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Original paper

Comparative analyses of coproscopical techniques to diagnose enteroparasites in a group of captive Indian peafowl (*Pavo cristatus*)

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ABSTRACT. Captive animals commonly have infections by direct life cycle parasites, since they are easily transmitted between individuals. However, diagnosing these infections in the laboratory is challenging due to the wide variety of parasite, their life stages and to the variety of available diagnose techniques, being difficult to choose the best one. The present study sampled a group of captive Indian peafowl (*Pavo cristatus*) from São Paulo Zoological Park Foundation, São Paulo, Brazil, to test and compare different coproscopical techniques commonly applied in veterinarian clinical analysis laboratories: direct smear, concentrations by sodium chlorite, sucrose, zinc sulphate, faecal sedimentation and formalin-ether followed by modified Ziehl-Neelsen staining. Sensitivity, specificity, predictive values (positive and negative) and Cohen's *kappa* index were calculated. In total 108 samples were processed and parasites found were: non-sporulated coccidian oocysts (91.7%), Capillarinae eggs (89.8%), unidentified nematode larvae (75%), Ascarididae eggs (63%), unidentified nematode adults (60.2%), unidentified nematode eggs (42.6%), strongylid-like eggs (42.6%), *Cryptosporidium* spp. (28.7%), flagellated (15.7%) and ciliated (10.2%) protozoans, trematode eggs (0.9%), Acanthocephala eggs (0.9%), Adeleidae oocysts (0.9%) and *Cruzia* sp. eggs (0.9%). Sensitivity and specificity varied considerably between parasite groups. Cohen's *Kappa* index reinforces the recommendation of applying more than one technique to diagnose enteroparasites infections.

Keywords: Capillarinae, coccidian, nematode, Ascarididae, parasites, *Pavo cristatus*

Introduction

Captive animals might have a high prevalence of parasitic infections, mainly because they live in restricted spaces and usually in high densities, which facilitates the transmission of parasites. In captivity, the presence of parasites with direct life cycles, such as nematodes, protozoans and coccidian, have been extensively reported and seems to be more common than parasites with indirect life cycles, such as trematodes, cestodes and acanthocephalans [1–7].

Parasitic infections may be asymptomatic, with no clinical symptoms, or have a symptomatic form, which most common symptoms include diarrhoea, anaemia, weight loss, growing problems in nestlings and juveniles and even death [8,9]. Many enteroparasites were described to infect wild birds (free-living and captive), the most frequently reported are: *Isospora* spp., *Atoxoplasma* spp., *Eimeria* spp., *Cryptosporidium* spp., *Sarcocystis* spp., *Giardia* sp., *Trichomonas gallinae*, *Histomonas meleagris*, *Toxoplasma gondii*, *Balantidium* spp., *Blastocystis* spp., *Entamoeba* spp., *Ascaridia* spp., *Heterakis* spp., *Capillaria* spp., *Baruscapillaria* spp., *Philophthalmus gralli* and *Taenia* sp. [1,2,8,10–16].

Diagnose of parasitic infections is essential to

guarantee the health of captive wild animals. Choosing the best technique to be used is an important part of diagnose process, since there are many different methods with different sensibility, specificity and indications [17]. According to the literature, it is recommended to use at least two different techniques when performing a parasitological diagnose, especially for enteroparasites due to the wide variety of parasite species and their life cycle [18]. Some techniques are broadly used, such as direct smear, flotation protocols (using different solutions) and faecal sedimentation [19].

In order to choose the best diagnose technique for coproscopical diagnosis, we studied a captive population of Indian peafowl *Pavo cristatus* that lives in São Paulo Zoological Park Foundation (FPZSP), São Paulo, Brazil. The aim of this study was (i) to identify parasite diversity infecting a captive population of Indian peafowl and (ii) to compare different coproscopical techniques of parasite diagnose: direct smear, concentration techniques with sodium chloride, sucrose and zinc sulphate solutions, faecal sedimentation and formalin-ether concentration followed by modified Ziehl-Neelsen staining.

