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Effect of closantel combined with fenbendazole on the eggs of gastrointestinal parasites in horses

Efecto del closantel combinado con fenbendazol sobre huevos de parásitos gastrointestinales en caballos

Cerna-Adame Roberto*[1] ID, Barrón-Bravo Oscar[2] ID, Angel-Sahagún Cesar[1] ID, Hernández-Marín Antonio[1] ID, Arredondo-Castro Mauricio[1] ID, Avila-Ramos Fidel[1] ID **

[1]División Ciencias de la Vida, Universidad de Guanajuato, Programa Educativo de Medicina Veterinaria y Zootecnia. [2]Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias, Campo Experimental las Huastecas, Carretera Tampico-Mante km 55, Villa Cuauhtémoc, Altamira, Tamaulipas, México. CP 89610. *Author for correspondence and responsible of investigation: Fidel Avila Ramos. Departamento de Veterinaria y Zootecnia. Universidad de Guanajuato. División de Ciencias de la Vida, ExHacienda El Copal, Km. 9 Carretera Irapuato-Silao, CP. 36500, Irapuato, Guanajuato, México. b.etobeto@live.com.mx*, barron.oscar@inifap.gob.mx, csahagun@ugto.mx, jahmarin@ugto.mx, arredondo.m@ugto.mx, ledifar@ugto.mx**

ABSTRACT

Gastrointestinal parasites should be periodical controlled in horses to maintain their productive performance and adequate health. The objective of the research was to know the effect and safety of closantel combined with fenbendazole on the eggs of gastrointestinal parasites in horses. The drug combination was administered to 102 horses of different breed, age, sex and body weight at a dose of 10 mg per kg each drug. Physiological constants were measured before and 90 minutes after the drug was administered. Additionally, a sample was collected directly from the rectum of the horses during the physical examination to determine the presence and quantity of eggs. In positive animals, samples were taken 15, 30 y 45 days after treatment to determine the time that the administrated drugs can protect the horses. In the results, 53 horses were positive for parasites, the drug showed efficacy of 99% at 15 days after treatment and 89.79 at 45 days, in the 102 treated horses there was no effect on the physiological variables evaluated. It is concluded that the combination of closantel with fenbendazole at 10 mg per kg of body weight has efficacy of 99% post application and is safe for horses.
Keywords: Anthelmintics, endectocides, adverse reaction, gastrointestinal eggs, innocuous.

RESUMEN

Los parásitos gastrointestinales deben controlarse periódicamente en los caballos para mantener su rendimiento productivo y adecuado estado de salud. El objetivo de la investigación fue conocer el efecto e inocuidad del closantel combinado con fenbendazol sobre huevos de parásitos gastrointestinales en equinos. La combinación del fármaco se administró a 102 caballos de diferente raza, edad, sexo y peso corporal a dosis de 10 mg por kg cada fármaco. Las constantes fisiológicas se midieron antes y 90 minutos después de administrado el gel. Adicionalmente, se colectó una muestra de heces directamente al recto de los caballos durante la exploración física para determinar la presencia y cantidad de huevos. En animales positivos se tomaron muestras a los 15, 30 y 45 días posteriores al tratamiento para conocer el tiempo que pueden proteger a los caballos los fármacos administrados. En los resultados 53 caballos fueron positivos a parásitos, el fármaco presentó eficacia del 99% a los 15 días post tratamiento y del 89.79% a los 45 días, en los 102 caballos que recibieron el tratamiento no presentaron ningún efecto sobre las variables fisiológicas evaluadas. Se concluye que la combinación de closantel con fenbendazol a 10 mg por kg de peso corporal tiene eficacia del 99% post aplicación y no causa efecto negativo para los caballos independientemente del sexo y la edad.
Palabras claves: Equinos, antihelmínticos, endectocidas, reacción adversa, huevos gastrointestinales, inocuidad.

INTRODUCTION

Internal parasites in horses must be controlled with safe, innocuous and efficient formulations to maintain their productive performance. Parasitic resistance is a global problem, the parasites that affect horses are resistant to the main commercial chemicals used for their control (García *et al*., 2013). To increase the efficacy of drugs and reduce the number of parasites, alternatives should be sought (Kaplan, 2002; Matthews, 2014); one proposal is to combine active principles such as closantel, combined with fenbendazole.

