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ELEMENTS OF COPROPARASITOLOGICAL DIAGNOSIS IN *Cyathostominae* INFESTATIONS IN DONKEYS

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Abstract. The study looked at the evolution of intestinal infestations with *Strongylidae* and *Cyathostominae* in donkeys under different maintenance conditions and service (work, recreation, recovery) by determining the number of larvae per gram (LPG), followed by the morphometric and structural differentiation of larvae in the stage L3. Larvhelmintoscopic examinations were performed by Harada-Mori and Dancescu (qualitative) and Euzeby modified (quantitative) methods, followed by the morphological characteristics of the larvae: dimensions (length and caliber), cuticle features, number and layout of the intestinal cells. The lots of donkeys were made up of five specimens in different locations in terms of maintenance, physiological status, application of periodic demulsification, climatic conditions at which LPG values ranging from 50 to 750 were identified. The morphological examination of the larvae indicated the presence of *Cyathostominae* (types A, D, G, *Gyalocephalus*), *Strongylidae* (*Strongylus vulgaris, Triodontophorus*) identified by the average dimensions of 800-860µ in length, 25-30 µ in width, 8 intestinal cells. At the level of cecum and colon, the adults were observed, fixed with the oral capsule and hemorrhagic infiltrative lesions, with the presence of crateriform ulcers of varying lengths of 2-3 mm caused by the larval stages.

Keywords: *donkey, strongyles, larvae, coproscopic exam.*

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1. Introduction

Preoccupation with *Cyathostominae* infestations on donkeys is primarily relatedto the large number of species encountered and the high level of intestinal contamination they can cause, both in adult and larval stages. Although the clinical implications of the *Strongylus* strain are more important, the *Cyathostominae* subfamily exhibits a lower pathogenic effect due to penetration of the L4 and L5 stage larvae into the cecal mucosa and colon with the formation of a variable number of nodules of hemorrhagic-necrotic character (Waqas *et al.*, 2014; Seri *et al.*, 2004; Mfitilodze & Rutchinson, 1990). Secondly, from a taxonomic point of view and by its morphostructural particularities, *Cyathostominae* infestations are a constant challenge for parasitologists, with identification of new species, even genres being possible due to molecular genetics. The existence of a large population variability creates the phenomenon of resistance to anthelmintics *specially to* benzimidazole and avermectin derivatives, which can be observed by maintaining or diminishing the level of OPG or LPG in faeces or soil (Cernea, 2008).

The morphological identification of *Cyathostominae* species can be made by: larvae typing by size and number of intestinal cells, the structure of the oral cavity in adults (papillary crowns, cephalic papillae, internal crowns, extracutaneous supports, esophageal

funnel), caudal chord structure in male (number and ratio of cutaneous ribs, lobes ratio, gubernaculum aspect, spicule aspect), posterior extremities structurein female (the vulvar opening) . In Romania, the field was opened and deepened by studies related to the incidence and diagnosis of strongylidosis in equine speciesin the north-west of the country (Madeira De Carvalho, 1999) supported by the studies in Portugal, which led to the development of specific identification models based on L3 larvae morphology (Lichtenfels *et al.,* 2002).

2. Materials and methods

The study was conducted between March 2019 and January 2020 aiming to identify *Strongylidae* and *Cyathostominae* larvae in two categories of donkeys that live under different conditions.

Fecal samples were collected from 45 donkeys (40 from Romania, 5 from central Scotland) which coproparasitologically examined by the Mini-FLOTAC method. The method was chosen due to the sensitivity and the possibility of retaining abundant plant debris in the feces, which in many cases alters the microscopic observation of the eggs and thus the establishment of the degree of infestation. In parallel, the climatic evolution specific to the donkey habitat was followed - the relative temperature and humidity that directly influence the evolution of the intestinal strongylides.

One group was made up of working donkeys reared in the household system, coming from different climatic zones: Miercurea Ciuc - Hargita County (-2-8°C average temperature, UR 90-95%) and Dimbovita County(average temperature 3-11°C, UR 80-90%), and the other group was consisted of donkeys belonging to Giurgiu communal association (average temperature 10-22°C, UR 77-85%) and Constanta County (average temperature 8-21°C, UR 77-85%).

Average air temperature values were recorded in order to be able to track the influence on larvae hatching and moulting.

