

Mello and Campos (1974) method adapted for the recovery of cestodes in birds (*Gallus domesticus*)

Método Mello e Campos (1974) adaptado para recuperação de cestódeos de aves (*Gallus domesticus*)

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ABSTRACT: The specific diagnosis and evaluation of the intensity of avian helminth infections are essential for efficacy studies and the determination of drug doses targeted to their control. This study evaluated the Mello and Campos method, originally described for parasitological diagnosis in dogs, in the recovery of scolices from cestode parasites of poultry (*Gallus domesticus*). A total of 52 naturally infected birds obtained from farms underwent parasitological necropsy using the Mello and Campos method. The method consisted of four steps: content, soaking, scraping and evaluation. The number of scolices recovered per bird ranged from 1 to 4,345, and the highest number of scolices was recovered from material derived from the soaking step. The cestodes species diagnosed were *Amoebotaenia cuneata*, *Choanotaenia infundibulum*, *Hymenolepis* sp., *Raillietina tetragona*, *Raillietina echinobothrida* and *Raillietina cesticillus*. The Mello and Campos method, originally used to test for helminths in dogs, was effective in avian cestode testing because it includes a soaking step, which enables a more efficient recovery of scolices.

KEYWORDS: *Gallus domesticus*; birds; cestoda; helminths; Mello and Campos.

RESUMO: O diagnóstico específico e a avaliação da intensidade da infecção helmíntica em aves são fundamentais em estudos de eficácia e determinação de doses de medicamentos direcionados ao seu controle. O presente trabalho avaliou a aplicação e adaptação da metodologia de Mello e Campos, descrita originalmente para diagnóstico parasitológico em cães, na recuperação de escólices de cestódeos parasitos de aves domésticas (*Gallus domesticus*). Foram empregadas 52 aves naturalmente infectadas e oriundas de produções rurais, as quais foram submetidas à necropsia parasitológica, adaptando-se a metodologia Mello e Campos. O método consistiu na realização de quatro etapas: conteúdo, imersão, raspado e avaliação. O número de escólices recuperadas por ave variou de 1 a 4.345, e o maior número de escólices foi recuperado do material oriundo da etapa de imersão. As espécies de cestódeos identificadas foram *Amoebotaenia cuneata*, *Choanotaenia infundibulum*, *Hymenolepis* sp., *Raillietina tetragona*, *Raillietina echinobothrida* e *Raillietina cesticillus*. Os resultados foram avaliados estatisticamente, concluindo-se que a metodologia adotada é eficaz para a recuperação de cestódeos de aves, uma vez que possui a etapa de imersão, que permite a recuperação mais eficiente de escólices.

PALAVRAS-CHAVE: *Gallus domesticus*; aves; cestódeos; helmintos; Mello e Campos.

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INTRODUCTION

The specific diagnosis and evaluation of the intensity of avian helminth infections are necessary to establish the best control and treatment strategies because helminthoses remain a key obstacle for aviculture. Furthermore, they are essential for efficacy studies and assessment of drug doses targeted at the control of helminth infections in birds (VICH TOPIC GL21, 2001; YAZWINSKI et al., 2003).

Poultry semi-extensive production systems have increased significantly, due to increased demand for natural and organic foods and the focus that society has given to animal welfare. In these systems and in the extensive production systems, the helminthoses still constitute an important aspect of sanitary management and must be considered in biosafety programs.

Helminthoses evaluation in poultry is part of national and international regulations (NURELHUDA et al., 1989; YAZWINSKI et al., 1992; 2003; SILVA et al., 1999; VICH TOPIC GL21, 2001; TUCKER et al., 2007; ALAM et al., 2014; BUTT et al., 2014). Methodologies owing to the cestodes recovery basically consist of collecting intestinal contents and materials scraped from the small intestine mucosa, followed by helminth screening.

Those methods are not fully effective, because scolices are extremely fragile, detaching from strobila, and being ultimately lost or damaged. Besides, the recovery is challenging when scolices are attached to the mucosal scraping fragments, complicating and lengthening the process. Such findings corroborate MELLO; CAMPOS (1974), who reported that intestinal helminth counts performed using the scraping method under estimate the number of helminths, and the examination of the material is laborious.

The thermo-hydrotropism method, described by MELLO; CAMPOS (1974) for the diagnosis of helminth infections in dogs, consists of four steps, named as follows: content and scraping, soaking, subsequent scraping, and examination of the contents of each step under a stereoscopic microscope. The authors concluded that the method is simple and that helminths are collected intact and at higher counts in the soaking step.

The objective of the present study was to adapt the application of the MELLO; CAMPOS (1974) to recover cestode parasites from birds (*Gallus domesticus*).

MATERIAL AND METHODS

Experimental birds

A total of 52 adult *G. domesticus* from farms of the municipality of Votuporanga, São Paulo, Brazil, raised under extensive and semi-extensive production systems, and a history of natural infection by cestodes was used. Infection was previously confirmed in 100% of ten birds from each site necropsied during the pre-experimental period.

