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A PRELIMINARY STUDY OF THE BIOLOGICAL CONTROL OF STRONGYLES AFFECTING EQUIDS IN A ZOOLOGICAL PARK

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SUMMARY

The main goal in this research was to determine the beneficial effect of incorporating biological procedures to the parasite control programs on equids from Zoological Parks. Two trials were developed on *Equus quagga*, *E. asinus* and *E. africanus asinus*. The first (September 2010 to August 2011) consisted of chemotherapy (ivermectin + praziquantel) only, and the second (September 2011 to September 2012) in the administration of chemotherapy and chlamydospores of the nematophagous fungus *Arthrobotrys (Duddingtonia) flagrans*. The effect of these measures was evaluated by the estimation of the reduction in the faecal egg-counts (FECR).

In the first trial, 100% FECR values were achieved fifteen days after treatment in all the animals. The Egg Reappearance Period (ERP) was 2-3 months for the equids, and all of them were passing strongyle eggs in the faeces 2-4 months after their deworming.

In the second experiment, the FECR values were 100% in the three species. An ERP of 3 months in the European donkeys, 4 months in the Africans and 6 in the zebras was recorded. All the equids were positive to the coprological flotation test 4-8 months after the anthelmintic administration.

This preliminary study demonstrates the incorporation of chlamydospores of nematophagous fungus as *A. flagrans* appears highly promising to reduce the infective stages of the strongyles affecting captive animals, but the experimental design precludes true determination of whether the treatment is fully efficacious.

Keywords: equids, zoological park, strongyles, biological control, Arthrobotrys flagrans

1. INTRODUCTION

The role of zoological parks has changed significantly in the last few decades. The former objective consisting of displaying wild animals for visitor entertainment has been replaced by the conservation of endangered species, education, and research [1]. This change has required large modifications to zoological parks to ensure animals have an appropriate habitat, thus making breeding possible in many cases. Besides the transformation of the exhibits into more adequate enclosures, attention is also given to enrichment, motivating animals to search for food and explore their environment, and improved veterinary care. As a consequence, preservation of several endangered or vulnerable species (African wild ass (*Equus africanus asinus*), Dromedary (*Camelus dromedarius*), plains zebra (*Equus quagga*), and eland (*Tragelaphus oryx*)) has been successful [2].

Animals in zoological parks may be more likely to become infected by parasites if they are housed on land previously occupied by domestic animals [3, 4]. Grazing animals are at an elevated risk of parasitic infection because of the presence of oocysts, eggs, cysts or larvae in the soil and/or herbage, which can be ingested simultaneously when feeding on pasture [5, 6]. Helminths (cestodes, trematodes and nematodes) are commonly reported in grazing horses, especially strongylid nematodes [7, 8]. Their generalized life cycle begins with the release of unembryonated eggs by adult worms located in the gastrointestinal tract, which are passed in the faeces. After their embryonation in the faecal pat, eggs hatch to first-stage larvae (L1), then moult into second-stage larvae (L2) and finally to third-stage larvae (L3), the infective phase [9]. Third stage larvae leave the faecal pats and move to adjacent plants, which are subsequently ingested by the animals on pasture. Strongyles in the equids are responsible for significant inflammation, unexplained weight loss, diarrhea, poor hair coat, and in extreme cases death.

Control of parasites in horses is most commonly based on anthelmintic treatment, but the absence of useful measures against the free-living stages in the environment makes it difficult to clear the infection. This becomes very important if pasture rotation can not be observed, due to a horse infected with strongyles can be passing millions of eggs/ day in the dung.

Arthrobotrys (formerly *Duddingtonia*) *flagrans* is a predatory fungus very frequently found in natural and agricultural soils [10]. This fungus can live either saprotrophically or predatorily (in the presence of larval nematodes). *A. flagrans* is characterized for producing thick-walled chlamydospores in abundance, which are eliminated intact in the faeces of herbivores after passing through their gastrointestinal tract [11]. As a consequence, the fungus establishes in the environment and develops an extensive hyphal system, together with traps at intervals along the hyphae, with the purpose to capture larval nematodes for obtaining N and C.

