doses of 10 ml in goats during three consecutive days and repeat this dosage was able to decrease the parasitic load by 60% especially nematodes, the authors also considered that the natural dewormer as well as being an anthelmintic is a rich source of vitamins.

Figure 3 shows a photo at the time the natural dewormer was supplied to the selected dogs



Figure 3. Supplied of the natural dewormer

Incidence of *Ancylostoma* before and after treatment

Table 3. Duncan test at 5% in the incidence variable of *Ancylostoma*

Incidence before deworming			Incidence after deworming		
Treatments	Means	Ranks	Treatments	Means	Ranks
T1	1.7	A	T1	1.5	A
T3	1.4	A	T2	0.8	AB
T2	1.3	A	T3	0.3	B
X= 1.4 Forms by field (NS)			X= 0.8 Forms by field (*)		
CV= 70.85%			CV= 79.75%		

Means with different letters are statistically different to 5%
 Means with the same letter are statistically equal to 5%

The presence of *Ancylostoma caninum* by means of coproparasitological examinations of a total of 36 canines the general average 1.4 forms / field before the administration of the dewormer and after deworming was determined, 0.8 forms / field was determined. It can be deduced that the infestation of parasites is slight considering that some animals are more parasitized than others, their normal range is one way / per field, these parasites are more resistant to anthelmintics.

After the Duncan test at 5% for the prevalence of *Ancylostoma* carried out before treatment, it was determined that the highest average was presented by treatment 1 (T1) followed by treatment 3 (T3). While for the average prevalence of *Ancylostoma* after the natural deworming supply, it was recorded that Treatment 1 (T1) presented a higher average, decreasing in 11.77% forms / field, followed by treatment 2 (T2) with a decrease of the 42.8% forms / field finally the treatment 3 (T3) is appreciated with a decrease of 76.9% forms / field.

In an investigation carried out by **Muñoz-Rodríguez** [17], he evaluated the deworming effect of a natural product based on seeds of cucurbit pepo, "flor del muerto", cusha, in poultry against nematodes in a dosage of 1 mL in young birds and 2 mL in adult birds, where the author concluded that with the application of the natural preparation it was possible to reduce the parasitic load in 33.33%, especially nematodes. When comparing our results with what was done by **Granados** [16], based on the results obtained, it can be considered that the different applied doses of dewormer in dogs, agree positively in reducing the incidence of these gastrointestinal parasites; However, the greatest reduction efficiency was obtained by applying 30 g of dewormer (T3) when reducing by 76%. This is because pumpkin seeds have an amino acid called cucurbitin, which weakens intestinal worms.

Toxocara incidence before and after treatment.

Table 4. Duncan test at 5% in the incidence variable of *Toxocara*

Incidence before deworming			Incidence after deworming		
Treatments	Means	Ranks	Treatments	Means	Ranks
T1	0.8	A	T1	0.6	A
T2	0.7	A	T3	0.2	A
T3	0.2	A	T2	0.2	A
X= 1.4 Forms by field (NS)			X= 0.3 Forms by field (NS)		
CV= 108.94%			CV= 173.35%		

The general average of the *Toxocara* nematode presented 0.5 (1) forms / field before the administration of the dewormer and after applying the treatment it was reduced to 0.3 (0) forms / field. The coefficient of variation is high because the response to treatments in animals depends on the biology and resistance of the dogs, it is a variable that is beyond the control of the researcher. When performing the Duncan test at 5%, for averages of the prevalence of *Toxocara* before the treatments on the dogs, the highest average incidence was determined. The treatment was one (T1), followed by treatment two (T2).

Muñoz-Rodríguez [17], in his research proved the effectiveness of a worming compound prepared in hot water with seeds of epazote, flower of the dead, pumpkin seeds in guinea pigs against nematodes whose dose was 2 mL per animal (mice), Drug application was efficient at 11.4%. In this work, there was slightly a decrease of *Toxocara* in treatment one (T1); but the most ostensible decrease was obtained by administering to the dogs 20 g per Kg of body weight, while the treatment (T3); there was no decrease in the parasitic load, this may be due to the fact that the canines of group three established greater resistance by remaining in an unsuitable site and being exposed to an earthen floor.

Incidence of *Capillaria* before and after treatment.

Table 5. Duncan test at 5% in the incidence variable of *Capillaria*

Incidence before deworming			Incidence after deworming	
Treatments	Means	Ranks	Treatments	Means
T3	0.1	A	T1	0.0
T2	0.1	A	T2	0.0
T1	0.0	A	T3	0.0
X= 0.1 Forms by field (NS)			X= 0 Forms by field (NS)	
CV= 314.64%				

In the present investigation it can be observed in the prevalence of *Capillaria* in general average, they presented 0.1. (0) forms / field before the administration of the dewormer and after performing the deworming, 0.0 forms / field was determined. When performing the Duncan test at 5%, for the prevalence averages of *Capillaria* before providing the dewormer, a single range of significance was determined, where Treatment one (T1) and Treatment two (T2) had shapes / field, while that Treatment three (T3) did not present / field forms.