The samples were examined using the Harada-Mori and Dancescu methods for qualitative examinations and using the Euzeby modified method for quantitative larvhelmintoscopy (Korna *et al.,* 2008).

Faecal samples were minced and distributed in plastic glasses due to the needof oxygenation of coprocultures, and a central tunnel in the faeces sample with a glass rod was recommended. This procedure is important to ensure optimal conditions for larval development (humidity, aeration). The plastic glasses were covered with an aluminium foil and punctured with a few holes with a thick needle to allow penetration of sprayed water into the foil and penetration of the air. The samples were placed in the thermostat at 26°C for 11-14 days. The coprocultures were daily sprayed with water to ensure the optimal humidity of larval development of 70-80%. Afterwards, the cup with the faecal sample is completely filled with tap water and the open top is covered with a concave adherent Petri dishand quickly turned upside down while avoiding the loss of water and faeces, and implicitly the loss of larvae population .

The remaining space between the glass and the edge of the Petri plate is filled with water, leaving this set up for while the larvae migrate to the peripheral fluid, which is to be harvested and placed in centrifuge tubes for 24hours.

In order to centre the larvae, the samples were centrifuged at 1,500 revs / minfor 3 minutes; 2/3 of the supernatant liquid is removed. The remaining aqueous solution was shaken before examination to obtain a homogenous suspension of larvae so the larvae

could be stored in the refrigerator.

Because their morphological structures are distorted and identification becomes difficult, it is necessary to do the examination as quickly as possible. Careshould be taken to adjust the light for the larval structures to be as clearly observable as possible. Generally, with few exceptions, the presence of a well- defined cuticle pod will confirm the identification of an infesting larva.

Due to the mobility of larvae that make identification difficult, bondingsolutions are used to immobilize them with formol solution or Lugol. The latter rapidly distorts the structure of intestinal cells that become vacuolated and difficult to differentiate. If faecal samples are harvested from the ground more than 3 hours before, one must avoid confusion with terrestrial nematode larvae, whose morphological structure is different (no distinguished intestinal cells, the intestine being a homogeneous, granular, translucent mass). There are at least 100 larvae on the slide and each larva is identified from the external morphological characteristics and species characteristics point of view. It is then expressed in percentages on basis of prevalence of the strongyles species.

The modified Dancescu method had the advantage of a shorter duration beingbased on the positive hydrotrope of the larvae. The faeces were mixed with a quantity of vegetable carbon powder and water into a thick paste and placed on a Petri dish so as to form a mound whose top touches the lid of the box. The water droplets formed by condensation attract the nematode larvae, which are found on the lid of the Petri dish that is raised and examined under a microscope with the x10, x20 lens. In order to speed up and maintain condensation droplets, it is advisable to cover the plates with a damp cloth that cools the lid and keep it cooler than the rest of the plate. The emergence of larvae in the droplets occurs after 2-3 days (Figure 1).

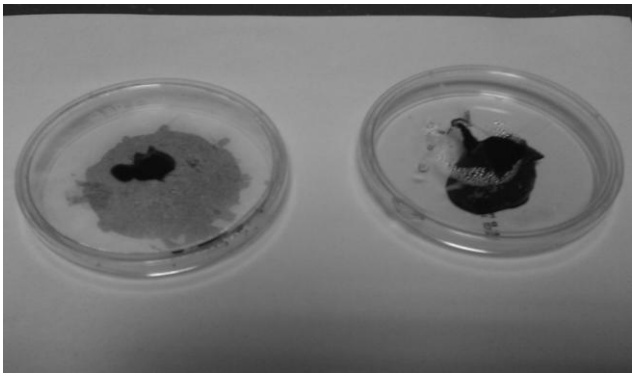

Figure 1. Implementing Dancescu method

Quantitative determinations used the modified Euzeby method for larvhelmintoscopy using 10 g of faeces, and 6 ml of liquid from the periphery of the Petri plates. The thus-obtained suspension was introduced into tubes, from which, after shaking and homogenization, 0.15 ml was collected, which was displayed on a blade and examined under a microscope with a 10x objective, counting the larvae population over the entire surface of the liquid.

Determining the morphological characteristics of larvae followed; dimensions (length and calibre), cuticle peculiarities, number and arrangement of intestinal cells. The dimensions and appearance of the cuticle cannot be considered as constant elements

because they are influenced by the temperature and humidity conditions during moulting (1, 4, 5).