The objective of this study was to investigate the effect of incorporating biological procedures to the parasite control programs for equids at a zoological park. In the first trial, an anthelmintic mixture was administered to the animals, whereas the second trial utilized anthelmintic treatment plus *A. flagrans* chlamydospores.

2. MATERIAL & METHODS

2.1. Marcelle Natureza Zoological Park

Marcelle Natureza is a 20 Ha zoological park located in NW Spain (Outeiro de Rei, Lugo) (43° 4' 14.71" N, 7° 37' 53.50" W). Collection animals live in fenced, semi-free ranging exhibits of various sizes. The animals are routinely dewormed in spring and autumn by adding granulated anthelmintic preparations to the concentrate (feedstuff) portion of the diet, on the basis of coprological determinations previously performed. Deworming is also provided when diarrhoea is observed, mainly from late spring to early autumn.

Removal of faecal material is performed daily in the paddocks, paying special attention to avoid or at least minimize their presence during the visiting hours.

2.2. Equids

Four equid species are maintained in the zoological park, including *Equus quagga* (plains zebra), *E. caballus* (Falabella miniature horse), *E. asinus* (European donkey) and *E. africanus asinus* (African wild ass). The Falabella miniature horse was not included in this study, as it is singly housed.

There are 3 adult zebras (3-10 yr; 1 stallion and 2 mares) housed in a 4038 m² meadow. The European donkeys (3-9 yr; 3 stallions and 3 mares) are housed in a 2015 m² pasture. The African wild assesses (3-6 yr; 1 stallion and 5 mares) are maintained in a 1000 m² parcel where sand is the main soil component throughout the year. For this reason, these animals are given herbage cut from areas both inside and outside of the park. All equids have access to pasture and are supplemented with pellets each two days. Water is available ad libitum.

2.3. *Arthrotrrys flagrans* (Af)

Chlamyospores were produced, harvested and managed in Petri dishes (9 cm diameter) containing wheat meal agar [12]. The medium was comprised of 20 g agar, 25 g wheat flour and 1 L distilled water.

2.4. Experimental design

Between September 2010 and September 2012, two trials were carried out. Faecal samples were collected directly from the soil of the paddocks where the zebras, European and African assesses were housed.

Excluding any type of immobilization of the animals due to their avoidance of stressful situations, faecal samples were collected from the soil. An equal number of stools to the animals in each enclosure were obtained. Collection of faeces was done early in the morning (0800-0900), prior to the daily cleaning of the paddocks by the animal keepers. A concise explanation about the need for taking the samples in different places of the paddocks was given to the keepers.

This experimental design makes difficult true determination of the treatment successfulness, because of the impossibility for having control animals under the same conditions than those receiving chlamydo spores with the feedstuff.

a) Anthelmintic only (September 2010 to August 2011)

In September 2010 and March 2011, after faecal analysis, the equids were dewormed by the application of ivermectin + praziquantel (Equimax[®], Virbac, Spain) at a dosage of 1.07 g gel / 100 kg body weight. Body weights were estimated by visual examination.

b) Anthelmintic + biological control (September 2011 to September 2012)

Anthelmintic therapy, as described previously, was administered to the equids in September 2011. *A. flagrans* chlamydo spores were then administered bi-weekly to the equids. By considering previous investigations [13], the total quantity of spores required for each individual was 2×10^6 *Af* chlamydo spores kg bw⁻¹. This was delivered orally by carefully dissolving the spores into 50 mL of water and then mixing with the feedstuff immediately prior to feeding the equids.

2.5. Faecal analyses

Faecal samples were applied in duplicate by using copromicroscopic techniques [14]. Five grams of faeces were analyzed by flotation to determine the presence of protozoan oocysts and eggs of cestodes and nematodes. The same quantity of faeces was processed by sedimentation to ascertain the existence of trematode infections. A McMaster technique with a sensitivity of 10 eggs/g faeces (EPG) was employed for the egg counting [14]. For determining the presence of lungworm infection, 10 g faeces were analyzed by means of the Baermann method.

Coprocultures were performed to determine the genera of strongyles affecting the equids [15]. Briefly, 20 g pools of the faeces were prepared from samples collected monthly from each enclosure. These were cultured at 22-24°C for 15 days, and then larvae were recovered by using the Baermann technique, and their identification done according to morphological keys [16, 17].