Materials and Methods

Studied population. This study was conducted in the Clinical Analysis Laboratory at Applied Research Department of São Paulo Zoological Park Foundation (FPZSP) (23°39′S, 46°37′W). The samples used in this study were collected from the enclosure where 28 Indian peafowl (*Pavo cristatus)* were kept in a semi-captivity environment, with a big wooded area where they can freely circulate. They were kept with water *ad libitum*, dry food for birds (produced at FPZSP), dry corn, dry oat and fresh chicory and catalonia.

Sample collection and parasitological analysis. Fresh faeces were randomly chosen and collected in a way to prevent soil contamination. Samples were collected between October 2017 and March 2018, twice a week in alternated weeks. After being collected, they were stored in clean containers with screw caps and kept at room temperature during transport to the laboratory, which did not take more than 30 minutes. Sample processing started as soon as samples arrived at the laboratory. The techniques used were: direct smear [17,19]; faecal flotation with sodium chloride (NaCl) solution with specific gravity of 1.20 g/ml, zinc sulphate ($ZnSO_4$) solution with specific gravity 1.18 g/ml and sucrose solution with specific gravity 1.27 g/ml [19–21]; faecal sedimentation [17,22,23], and formalin-ether sedimentation followed by modified Ziehl-Neelsen staining technique to identify the presence of *Cryptosporidium*-like oocysts [19,24,25]. Parasites were identified according to Foreyt [11], Greiner [9] and Henriksen et al. [26]. Preparations were analysed in their totality under 100× magnification. Slides stained with modified Ziehl-Neelsen technique were also analysed in their totality and under high magnification (1000×) in oil immersion. All samples were screened using a Zeiss PrimoStar light microscope (Carl Zeiss MicroImaging GmbH, Jena, Germany).

Statistical analysis. The flotation technique using NaCl solution was considered as *gold standard* because it is used in the routine coproscopical exams of the Clinical Analysis Laboratory at FPZSP. The results were compared among techniques and separated by the type of parasite found (protozoans, coccidian, nematodes, cestodes, trematodes and acanthocephalans), not taking into account the parasite stage that was found, such as larvae, eggs and/or adults. For parasites diagnosed in all applied techniques, sensitivity, specificity, positive predictive values (PPV) and negative predictive values (NPV) were calculated, along with their respective 95% confidence interval (95% CI). Cohen's *Kappa* index (κ) was calculated to evaluate the concordance between applied techniques and the gold standard method, being considered as follow: $\kappa<0$, no agreement; $\kappa=0–0.20$, poor agreement; $\kappa=0.21–0.40$, fair agreement; $\kappa=0.41–0.60$, moderate agreement; $\kappa=0.61–0.80$, substantial agreement; $\kappa=0.81–1.00$, almost perfect agreement [27,28].

Results

Diversity of parasites

In total, one hundred and eight samples were analysed during the present study and all of them were positive for at least one parasite (Table 1). The higher prevalence was reported for non-sporulated coccidian oocysts (Fig. 1B), followed by Capillarinae eggs (Fig. 1D,E). Different life stages of unidentified nematodes were found, such as eggs (Fig. 1F), larvae and adults. The presence of Ascarididae (Fig 1J) and strongylid-like (Fig. 1I) eggs were also detected in the present study. Among samples that were positive for Capillarinae eggs, it was possible to observe two

Table 1. Diversity and prevalence of infections in captive Indian peafowl (*Pavo cristatus*), São Paulo Zoo/Brazil, October 2017 and March 2018

