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Biological control of soil transmitted helminths (STHs) in a zoological park by using saprophytic fungi

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ABSTRACT

Toxascaris leonina and *Trichuris* sp. are soil-transmitted helminths (STHs) infecting domestic and wild mammals. The antagonistic effect of the saprophytic filamentous fungi *Mucor circinelloides* and *Verticillium* sp. was examined on eggs of *T. leonina* passed in the feces of captive lynxes (*Lynx lynx*) kept in a zoological park. The activity of *M. circinelloides* and *Trichoderma atrobrunneum* was tested on eggs of *Trichuris* sp. shed by captive dromedaries (*Camelus dromedarius*). The parasiticide activity was assessed by measuring the ovistatic (delayed development) and ovicidal (non-viability) effects on eggs placed in Petri plates, and by spraying spores directly onto fecal samples. Based on the observation of that hyphae of *M. circinelloides*, *Verticillium* sp. and *T. atrobrunneum* adhered to the eggshells, penetrated and destroyed the inner embryo, an ovicidal type 3 effect was concluded. Development of eggs of *T. leonina* and *Trichuris* sp. in the feces was delayed in the presence of all fungi, and one third remained at the stage of zygote. A significant reduction of *T. leonina* viable eggs was recorded in the feces sprayed spores of *M. circinelloides* (58%) or *Verticillium* sp. (67%). Fifty percent of the eggs of *Trichuris* sp. became into non-viable by 30 days after the exposure to either *M. circinelloides* or *T. atrobrunneum*. It is concluded that distribution of the filamentous fungi *M. circinelloides*, *Verticillium* sp. and *T. atrobrunneum* constitutes a novel approach to conduct the biological control of the STHs (*T. leonina* and *Trichuris* sp.) affecting wild animals captive in a zoological park.

1. Introduction

Soil-transmitted helminths (STHs) are parasites involving roundworms (ascarids), hookworms and whipworms which can affect animals and humans. Infection occurs through the accidental ingestion of infective eggs in contaminated soil or food (Vandemark et al., 2010; Elsheika, 2011), which develop in the feces from unembryonated eggs shed by adult female worms localized in the intestine (Mateus et al., 2014; Hoopes et al., 2015). Embryonation happens in the feces or ground after several weeks, resulting in that a second stage larva (L2) originates inside the eggs of ascarids, which becomes into infective (Hendrix, 2014), whereas in the case of whipworms the eggs are infective when a L1 larva develops inside (Felsmann et al., 2017). The role of rodents as paratenic hosts has been indicated in the transmission of *Toxocara* and *Toxascaris* (Okulewicz et al., 2012), and thus it has been pointed that the cycles of these nematodes could be considered as non-strictly monoxenous (Reperant et al., 2007).

Some ascarid species have zoonotic potential, as *Toxocara canis*, *T. cati*, *Baylisascaris procyonis*, *Ascaris suum* or *Toxascaris leonina*, frequently detected in domestic (cats, dogs, pigs) and wild mammals (foxes, wolves, lynxes, raccoons) (Okulewicz and Buńkowska, 2009; Dado et al., 2012; Carver et al., 2012; Beiromvand et al., 2013; Neves et al., 2014; Figueiredo et al., 2016). The zoonotic role of *Trichuris* spp. has been also reported (Gałęcki et al., 2015; Felsmann et al., 2017; Gawor and Borecka, 2017).

As indicated for other STHs, control of ascarids regularly comprises the administration of anthelmintics together with adequate hygiene (Alemu et al., 2011). Despite successful deworming is frequently administered among captive wild carnivores in zoo gardens (lions, lynxes, foxes, wolves), they infected again by helminths because their maintenance in the same parcels enhances that ground is permanently contaminated by eggs passed in the feces (Fagiolini et al., 2010; Maesano et al., 2014). In the same way, frequent infection by *Trichuris* sp. in dromedaries has been also described (Gurler et al., 2010; Eo et al., 2014).

The eggs of ascarids and whipworms are very resistant to chemical and climatic factors, thus can remain infective in the soil for several years (Gavin et al., 2005; Overgaauw and van Knapen, 2013; Gałęcki

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et al., 2015; Gawor and Borecka, 2017). Nevertheless, there is insufficient information regarding measures to reduce the presence of infective stages in the ground. Prior investigations reported that certain saprophytic soil fungi perform an antagonistic activity on eggs of *T. canis* and *A. suum*, based on their ability to adhere to the eggshell, penetrate and destroy the embryo inside (Carvalho et al., 2010; Cortiñas et al., 2015). In this way, significant reductions on egg-viability have been recorded both in the soil and in feces of parasitized animals by using *Pochonia chlamydosporia*, *Trichoderma* sp. and *Mucor circinelloides*, saprophytic filamentous fungi harmless for humans, animals and plants (Maciel et al., 2012; Arias et al., 2013a; Cazapal-Monteiro et al., 2015).