2.6. Efficacy of deworming procedures

The success of the two trials was determined by faecal analysis. Eggs per gram (EPG) values were determined on the day of treatment, 15 days after treatment, and then once per month. The Faecal Egg Count Reduction (FECR) was calculated by using the following formula [15]:

$$\text{FECR (\%)} = [1 - (\text{FEC}_{\text{post-treatment}} / \text{FEC}_{\text{pretreatment}})] \times 100$$

2.7. Climatic pattern

For gaining information about possible climatic variations between the two years of study, the values of maximal temperature, minimal temperature and relative humidity were monthly obtained from an automated meteorological station near (10 Km) to the Park.

2.8. Statistical analyses

The values of FECR were expressed as percentages (Table 1). ANOVA was utilized to examine total differences among the different equids. Differences were considered significant when $P < 0.05$.

3. RESULTS

3.1. Climatic pattern

As presented in Figure 2, a similar climatic pattern was established in the two years of study, and significant differences in the values of maximal temperature, minimal temperature and relative humidity were not observed.

3.2. Parasitological examinations

At the beginning of the study, strongyle eggs were observed in the faeces of the equids. No protozoa, trematodes, cestodes or lungworms were found in any of the examined stool samples. Specimens belonging to the genera *Cyathostomum sensu lato*, *Trichostrongylus*, *Gyalocephalus* and *Poteriostomum* were identified in the coprocultures.

3.2. Administration of anthelmintics only (September 2010 to August 2011)

As shown in Figure 3, egg-output values higher than 500 EPG were recorded in September 2010 for all the equids. The FECR showed a 100% efficacy 14 days after the administration of anthelmintics to the equids. The Egg Reappearance Period (ERP) was 2 months for the European donkeys, and 3 months for the African asses and the zebras (Table 1). All the equids were positive to strongyle egg-excretion 3 months after their deworming.

The EPG numbers increased to March 2011, and EPG numbers ≥ 500 were recorded again. Chemotherapy suppressed the egg-output in all the treated animals, and a 100% value for the FECR 15 days after treatment was achieved (Table 1). An ERP of 1 month for the African and the European donkeys, and 2 months for the zebras were observed (Table 1). All the African asses had strongyles in their faeces by 3 months after chemotherapy, while 2 months were required for the European donkeys and 4 months for the zebras.

The egg-output values rose markedly in the donkeys from May 2011 and peaked in July (Africans) or August (Europeans). A slight increase in the number of strongyle eggs in the zebras was noted, with the highest values in August (Fig. 3).

According to the equid species, statistical differences between the European donkeys and the zebras were observed ($F= 14.287$, $P= 0.001$).

b) Anthelmintic + biological control (September 2011 to September 2012)

After the application of anthelmintics and *Af* chlamydospores in September 2011, no strongyle eggs were observed in the zebras until March 2012 (ERP= 6 months) (Fig. 4), while all of them were passing eggs by faeces 8 months after the anthelmintic administration (May 2012) (Table 1).

The ERP was 3 months for the European donkeys and 4 months for the African asses. All the Africans were positive to the flotation 4 months after their deworming, while 5 months were required in the Europeans and 8 in the zebras (Table 1).

From October 2011 to September 2012, the egg-output counts ranged between 50 and 250 EPG for all the equids, except the European donkeys in February. No differences were recorded in the strongyle egg counts among the three equids ($F= 1.684$, $P= 0.190$).

Statistical differences between the egg-output in the two trials for the African asses ($F=18.114$, $P=0.001$), European donkeys ($F=18.194$, $P=0.001$) and zebras ($F=10.420$, $P=0.002$) were demonstrated. No differences regarding the age or gender were obtained.

4. DISCUSSION

Similar to that described in domestic animals, anthelmintic therapy is the main applied measure for the control of parasites in Zoological Parks. In the current study developed in Marcelle Natureza Zoological Park, deworming of equids by administering a granulated mix of ivermectin + praziquantel in the feedstuff provided a 100% efficacy. The strongyle egg-output appeared again 2-3 months later in the donkeys and in the zebras. These results agree with previous studies on domestic horses [18].

