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Eimeria control in baby alpacas using toltrazuril as a prophylactic measure in humid Puna

Control de Eimerias en crías de alpacas con toltrazuril como medida profiláctica, puna húmeda

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Abstract

An effective concentration of toltrazuril to prophylactics in the control of eimerias and determine the prophylactic dose against eimerias species was evaluated in cria alpacas of the La Raya research center in Cusco Peru. Fifty cria alpacas of 3-4 months of age. distributed in 5 groups of 10 animals each: G_1 treated with 15 mg/kg of live weight (LW) of toltrazuril orally (VO), G_2 with 18.7 mg/kg BW/PO, G_3 with 22.5 mg/kg, G_4 with 30 mg/kg and G_5 without any dosage (control group). All dosage was given orally. Fecal analysis was performed using the modified McMaster technique at time 0 ad seven days later. Data were analyzed using analysis of variance and a Fisher test was done to determine statistical significance. The results indicate that using toltrazuril 15 mg/kg BW and 18.7 mg/kg BW, only small species such as *Eimeria punoensis E. alpacae* and *E. lamae* are controlled, but not the large species (*E. macusaniensis*) and with 22.5 and 30 mg/kg LW, in addition to controlling small species, the elimination of *E. macusaniensis* oocysts is also significantly reduced ($p \leq 0.05$). It is concluded that the prophylactic doses to control eimerias in baby alpacas is 22.5 and 30 mg/kg BW, with a single oral application, which manages to control the four species registered in this work.

Resumen

Con los objetivos de determinar la eficacia del toltrazuril como profilácticas sobre el control de eimerias y determinar la dosis profiláctica frente a las especies de eimerias en crías de alpacas situadas en puna húmeda, se realizó un estudio en el Centro de Investigación de Camélidos Sudamericanos La Raya Cusco Perú, se identificó 50 crías de alpaca de 3 a 4 meses de edad distribuidas en 5 grupos de 10 animales cada uno: G_1 tratadas con 15 mg/kg de peso vivo (PV) de toltrazuril por vía oral (VO), G_2 con 18.7 mg/kg PV/VO, G_3 con 22.5 mg/kg, G_4 con 30 mg/kg y G_5 sin dosificación alguna (grupo control). Se realizó el análisis coproparasitológico antes de dosificar (día 0) y a los siete días post-tratamiento. El análisis se realizó por el método de MacMaster modificado. Los datos se sometieron a análisis de varianza y a la prueba de Fisher. Los resultados señalan que utilizando 15 mg/kg PV y 18.7 mg/kg PV de toltrazuril se controla solamente especies pequeñas como *Eimeria punoensis E. alpacae* y *E. lamae*, mas no la especie grande (*E. macusaniensis*) y con 22.5 y 30 mg/kg PV además de controlar especies pequeñas también se reducen significativamente la eliminación de ooquistes de *E. macusaniensis* ($p \leq 0.05$). Se concluye que la dosis profiláctica para controlar eimerias en crías de alpacas fue de 22.5 y 30 mg/kg PV, con una sola aplicación por vía oral, logra controlar las cuatro especies registradas en este trabajo

Introduction

Alpacas are naturally distributed between 3600-5400 meters' altitude. In South America, more than a million small producers have alpacas and llamas, as their main means of subsistence; these animals provide meat, milk, fiber, transport energy, fertilizer, and they are also an important element of their cultural identity of the high Andean[1,2].

Parasitism becomes a common threat to the health and productivity of alpacas[3], they have had a direct impact on the economic income of farmers, some protozoa the genus Eimeria, these have affected the health of neonates, young alpacas from 2-3 weeks of age, causing the latter up to 80 % diarrhea[4], death 43.3 % of offspring between 1 to 2 months of age[5]. Among the species are small eimerias such as *Eimeria punoensis*, *E. alpacae* and *E. lamae*[6], large eimerias, such as *E. macusaniensis*, *E. ivitaensis* were also described, especially the former alone or associated with *E. lamae* or *E. ivitaensis* are considered highly pathogenic, *E. macusaniensis* being found in diarrheal processes that cause serious intestinal lesions in alpacas[7].