Few investigations have been carried out concerning biological control of parasites among captive wild animals kept in zoo gardens, involving some ovicide fungi (*Mucor circinelloides*, *Paecilomyces lilacinus* and *Verticillium* sp.) against *Baylisascaris procyonis* (Cazapal-Monteiro et al., 2015) or a trapping nematophagous fungus (*Duddingtonia flagrans*) against strongyles (Terry, 2009; Arias et al., 2013b). The usefulness of the fungi *M. circinelloides* and *Verticillium* sp. to conduct biological control of *T. leonina* eggs excreted in the feces of lynxes captive in a zoological park has been assayed in the present study. The activity of *M. circinelloides* and *Trichoderma atrobrunneum* was tested on eggs of *Trichuris* spp. shed in the feces of confined dromedaries.

2. Material and methods

2.1. "Marcelle Natureza" zoological park

The present investigation was conducted in the "Marcelle Natureza" Zoological Park (43°4′14.71″ N, 7°37′53.50″ W; Outeiro de Rei, Lugo, NW Spain), where a number of 150 animal species are maintained, most of them in fenced parcels.

Three adult Eurasian lynxes (*Lynx lynx*; one male and two females) are housed in a 0.5 ha plot with vegetation and trees. Food is provided to lynxes in feeders placed in individual cages of concrete soil and iron walls, and each one takes it from the same cage.

Four adult dromedaries (*Camelus dromedarius*; one male and three females) are maintained in a 0.6 ha sandy area with palm trees, feeders and drinkers. There is also a large shelter for the dromedaries can keep under adverse climatic conditions. All the cages and plots in the zoo are cleaned daily with water, and feces removed prior to the visitors arrive.

2.2. Control of parasites

Two parasiticide treatments are yearly administered in March and in September, unless additional treatment is considered needed on the basis of the coprological tests.

Fecal analyses are performed monthly in the COPAR Lab (Control of Parasites, Faculty of Veterinary, University of Santiago de Compostela, Spain), located at a distance of 12 Km from the zoological park. The apical region of the feces is taken directly from the ground of the paddocks and then examined by means of flotation technique with saturated sodium chloride solution ($\rho = 1.20\ g/cm^3$), sedimentation and migration tests (Arias et al., 2013b).

Deworming of lynxes consists of giving, for three consecutive days, anthelmintic granules (10 mg Fenbendazole/Kg body weight (bw); Panacur® Granules 22.2%, Intervet GesmbH, Vienna, Austria) previously mixed with the food. Consequently, lynxes remain briefly caged every day until all the premixed food is eaten to ensure that each individual ingests the prescribed dosage. Despite *T. leonina* eggs are not observed by 15 days post-treatment and successful of deworming is concluded, eggs appear again by 2–3 months after treatment and counts increase until lynxes are dewormed again (unpublished data). It has been observed that lynxes defecate mainly in two zones of the parcel, under a tree.

Dromedaries are provided an oral dosage of 10 mg Fenbendazole/Kg bw (Panacur® suspension 10%; MSD Animal Health, Madrid, Spain) during five consecutive days.

2.3. Fungal specimens

The CECT 208,724 strain of *Mucor circinelloides*, CECT strain of *Trichoderma atrobrunneum* and a wild-type strain of *Verticillium* sp. were utilized in the present study. These specimens were isolated from feces of domestic and wild animals (Hernández et al., 2017), identified according to their morphology (de Hoog, 2000) and cultured individually in a submerged medium for the production of spores of different fungal species (COPFr) (Cazapal-Monteiro et al., 2015).

2.4. Collection of feces

Fresh fecal samples were collected directly from the soil in the two parcels, early in the morning. The samples were kept at 4 °C and brought to the Lab. Eggs of *T. leonina* were detected in all the samples from the lynxes, and eggs of *Trichuris* sp. in those of dromedaries.

2.5. Purification of eggs of helminths

For each of the animal species, a total of 16 samples of feces (200 g approximately) taken from the ground were pooled and mixed with 2 L distilled water (Cazapal-Monteiro et al., 2015). This solution was filtered consecutively through wire sieves of 300, 150 and 40 μm in a decreasing order. The sediment obtained between the two last wire sieves was collected and resuspended in distilled water, then transferred to acetate tubes and centrifuged at 2500 rpm for 5 min. The supernatant without eggs was removed by aspiration and distilled water added for sediment resuspension. These steps were performed until the supernatant was fully transparent, then acetate tubes were filled with saturated NaCl solution and centrifuged at 2500 rpm for 5 min. The exceeding supernatant with eggs (around 2 mL) was collected by repeated pipetting, washed with distilled water and centrifuged again at 2500 rpm for 5 min. After discarding the supernatant without eggs, the sediment was cleaned with distilled water and centrifuged three times to eliminate NaCl residues. Finally, 50 μL aliquots were placed on glass slides with glass covers and observed under an optical microscope (Leica DM2500; 20–40×) to estimate the number of eggs per mL. The aqueous solutions were adjusted to 400 eggs of *T. leonina* or *Trichuris* sp./mL and kept at 4 °C.