With the aim to achieve a more reasonable and efficient control of equine strongyles, selective treatment has been proposed for horses with faecal egg counts exceeding a predetermined cut-off value, as 300 EPG [19]. In this way, two additional dewormings seem required, in December 2010-January 2011 and in June-August 2011. Development of anthelmintic resistance has been associated to increase of deworming frequency [20], even in captive giraffes [21]. Reduced insect activity as a consequence of ivermectin administration to livestock has been demonstrated, which might retard dung degradation and cause the accumulation of non-degraded manure on pastures [22].

The findings of 100% FECR values, together with ERPs higher than 8 weeks, point the successful of the treatment in all the equid species, and highlight the absence of anthelmintic resistance [23]. Nevertheless, they appear to be at a high risk for reinfection by the infective (L3) larval stages in the soil, as demonstrated by the observation of elevated egg-output numbers 2-3 months after treatment.

Some measures have been suggested for controlling free-living stages, including rotation of pastures or regular collection of faeces. The first option may not be viable in a zoological setting, but as mentioned previously manure is removed daily by the animal keepers. In the last decade, research advancements have been made on reducing pasture contamination through biological control methods [24]. A second trial consisting of incorporating anthelmintic treatment and bi-weekly administration of chlamydo spores of *Arthrobotrys flagrans* was performed in the current work. An increment in the ERP with respect to the only application of chemotherapy was observed, especially for the zebras. The numbers of egg-output ranged between 50 and 250 EPG till the end of study, except for the African asses in February 2012. These findings are consistent with previous studies in horses, where by adding *A. flagrans* chlamydo spores to nutritional pellets, a significant reduction (35-73%) of the EPG was reported [25].

Contamination of paddocks by infective parasite stages depends on the numbers of animals passing eggs by faeces, the pasture surface, quantity of faeces and frequency of deworming. By considering the animals are confined to a paddock, reduction of parasitized animals should be taking into account when assessing the successfulness of control measures. After combining the effect of anthelmintic and fungal spores, a period of 4, 5 and 8 months for all the African asses, Europeans and zebras (respectively) were observed, and FEC counts lower than 300 were maintained. By comparison, all the African donkeys, Europeans and zebras were positive to the flotation test 2-4 months after the administration of anthelmintics only.

It should be emphasized that *A. flagrans* provides a prophylactic measure since its action focuses on free-living stages, and thus it should be combined with other strategies acting against the parasitic stages infecting herbivores, as chemotherapy [26]. Nevertheless, reduction of the presence of infective stages in the soil will help to lessen the frequency of deworming animals.

5. CONCLUSIONS

Equids maintained in zoological parks such as Marcelle Natureza share the same problems as horses regarding the control of strongyles. While effective anthelmintics are available, this is a temporary solution, and animals may be reinfected as soon as one month after treatment. This situation often worsens as pasture rotation is seldom possible, and thus the level of contamination increases. This underlines the need for appropriate treatment against the strongyle free-living stages.

The incorporation of chlamyospores of the nematophagous fungus *Arthrobotrys flagrans* to the feedstuff appears highly promising to reduce the infective stages of the strongyles affecting captive animals, but the experimental design precludes true determination of whether the treatment is fully efficacious. With the aim to enhance their utilization, some procedures are actually in progress for incorporating the chlamyospores to the feedstuff manufacturing process.

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7. CONFLICT OF INTEREST

All the authors declare the absence of any financial or personal relationships with other people or organizations that could inappropriately influence (bias) the current work. The final article has been approved for all their authors.

8. POLICY AND ETHICS

The current investigation complies with the current laws for Animal Health Research in Spain and with the EC Directive 86/609/EEC for animal experiments.

9. FUNDING SOURCE

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11. CAPTION FIGURES

Figure 1: Values of climatic parameters from September 2010 to September 2012 in the Marcelle Zoological Park (Outeiro de Rei, NW Spain).

Figure 2: Dynamics of strongyle egg-output in equids at the Marcelle Natureza Zoological Park (Outeiro de Rei, NW Spain). IVM: ivermectin; PZQ: praziquantel. \mathfrak{z} : Zebra (*Equus quagga*); \mathfrak{a} : African Wild Ass (*E. africanus asinus*); \mathfrak{d} : European Donkey (*E. asinus*). Values are means plus 2 SD.