To avoid the consequences of eimeriosis, prevention is important, through management and hygiene, accompanied by the prophylactic use of like sulfonamides, ionophores, amprolium, halofuginone, toltrazuril, nicarbazine[8]. Dubey et al.[9] They mention that the efficacy of several drugs to treat clinical coccidiosis is unknown. And that no anticoccidial has a measurable effect on the late stages of gamontes and oocysts that have been commonly related to clinical coccidiosis associated with *E. macusaniensis* and *E. ivitaensis*. However, the use of preventive drugs was studied in alpacas such as decoquinate at 0.5 mg/kg LW for 28 days and amprolium at 5 mg/kg LW for 21 days in alpacas[10]. Toltrazuril was also used at low doses, as it is a drug that it acts on all intracellular stages of the parasite[11]. Similarly, Ballweber[10] performed treatments with 15 to 20 mg/kg LW of toltrazuril in alpacas, the response to the use of toltrazuril for other animal species is efficient, thus. In goats it is induced the complete elimination of eimerias oocysts 7 days after treatment with a single dose of 20 mg/kg LW[11]. In calves an anticoccidial response is also reported with 15 mg/kg LW[12] and 20 mg/kg LW of toltrazuril[13]. The same occurs in sheep at 20 mg/kg LW[14]. Because, toltrazuril acts against all intracellular development stages of coccidia, this drug interfering in the division of the nucleus, the activity of mitochondria and wall-forming bodies in microgametes, to result in the death of the organism[15,16] cataloged as a promising drug for the control of eimerias in different animal species.

Considering the antecedents described, the study was designed with the aim to determine the efficacy of toltrazuril as a prophylactic for the control of eimerias, to determine the prophylactic dose against eimerias species in baby alpacas in humid puna, because it has not yet. Reference data have reported effective doses of toltrazuril on the control of eimerias. And whose results will be useful in the prevention of coccidiosis.

Materials and methods

Place of study. The study has developed long the month of May 2017, at the South American Camelidae Research Center (CICAS) La Raya of the National University of San Antonio Abad del Cusco (UNSAAC) located at 4130 altitude meters. Located in Marangani, Cusco-Peru.

Animals. In this study. Has been used 50 baby Huacaya alpaca of 3 to 4 months. They had an average live weight (LW) of 10 kg. The animals were in apparent good clinical condition, all the animals belong to the CICAS “La Raya".

Figure 1 Oral application of toltrazuril

The 50 offspring have randomly distributed into 5 groups (4 treatments and 1 control). Each group had 10 animals. They have based on the doses of toltrazuril (mg/kg LW) administered orally (AO) (Figure 1). Group 1 G_1 were administered 15 mg/kg PV, group 2 G_2 18 mg/kg LW, group 3 G_3 22.5 mg/kg LW, group 4 G_4 30 mg/kg PV and group 5 G_5 control group without application of any placebo, but subjected to the same manipulation for the collection of samples. After the application of the drug, all the animals remained under the same management and feeding conditions, consuming native pastures of CICAS La Raya. The 15 mg dose recommended by the manufacturer of the drug (Tolcocx®) and the higher doses by experiences carried out by other authors[17] and by previous experiments.

The drug. Tolcocx®, Laboratorios Biomont Perú, with a chemical base of toltrazuril at 50 mg/mL, production date 07-2015, batch 074095. We assume this dose because there is not data about this use in the treatment of coccidiosis in camelids[10].

Collection of samples. The collection of fecal samples has made directly the rectal ampulla between 6 and 7 hour of day. This have done before to herding, consecutive to the administration of the drug (Figure 2). Samples were put into labeled polyethylene bags. This samples without the use of preservatives, their transfer was immediate for subsequent analysis on the same day of collection. Samples were obtained prior to treatment (day 0), to determine the initial burden of eimerias. And the effect of toltrazuril doses was corroborated, 7 days after treatment.

Figure 2 Collection of fecal samples

Sample analysis. The coproparasitological analysis was carried out in the Parasitology Laboratory of the School of Veterinary Medicine, Canchis Headquarters of the UNSAAC. To determine the amount of eimerias, the oocyst count per gram of stool (OPG) was performed, using the modified MacMaster technique using Sheather's solution and applying the correction factor of 100[18,19].