2.6. Experimental design

The fungal activity was assayed in Petri plates to establish the antagonistic effect on the eggs of the helminths, and in fecal pats to ascertain the activity of the filamentous fungi on eggs in fecal samples. Plate assays were performed by triplicate.

2.6.1. Plate assays

Thirty Petri plates with agar-water medium (2%) were added 200 eggs of *T. leonina* in one edge, and according to previous investigations (Cazapal-Monteiro et al., 2015) 0.5 mL of liquid culture containing $2 \times \cdot 10^6$ spores of each fungus ($n = 10$ plates *M. circinelloides*, and $n = 10$ *Verticillium* sp.) on the opposite side. Ten plates were also placed 200 eggs in one edge, plus 0.5 mL distilled water on the opposite side (control plates).

Similarly, 30 Petri plates with agar-water medium (2%) were placed 200 eggs of *Trichuris* sp. Ten plates were also provided with 0.5 mL of liquid culture containing about 2×10^6 spores of *M. circinelloides* and other 10 dishes 2×10^6 spores of *T. atrobrunneum*. Other 10 plates were placed eggs of *Trichuris* sp. and 0.5 mL distilled water (control plates).

Petri dishes were maintained at room temperature (15–20 °C) and darkness for 22 days. During this period, plates were examined daily under an optical microscope at 10× and 40× (Leica DM2500) until a

minimum of 120 eggs were visualized in each. The fungal effect was defined as type 1 when eggshells remained unaltered with hyphae adhered; type 2 if disrupted eggshells without hyphal penetration were observed, and type 3 in the presence of eggshell injured, infiltration of fungal hyphae and embryo destruction (Lýsek and Krajcí, 1987; Cazapal-Monteiro et al., 2015).

2.6.2. Fecal pats assays

The feces collected from the soil of the lynxes parcel were pooled and divided into two groups. Five grams of feces were placed in 38 polypropylene translucent boxes (15 × 6 × 15 cm) (1.3 L volume) with a cover (Cazapal-Monteiro et al., 2015); twelve boxes (group TlMc) were added 3 mL of liquid medium with 2×10^6 spores of *M. circinelloides*, other 12 boxes (group TlVe) received 3 mL of medium containing 2×10^6 spores of *Verticillium* sp.; fourteen boxes were added 3 mL distilled water as controls (group TlC).

Similarly to that described before, after pooling the fresh samples of feces of dromedary, a quantity of 5 g were placed in 30 boxes which were divided into three lots of 10 each. Group TcMc received 3 mL of medium with 2×10^6 spores of *M. circinelloides*, group TcTa was added 2×10^6 spores of *T. atrobrunneum*, and group TcC was added 3 mL distilled water as controls.

All the boxes were maintained outdoors in a wooded grass, and feces examined after 10, 20 and 30 days through the flotation test and sucrose saturated solution ($\rho = 1.25\ g/cm^3$). Aliquots of 50 µL placed between a glass slide and a glass coverslip were examined at 20× and 40× under a light microscope (Leica DM2500), until a minimum of 150 eggs were visualized in each. The fecal assays were each conducted in triplicate.

2.7. Ovicide and ovistatic effects

The effect of the soil fungi on the eggs of *T. leonina* and *Trichuris* sp. was ascertained by measuring their viability and development rate. Eggs were considered viable when showing no apparent damage and/or unaltered eggshells with attached hyphae (type 1 effect) (Cazapal-Monteiro et al., 2015), and the percentage of reduction estimated according to the formula:

$$\%\ \text{Viability reduction} = [1 - (\text{mean viable eggs}_{day0}/\text{mean viable eggs}_{day\ of\ assay})] \times 100$$

With the aim to assess the ovistatic effect, the development of the eggs of *T. leonina* and *Trichuris* sp. was measured. Therefore, viable eggs without cellular division (zygote) were classified as non-developed, whereas eggs were considered as developed if contained a morula, blastula, gastrula or larva inside.

2.8. Statistical analysis

Normality of data collected in the current investigation was assessed by performing the Kolmogorov-Smirnov test. Because Z values resulted lower than 0.05, it was concluded that data do not have a normal distribution. Besides this, the Levene's test demonstrated the variances were not homogeneous ($P < 0.05$). Thus, non-parametric tests (Kruskal-Wallis and Mann-Whitney U) were performed at a significance level of $P < 0.05$ (Thrusfield, 2007).