3. Results and discussions

The larvae have been morphologically assessed using Cyathostomum type grading (A, B, C, D, E, F, G, R) recommended by Cernea, the differentiation being based on the assessment of the average length, width, number and layout of the intestinal cells.

From the *Cyathostominae* subfamily, L3 larvae belonging to the genus

Gyalocephalus and *Cyathostomum spp.* (type A, D, G) from the *Strongylidae* subfamily, *Strongylus vulgaris* larvae have been identified.

Cytostomus spp. type A larvae have an average length of 800/25 μ intestinal cells, the first 2 disposed parallel, 6 continuously (Figure 2).

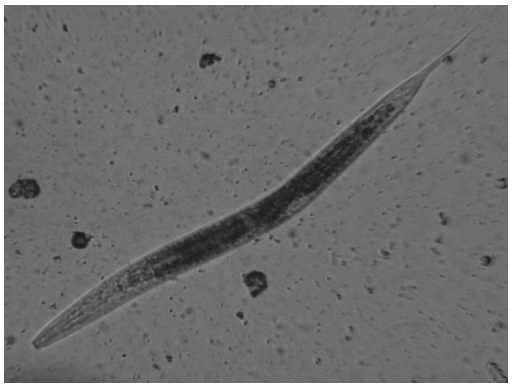

Figure 2. Larvae *Cyathostominae* tip A

Cythostomus spp. type D larvae have an average lenth of 850- 26 μm wide, 8 intestinal cells displayed in a continuous row.

Cythostomus spp. G-type larvae have an average length of 860- 25 μm wide, 8 intestinal cells displayed randomly.

Gyalocephalus larvae have an average length of 720 μm, 30 μm wide, 12 intestinal cells, 6-10 displayed parallel, the remainder in continuous row (Fig. 3).

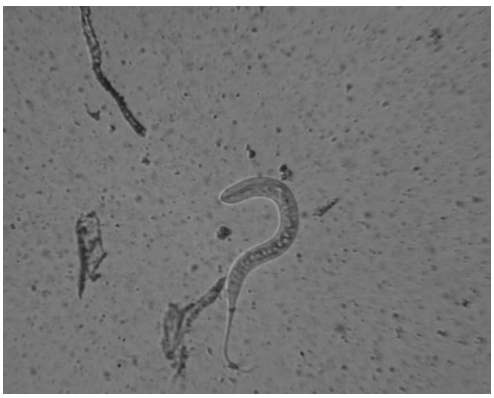

Figure 3. *Gyalocephalus larvae*

In a study carried out in 2008 (8) comparing the results from Portugal and Romania, 8 morphological types of L3 *Cyathostomum sensu lato* were identified: five types (A, B,

C, D and G) with 8 intestinal cells, and three (E, F and R) with 6, 7 and 9 cells.

In addition to the Cyathostominae subfamily larvae, Strongylidae subfamilies were identified, particularly Strongylus vulgaris, with size μ of 900-940 μ numerous intestinal cells (about 30), laid out in parallel rows, and *Trichodontophorus* spp. (850 μ long, 20 triangular-shaped intestinal cells laid out in parallel rows).

According to a classification scheme by morphometric L3 stage, distribution of types could possibly be (1, 4):

- type A - *Cylicocyclus (insigne, nassatus, radiatus), Cylicostephanus(poculatus, minuteus, longibursatus), Cyathostomum (catinatum, pateratum);*
- type B - *Cyilicocyclus (brevicapsulatus, ultra}ectinus), Cylicodontophorus bicoronatus;*
- type C - *Cyilicostephanus (calicatus, hybridus, longibursatus).*

to a classification scheme by morphometric L3 stage, distribution of types could possibly be (1, 4):

Morphopathological examinations in the cecal mucosa and the colon revealed lesions produced by adult and larval stages with different morphological presentations. In the case of lesions caused by adults, hemorrhagic infiltration processes are detected with the presence of crateriform ulcers with variable lengths of 2-3 mm, caused by the oral capsule accompanied by a hyperplasic reaction that generates a circumferential appearance, the ability of attachment being particularly accentuatedat the ileocecal valve location (Figures no. 4, 5, and 6).