Mello and Campos method, adapted to birds

The selected birds were transferred to the Laboratory of Animal Parasitology of the Instituto Biológico, São Paulo, Brazil, and were maintained in cages with water and feed free of anthelmintic medication for a seven-day period of adaptation (YAZWINSKI et al., 2003). Following this period, the birds were euthanized (YAZWINSKI et al., 2003) and necropsied, and the Mello and Campos method was adopted (Fig. 1). A modification was performed in the first step, and it consisted of collecting only intestinal contents, without performing the mucosal scraping. This step was named “content”. The content was collected by passing the fingers over the intestinal mucosa, but without pressure or scraping (Fig. 1, step 1). The second step (soaking) and the third one (named “scraping” instead of “subsequent scraping”) were originally accomplished as described in the Mello and Campos method (Fig. 1).

To confirm the effectiveness of the method at different infection levels, after counting the scolices present in the materials collected at each step of the method applied, the data were tabulated and distributed randomly into four groups of 13 birds each, with different infection levels: Group 1 (G1: 1 to 32 scolices), Group 2 (G2: 135 to 354 scolices), Group 3 (G3: 473 to 994 scolices), and Group 4 (G4: 1,044 to 4,345 scolices).

The effectiveness of the method was evaluated at different infection levels, because the guidelines for avian cestode studies only advocate the use of at least 10 infected birds, without defining the mean number of cestodes (VICH TOPIC GL21, 2001; YAZWINSKI et al., 2003).

Stereomicroscopy was used to evaluate the collected materials, and light microscopy was used to identify the species based on their morphological characteristics (YAMAGUTI, 1959; SCHMIDT, 1970; 1986; REID; McDUGALD, 1997; RUFF; NORTON, 1997).

Statistical analysis

The scolice count data were tested using the Kruskal-Wallis test at 95% reliability (SAS, version 9.0).

RESULTS AND DISCUSSION

The number of scolices recovered per bird ranged from 1 to 4,345. The following groups were formed according to the infection level: G1, G2, G3, and G4. For all infection levels, the soaking step allowed an efficient recovery of scolices, ranging from 77.62% for G1 to 95.4% for G3.

The scolice count data at each step, indicating that the highest number of scolices (2,630) was recovered from the material derived from step 2 (soaking). The mean number

of scolices recovered in this step was not different ($p \geq 0.05$) from the sum of the total number found in the three steps at any infection level (Table 1).

The mean number of scolices recovered in step 2 (soaking) was significantly higher ($p \geq 0.05$) than the numbers in steps 1 (content) and 3 (scraping), with the single exception