All the tests were carried out by using the statistical package SPSS, version 20 (IBM SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Fungal effect

In the Petri dishes added spores of *M. circinelloides* or *Verticillium* sp., hyphae developed towards the *T. leonina* eggs surface at the 1st day.

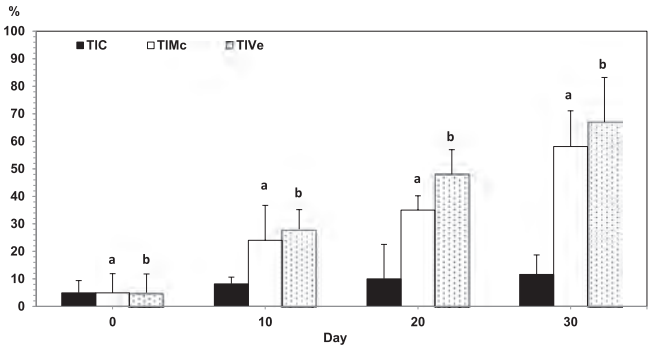

Fig. 1. Percentages of non-viable eggs of *Toxascaris leonina* in the feces of lynxes captive in a zoological park. TlC: control (untreated) group; TlMc: feces hand sprayed spores of *Mucor circinelloides*; TlVe: feces hand sprayed spores of *Verticillium* sp. Bars represent the 95% Confidence Interval for mean. Statistical analysis: a means statistical differences between TlMc and TlC; b indicates significant differences between TlVe and TlC.

After attaching to the eggshells and penetrating them (3 ± 2 days), the interior was colonized (5 ± 3 days) and the embryo destroyed (17 ± 5 days). No differences were ascertained between the effects of the two fungal specimens. Fungal growth was not observed in the control plates, and the eggs of *T. leonina* remained unaltered.

When spores of *M. circinelloides* or *T. atrobrunneum* were added to Petri plates containing eggs of *Trichuris* sp., a very fast growth of mycelium was recorded (1 day). Hyphae attached to the eggshell (3 ± 1 days), entered (6 ± 2 days) and destroyed the inner embryo (24 ± 7 days). Differences between the two filamentous fungi were not observed, or mycelium development in the control dishes.

3.2. Ovicidal activity on T. leonina in lynx feces

At the beginning of the assay, counts of 3912 ± 1278 viable unembryonated eggs of *T. leonina* per gram of lynx feces (EPG) were recorded in the controls (TlC), which reduced by 8% after a period of 10 days, and by 12% on the 30th day (Fig. 1). In group TlMc (eggs exposed to *M. circinelloides*) hyphae attached and penetrated the eggshells, and the numbers of non-viable eggs increased significantly throughout the study until reaching values of 58% (day 30) (Fig. 2). In group TlVe (eggs in the presence of *Verticillium* sp.) percentages of non-viable eggs of *T. leonina* between 28% (10th day) and 67% (30th day)

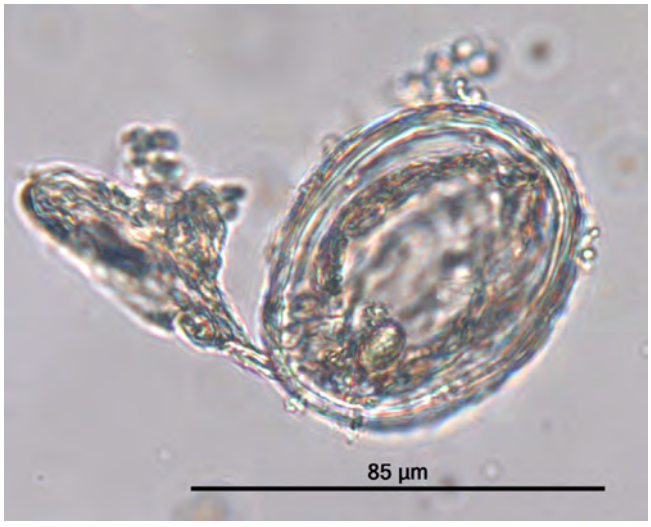

Fig. 2. Non-viable egg of *T. leonina* due to hyphae of the saprophytic fungus *Mucor circinelloides* attached to the shell, penetrated and destroyed the inner embryo.