Parasite	No+ (%)	Direct (%)	NaCl (%)	$ZnSO_4$ (%)	Sucrose (%)	Sedimentation (%)	Ether-formalin (%)
Non-sporulated coccidian oocysts	99 (91.7)	30 (30.3)	80 (80.8)	68 (68.7)	79 (79.8)	40 (40.4)	–
Capillarinae eggs	97 (89.8)	32 (33.0)	74 (76.3)	58 (59.8)	86 (91.8)	40 (41.2)	–
Unidentified nematode larvae	81 (75.0)	42 (51.9)	16 (19.8)	72 (88.9)	54 (66.7)	41 (50.6)	–
Ascarididae eggs	68 (63.0)	16 (23.5)	39 (57.4)	44 (64.7)	55 (80.9)	34 (50.0)	–
Unidentified nematode adults	65 (60.2)	26 (40.0)	6 (9.2)	53 (35.4)	36 (55.4)	25 (38.5)	–
Unidentified nematode eggs	46 (42.6)	7 (15.2)	23 (50.0)	25 (54.4)	29 (64.4)	13 (28.3)	–
Strongylids-like eggs	42 (38.9)	1 (2.4)	17 (40.5)	21 (50.0)	28 (66.7)	6 (14.3)	–
Cryptosporidium spp.	31 (28.7)	–	–	–	–	–	31 (100)
Trophozoites of flagelated protozoans	17 (15.7)	17 (100)	–	–	–	–	–
Trophozoites of ciliated protozoans	11 (10.2)	5 (45.5)	–	–	–	8 (72.7)	–
Non-sporulated coccidian oocysts (Adeleidae)	1 (0.9)	–	1 (100)	1 (100)	1 (100)	–	–
Cruzia sp. eggs	1(0.9)	–	–	1 (100)	–	–	–
Acanthocephala egg	1(0.9)	–	1 (100)	–	–	–	–
Trematode eggs	1(0.9)	–	–	–	–	1 (100)	

Explanations: No+: number of positive samples; (%): prevalence of positive samples

morphological distinctive types of eggs (Fig. 1D,E). Trophozoites of ciliated (Fig. 1A) and flagellated protozoans were identified. *Cryptosporidium*-like structures were also observed (Fig. 2). Other coccidian oocysts and helminth eggs were identified in the present study in a low prevalence. These are *Cruzia* sp. (Fig. 1K), trematode (Fig. 1G), Acanthocephala eggs (Fig. 1H) and Adeleidae oocysts (Fig. 1C). Noteworthy, some of the parasites with low prevalence detected in the present study can be considered as pseudoparasites/contamination, and do not represent *P. cristatus* parasite diversity, these are: Acanthocephala and *Cruzia* sp. eggs, and Adeleidae oocysts.

Comparison between techniques

Direct smear technique was the only one that detected the presence of flagellated protozoan parasites. It is also interesting to note that this technique could detect the majority (nine out of the 14) of the parasites found in the present study (Table 1), however, in a lower prevalence when compared to other techniques used. Direct smear presented an intermediate sensitivity (75%) for detection of unidentified nematodes but, for all the other parasites, this method had a low sensitivity (< 30%). The specificity of direct smear was high (> 92%) for strongylid-like and Ascarididae parasites. This technique detected the smallest number of infections (Table 2).

Flotation with NaCl solution was capable to detect the majority of the infections by non-sporulated coccidian oocysts and Capillarinae eggs. But it is not recommended to diagnose the presence of adults or larvae of nematodes (Table 1). $ZnSO_4$ solution was the best one to diagnose the presence of unidentified nematodes in larvae and adult stages (Table 1). This method has a high sensitivity (>90%) to unidentified nematodes and an intermediate specificity (82.5%) to strongylid-like