Closantel belongs to the group of salicylanides, with the chemical formula $C_{22}H_{14}Cl_2I_2N_20_2$ and a molecular weight of 663.07 g/mol, its LD_{50} is 50 mg/kg IM via. It is administered at doses of 10 to 40 mg/kg to control *Strongylus edentatus*, *Strongylus vulgaris*, *Triodontophorus* spp, *Gasterophilus intestinalis*, and *Anaplocephala perfoliata* (Gokbulut and McKellar, 2018). In horses, the suggested dose is 10 mg/Kg orally with 40 days of withdrawal time (Guerrero *et al*., 1983; Guerrero, 1984). In the bloodstream, closantel binds to albumin and reaches its maximum concentration at 40 hours, its half-life is three weeks with oral bioavailability of 50%. The compound blocks oxidative phosphorylation at the mitochondrial level, preventing the availability of energy, causing the death of parasites (Hennessy and Ali, 1997).

Fenbendazole is a white powder with the chemical name 5- (phenylthio) -1H-benzimidazol-2-yl carbonic acid methyl ester, molecular weight 299.34 g/mol and its formula is $C_{15}H_{13}N_30_2S$ (Hennessy and Ali, 1997). It is used to control parasites in cattle, horses, pigs, dogs and cats. In horses it is indicated for large and small *Strongylus* spp and *Oxyuris equi*, it is recommended at a dose of 10 mg/kg orally for horses (Guerrero *et al*., 1983; Guerrero, 1984). The LD_{50} in horses is given when it exceeds 10 g/kg administered orally, it has no negative reports; however, hypersensitivity reactions may occur.

The objective of the present investigation was to know the effect and safety of closantel, combined with fenbendazole, at a dose of 10 mg/kg on the eggs of gastrointestinal parasites in horse feces.

MATERIAL AND METHODS

Place of study

The study was carried out in Guanajuato state, Mexico, in Irapuato municipality; located at 101° 20'48" West Longitude of the Greenwich meridian, at 20° 40'18" North Latitude; and in Pénjamo municipality, located at 101º 42 '22" West Longitude of the Greenwich Meridian and at 20º 25' 44" North Latitude; both locations at 1,730 m above sea level (INEGI, 2015).

Experimental units

Closantel was administered in combination with fenbendazole, at a dose of 10 mg/kg of body weight for each compound; its presentation in paste with applicator-dispenser for 600 kg of live weight to 102 horses (Productos Farmacéuticos S.A. de C.V.). The treated animals were of different breed, variable age from 1-30 years and different body weight, infested or not with gastrointestinal parasites in a natural way. The experiment was supervised and the protocol authorized by the Research Committee AG (OFAG02-2019).

Physical exam

All horses were approximated their dental age, a physical examination was carried out through their heart rate, respiratory rate, peristaltic movements and capillary filling time, before and 90 minutes after the drug was administered.

Drug administration

To administer the paste, the live weight of the horse was calculated in kilograms, with the morphometric procedure of Martinson *et al*. (2014). Subsequently, the Vermi-Horse Silver® applicator with the graduating ring was adjusted to the respective weight of the horse fasted to introduce the applicator into the interdental space. The paste was placed on the back of the tongue, lifting the horse's head for five seconds so that he could swallow the medicine.

Laboratory analysis

30 g of feces obtained directly from the rectum of the horses were collected to examine the presence or absence of gastroenteric parasite eggs through the count of eggs in the feces (CEF). Positive samples were counted for the number of parasite eggs in the stool, using the McMaster chamber procedure. The positive horses underwent three consecutive samplings after the application of the drug at 15, 30 and 45 days.

Statistical analysis

The amount of eggs found in the feces of the males were compared with those found in the feces of the females, using the test of independent means with the Student's t-statistic with the PROC TTEST procedure of SAS and a significance level of $P \leq 0.05$. To know the level of infestation and locality effect, a Pearson correlation was performed between the number of eggs and the locality, using the PROC CORR procedure of SAS at a significance level of $P \leq 0.05$. The physiological variables evaluated before and after applying the drug; as well as the amount of eggs in the stool at 15, 30, and 45 days, were compared with paired means with the Student's t test, using the PROC T procedure of SAS. All data were analysed with the statistical package SAS (2012).

RESULTS

In the investigation 102 horses were sampled; 53 animals tested positive for *Strongylus* Spp., and two horses of the 53 also presented *Parascaris* Spp. eggs, the prevalence was 51.9%. The presence of eggs in feces decreased due to the effect of the treatment ($P \leq 0.05$); in males, the efficacy was 98.87, 83.56 and 91.68% and in females 99.23, 93.11 and 87.90%, at 15, 30 and 45 days (table 1), respectively. Furthermore, the amount of eggs in fecal feces of females was higher compared to males ($P \leq 0.05$). The Irapuato horses presented more eggs (25.52) in the feces ($P \leq 0.05$), compared with the Pénjamo horses (10.58) (Table 1). The age of the horses did not show any relationship with the amount of eggs found in the feces (data not shown).