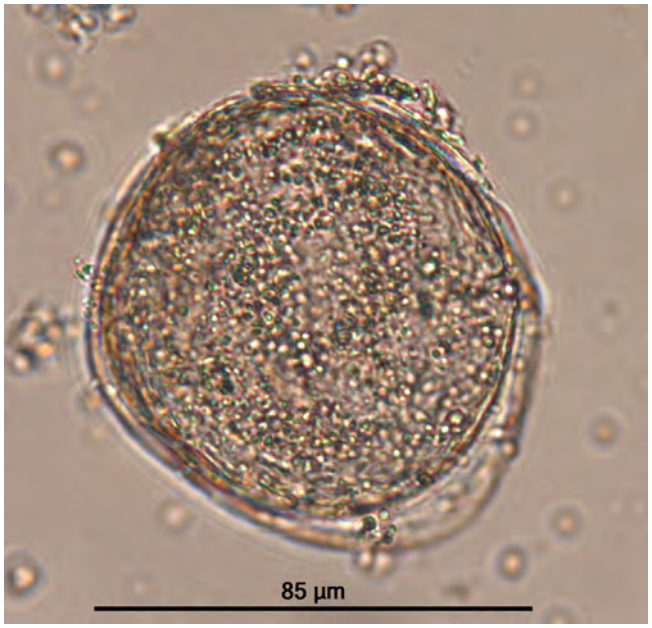

Fig. 3. Non-viable egg of *T. leonina* in feces of captive lynxes hand sprayed spores of *Verticillium* sp.

were recorded (Fig. 3).

Significant differences were obtained among the controls and the fecal pats added spores of *M. circinelloides* or *Verticillium* sp. ($\chi^2 = 48.619$, $P = 0.001$). No differences were observed between the two groups of pats added fungal spores.

3.3. Ovicidal activity on Trichuris sp. in dromedary feces

The counts of viable unembryonated eggs of *Trichuris* sp. were 500 ± 113 EPG in the feces of dromedaries. In the controls (group TcC), non-viable eggs oscillated between 4% (day 0) and 10% (day 20) (Fig. 4). After spreading spores of *M. circinelloides* on the feces (group TcMc) the percentages of non-viable eggs ranged from 11% (day 10) to 50% (day 30) (Fig. 5). Viability of eggs of *Trichuris* sp. (group TcTa) decreased by 13% after when spraying spores of *T. atrobrunneum* (day 10), and then by 50% (day 30) (Fig. 6). The differences among the three groups were significant ($\chi^2 = 9.374$, $P = 0.009$), opposite to that observed between the groups added spores.

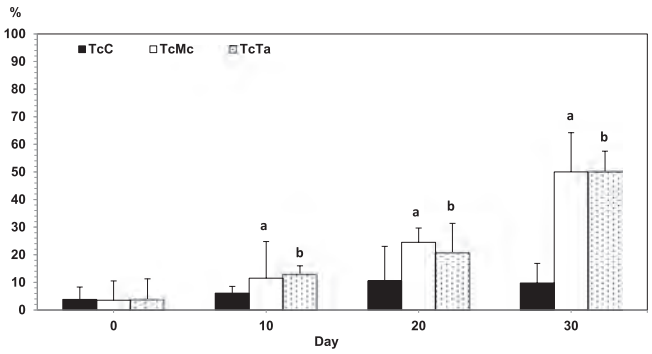

Fig. 4. Percentages of non-viable eggs of *Trichuris* sp. in the feces of dromedaries captive in a zoological park. TcC: control (untreated) group; TcMc: feces hand sprayed spores of *Mucor circinelloides*; TcVe: feces hand sprayed spores of *Verticillium* sp. Bars represent the 95% Confidence Interval for mean. Statistical analysis: a means statistical differences between TlMc and TlC; b indicates significant differences between TlVe and TlC.

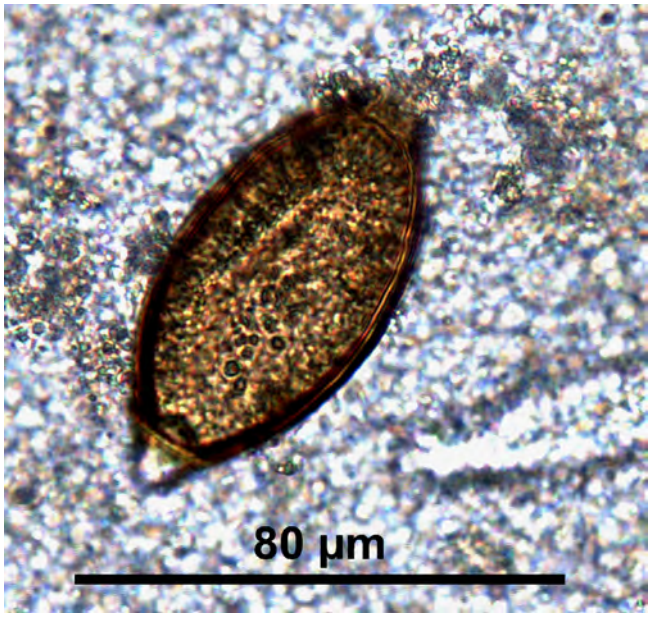

Fig. 5. Hyphae of *Mucor circinelloides* were capable to penetrate eggs of *Trichuris* sp. by removing one of the polar plugs.

