ACTIVITY OF A PASTE CONTAINING ALBENDAZOLE AND TRICHLORPHON AGAINST SMALL STRONGYLES (*CYATHOSTOMINAE*) AND OTHER EQUINE PARASITES.

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SUMMARY: Based on the mean of three consecutive faecal egg counts (EPG), fourteen male adult crossbred equines were selected and distributed into two groups of seven. One group was given an oral paste combining trichlorphon (25.0 mg/kg) and albendazole (10 mg/kg) (PVN 085 - VALÉE S.A.), while the other one, the control group, received oral placebo. Faecal EPG, coprocultures and biochemical analysis (alanine transferase, aspartate transferase, alkaline phosphatase, creatinine, urea and glucose) were performed 0, 1, 3, 5 and 7 days after treatment. All animals were necropsied on the seventh day and helminths were collected, counted and identified. The following post-treatment reductions in faecal EPG were displayed by the drug combination: 61.96%, 100.00%, 100.00%, 97.71%. Post-mortem examination showed that the albendazole and trichlorphon combination was 100% effective in removing *Strongylus edentatus*, *Triodontophorus* and *Oxyuris equi*; 75.15% and 42.71%, respectively, in removing *Habronema muscae* and *Gasterophilus nasalis*. With respect to small strongyles (Cyathostominae), drug efficacy was above 97% against *Cylicostephanus minutus*, *C. calicatus*, *C. longibursatus*, *C. goldi*, *Cylicoclyclus insigne*, *C. leptostomus*, *C. nassatus*, *Cyathostomum labiatum*, *C. labratum*, *C. coronatum*, *C. pateratum*, *C.catinatum*, *Gyalocephalus capitatus* and *Cylicodontophorus euproctus*. The combination was not effective against immature forms of cyathostomes (8.68% reduction). Haematological analysis did not reveal alterations compatible with any toxic effect of drug combination.

KEY WORDS: Albendazole, triclorphon, nematodes, cyathostomes, equine.

INTRODUCTION

According to LICHTENFELS (1975), 56 out of the 75 equine parasite nematode species belong to the Strongylidae family. 41 of them, distributed in eight genera, are small strongyles (Cyathostominae).

There has been an increasing scientific interest in cyathostomes in recent years, with particular attention to clinical parasitism (REINEMEYER, 1986). Different authors consider this group of nematodes as the major cause of equine colic of parasitic origin (HERD, 1990; UHLINGER, 1990; HERD, 1991). As these small strongyles encyst in the host's large intestine wall (larval cyathostomiasis) they become more relevant to this pathology. Faecal egg counts in these cases may be zero, even though the host might be dying of colic (HERD, 1990; 1991).

Many anthelmintics proved to be ineffective against cyathostomes, in particular in larval stages. Both resistance to drugs and the encysting capacity of small strongyles has, thus, made this equine parasitism difficult to treat. (EYSKER *et alii*, 1988; HERD, 1991; KLEI, *et alii*, 1993; XIAO *et alii*, 1994).

The main chemical groups of anthelmintics used in the treatment of equine nematodes are: benzimidazoles, probenzimidazoles, organophosphates, pirimidins and avermectins (SLOCOMBE *et alii*, 1982; DUNCAN, 1983; REINEMEYER, 1986; HERD, 1991). The use of albendazole in equines is relatively less known than that of other benzimidazoles. A few critical tests showed that albendazole can have an effective action upon both large and small strongyles when they are not in their larval stages (COLGLAZIER *et alii*, 1977; DRUDGE *et alii*, 1984;

EGRI & SÀRKOZI, 1990). Biochemical tests and clinical examinations have shown that albendazole is somewhat toxic to equines (EGRI & SÁRKOZI 1990).

Trichlorphon, the organophosphate included in the paste, has been widely used in combination with benzimidazoles, mainly for removing *Gasterophilus* larvae (DUNCAN, 1983; HONER & BIANCHIN, 1985).