Statistical analysis. Average and standard error of the mean were used to describe the quantitative OPG data of eimerias. While to determine the effect of

toltrazuril doses in the control of small eimerias (*E. punoensis, E. alpacae, E. lamae*) and large eimeria (*E. macusaniensis*), the data were subjected to an analysis of variance. To discriminate the mean difference between treatment groups, Fisher's least significant difference (LSD) was performed. For the statistical analysis, the statistical package Statgraphics Centurion version 1820 was used.

Results

The effectiveness of toltrazuril doses on the oocyst amount of small and large eimerias in baby alpacas, these is illustrated in Table 1. It is observed that the amount of oocysts in both small and large eimerias before treatment (day 0) was similar (p> 0.05). At 7 days of treatment, the small eimerias (*E. punoensis, E. alpacae, E. lamae*) with doses of 15 mg/kg LW maintain a population similar to the control group (p> 0.05). While when applying 18.7 and 22.5 mg/kg LW the amount of oocysts decreased, being the value of 0 OPG with the dose of 30 mg/kg LW (p≤0.05). Regarding large eimeria (*E. macusaniensis*) at 7 days it is evidenced that there was a significant reduction in OPG with doses between 22.5 mg / kg LW and 30 mg / kg LW (P≤0.05).

Table 1 Effectiveness of toltrazuril doses on the oocyst count of small eimerias and large eimeria (mean±SE) in baby alpacas

Groups	n	Toltrazuril dose mg / kg LW	OPG Small eimeria		OPG Large eimeria	
			Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
			Day 0	Day 7	Day 0	Day 7
G_1	10	15.0	5520±1721	4680^{c} ± 1767	3620±1633	3080^{b} ± 1559
G_2	10	18.7	5280±2231	3050^{b} ± 2274	3950±2444	2810^{b} ± 2376
G_3	10	22.5	8570±3401	1200^{ab} ± 875	5710±3071	1200^{a} ± 875
G_4	10	30.0	7720±3267	0^{a} ± 0	2130 ±1310	0^{a} ± 0
G_5	10	**Control**	4970±2345	5110^{c} ± 1325	3390±2386	2710^{b} ± 1105

Table 2 Presence of eimerias species, in oocysts per gram of feces (OPG), in baby alpacas before and after treatment according to toltrazuril dose

Especies	Before treatment (OPG)	After treatment (OPG)				
	Average	Toltrazuril dosage (mg/kg PV)				Control
		15	18.7	22.5	30	
Small eimerias						
E. punoensis	1936	460	240	0	0	1330
E. alpacae	60	460	0	0	0	0
E. lamae	652	755.56	0	0	0	800
Big eimeria						
E. macusaniensis	3760	3080	2810	1200	0	2800
Summation	**6408**	**4755.56**	**3050**	**1200**	**0**	**4930**

The presence of eimerias species, in alpaca offspring before and after treatment according to toltrazuril dose are presented in Table 2. It is evidenced that of the total of baby alpaca examined prior to treatment, 94 % were parasitized (47/50), with an average of 6408 OPG of *Eimerias* spp. With a more predominance of *E. macusaniensis* than rest (P≤0.05). And the presence of oocysts of *E. ivitaensis* was not evidenced.

The efficacy of toltrazuril on the species of eimerias, in Table 2. It was observed that when orally administering 15 mg/kg LW of toltrazuril, the oocysts of *E. punoensis* are in lower quantity than control group, but the species of *E. alpacae, E. lamae* and *E. macusaniensis* are not affected. Considering an insufficient dose for their control. By applying 18.7 mg, the number of oocysts is reduced in all species, but the high number of 2810 oocysts of *E. macusaniensis* is still maintained. The effect of 22.5 mg/kg PV reduces the presence of small eimeria oocysts to zero. However, *E. macusaniensis* is present with 1200 OPG, an amount that is reduced to zero by providing 30 mg/kg PV, which is similar in the rest of the species.

Discussion

The efficiency of the toltrazuril dose to the control eimerias in baby alpacas, is observed in Table 1, 15 mg/kg LW. There was not reduction in the amount of oocysts. These is referring to what was observed in the control group. Despite the fact that Ballwever[10] reports this amount as a preventive dose for cattle, as well as the manufacturer suggests it for cattle (Tolcocx®, Laboratorios Biomont Perú). The difference in its effect on alpacas, because it is another species. That is due to the different physiological mechanisms that arise in each animal species[21].