Table 1. Amount of gastrointestinal parasite eggs in g of feces in males and females at 0, 15, 30 and 45 days of the experiment (N = 53)

Sex	Day			
	0	15	30	45
Males (31)	14.35[b]	0.16	2.35	1.19
P*		0.0202	0.0415	0.0211
Females (22)	29.72[a]	0.22	2.04	3.59
P*		0.0096	0.0101	0.0051

*P= Probability.
[a-b]= Different literal in the same column indicates statistical difference, Tukey (P≤0.05).

In the physiological variables of the horses there were no changes due to the effect of the drug at 90 minutes after application (Table 2).

Table 2. Physiological variables of horses treated with oral closantel-fenbendazole paste at 90 minutes (N = 102).

Variable	**Measurement**		
	Initial *	Final**	Probability
Heart rate	40.0 ± 9.6	39.5 ± 8.1	0.3699
Respiratory rate	20.8 ± 9.4	21.1 ± 8.0	0.7195
Body temperature	37.8 ± 0.8	37.9 ± 0.8	0.0705
Capillary filling time	2.8 ± 0.6	2.8 ± 0.5	0.5663
Peristaltic movements	2.9 ± 0.4	2.9 ± 0.4	0.3197

* Initial = before drug administration.
** Final = 90 minutes after drug administration.

DISCUSSION

The control of gastrointestinal parasites in horses is a common activity integrated into the management calendar, but a diagnosis of the type and quantity of parasites in the locality or the place where the productive unit is located is not obtained (García *et al*., 2013). Bedoya *et al*. (2011) reported prevalence of 92% and Cala-Delgado *et al*. (2016) of 71%, high percentages compared to the results obtained in the study of 51.9%, but they are percentages close to those reported by Aromaa *et al*. (2018) of 57.6% in different breeds and types of equines. The variation in the prevalence and quantity of parasites found can be caused by the zootechnical purpose, the locality, type of feeding, sanitary management of the animals and the diversity of the parasites in the study areas. In the investigations the most reported genera are *Trichostrongylus*, *Trichonema* spp. and *Strongylus* Spp (Cala-Delgado *et al*. (2016). However, in the study carried out, all positive horses presented *Strongylus* Spp eggs, and only two horses also presented *Parascaris* Spp.
The study only has the purpose of knowing the effect of pasta on the number of eggs and the species of parasites were not considered, but two were identified.

In horses, the control of gastrointestinal parasites is carried out on a daily basis; Buzatu *et al*. (2015) determined prevalence greater than 50 parasite eggs per gram of feces in 137 horses out of 195 sampled. At 10 weeks after treatment, they determined that the horses were only protected for less than three months. Sanna *et al*. (2016) used macrocyclic lactones and benzimidazoles to evaluate their effect from day 0 to 150 days after their application. The results obtained to eliminate total parasite eggs were 99.7% and at 14 days 99%. Similar results show the study presented with the proposed combination at the dose indicated by the manufacturer.

The active principle used can determine the efficiency of treatment and protection time in horses. The treatment of choice are paste drugs with ivermectin, pyrantel pamoate, moxidectin with praziquantel; or its ivermectin-praziquantel combination. Caffe *et al*. (2018) evaluated ivermectin at a dose of 0.2% mg/kg as the treatment of choice with efficacy to reduce the fecal egg count of 99.8% in mares and 100% in fillies at 15 days, results similar to those found in the present study combining closantel-fenbendazole. In no horse was the age effect in the samplings on the amount of eggs in feces; the youngest horses were 1.5 to 2 years old and the older ones about 30 years old.

In pack-horses or draft horses the parasite incidence is higher, compared to animals used for sports, entertainment or therapy. Tedla and Abichu (2018) determined 72.2% incidence in pack animals, percentages higher than those found in our study, due to the work environment and type of care they receive. In the stables, canvases and police groups the animals have periodic care, this means that they have preventive medicine programs generally established but horses destined for field work do not receive the same care, their metabolic wear is greater due to their general living conditions. Therefore, it is common to find results that exceed those reported in the present study, due to poor management, working conditions and nutritional status; as well as the grazing carried out in pastures or workplaces, age of the hosts, and even race and sex can affect the results of studies (Caffe *et al*., 2018).

Horse sex can be a predisposing factor in describing and reporting results in equid investigations. Bedoya *et al*., (2011) report that the horse sex has no effect on the incidence of parasites; but our results indicate that females have 48% more parasite eggs per gram of feces, compared to males. Evident findings that may suggest that the number of parasite eggs may be related to the equine sex.

CONCLUSION

The efficacy found in the study confirms that closantel combined with fenbendazole at 10 mg per kg of each drug is 99.05% at 15 days, and its administration is safe for horses.

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