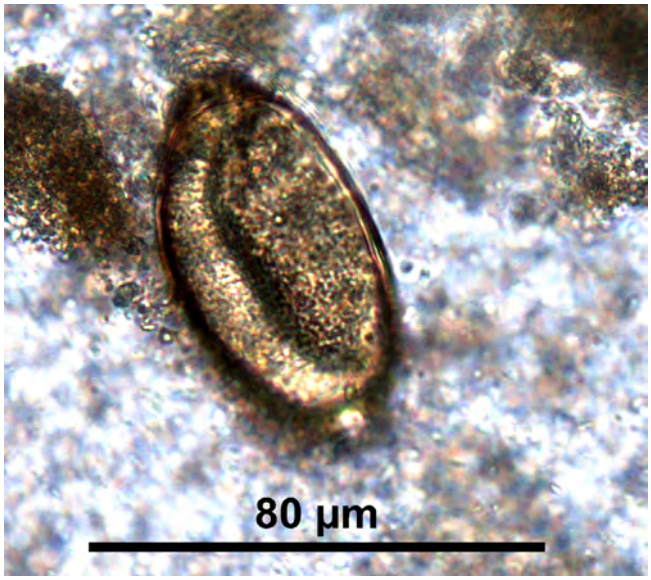

Fig. 6. Non-viable egg of *Trichuris* sp. in feces of captive lynxes hand sprayed spores of *Trichoderma atrobrunneum*.

3.4. Ovistatic effect on T. leonina in lynx feces

Among the viable eggs of *T. leonina*, high percentages of developed eggs were observed in the controls (81% by day 10 and 99% by day 30), while one third of the eggs exposed to *M. circinelloides* were at zygote stage on days 20 and 30 (Table 1). Significant differences were obtained throughout the assay between control and *Mucor*-treated fecal pats ($F = 48.729$, $P = 0.001$).

Ten days after the exposure to *Verticillium* sp., a percentage of 42% *T. leonina* viable eggs remained at zygote stage, and 31% did it by day 30 (Table 1). Significant differences were obtained between controls and *Verticillium*-treated fecal pats ($F = 21.792$, $P = 0.001$).

The percentage of infective eggs (containing one L2 inside) increased throughout the assay, and values of 86% were reached in the controls at the end of the study (Table 1). In the eggs exposed to *M. circinelloides*, 32% reached the L2 stage, and 29% in those in contact

Table 1
Percentages of the different stages of development of eggs of *Toxascaris leonina* in the feces of lynxes captive in a zoological park. TlC: control (untreated) group; TlMc: feces hand sprayed spores of *Mucor circinelloides*; TlVe: feces hand sprayed spores of *Verticillium* sp.

Group	Day	Undeveloped	Developed			
		Zygote	Morula	Blastula	Gastrula	Larva 2
TlC	0	100	0	0	0	0
	10	19	15	12	16	38
	20	9	7	13	10	61
	30	1	3	3	7	86
TlMc	0	100	0	0	0	0
	10	39	15	17	14	15
	20	33	17	14	16	20
	30	30	19	10	9	32
TlVe	0	100	0	0	0	0
	10	42	15	16	16	11
	20	37	17	14	12	20
	30	31	13	18	9	29

with *Verticillium* sp.

3.5. Ovistatic effect on Trichuris sp. in dromedary feces

The percentages of developed eggs rose throughout the study, and those surpassing the zygote phase ranged between 38% (day 10) and 66% (day 30) in the controls (Table 2). Ten days after the exposure to *M. circinelloides* one quarter of the eggs passed the zygote stage, and half after 30 days. Significant differences were obtained between controls and *M. circinelloides*-treated fecal pats ($F = 21.792$, $P = 0.001$).

As represented in Table 2, in the eggs exposed to *T. atrobrunneum*, values of viable and developed eggs of 22% and 49% were observed at days 10 and 30, respectively. Significant differences were obtained between controls and *T. atrobrunneum*-treated fecal pats ($F = 21.792$, $P = 0.001$).

At the end of the study, the presence of infective eggs (with a L1) was detected in one third of the controls, 11% of *M. circinelloides*-exposed, and 9% of *T. atrobrunneum*-exposed (Table 2).

