Results And Epidemiological Impact of The Directly Observed Antiparasitic Treatment For Canine Echinococcosis.

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Abstract

Cystic echinococcosis (CE) continues to be one of the main health problem in cattle regions. Controlling the disease requires new therapeutic strategies. The objective was evaluated the results of an intervention program based on the directly observed antiparasitic treatment to dogs in an endemic area of CE.

Methods: A quasi-experimental study of a single cohort of dogs from a CE endemic area was conducted. To identify infected dogs, stool samples were analyzed for coproantigens by enzyme-linked immunosorbent assay, before and after the direct and observed administration of praziquantel 5 mg per kg orally every 30 days for 3 consecutive months once a year for two years. Results: A total of 252 dogs entered the study, of which 119 (47%) dogs presented positive results for *E. granulosus* at the beginning of the intervention. The adverse effects that occurred with the administration of praziquantel were vomiting (13%), diarrhea (2.4%) and lethargy (2%); 229 dogs completed the 2 treatment cycles with praziquantel. The presence of some positive coproantigen was evident in 03 (1.3%) dogs after the intervention. In conclusion: Antiparasitic chemotherapy administered directly to dogs was able to reduce the prevalence of canine echinococcosis after 2 years of intervention.

Introduction

Echinococcus granulosus is the causal agent of cystic echinococcosis (CE), it is located in the small intestine of the definitive host (the dog), this adult parasite can eliminate up to a quarter of a million eggs during its entire life (Craig et al., 2019; Boufana et al., 2015). Cattle that are fed grass contaminated with dog feces have a high probability of becoming infected. The eggs enter orally and in the stomach intestine the dissolution of the outer covering occurs, releasing the oncosphere that migrates to the visceral tissues and there the cysts develop. The dog is reinfected by ingesting the raw viscera of infected sheep, and humans are accidentally infected by ingesting the eggs released by the parasitized dogs (Merino et al., 2017; Quispe et al., 2018). CE is common in the population with poor hygienic habits, poor sanitary services, lack of drinking water, poor disposal of excrement and litter (Pavletic et al., 2017).

In many rural areas of Peru, dogs are allowed to feed on infected viscera, creating the ideal conditions for the persistence of this disease⁶ and in these rural areas, the prevalence of canine infection can reach up to 50% (Larrieu et al., 2019; Montalvo et al., 2018; Larrieu et al., 2014).

Programs that attempted to control canine echinococcosis with the massive administration of praziquantel have managed to reduce the prevalence of CE from 22.1 per 100,000 to 6.2 per 100,000, with persistence of high rates of transmission in some focal areas^{8,9}. While, in other areas, studies have obtained disappointing results, because it has not been possible to raise awareness about the need for canine prophylaxis and the lack of permanent control programs (Van et al., 2015; Farias et al., 2004; Andersen et al., 1991; Zhang et al., 2015).

Directly Observed Treatment is the act of administering the antiparasitic drug, observing, in physical presence, how the dog tolerates it, subsequently controlling and inspecting that it has been ingested,

ensuring the correct dose and the correct moment.

Objective

This study was planned to evaluate the results and epidemiological impact of an intervention program based on the directly observed antiparasitic treatment for the control of canine Echinococcosis in an endemic area of CE.

Graph 1. Map of the intervention area for the evaluation of therapy administered directly with praziquantel to dogs, Junín-Peru.

Material And Methods

Population

Two communities are visited in the period May 2018 to December 2019: Usibamba and Chaquicocha, belonging to the San José de Quero district, Concepción province, located in central Peru at 4000 meters above sea level with temperatures ranging between -4 ° C and 16 ° C. These two communities have 2,193 inhabitants and are endemic to CE (Graph 1).

There is not enough information on the number of dogs living in the study area to evaluate the size of the sample, this was calculated on an unknown population with an expected prevalence of 28% of dogs infested with *E. granulosus* in endemic áreas (Acosta et al., 2014), 95% as confidence interval, 80% power and adding 10% for probable losses. The analyzed sample was 252 stool samples, using the proportions method with the statistical program Stata v. 13.1 (Stata Corporation, Texas, USA).

Design and Procedures

A quasi-experimental study of a single cohort of dogs from a CE endemic area was conducted. Before the intervention, the dwellings were randomly chosen by strata according to the proportion of inhabitants of each community and each street, with the support of the demographic survey of the municipality of San José de Quero, satellite images and a geographical map.