Figure 3: Dynamics of strongyle egg-output in equids at the Marcelle Natureza Zoological Park (Outeiro de Rei, NW Spain) receiving a bi-weekly dosage of 2×10^6 *Arthrotrys flagrans* chlamydospores kg bw⁻¹. \mathfrak{z} : Zebra (*Equus quagga*); \mathfrak{a} : African Wild Ass (*E. africanus asinus*); \mathfrak{d} : European Donkey (*E. asinus*). Values are means plus 2 SD.

Table 1: Strongyle Faecal Egg Count Reduction (FECR) and Positive Equids Reduction (PER) from equids housed at the Marcelle Natureza Zoological Park (Outeiro de Rei, NW Spain).

12. CONTRIBUTORS

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1) Conception and design of the study

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2) Acquisition of data

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3) Analysis and interpretation of data

Mariasol Arias, María Eugenia López-Arellano, Luis Madeira de Carvalho, Pedro Mendoza de Gives, Rita Sánchez-Andrade, Adolfo Paz-Silva.

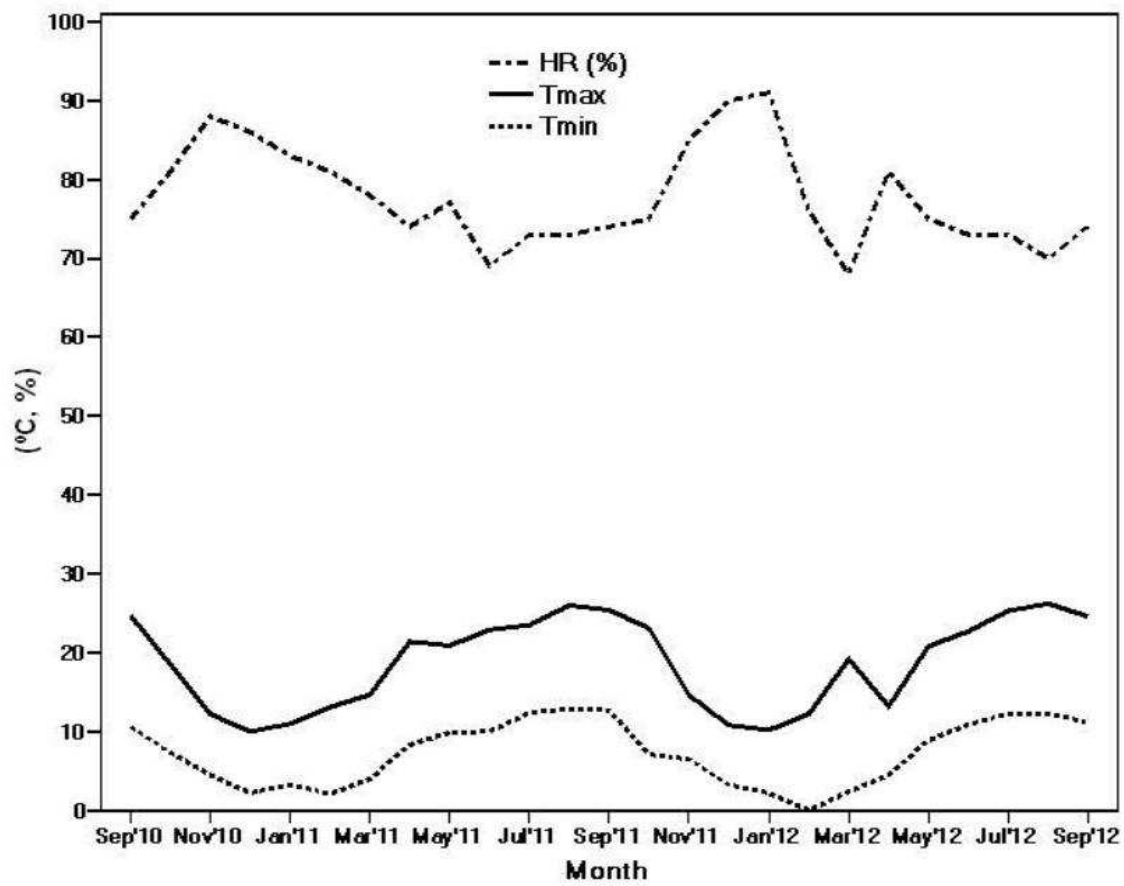
3) Redaction and revision of the article