3.6. Morphological damage on eggs of exposed to fungi

As represented in Fig. 7, blastomeres with different size were observed in eggs of *T. leonina* exposed to *M. circinelloides* or *Verticillium* sp. Broken eggshells were observed in 53% eggs exposed to *M. circinelloides*

Table 2
Percentages of the different stages of development of eggs of *Trichuris* sp. in the feces of dromedaries captive in a zoological park. TcC: control (untreated) group; TcMc: feces hand sprayed spores of *Mucor circinelloides*; TcTa: feces hand sprayed spores of *Trichoderma atrobrunneum*.

Group	Day	Undeveloped	Developed			
		Zygote	Morula	Blastula	Gastrula	Larva 1
TcC	0	100	0	0	0	0
	10	62	24	14	0	0
	20	53	25	12	8	2
	30	34	6	14	14	32
TcMc	0	100	0	0	0	0
	10	76	14	10	0	0
	20	65	19	9	7	0
	30	52	16	11	10	11
TcTa	0	100	0	0	0	0
	10	78	13	9	0	0
	20	67	18	7	8	0
	30	51	17	14	9	9

Fig. 7. Egg of *T. leonina* in feces of captive lynxes hand sprayed spores of *Verticillium* sp., showing two different sized blastomeres.

and 58% of those in contact with *Verticillium* sp., even in those completing their development and containing one L2. Finally, the occurrence of L2 larvae exiting off the egg spontaneously was recorded by 30 days after the addition of the fungal spores.

Eggs of *Trichuris* sp. in the controls showed a barrel-shape with mucoid polar plugs at each end. Exposure to *M. circinelloides* or *T. atrobrunneum* resulted in the disappearance of one of the polar plugs in 30–35% eggs, with penetration and destruction of the embryo inside. Vacuolization was detected in 12–24% eggs (Fig. 8), but broken eggshells were rarely observed.

4. Discussion

Control of soil-transmitted helminths (STHs) among wild captive species in zoological parks remains unsolved, as occurs in domestic animals, due to the occurrence of viable stages in the soil enhances their persistent infection (Arias et al., 2013b; Mazurkiewicz-Zapałowicz et al., 2014). Roundworms and whipworms are STHs characterized by their infective stages (eggs containing one L2 and L1, respectively) can survive for months or even years until ingested (Dąbrowska et al., 2014; Traversa et al., 2014). With the aim to gain information on the possibilities of lowering the viability of eggs of *Toxascaris leonina* and *Trichuris* sp. shed by lynxes and dromedaries captive in a zoological park,

Fig. 8. Vacuolization was observed in eggs of *Trichuris* sp. exposed to *Mucor circinelloides*.

spores of three saprophytic filamentous fungi (*Mucor circinelloides*, *Verticillium* sp. or *Trichoderma atrobrunneum*) were hand sprayed directly on their feces in the current investigation. Less than half of the eggs of *T. leonina* remained viable one month after being exposed to *M. circinelloides*, and one third in the presence of *Verticillium* sp. It has been reported the viability of eggs of *Baylisascaris procyonis* lessened significantly after hand spraying spores of the saprophytic fungi *M. circinelloides*, *Paecilomyces lilacinus* and *Verticillium* sp. on feces of captive raccoons, due to their ability to develop hyphae in the presence of eggs of the parasite, adhere to the shells and destroy the inner embryo (Cazapal-Monteiro et al., 2015). Similar findings were obtained by spreading spores of *M. circinelloides* on swine feces with eggs of *Ascaris suum* (Cortiñas et al., 2015). In the present study, addition of spores of *M. circinelloides* or *T. atrobrunneum* to the feces of dromedaries was associated to a 50% reduction of the viability of eggs of *Trichuris* sp. There is only *in vitro* demonstration of the ovicide *activity* of *Pochonia chlamydosporia* on eggs of *T. trichiura* in Petri plates (2.95–94.8%) (Silva et al., 2010).

Eggs of roundworms or whipworms are passed unembryonated in the feces of parasitized individuals, and the infective stage is reached after a variable period in the soil (Okulewicz et al., 2012). Because of delaying the embryogenesis of eggs could be helpful to decrease their viability and/or the presence of infective stages in the ground, the possible ovistatic effect of *M. circinelloides* and *Verticillium* sp. on eggs of *T. leonina* was analyzed in the present study. The observation of higher percentages of viable eggs remaining undeveloped (at zygote stage) in the presence of the fungal spores seems to confirm a delay on their evolution to the infective stage, as suggested in previous studies conducted on eggs of roundworms as *T. canis* and *A. suum* (Kuźna-Grygiel et al., 2001). Similar records were obtained in the present investigation when spores of *M. circinelloides* and *T. atrobrunneum* were hand sprayed over dromedary feces containing eggs of *Trichuris* sp.