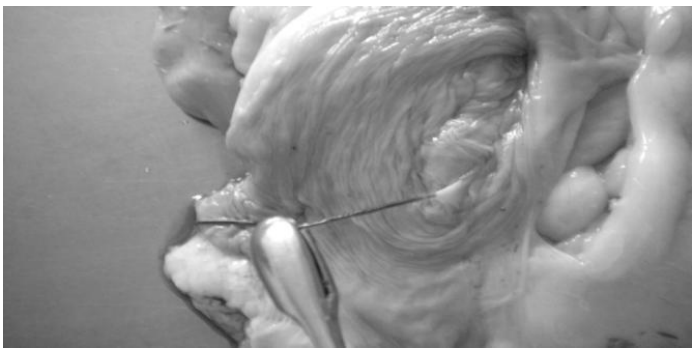

Figure 4. Adults worms in the ileocecal location

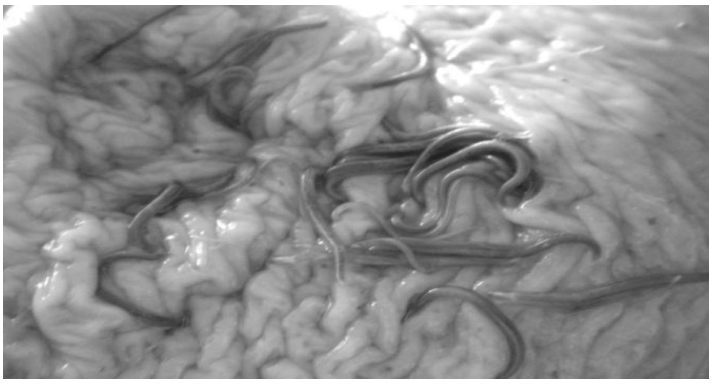

Figure 5. Adult firmly attached on colonic mucosa

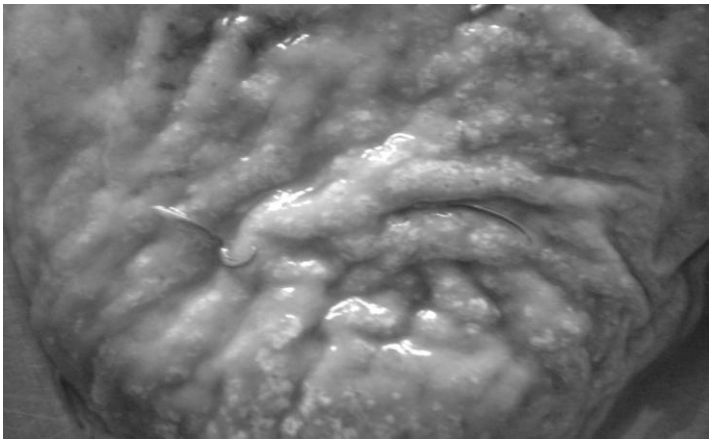

Figure 6. *Prolypherative-haemoragic typhlocolotis induced by larvae*

4. Conclusions

The results indicated a 100% degree of contamination, with EPG values between 1100 and 600, in which *Cyathostomins, Trichonema* and *Strongylus* predominated – the differentiations being based only on the morphological aspects of the eggs. The degree of infestation was high between November and April, when the average temperature was 5-6˚C with the absence of consistent snow layers. Lower values were recorded during the summer with a thermal average of 26˚C and the absence of rain. It should be noted that the observations were made in the conditions in which no antihelminthics were administered.

By quantitative larvoscopic quantitative larvoscopic methods, in a number of 40 donkeys in 2 heterogeneous lots, the level of infestation with *Cyathostomina*e and *Strongylidae* larvae was between 50-750 LPG.

From the *Cyathostominae* subfamily, L3 larvae belonging to the genus *Gyalocephalus* spp. and *Cyathostomum* spp. (type A, D, G) have been identified and from the *Strongylidae* subfamily, *Strongylus vulgaris* larvae have been identified.

Determining the morphological characteristics of larvae was based on: the size (length and diameter), cuticle particularities, the count and the arrangement of the intestinal cells. *Cyathostomum* types A, D, G larvae, *Gyalocephalus, Strongylus vulgaris* and *Trichodontophorus* spp. Larvae have been identified. Morphopathological examinations in the cecal mucosa and the colon revealed injuries caused by the adult stage: hemorrhagic infiltration with the presence of crateriform ulcers of 2-3 mm variable sizes, caused by the oral capsule and larval stages: nodular triflocolitis with nodules of variable sizes with bleeding, blackish or white-gray appearance.

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