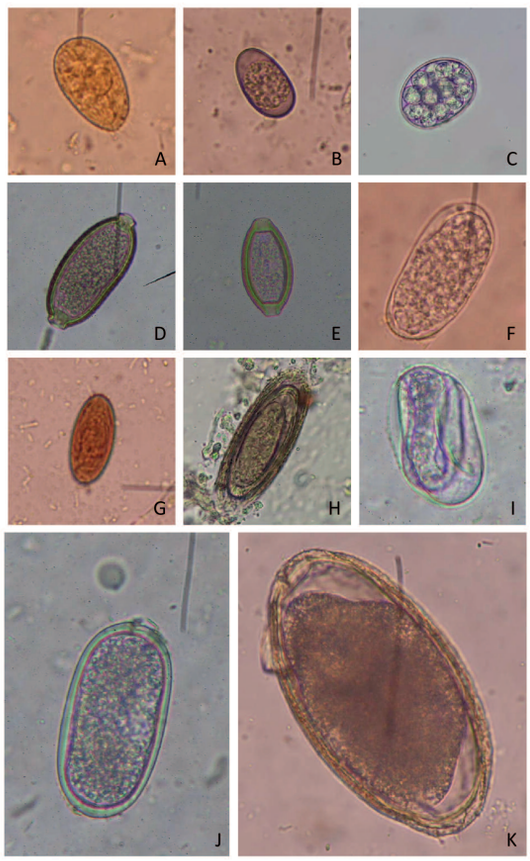

Figure 1. Protozoan and helminths found in *Pavo cristatus*. Trophozoites of ciliated protozoan (A), non-sporulated coccidian oocysts (B), sporulated coccidian oocyst, suggestive of belonging to Adeleidae (C), Capillarinae eggs (D,E), unidentified nematode egg (F), Trematode egg (G), Acanthocephala egg (H), strongylid-like egg (I), Ascarididae egg (J) and egg suggestive of *Cruzia* sp. (K). 400× magnification.

(Table 2). While, sucrose solution seems to be the best one to diagnose nematode infections (Capillarinae, Ascarididae, unidentified nematode and strongylid-like eggs) (Table 1). This technique presented a high sensitivity (>90%) to diagnose nematodes unidentified nematodes and Capillarinae, however with low specificity (58.3% and 47.1%, respectively) (Tab. 2).

Sedimentation technique was also able to detect nematode and coccidian infections, usually in the same rate as the direct smear, and sometimes even better than this one (Table 1). However, it was the only one capable to detect the presence of trematode eggs, that was found in only one sample. This technique had a high sensitivity to detect unidentified nematodes (94.4%) and a high specificity (89.3%) to coccidian oocysts (Table 2).

For the studied population, PPV values were high (>90) only for Ascarididae eggs with $ZnSO_4$ and for coccidian oocysts using faecal sedimentation. In regards of NPV, the majority present a moderately high value (>80), however, low values were observed for Capilariinae eggs and coccidian oocysts (Table 2).

Cohen's *Kappa* index showed that the agreement between techniques were considered mainly moderate or fair. There was no agreement considered as substantial or almost perfect. Combination with NaCl and sucrose was the one that presented the highest Cohen's *Kappa* index for all parasite groups, with exception of unidentified nematodes, which combination among gold standard and faecal sedimentation had a better agreement between techniques (Table 3).

Discussion

This study was able to diagnose infections by Capillarinae, Ascarididae, unidentified nematodes, strongylid-like, coccidian and trematodes in captive Indian peafowl population of FPSZP. Many parasites have already been reported in Indian peafowl such as: *Giardia* spp., *Eimeria* spp., *Cryptosporidium* spp., *Strongyloides pavonina*, *Strongyloides* sp. *Heterakis* spp., *Capillaria* spp., *Ascaridia* spp. and cestodes [12,15,16,26–31]. Even though other authors have reported the presence of cestodes in *P. cristatus* [8,12], this group of parasites is rarely mentioned for captive birds and it was not found in the present study. This is probably due to the captivity condition that the studied group lives, which do not favour the development of these parasites, that, in general, have a complex life cycle that requires at least one intermediate host to complete their development [22]. In general, captive environment favours the presence of direct life cycle parasites [32]. Unfortunately, it was not possible to identify the parasites found till the species level. It is interesting to note that the techniques applied in the present study might not be the most appropriated ones for the diagnose of nematode larvae and adults [17]. However, we could detect the presence of these nematode parasite stages in our study, showing that these techniques might be applied in order to have a fast parasite diagnoses in veterinary laboratories.

Despite the carefull sampling, some of the parasites found in this study might be considered as pseudoparasites/contamination for the studied population. Acanthocephala and *Cruzia* sp. eggs reported are similar to parasites found during the

Table 2. Prevalence, sensitivity, specificity, positive and negative predictive values obtained with coproscopical methods in samples from captive population of Indian peafowl, São Paulo Zoo/Brazil, October 2017 and March 2018. Concentration with NaCl solution was considered as gold standard method.