Periodical deworming is the only measure for the control of parasites among animals captive in zoos, and preventive procedures are seldom observed. Different measures are frequently advised to limit the risk of infection by STHs, mainly focused on hygiene behaviors which include collection and appropriate elimination of pet feces, besides their frequent deworming (Arias et al., 2013a; Traversa et al., 2014). Despite feces are removed daily from the paddocks in zoos, early in the morning before visitors arrive and 1–2 times during the visiting hours, the persistent infection of animals points that eggs of parasites pass to the soil and develop to infective stages. Direct flaming on raccoon feces has been advised to prevent infection by the roundworm *B. procyonis* (Page et al., 2011), although this solution appears tricky to apply on captive animals. Rotational grazing is currently recommended for trying to limit infection by parasites among livestock (Relf et al., 2013), but this measure is often intractable to employ in zoological parks (Hernández et al., 2017). In the present assay, addition of spores of filamentous fungi to feces containing eggs of *T. leonina* or *Trichuris* sp. resulted in a reduction of viability $\geq$50%, and development was delayed in more than one third of the viable eggs.

Some saprophytic filamentous fungi, frequently present in the soil, can turn into predatory in the nearness of eggs of parasites even under aqueous environments (Arroyo et al., 2017). It has been shown that *Verticillium* spp. and *M. circinelloides*, in contact with the eggshells, develop hyphae able to penetrate and destroy the embryo inside (Lýsek and Stěrba, 1991; Arias et al., 2013a). By opposite, no signs of destruction of shells or penetration of mycelium have been stated into eggs of *Ascaris suum* exposed to *Paecilomyces frequentans*, and eggs of *Toxocara canis* in contact with *P. fumosoroseus* or *T. viride*; accordingly, morphological damage together with slowing embryonic development have been attributed to certain metabolites released by these fungi (Mazurkiewicz-Zapałowicz et al., 2014). One outstanding result in the present investigation was the observation of an important percentage of broken shells of eggs of *T. leonina*, as well as L2 larvae outside the eggs. A possible explanation could be that, in the presence of the soil fungi, eggshells weaken until break, which could facilitate the L2s could abandon the eggs. It should be remembered that these larvae leave the eggs after being ingested and reach the intestinal lumen in the hosts, but they are not able to survive in the environment. Silva et al. (2010) reported that hyphae of *Pochonia chlamydosporia* might penetrate the eggshell of *Trichuris trichiura*, but in the current research damage of eggs of *Trichuris* sp. was mainly caused by the ability of hyphae of *M. circinelloides* and *T. atrobrunneum* to remove one of the polar plugs and penetrate inside.

Fungi are widely employed as biological agents for the control of plant pests, but there is scarce number of assays against parasites affecting animals, especially under their fecal natural environment. There is also limited information regarding the biological control of parasites on captive animals. A significant reduction of infection by strongyles among captive equines in a zoological park was demonstrated, by giving them commercial food merged previously with spores of *D. flagrans* (Arias et al., 2013a). Different investigations among livestock provided successful results through the oral administration of spores of *P. chlamydosporia*, *M. circinelloides*, *D. flagrans* or *Monacrosporium thaumasium* in water solutions, mixed with commercial food or even in industrially manufactured pellets (Braga et al., 2010; Araujo et al., 2012; Arroyo et al., 2016). It should be emphasized the absence of side effects regarding respiration, digestion, reproduction or even the skin, among domestic and wild captive animals provided spores of *M. circinelloides* and/or *D. flagrans* (Arias et al., 2013a; Hernández et al., 2016).

Biological control of STHs appears very useful in zoological parks, because of data in the present investigation showed the capability of three innocuous saprophytic fungi, *M. circinelloides*, *T. atrobrunneum* and *Verticillium* sp., to reduce the presence of infective stages of *T. leonina* and *Trichuris* sp. by more than half, as well as to delay the development of the infective stage by more than one third, in feces of captive lynxes and dromedaries. One interesting question relies on the appropriate way to ensure that fungal spores are properly spread in the feces (Despommier, 2003). Due to fungal spores in the current study have been obtained in a submerged culture, direct spraying on feces appears very useful especially when animals are maintained in small/medium size parcels.

5. Conclusions

Proper preventive strategies are needed to support the control of STHs in animal species captive in zoological parks. Results obtained in the current research led us to conclude the usefulness of hand spraying spores of filamentous fungi as *M. circinelloides*, *T. atrobrunneum* or *Verticillium* sp. directly on feces of infected animals to reduce the viability of their eggs and/or their development to the infective stages. Moreover, spores should be also sprayed on the ground after the routine collection of feces from the plots, minimizing thus the risk of infection by soil transmitted helminths.

6. Conflict of interest

The final article has been approved by all authors, whose assert the absence of any financial or personal interests that could improperly influence the present paper.

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