All the dogs present in each selected house were sampled, if the occupants of a house were absent, refused to participate in the study or did not have a dog, we selected a neighboring house and previously asked about the presence or absence of dogs. The process continued until the sample size was completed. With prior informed consent to the owners, the information was collected using a structured and validated form. A Bulletin was delivered with information on the importance of deworming and the prevention measures for CE. Fresh stool samples were obtained, maintaining biosafety measures. The fecal samples were deposited in a bottle with 10% formalin and kept refrigerated at 4 ° C. Then, they were transported to the microbiology department of the Institute of Tropical Medicine - National University of San Marcos for microscopic identification. Fresh feces samples were also collected from the fields and the main square from the stray dogs in order to microbiologically compare the results.

The microscopic examination of fecal samples preserved in 10% formalin, the direct method was carried out, which consists of dissolving the sample well, keeping it at rest for 5 minutes and with the help of a Pasteur pipette, took a drop from the bottom and place it in a slide, protected with a coverslip and observed under a microscope at 10 and 40X magnification. The objective of this procedure was to identify the presence of other parasites.

The diagnosis of canine echinococcosis was made by analysis of coproantigens, using the commercial immunoenzymatic technique (ELISA) for *E. granulosus* (Cestodas Zoonoses Research Group, University of Salford) (Verastegui et al., 1992). The same technique was used for both stool samples.

Intervention

Due to the unevenness of the working area surface, an electronic scale was used to calculate the dog's weight, the owner loaded the dog on the scale, and then the difference from the owner's weight was subtracted to obtain the dog's weight. For stray dogs, the weight of the dogs was roughly calculated according to external characteristics.

Dogs received 2 cycles of treatment, with a 12-month interval between each cycle, in each cycle received three doses of praziquantel, one dose per month. The first treatment cycle began in May 2018 and the last cycle began in June 2019. Dog stool samples were collected at the beginning of the first cycle and at the end of the second treatment cycle.

Praziquantel 5 mg / kg was administered orally to dogs every 30 days for three consecutive months, every 12 months for two consecutive years, dogs received 6 doses in 2 years. The stray dogs found in the parks were also dewormed, the antiparasitic praziquantel was combined with bread or chicken, the stray dogs received a dose and frequency similar to other dogs, a photograph was selected in each deworming process to identify the dog. The administration of the medication went directly to the dog and under supervision, the medication was not delivered to the owner or to the health center.

During each home visit, the messages on prevention of CE were reinforced and the delivery of informative material, in addition, an antiparasitic card was given to the owner and it was marked each time the dog received antiparasitic treatment, in the same way that the information on the effects was obtained adverse presented by the dog. Not finding the owner, we visited him the next day to complete the canine deworming process.

One month after the last deworming cycle, in September 2019, stool samples were collected again in the same way as before the intervention and transferred to the microbiology laboratory to identify the parasite, maintaining the same detection technique for antigens.

Statistic analysis

Microsoft Excel for Windows was used for the double data entry process. STATA version 13.0 for Windows (STATA Corp, College Station, TX, USA) was used for the analysis. Initially, a description of the

demographic characteristics was tabulated according to the general characteristics of the dogs before the intervention. Categorical variables were compared using the Chi square test or Fisher's exact test as appropriate. While the numerical variables were compared by Student's t test. In addition, the mean of quantitative variables such as the age of the dog and the number of dogs per house was calculated for each group according to the positive or negative result of *E. granulosus*.

Results

A total of 252 samples of faeces from dogs chosen at random were analyzed before administering the antiparasitic, 119 (47%) dogs showed positive results for *E. granulosus* in the stool sample by detecting coproantigens; while 133 (53%) dogs showed negative results. Other parasites found on additional microscopic examination of feces at the beginning of the study were *Toxocara canis*, *Balantidium coli*, *Entamoeba coli*, *Blastocystis hominis*, *Endolimax nana*, *Entamoeba coli*, *Giardia canis*, *Taenia* spp eggs, *Fasciola hepatica*, *Urbanidum canppin*, *Dipylla hepatica*, *Urbanidum canppin*, *canppin*, *Ancylostoma* spp; 9 out of 10 dogs had at least one of these parasites.

The average age of the dogs was 33 months, the male sex was the most frequent, representing 72% of the dogs, and the number of dogs chosen was related to the proportion of inhabitants of each community. Overall, the mean number of dogs per household was 2.4; presenting the highest average in infected dog houses (2.5) compared to uninfected dogs whose average number of dogs per house was 2.2 ($p = 0.0025$).