Group of infections/methods	No. (%)	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
unidentified nematodes (eggs, larvae, adults)					
Zinc Sulfate	79 (73.2)	94.4 (87.0-100)	37.5 (26.3-48.7)	43.0 (32.1-54.0)	93.1 (83.9-100)
Sucrose	64 (59.3)	94.4 (87.0-100)	58.3 (47.0-69.7)	53.1 (40.9-65.4)	95.5 (89.3-100)
Faecal sedimentation	50 (46.3)	80.6 (67.6-93.5)	70.8 (60.3-81.3)	58.0 (44.3-71.7)	87.9 (79.6-96.3)
Direct	49 (45.4)	75.0 (60.9-89.2)	69.4 (58.8-80.1)	55.1 (41.2-69.0)	84.8 (75.6-93.9)
Capillarinae (eggs)					
Zinc Sulfate	58 (53.7)	64.9 (54.0-75.5)	70.6 (55.3-85.9)	82.8 (73.0-92.5)	48.0 (34.2-61.9)
Sucrose	86 (79.6)	91.9 (85.7-98.1)	47.1 (30.3-63.8)	79.1 (70.5-87.7)	84.8 (54.1-91.3)
Faecal sedimentation	40 (37.0)	43.2 (32.0-54.5)	76.5 (62.2-90.7)	80.0 (67.6-92.4)	38.2 (26.7-49.8)
Direct	32 (29.6)	29.7 (19.3-40.1)	70.6 (55.3-85.9)	68.8 (52.7-84.8)	31.6 (21.1-42.0)
Strongylid-like (eggs)					
Zinc Sulfate	21 (19.4)	35.3 (12.6-58.0)	82.5 (75.9-91.1)	28.6 (9.6-47.9)	87.4 (80.4-94.3)
Sucrose	28 (25.9)	58.8 (35.4-82.2)	80.2 (72.0-88.4)	35.7 (18.0-53.5)	91.3 (85.1-97.4)
Faecal sedimentation	6 (5.6)	23.5 (3.4-43.7)	97.8 (94.8-100)	66.7 (29.0-100)	87.3 (80.8-93.7)
Direct	2 (1.9)	0 (-)	97.8 (94.8-100)	0 (-)	84.0 (77.0-91.0)
Ascarididae (eggs)					
Zinc Sulfate	44 (40.7)	74.4 (60.7-88.1)	78.3 (68.5-88.0)	95.6 (51.9-79.9)	84.4 (75.5-93.3)
Sucrose	55 (50.9)	89.7 (80.2-99.3)	71.0 (60.3-81.7)	63.6 (50.9-76.4)	92.5 (85.3-99.6)
Faecal sedimentation	34 (31.5)	64.1 (49.1-79.2)	87.0 (79.0-94.9)	73.5 (58.7-88.4)	81.1 (72.2-90.0)
Direct	16 (14.8)	28.2 (14.1-42.3)	92.8 (86.6-98.9)	68.8 (46.0-91.5)	69.6 (60.2-79.0)
Coccidian (oocysts)					
Zinc Sulfate	65 (60.2)	68.8 (58.5-79.2)	57.1 (38.8-75.5)	81.5 (72.1-91.0)	40.0 (24.8-55.2)
Sucrose	79 (73.2)	83.8 (75.7-91.8)	57.1 (38.8-75.5)	84.8 (76.9-92.7)	55.2 (37.1-73.3)
Faecal sedimentation	39 (36.1)	45.0 (34.1-55.9)	89.3 (77.8-100)	92.3 (83.9-100)	36.2 (24.9-47.6)
Direct	30 (27.8)	28.8 (18.8-38.7)	75.00 (59.0-91.0)	76.7 (61.5-91.8)	26.9 (17.1-36.8)

Explanations: %: prevalence of infections; CI: confidence interval; PPV: positive predictive value; NPV: negative predictive value

processing of samples from free-living *Didelphis aurita* encountered in the study site (personal communication; [33]). Another parasite found during the present study, that can be classified as a pseudoparasite/contamination are the Adeleidae oocysts, they are parasites of invertebrates and have around ten sporocysts on each oocyst [34], being readily distinguished from the other oocysts found. The presence of this coccidian in our samples can be the result not only of contamination, but also due to the feeding habits of Indian peafowls that eat small invertebrates that may enter their enclosure.