The purpose of this study was to evaluate the efficacy of the albendazole and trichlorphon association, in paste, for the removal of nematodes, cyathostomes in particular, *Gasterophilus* larvae, as such information cannot be found in the Brazilian literature.

MATERIALS AND METHODS

Fourteen male equines of mixed breed, five to seven years old were selected based on worm egg counts per gram of faeces (EPG) and coprocultures. Animals were randomly allocated into two groups: the control group was given oral placebo, and the test group was given an association of albendazole and trichlorphon in paste at 10.0 mg and 25.0 mg/kg of body weight, respectively. Fecal samples were collected for EPG counts (GORDON & WHITLOCK, 1939) and coprocultures one, three, five and seven days after the medication. Infective larvae were obtained and identified as described by THIEPONT *et alii* (1979). Faeces eliminated during the first seven days after medication were individually examined for *Gasterophilus* larvae. Blood samples were taken from all animals for alanine transferase, aspartate transferase, alkaline phosphatase, creatinine, urea and glucose determinations. Blood samples were tested in accordance with Labtest-Sistemas Diagnósticos Ltda. and spectrophotometric readings (Coleman, 295-Perkin-Elmer model, SP).

All animals were necropsied seven days after treatment. Stomach, small intestine, caecum, colon and rectum were carefully opened, their contents collected and the mucosa was scraped. *Gasterophilus* larvae were collected "in totum". Search for parasites was performed with 10% of the total contents and scrapings for each organ. Qualitative and quantitative parasitologic tests were performed in each organ. Specific diagnosis for those helminths collected, particularly cyathostomes, was performed according to taxionomic criteria established by YAMAGUTI (1961), LICHTENFELS (1975) and LANFREDI (1983).

For the purpose of statistical analysis data related to counts and EPG reduction were transformed into x+0.5 and Arc. sen %, respectively. For the purpose of variance analysis the F test was used for treatment effects, and Tukey test was used to compare treatments (PIMENTEL GOMES, 1987). Anthelmintic efficacy of the drug combination was calculated comparing geometric means transformed into log (x+1) of helminths collected from necropsied animals (DUNCAN *et alii*, 1988).

RESULTS AND DISCUSSION

Chronological testing results left no doubt about the anthelmintic effect of the drug combination used (Table 1). Except for the first day after treatment, the other dates (3rd, 5th, 7th, and mean) presented statistically significant reduction in EPG counts for the treated animals ($p<0,01$) when compared to the control group. Albendazole + thichlorphon showed the following reduction in EPG counts expressed as percentages in the respective dates: 61.96; 100.00; 100.00; and 97.71%.

Coproculture tests showed a marked dominance of small strongyles larvae in all animals and in all dates in

Table 1 - Mean values and results obtained in the variance analysis por faeces EPG counts, number of nematodes collected in necropsy (transformed in to $\sqrt{x+0.5}$)and in EPG reduction percent (transformed into Arc sen $\sqrt{\%}$).

TREAT-MENTS /GROUPS	FAECAL EPG COUNT			EPG REDUCTION		Number of nematodes collected		
	Before Treat.	1 Day Post-treat.	mean EPG Post-treat.	1 Day Post-treat.	Mean Post-treat.	*Habronema muscae*	Cyathostos-tominae	TOTAL
Treated	42,2161a(1)	11,9906a	6,1499b	72,9996a	81,6070a	1,2058a	12,1750b	12,1929b
Control	41,8819a	20,0610a	16,6934a	59,8902a	66,3370b	2,1600a	134,8441a	134,9209a
F Teste	0,02 NS	2,52 NS	33,91**	3,14 NS	35,43**	3,43 NS	25,14**	25,17**
D. M. S.	5,9286	11,0617	3,9431	16,1207	5,5868	1,1227	53,2824	53,2739
C. V.(%)	12,11	59,29	29,66	20,84	0.06	05.7	62,26	62,21

1 Means followed by at least one common letter do not differ from each other according to the Tukey Test
NS Not significant at the level of 5% probability
** Significant at the level of 1% probability.
D.M.S. Minimum significant difference.
C.V. Variation Coeficient.

which EPG counts were positive for Strongilydea eggs (YAMAGUTI, 1961).