There were 21 (8.3%) street or homeless dogs, of which 17 (80.9%) dogs tested positive for coproantigens ($p = 0.001$). There were 231 (91.7%) dogs owned, of which 102 (44.2%) presented positive results to the study of the coproantigen for *E. granulosus* (Table 1).

In total, 73 dogs (29%) have received antiparasitic treatment in the last year, of which 27 (36.9%) tested positive for echinococcosis infection. It is characteristic in these rural areas that dogs share their home with sheep farming, it was found that 79 (31%) dogs live with sheep.

The treatment administered was independent of the result of the coproantigen test obtained. All dogs received 01 cycle of antiparasitic treatment with praziquantel per year for two consecutive years, each cycle consisted of 03 doses with a difference of 30 days between each dose. Complications were reported: 13 (5.2%) dogs had vomiting after receiving the dewormer, 6 (2.4%) dogs had diarrhea, 5 (2%) had drowsiness or lethargy. However, the most serious complication was the death of 02 (0.8%) dogs 7 days after the first dose, these dogs came from the same house, it is very difficult to associate this death of dogs with Praziquantel.

During the follow-up, some dogs left the study because they died (2 dogs), the owners moved to another community (4 dogs), the owners refused to continue the study (3 dogs), the owner or dog was not found by Se observed more two-month-old children (6 dogs) and some stray dogs during follow-up (8 dogs) Figure 2.

After two years, 229 dogs completed the 2 cycles of praziquantel administration, then they were evaluated by studying stool samples to verify the presence of *E. granulosus* infection, the procedure was performed in the same way as before the intervention. From the program. Of the 229 samples analyzed, 03 (1.3%) dogs presented positive coproantigen after direct antiparasitic intervention, these 03 infected dogs were stray dogs, 02 came from Usibamba and one from Chaquicocha (Table 2).

Table 1. General characteristics of the dogs at the beginning of the intervention.

Characteristic	Total n = 252	<i>Positive echinococcus</i> 119 (47%)	<i>Negative echinococcus</i> 133 (53%)	p &
Average age*	33,4	33,8	33,1	0,87
Sex:				0,88
Male	183 (72,6%)	86 (46,9%)	97 (53,1%)	
Female	69 (27,4%)	33 (53,1%)	36 (46,9%)	
Provenance				0,011
Usibamba	141 (55,9%)	77 (54,6%)	64 (45,4%)	
Chaquicocha	111 (44,1%)	42 (37,8%)	69 (62,2%)	
Average dogs per household	2.4	2.5	2.2	0,025
Lifestyle				0,001
Street dog	21 (8,3%)	17 (80,9%)	4 (19,1%)	
Dogs with known address	231 (91,7%)	102 (44,2%)	129 (55,8%)	
Previous antiparasitic treatment				0,09
Yes	73 (28,9%)	27 (36,9%)	46 (63,1%)	
No	179 (71,1%)	92 (51,4%)	87 (48,6%)	
The dog lives together with the sheep.	79 (31,34%)	42 (53,2%)	37 (46,8%)	0,011
*Age in months. &The Fisher exact test was used to calculate the p-value in the case of categorical variables and the Student's t test for numerical variables.				

Table 2

Prevalence of dogs infected by *Echinococcus granulosus*: comparison before and after the intervention.

	Infected dogs before the intervention.	Infected dogs After the intervention.
Positive <i>Echinococcus</i>	119 (47,2%)	3 (1,3%)
Negative <i>Echinococcus</i>	133 (52,9%)	226 (98,7%)
Provenance		
Usibamba	77 (54,6%)	2 (66,6%)
Chaquicocha	42 (37,8%)	1 (33,4%)
Lifestyle		
Street dog	17 (14,3%)	3 (100%)
Dogs with known address	235 (85,7%)	226 (0%)

Discussion

CE continues to be considered a neglected zoonotic disease with great economic impact in rural áreas (Montalvo et al., 2017) and communities with a cold climate favor the viability of parasite eggs in the environment for several months (Schurer et al., 2015; Han et al., 2018). In some countries it is considered a re-emerging disease, because it is very difficult to control or eliminate, mainly in areas of difficult access, where social factors related to certain zootechnical practices are determining factors in the prevalence of this disease. The absence of sustained canine deworming programs, the dog's eating habits based on viscera and the delay in diagnosis are the most important factors that currently maintain the endemic disease in livestock regions.