Trematode eggs were found in only one analysed sample. Birds can harbour infections by these group of parasites, however it has never been reported in Indian peafowls before. Trematode infections were also seen in samples of free-living *Didelphis aurita* the lives in the study site (personal communication). Without knowing to which species these trematodes belong to, it will be difficult to confirm if this is a true parasite of Indian peafowls or pseudoparasite/contamination. More studies are required in this matter.

Cohen's *Kappa* index calculated in the present

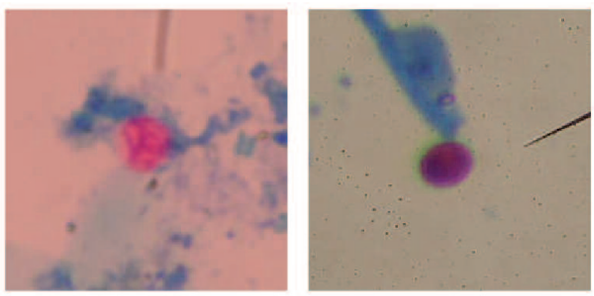

Figure 2. *Cryptosporidium*-like structures. Pink round structures, in contrast with the blue background staining, suggestive of being *Cryptosporidium* spp. Stained by modified Ziehl-Neelsen technique. 1000× magnification.

study shows that there is a small degree of agreement between the techniques applied in this study. This is probably due to the difference between the specific gravid of the flotation solutions used and the diversity of parasite stages that can be found in the studied host. The present study corroborates with previous ones that recommends using more than one diagnose technique when it is necessary to diagnose parasitic infections [17,18].

Among all methods used in this study, direct smear was the only one capable to detect the presence of flagellated protozoans in samples. This data corroborates with previous studies, indicating that it is possible to observe the presence of protozoan in a relatively simple technique, that can be easily applied in veterinarian diagnose laboratories [11,22]. Despite that, this is not the best method to detect infections by other parasites, specially trematodes and strongylid-like eggs (Table 1).

Flotation solutions are indicated to be used for light eggs, such as nematodes and cestodes, oocysts and some protozoan cysts [20,22]. Using the NaCl flotation solution was possible to detect all nematodes and coccidian infections, but it seems to be better for diagnosing coccidian infections (Table 1). Egbetade et al. [35] used this technique in

Table 3. Concordance between coproscopical techniques in captive population of Indian peafowl, São Paulo Zoo/Brazil, October 2017 and March 2018

Group of infections/methods	No+	*Kappa* (95% CI)
Unidentified nematodes (eggs, larvae, adults)		
NaCl*Zinc Sulfate	34	0.3 (0.1-0.4)
NaCl* Sucrose	34	0.4 (0.3-0.6)
NaCl* Faecal sedimentation	29	0.5 (0.3-0.6)
NaCl*Direct	27	0.4 (0.2-0.6)
Capillarinae (eggs)		
NaCl*Zinc Sulfate	48	0.3 (0.1-0.5)
NaCl* Sucrose	68	0.4 (0.3-0.6)
NaCl* Faecal sedimentation	32	0.2 (0.0-0.3)
NaCl*Direct	22	0.0 (0.0-0.1)
Strongylid-like (eggs)		
NaCl*Zinc Sulfate	6	0.2 (0.0-0.4)
NaCl* Sucrose	10	0.3 (0.1-0.5)
NaCl* Faecal sedimentation	4	0.3 (0.0-0.5)
NaCl*Direct	0	-
Ascarididae (eggs)		
NaCl*Zinc Sulfate	29	0.5 (0.4-0.7)
NaCl* Sucrose	35	0.6 (0.4-0.7)
NaCl* Faecal sedimentation	25	0.5 (0.4-0.7)
NaCl*Direct	11	0.2 (0.1-0.4)
Coccidian (oocysts)		
NaCl*Zinc Sulfate	56	0.2 (0.1-0.4)
NaCl* Sucrose	67	0.4 (0.2-0.6)
NaCl* Faecal sedimentation	36	0.2 (0.1-0.4)
NaCl*Direct	23	0.0 (0.0-0.1)