Related to effect on small eimerias. Doses of 18.7 and 22.2 mg/kg LW produce more reduction in oocysts than control group. Same to 20 mg/kg, dose was recommended to pigs for Ballweber[10]. Also, Sánchez et al.[13] to cattle. And for goats Iqbal et al.[11] Meanwhile. To control large eimerias like *E. macusaniensis*, dose of 18.7 mg/kg LW was insufficient. Possibly due to the resistance of its thick wall[22] face several chemical agents[17]. But, with 22.2 and 30 mg/kg LW it is possible to control large eimerias, which are considered the most pathogenic for alpaca babys[23].

With reference to the effect of the drug among eimerias species, 94 % (47/50) of alpaca offspring have the presence of some eimeria species, a higher percentage than that reported by Salazar Robayo[24], Camareno et al.[25] and Kultscher et al.[26] who mention values of 70.7, 52.4 and 75.1 %, respectively, which is probably due to the fact that these authors worked with older animals. Before treatment, the average the four species of eimerias found in baby alpacas was 6408 OPG, a figure higher than that reported by Quina Quina[27] who reported 3883 OPG of eimerias in 3-month-old alpacas, a difference that we attribute to the inclusion in their work in adult animals, the presence of eimerias is lower than those found in offspring[28].

The preponderant eimerias are *E. macusaniensis* and *E. punoensis* that are found in 78 % (30/50) and 58 % (29/50) in the offspring before treatment, similar to Diaz[2] who report that *E. macusaniensis* is superior to the rest of species, in 38 % of three-month-old offspring, on the other hand Salazar Robayo & Galecio[24] mention 29.3 % in 5-month-old and adult alpacas as carriers, with respect to the amount Quina Quina[27] they found a maximum peak of 1304 OPG of *E. macusaniensis*. For their part, Camareno et al.[25] reported the prevalence of *E. macusaniensis* in alpacas from 5 to 12 months in relation to the rest of the species ($P \leq 0.05$) and among small species, 66.2 % *E. punoensis*, the same way Díaz et al.[2] found 78 % of offspring infected with *E. punoensis*.

It is important to distinguish the species of parasites, because they can be simultaneously infecting a host, and vary in pathogenicity in their biology[29], so we take as a reference for pathogenicity *E. macusaniensis*. This specie causes moderate to severe lesions

in the gut alpaca and can kill them with signs diarrhea[23]. The findings indicate that doses lower than 22 mg/kg LW were necessary to reduce of oocysts from small eimerias, but insufficient to control *E. macusaniensis* (Table 2). It could be due to the larger size and structures of parasite[22]. That does not allow the effect elicited in small species[17], this finding would indicate that higher doses of toltrazuril, than those recommended by Ballweber[10] (15-20 mg/kg/LW), are effective to control of eimerias. *E. macusaniensis* contrary to OPG from *E. alpacae, E. lamae* and *E. macusaniensis* are not affected. To date, no studies have yet been, reported regarding prophylactic doses of toltrazuril in alpacas, nor its effect on the different species of eimerias.

The present work reports that by applying 22.5 and 30 mg/Kg LW of toltrazuril in single doses. It acts as a prophylactic, managing to control elimination of oocysts of *E. punoensis, E. alpacae, E. lamae* and *E. macusaniensis*. The drug could be used to reduce the spread of eimerias and the presentation of eimeriosis in baby alpacas.

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Conflicts of interest

The authors declare that they have no conflicts of interest with respect to the research, authorship and/or publication of this article.

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Ethical considerations

The study had the approval of the Director of the Professional School Dr. Walter Bravo Matheus and Dr. Virgilio Alarcón (RIP), because at that time the Faculty of Agrarian Sciences of the San Antonio Adab University of Cusco did not yet have the organization of an Ethics committee.

Authors' contribution to the article

Sánchez-Herencia Diana, wrote the manuscript and the experimental part of the research. *Mamani-Mango Guiulfo*, performed the experimental design and writing of the manuscript. *Coila-Añasco Pedro*, carried out the writing and final revision of the manuscript.

Limitations in the research

There were no limitations for the development of the research.

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