Vélez (3), considers that it is necessary to consider that, a negative coproparasitoscopic examination has no predictive value, for reasons such as: The analysis may be negative, however, the patient may be parasitized. So, it is recommended to perform at least three coproparasitoscopic exams. At the end of the test it was determined that there was no presence of *Capillaria* in the animals under study; this may be due to the scarce presence of this gastrointestinal parasite at the beginning of the test in the animals, so the actual effectiveness of the dewormer could not be assessed.

CONCLUSION

Of the 36 dogs analyzed in the shelter 2 "O" distributed in three groups and subjected to a dewormer based on *Cucurbita ficifolia* seeds, decreases the parasitic load, which allows to conclude that, to major concentration of natural dewormer, low level of parasites presence, besides, could be used as an alternative in deworming domestic dogs.

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REFERENCES

- [1] Sánchez, C. Crianza, razas y entrenamiento de perros. Edit. Ripalme. Lima, Perú. (2003). Pp. 12,92.
- [2] Rodríguez, F Franco, Carrasco, V., Rodríguez, A., Canfrán, S., García-Sancho, M., Sainz, Á, Canine gastric carcinoma: endoscopic localization and macroscopic appearance. Revista Complutense de Ciencias Veterinarias 2009; 3(1): 72-80.
- [3] Vélez, R.A. (2009) "Guías en Parasitología Veterinaria". Exitodinámica Editores. Medellín Colombia. Identificación de huevos de nemátodos en carnívoros y primates ubicados en el Zoológico Santa Fe de Medellín, mediante método coprológico directo y de flotación REVISTA SPEI DOMUS 2009; 5 (10): pp36.
- [4] Gallego J. (2006) Manual de parasitología, Edit. Universidad de Barcelona, España., pág. 36.
- [5] Cordero C, Vásquez R. (1999). "Parasitología Veterinaria". Edit. Interamericana. Madrid, España, pp. 636, 637.
- [6] Manual MERCK of veterinary. 5ta Edition. Océano. Barcelona, España. (2000) p. 348, 349.
- [7] Seguel, M., Gottdenker, N. The diversity and impact of hookworm infections in wildlife. Int. J. Parasitol. Parasites Wildl. 2017; 6: 177–194. <https://doi.org/10.1016/j.ijppaw.2017.03.007>.
- [8] Basso W, Spänhauer Z, Arnold S, Desplazes P. *Capillaria plica* (syn. *Pearsonema plica*) infection in a dog with chronic pollakiuria: challenges in a diagnosis and treatment. Parasitol Int 2014; 63(1):140–142. <https://doi.org/10.1016/j.parint.2013.09.002>

- [9] Chang S, Lim J.H., Choi, D., Park, C.K., Kwon, N.H., Cho, S.Y. et al. Hepatic visceral larva migrans of *Toxocara canis*: CT and sonographic findings. *AJR*. 2006; 187: W622–W629.
- [10] Jiang, P, Zhang X. Liu R. D, Wang Z.Q, Cui J. A Human Case of Zoonotic Dog Tapeworm, *Dipylidium caninum* (Eucestoda: Dilepidiidae), in China. *The Korean Journal of Parasitology* 2017; 55(1): 61–64. <http://doi.org/10.3347/kjp.2017.55.1.61>
- [11] Narasimham MV, Panda P, Mohanty I, Sahu S, Padhi S, Dash M. *Dipylidium caninum* infection in a child: a rare case report. *Indian J Med Microbiol* 2017; 55(1): 61–64.
- [12] Kirkbride J.H. and Dallwitz M.D. *Cucumis* and *Cucumella* (Cucurbitaceae): Cucumbers and Melons, *Systematic Botany and Mycology Laboratory* 2016; 1: pp.1.
- [13] Barrietos L. *Fito fármacos chilenos* (en línea). Chile, Universidad de Chile, 2001.
- [14] Bayat A, Jamali Z, Hajianfar H, Heidari Beni M. Effects of *Cucurbita ficifolia* Intake on Type 2 Diabetes: Review of Current Evidences, *Shiraz E-Med J*. 2014; 15(2):e20586. doi: 10.17795/semj20586.
- [15] Castillo-Cuenca J, Iannacone J, Fimia R, Cepero O, Morales O. Prevalence and risk factors associate with *toxocara canis* and *ancylostoma caninum* infection in companion dogs. *The Biologist (Lima)* (2016).. 14(1), jan-jun: 103-108.
- [16] Granados I. (2004). Evaluación del efecto desparasitante de un producto natural a base de apazote (*Chenopodium ambrosioides*) semillas de ayote (*Cucurbita pepo*) y flor de muerto (*Tagetes erecta*) al ser comparado con productos comerciales, en dos grupos caprinos en la ciudad de Guatemala. Tesis de grado, Universidad San Carlos de Guatemala, pp 79.
- [17] Muñoz M. Rodríguez. Evaluación del efecto de un desparasitante natural, contra nematodos de aves de traspatio, comparado con un desparasitante comercial, en la aldea el paraíso, municipio de Palencia, Guatemala. Tesis de grado, Universidad San Carlos de Guatemala, 2004; pp 60.