In this study, direct administration ensured the correct dose and frequency or administering a second dose in case the dog vomits the antiparasitic in order to ensure compliance with the antiparasitic treatment, since the objective of the study was to evaluate the results of a program intervention based on antiparasitic therapy administered directly for the control of canine echinococcosis. The initial prevalence of 47% was evidenced and after the intervention, two years later, the prevalence was reduced to 1.3% in the two rural communities. Increasing the continuous cycles of canine deworming with praziquantel ensures a decrease in the prevalence of reinfection by *E. granulosus* after two years of intervention.

Massive treatment with praziquantel has proven its efficacy in some countries and in others not so much because of the different regimens they use, non-compliance with doses, non-supervision of drug administration, non-permanent and non-periodic programs. Many of these programs are costly in financial terms, but beneficial in terms of sickness expenses, but they are poorly evaluated in relation to impact (Irbabedra et al., 2016; Van et al., 2017; Craig et al., 2017).

Praziquantel is effective in the treatment of echinococcosis, reduces the burden of infection in dogs and indirectly in sheep, but does not protect against future reinfections if treatment is not continuous and cyclical, which may explain the low decrease in prevalence in places where deworming is of short duration and the schemes are prophylactic and discontinuous.

The use of praziquantel as preventive chemotherapy, that is, to administer periodic treatments in populations at risk (endemic areas), is approved by the World Health Organization to reduce the burden of disease and parasite biomass and thus prevent the hosts from infected develop chronic disease and achieve cure of mild symptoms (WHO 2006; Zhang et al., 2007; Quedraogo et al., 2016). Although reinfection can occur after treatment, this procedure is still considered the main effective measure in the fight against echinococcosis.

Praziquantel is the treatment of choice against *Echinococcus*, it seems to be a safe drug with good tolerance levels, safety, easy to administer, but with little effect in young stages of the parasite, with some adverse effects evidenced in the study such as vomiting, diarrhea and lethargy, which were self-limited (Yue et al., 1985; Sabah et al., 1986); However, the efficacy of praziquantel depends on the host's immune response, and the severity of side effects may increase in hyperinfested dogs after administration of the antiparasitic causing a severe inflammatory response (Xiao et al., 2018), this may explain the death of two dogs that received praziquantel in the first dose.

Attempts to eliminate canine echinococcosis have been going on for years and effective control has yet to be achieved. However, some countries have reduced infection in dogs to undetectable levels thanks to the sustained effort of programs based on continuous deworming and health education aimed mainly at groups directly related to the transmission of the disease.

Some limitations found in the study are that the positive coproantigen test used does not usually detect low parasite loads in feces (Allan and Craig, 2006), so there may be dogs infected with negative coproantigen; However, to compare the response to the antiparasitic, the same test was used before and after the administration of praziquantel, the possibility of future intervention studies using molecular diagnostic methods remains open.

The advantages of this interventional study is that the surveillance of infections in dogs was not invasive, since the fresh stool samples were collected from the ground or with rectal swabs when the samples were not possible (Pierangeli et al., 2010). This control program only was applied in two rural communities and we do not have data to evaluate the gradual response after the two years of intervention, because the study ended, requiring the application of the program on longer and sustained time scales to evaluate response rates and reinfection.

Conclusions

Antiparasitic chemotherapy administered directly to dogs on a sustainable basis and managed to reduce the prevalence of canine echinococcosis and is considered as the main control measure due to its

efficacy.

Declarations

Contribution roles: RM, AH, JB, VN, PQ, MC, JM, SO: Conceptualization, Methodology, software, Validation, Formal analysis, Research, Resources, Data curation, Writing- Preparation of the original draft, Writing- Review and editing, Visualization, Supervision, Project management.

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Ethics approval: For the present study, the International Ethics Guide for Epidemiological Studies, prepared by the Council for International Organizations of Medical Sciences (CIOMS) in collaboration with the World Health Organization (WHO) in Geneva, 2009 was taken into account. ethical principles of epidemiological studies involving human beings, especially to safeguard the confidentiality and veracity of the data. Likewise, all procedures conducted in this study were reviewed and approved by the ethics and research committee of Hospital Carrion, Huancayo, Peru under protocolo number 039-2017-OCEI-HRDCQDAC.

Conflict of interest All authors declare that they have no conflict of interest.

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Figures

Figure 1

Map of the intervention area for the evaluation of therapy administered directly with praziquantel to dogs, Junín-Peru.

Figure 2

Recruitment and follow-up of the dogs that entered the study.

Figure 3

Graph N ° 2 Percentage of dogs infested by *Echinococcus granulosus* before and after the intervention, San José de Quero district, Concepción - Perú.