Explanations: No+: number of positive samples; CI: confidence interval

samples from captive animals, including mammals, reptiles and birds, and found a high diversity of nematode parasites, however did not reported the detections of any coccidian oocysts, while Marques et al. [36] found nematode eggs (Capillarinae, Ascarididae, Strongylidae) and cysts of amoeba and coccidian oocysts. When $ZnSO_4$ solution was used, it was possible to diagnose the same parasites as the previous technique but seems to be the most suitable for diagnosing larvae and adults of unidentified nematode (Table 1). Previous studies using this technique were able to diagnose infections of coccidian oocysts, nematode eggs and larvae, cysts of amoeba, Ascarididae and Capillarinae eggs in dogs and cats [37] and in Galliformes, Anseriformes and Struthioniformes [38]. In regards of sucrose solution, it is recommended mainly to diagnose infections by Capillarinae and Ascarididae. In the literature other studies reported the presence of nematode eggs, *Heterakis* sp., strongylid, *Capillaria* sp. and cestodes in domestic chicken [39], Psittacidae, Cracidae and Ramphastidae [40].

The preparation of flotation solution is a very important step during diagnostic procedures in veterinarian clinical analysis laboratories. Other important step when performing these techniques is the interval between preparing the samples and performing microscopic analysis, since each flotation solution has some peculiarities such as being able to deform eggs and larvae stages due to their high density and different drying out properties. NaCl flotation solution was the easiest one to prepare, but preparations can dry out quite fast, making difficult to perform microscopic analysis when many preparations are processed at the same time. To avoid this, it would be recommended to prepare few samples at the same time, or keeping them in a humid chamber until microscopic analysis. Concerning $ZnSO_4$ solution, it is very fast to prepare, and the salt is easily diluted; preparations took more time to dry out when compared to NaCl solution. Sucrose solution was the most difficult to prepare, it requires the use of warm/boiled water, so the sugar can be easily diluted, which takes a long time to be done; this solution has high viscosity leading to a very low drying out rate after slides are prepared. Besides that, it is recommended to add formalin to avoid that bacteria and fungi contaminate it, which require extra care to discard this solution after being processed, in order to prevent environmental contamination.

Faecal sedimentation technique is indicated for concentration of some protozoan cysts and heavy eggs such as Trematoda and Acanthocephala [22]. Despite of this indication, the detection of light eggs was possible in the present study, which corroborates with previous studies [41,42]. It is interesting to note that this technique was applied before in samples from dogs, being able to diagnose coccidian oocysts, nematode and cestode eggs [42]. In another study with captive Brown capuchin (*Cebus apella*) it detected *Ancylostoma* sp. and *Strongyloides* sp. [41].

Cryptosporidium-like oocysts can be very difficult to be diagnosed, which is mainly due to their small size (between 3 to 5 µm), similar to some fungi structures that can be found in bird faeces [43]. In order to increase the chance of diagnosing these infections it is important to apply not only concentration techniques, such as formalin-ether sedimentation, but also staining techniques, as is the case of the modified Ziehl-Neelsen staining. These two techniques applied together makes easier to find *Cryptosporidium*-like oocysts. Although, not only *Cryptosporidium*-like oocysts can be easily seen, other structures have similar size and stain in a similar way, such as fungi. The recommendation is to apply these techniques as a first screening, in order to select the samples to be analysed using more sensitive technique, such as PCR-based protocols.

With this study, we were able to conclude that direct smear is suitable for diagnosing flagellated protozoan infections. Sucrose and zinc sulphate solutions presented good results when compared to the NaCl solution. Despite of the sedimentation technique being able to diagnose nematodes and coccidians infection, more sensitive and specific techniques should be applied. The combination of formalin-ether and Ziehl-Neelsen techniques were the only one capable to indicate the presence of *Cryptosporidium*-like oocysts, despite of that, it is still necessary to apply complementary tests to confirm the infection.

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