Necropsy findings showed that cyathostomes markedly prevailed over other nematodes diagnosed. Out of the 146,550 specimens found, 143,070 were small strongyles, i.e., this group represented 97.62% of the parasite infection. It is thus evident that in equines of this age and raised under the prevailing conditions and possibly in many others regions of the country, cyathostominae are, quantitatively, the major group of parasitic helminths. Several studies carried out in other countries also point to the relevance of the small strongyles (REINEMEYER, 1986; HERD, 1990; HERD, 1991).

Based on those taxionomic criteria established by LICHTENFELS (1975) and LANFREDI (1983), this study identified 14 species of cyathostominae: *Cylicostephanus longibursatus, C. goldi, C. minutus, C. calicatus, Cylicocyclus insigne, C. nassatus, C. leptostomus, Gyalocephalus capitatus, Cylicodontophorus euproctus, Cyathostomum labiatum, C. labratum, C. coronatum, C. pateratum, C. catinatum.*

Fig. 2 shows that, apart from immature forms, the drug combination used showed high efficacy (> 97%) against all species of small strongyles diagnosed in the 14 necropsied animals. Such results are in agreement with those obtained by DRUDGE *et alii*, (1984).

The poor efficacy of the drugs used, albendazole in particular, against immature forms of cyathostominae, was also observed by COLGLAZIER *et alii*, (1977), DRUDGE *et alii*, (1984) and by EYSKER *et alii*, (1988). It seems evident, therefore, that this benzimidazole lacks action solely on immature stages of small strongyles, and it is also uneffective against larval cyathostomiasis. However, further studies should be performed and specially designed to shed more light on albendazole action upon small strongyles larvae.

As to other nematodes diagnosed (Fig. 1), the drug mixture assessed, in the used dose, showed high effectiveness (>90%) against *Trichostrongylus axei, Strongylus edentatus, Craterostomum acuticaudatum, Triodontophorus* and *Oxyuris equi*. The parasite's reduction produced by the mixture against *Habronema muscae* (75.15%) was not statistically significant at the level of 5% probability (Table 1). Briefly, general efficacy results, particularly those related to adults Cyathostominae, clearly demonstrated that the combination of active ingredients (albendazole and thichlorphon) used has a therapeutic value (> 97%) against equine helminthosis in Brazil.

The degree of efficacy presented in this study resulted from the following formula: 100x (C-T)/C, where C and T

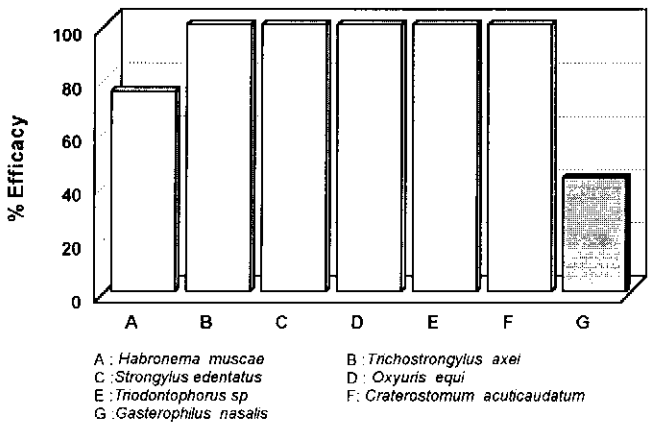

Fig. 1 - Percent reduction (efficacy) of nematodes and *Gasterophilus nasalis* larvae collected from equines treated with Albendazole + Trichlorphon, and from untreated animals (control group).

