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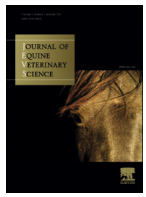

Original Research

# A Preliminary Study of the Biological Control of Strongyles Affecting Equids in a Zoological Park

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ABSTRACT

The main goal in this research was to determine the beneficial effect of incorporating biological procedures in parasite control programs for equids in zoological parks. Two trials were developed for *Equus quagga*, *E asinus* , and *E africanus asinus*. The first trial (September 2010 to August 2011) consisted of chemotherapy only (ivermectin plus praziquantel), and the second trial (September 2011 to September 2012) consisted of 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 fecal egg counts (FECR). In the first trial, 100% FECR values were achieved 15 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 feces at 2-4 months after their deworming. In the second experiment, the FECR values were 100% in the three species. ERPs of 3 months in the European donkeys, 4 months in the Africans, and 6 months in the zebras were recorded. All the equids had positive results for the coprological flotation test 4-8 months after anthelmintic administration. This preliminary study demonstrates the incorporation of chlamydospores of nematophagous fungus, as *A flagrans* appears highly promising for reduction of the infective stages of the strongyles affecting captive animals, but the experimental design precludes true determination of whether the treatment is fully efficacious.



## 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 The former objective consisting of displaying wild animals for visitor entertainment has been replaced by the conservation of endangered species, education, and research [1].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 to improved veterinary care. As a consequence, preservation of several endangered or vulnerable species (eg, African wild ass [*Equus africanus*

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*asinus*], dromedary [*Camelus dromedarius*], plains zebra [*E 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 feces. After their embryonation in the fecal 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 fecal pats and move to adjacent plants, which are subsequently ingested by the animals on pasture. Strongyles in 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 cannot be observed, because a horse infected with strongyles can pass millions of eggs each day in the dung.

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

The objective of this study was to investigate the effect of incorporating biological procedures in 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 used anthelmintic treatment plus *A flagrans* chlamydospores.

## 2. Material and Methods

### 2.1. Marcelle Natureza Zoological Park

Marcelle Natureza is a 20-ha zoological park located in northwest Spain (Outeiro de Rei, Lugo; 43°4'14.71" N, 7°37'53.50" W). Collection animals live in fenced, semifree 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 diarrhea is observed, mainly from late spring to early autumn.

Removal of fecal 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 *E 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 years old; 1 stallion and 2 mares) housed in a 4038-$m^2$ meadow. The European donkeys (3-9 years old; 3 stallions and 3 mares) are housed in a 2015-$m^2$ pasture. The African wild assesses (3-6 years old; 1 stallion and 5 mares) are maintained in a 1000-$m^2$ 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 2 days. Water is available ad libitum.

### 2.3. Arthrobotrys flagrans

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

### 2.4. Experimental Design

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

Excluding any type of immobilization of the animals to avoid exposing them to stressful situations, fecal samples were collected from the soil. The numbers of stool samples equal the number of animals in each enclosure were obtained. Collection of feces was done early in the morning (8:00 AM-9:00 AM), 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 true determination of treatment success difficult because of the unavailability of control animals under the same conditions as those receiving chlamydospores with the feedstuff.

#### 2.4.1. Anthelmintic Only (September 2010-August 2011)

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

#### 2.4.2. Anthelmintic Plus Biological Control (September 2011-September 2012)

Anthelmintic therapy, as described previously, was administered to the equids in September 2011. *A flagrans*