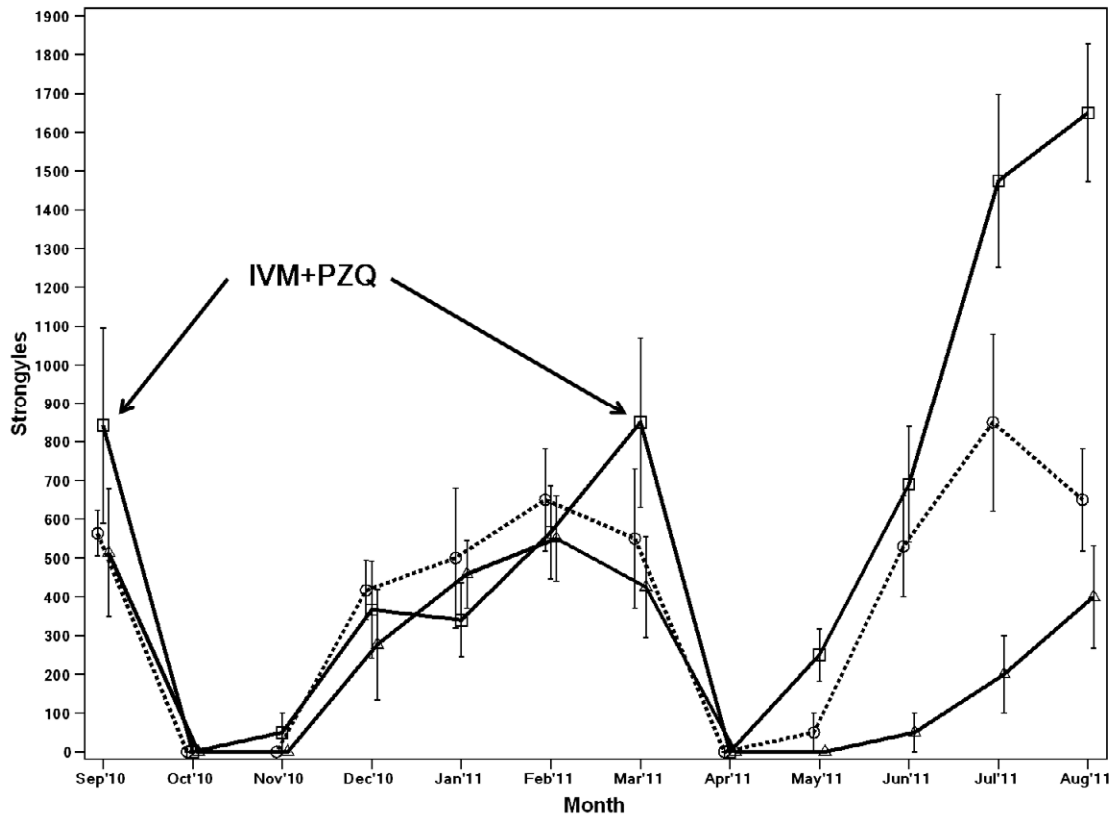
Mariasol Arias, Cristiana Cazapal-Monteiro, Esther Valderrábano, Silvia Miguélez, José Luis Rois, María Eugenia López-Arellano, Luis Madeira de Carvalho, Pedro Mendoza de Gives, Rita Sánchez-Andrade, Adolfo Paz-Silva.