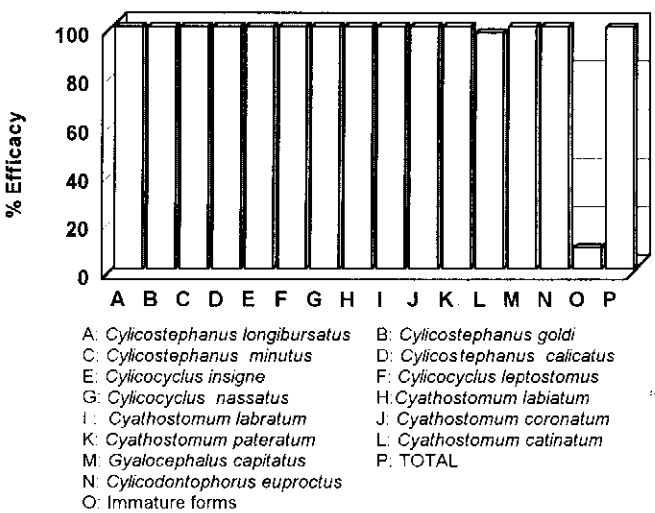

Fig. 2 - Percent efficacy of the Albendazole + Trichlorphon combination against "small strongyles" (Cyathostominae) collected from necropsied equines.

are the geometric means of the numbers transformed into log (x + 1) of nematodes in the control group and in the treated group, respectively (DUNCAN *et alii*, 1988).

The trichlorphon dose used (25.0 mg/kg) was not effective (42.71%) to remove *Gasterophilus nasalis* larvae from the treated animals. It is, probably, necessary to increase the dose of this organophosphate in order to obtain such effect.

With respect to the biochemical tests performed, no changes were detected that could suggest toxicity resulting from the drug combination. This finding is reinforced by the results obtained by EGRI & SÁRKOZY (1990), who did not observe clinical or biochemical alterations in equines given as much as five times the therapeutic dose (10.0 mg/

kg) of albendazole.

SUMÁRIO

Com base na média de três contagens de ovos por grama de fezes consecutivas (OPG), foram selecionados quatorze machos equinos adultos mestiços e distribuídos em dois grupos de sete. Um grupo recebeu uma pasta oral que combinava triclorphon (25,0 mg/kg) e albendazole (10 mg/kg) (PVN 086 - VALÉE S.A.), enquanto o outro grupo, o de controle, recebeu placebo via oral. Análises de exames de fezes (OPG), coproculturas e bioquímicas (alanina transferase, aspartato transferase, fosfatase alcalina, creatinina, uréia e glicose) foram realizadas 0, 1, 3, 5 e 7 dias após o tratamento. Todos os animais foram necropsiados no sétimo dia e os helmintos foram coletados, contados e identificados. As seguintes reduções pós-tratamento nos exames de fezes OPG foram obtidas com a combinação das drogas: 61,96%, 100,00%, 100,00%, 97,71%. Exames post-mortem mostraram que a combinação de albendazole e triclorphon teve uma eficiência de 100% na remoção de *Strongylus edentatus*, *Triodontophorus* e *Oxyuris equi*; 75,15% e 42,71%, respectivamente, na remoção de *Habronema muscae* e *Gasterophilus nasalis*. Com relação a pequenos estrongilídeos (Cyathostominae), a eficácia da droga foi acima de 97% contra *Cylicostephanus minutus*, *C. calicatus*, *C. longibursatus*, *C. goldi*, *Cylicocyclus insigne*, *C. leptostomus*, *C. nassatus*, *Cyathostomum labiatum*, *C. labratum*, *C. coronatum*, *C. pateratum*, *C.catinatum*, *Gyalocephalus capitatus* e *Cylicodontophorus euproctus*. A combinação não foi eficaz contra formas imaturas de ciatóstomos (redução de 8,68%). A análise hematológica não revelou alterações compatíveis com qualquer efeito tóxico de combinação de drogas.

PALAVRAS-CHAVE: albendazole, triclorphon, nematóides, ciatostomíneos, equino.

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(Received 19 September 1995, Accepted 09 November 1995)