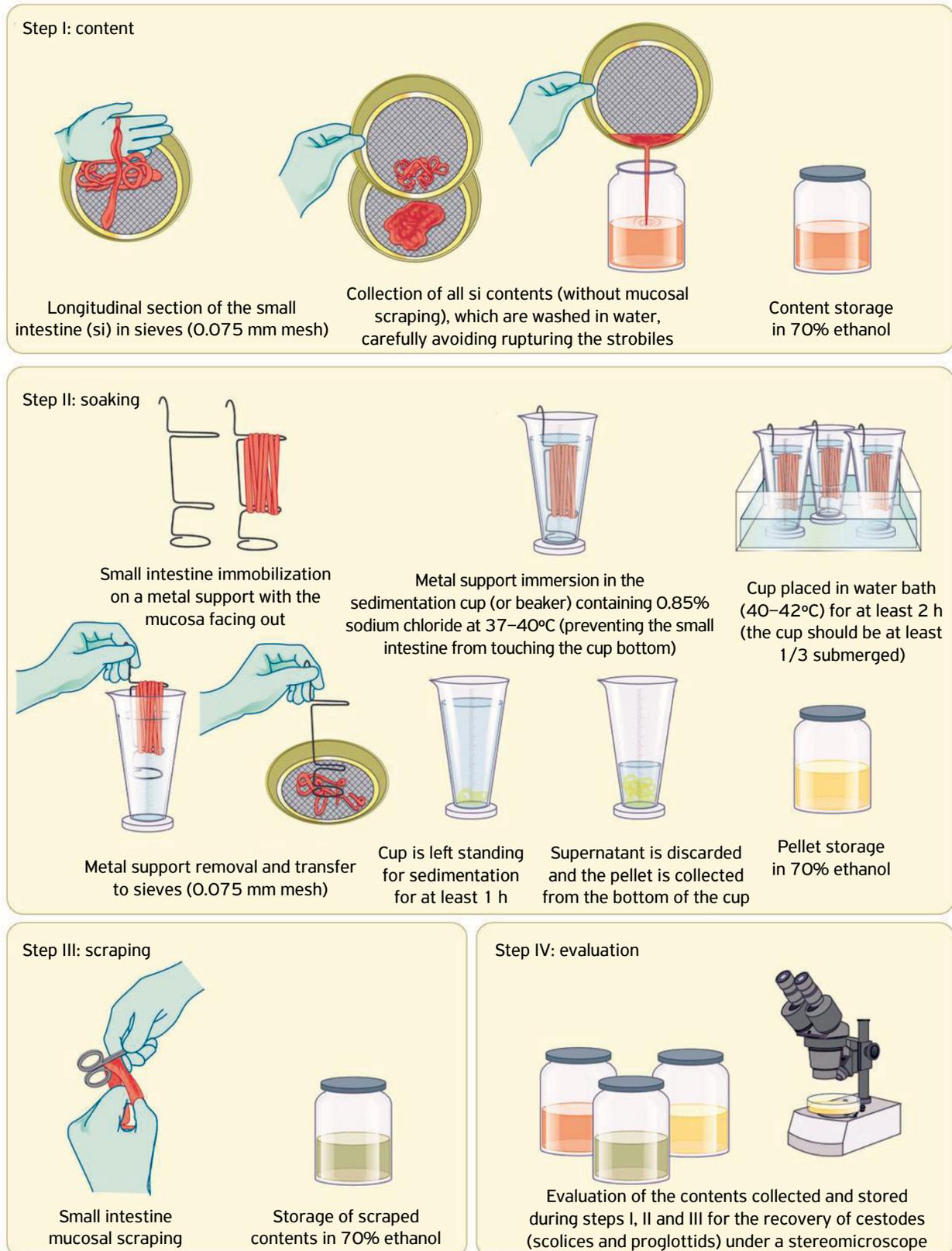


Figure 1. Mello and Campos method steps applied to recover cestode parasites from birds (*Gallus domesticus*).

Table 1. Results of multiple comparisons of scolices recovered during bird necropsy.

STEP	Infection Levels/Means and Standard Deviations ¹									
	G1	%	G2	%	G3	%	G4	%	Total	%
1: Content	1,15 ± 2,23 ^C	10,49	3,54 ± 7,88 ^B	1,74	18,38 ± 42,94 ^B	2,48	45,77 ± 44,35 ^B	2,24	68,85 ^B	2,30
2: Soaking	8,54 ± 10,20 ^{AB}	77,62	190,08 ± 71,96 ^A	93,67	707,46 ± 159,69 ^A	95,40	1724,85 ± 792,75 ^A	84,60	2630,92 ^A	87,86
3: Scrapping	1,31 ± 1,65 ^{BC}	11,89	9,31 ± 9,88 ^B	4,59	15,69 ± 31,57 ^B	2,12	268,31 ± 517,10 ^B	13,16	294,62 ^B	9,84
Total	11,00 ± 11,31 ^A	100,00	202,92 ± 68,29 ^A	100,00	741,54 ± 165,80 ^A	100,00	2038,92 ± 958,10 ^A	100,00	2994,38 ^A	100,00
Test Value	18,94		40,23		38,73		34,96			
Prob. < H ²	0,0003		<0,0001		<0,0001		<0,0001			

¹Values followed by the same letter in columns are not different from each other according to ANOVA and the Kruskal-Wallis Test ($p \geq 0.05$)

²Probability of significance

of the scraping step at the lowest infection level (step 3, G1; Table 1). The results confirmed the report by MELLO; CAMPOS (1974), who also observed greater recovery of scolices in the soaking step compared to the usual methods in a study involving dogs. The mean numbers of scolices from material derived from steps 1 (content) and 3 (scraping) were not different from each other ($p > 0.05$; Table 1).

Cestodes recovery methods involving only content collection and mucosal scraping (NURELHUDA et al., 1989; YAZWINSKI et al., 1992; 2003; SILVA et al., 1999; VICH TOPIC GL21, 2001; TUCKER et al., 2007; ALAM et al., 2014; BUTT et al., 2014) hinder the recovery of scolices because strobiles rupture. In addition, the recovery of the remaining scolices is difficult owing to the presence of mucus and mucosal fragments and may be damaged upon removal or may even be undetected. The modification in the step 1 (content) was adopted to preclude strobiles rupture observed in the scraping step of the original Mello and Campos method.

Furthermore, the soaking phase of the Mello and Campos method allowed increased recovery and faster detection of scolices, because the material was clearer and could be collected in less volume. It is also noteworthy that intact cestodes (scolice + strobilus) were only recovered from material derived from the soaking phase, which significantly contributes to classify the species. MELLO; CAMPOS (1974) also reported that intestinal helminth counts performed using only the scraping method

underestimate the number of helminths, and their examination is laborious.

The cestodes species diagnosed in birds in this study were *Amoebotaenia cuneata*, *Choanotaenia infundibulum*, *Hymenolepis* sp., *Raillietina tetragona*, *Raillietina echinobothrida* and *Raillietina cesticillus*.

CONCLUSION

The Mello and Campos method, originally used to test for helminths in dogs, was effective in avian cestode testing, because it includes a soaking step, which enables a more efficient recovery of scolices for all infection levels.

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