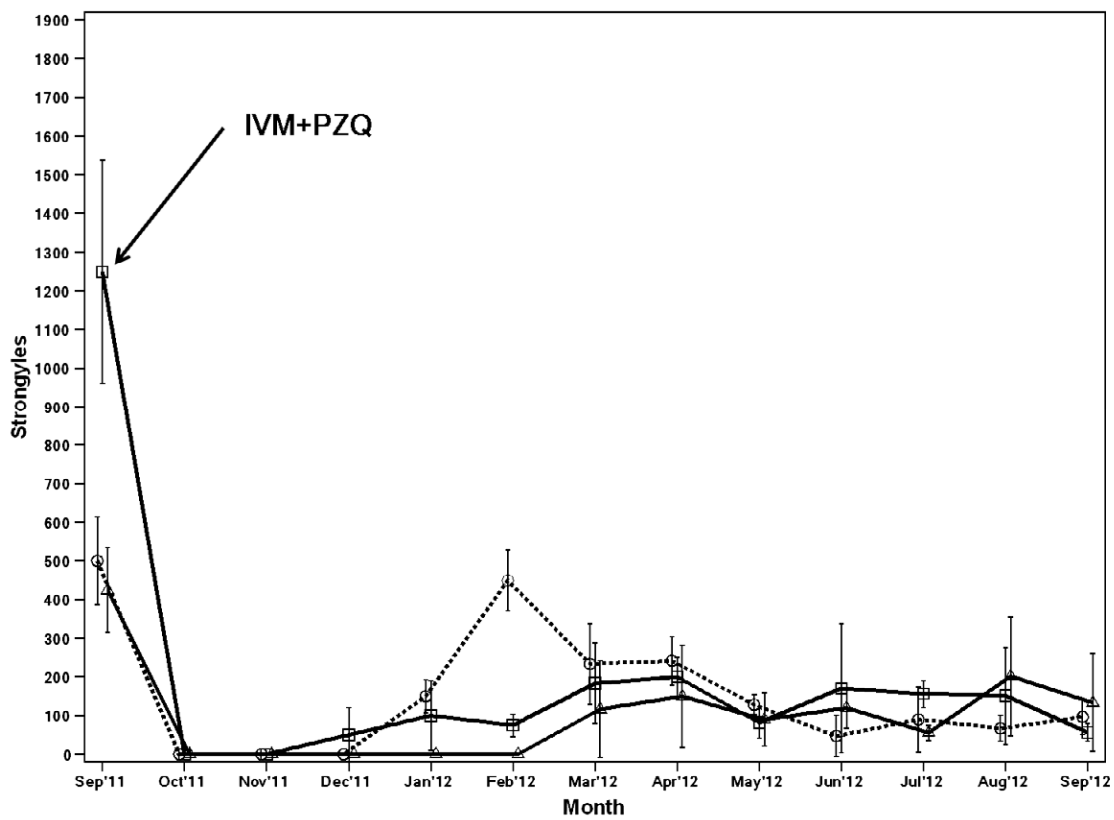
4) Final approval of the version to be submitted

**Mariasol Arias, Cristiana Cazapal-Monteiro, Esther Valderrábano, Silvia Miguélez,
José Luis Rois, María Eugenia López-Arellano, Luis Madeira de Carvalho, Pedro
Mendoza de Gives, Rita Sánchez-Andrade, Adolfo Paz-Silva.**

ACCEPTED MANUSCRIPT







Month	Strongyle Control	African donkeys (n = 6)		European donkeys (n = 6)		Zebras (n = 3)	
		FECR	PER	FECR	PER	FECR	PER
Sep 10	Anthelm.						
Oct	-	100	100	100	100	100	100
Nov	-	100	100	94	33	100	100
Dec	-	26	0	56	0	46	0
Jan 11	-	11	0	60	0	11	0
Feb	-	0	0	33	0	0	0
Mar	Anthelm.	15	0	0	0	23	0
Apr	-	100	100	100	100	100	100
May	-	91	33	71	0	100	100
Jun	-	4	0	20	0	88	33
Jul	-	0	0	0	0	53	0
Aug	-	0	0	0	0	6	0

Sep	Anthelm.+ <i>Af</i>	23	0	24	0	0	0
Oct	<i>Af</i>	100	100	100	100	100	100
Nov	<i>Af</i>	100	100	100	100	100	100
Dec	<i>Af</i>	100	100	96	50	100	100
Jan 12	<i>Af</i>	70	0	91	33	100	100
Feb	<i>Af</i>	10	0	94	0	100	100
Mar	<i>Af</i>	53	0	85	0	73	33
Apr	<i>Af</i>	52	0	84	0	65	33
May	<i>Af</i>	74	0	93	0	79	0
Jun	<i>Af</i>	91	33	86	0	72	0
Jul	<i>Af</i>	82	33	87	0	87	0
Aug	<i>Af</i>	87	0	88	0	53	0
Sep	<i>Af</i>	81	0	95	0	69	33