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Sensitivity and efficiency of selected coproscopical methods—sedimentation, combined zinc sulfate sedimentation-flotation, and McMaster method

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Abstract Coproscopical methods used in veterinaryparasitological diagnostics were validated according to their sensitivity (Se) and egg recovery rate [efficiency (Ef)]. Validation of the combined sedimentation-flotation method and the modified McMaster method was performed by using feces spiked with eggs of Ancylostoma caninum, Uncinaria stenocephala, Cooperia oncophora, cyathostomins, Ascaris suum, Toxascaris leonina, Toxocara canis, Trichuris vulpis, Moniezia expansa, and Anoplocephala perfoliata. For validation of the sedimentation method, Fasciola hepatica eggs were used. With the combined sedimentation-flotation method using ZnSO4 as flotation medium [specific gravity (SG) 1.30], 5 g fecal samples of all tested parasite species (concentration levels 1, 5, 10, 20, 40, 60, and 80 epg) were reproducibly detected "positive" (100 % Se) as of 80 epg. The Ef of the combined sedimentation-flotation method, defined as percentage of rediscovered eggs, revealed clear differences between parasites and showed the highest value for cyathostomins and the lowest for U. stenocephala and T. leonina eggs. The average Ef for all parasite species at 80 epg was 1.50 %. With the McMaster method (concentration levels 1, 30, 50, 80, 100, 500, and 1000 epg), all tested parasite species were detected reliably positive as of 500 epg with a mean Ef of 46.4 %. When evaluating the sedimentation method (concentration levels 1, 5, 10, 15, 20, 25, and 30 epg), F. hepatica eggs were reproducibly found in 5 g fecal

Christina Strube christina.strube@tiho-hannover.de samples as of 20 epg with 20.0 % Ef. The result that the combined zinc sulfate sedimentation-flotation method (SG 1.30) as flotation medium provides diagnostic certainty only as of 80 epg has to be considered at preventing zoonoses. If pet owners wish to prevent any zoonotic infection ("zero tolerance"), a monthly anthelminthic treatment should be advised instead of monthly fecal examinations.

Keywords Diagnostics · Coproscopical examination · Coproscopy · Fecal examination · Parasitological examination · Sedimentation-flotation method · McMaster · Sedimentation

Introduction

Microscopic examinations of human or animal fecal samples are an integral part of medical diagnostics and are performed in most laboratories throughout the world (Ward et al. 1997). Quality assurance and management systems in parasitological laboratories raise the necessity of standardized validation of the methods used in those laboratories. In search of reliable, fast, quantitative, and qualitative possibilities to detect helminth parasite stages, many methods have been developed. The methods most frequently used to detect eggs in fecal samples are the combined sedimentation-flotation method or sedimentation alone in the case of parasitic eggs with higher density (e.g., trematode eggs). For quantitative determination of cestode and nematode eggs in fecal samples, the McMaster technique (Gordon and Whitlock 1939; Whitlock 1948) and its several modifications (MAFF 1986; Dunn and Keymer 1986; Cringoli et al. 2004) are most commonly used. These approved methods are partly

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several decades old and, despite some modifications, rely on the same protocol. Surprisingly, validations of these methods were rarely carried out systematically using eggs of different parasite species and if done, only on a small scale with either one or few parasite species. Questions regarding precision became progressively aware in times of quality assurance and quality management systems. In addition, fecal egg shedding intensity-based selective treatment approaches in ruminants and particularly horses arose over recent years due to anthelmintic resistance. In companion animals, veterinarians and pet owners become more and more aware of zoonotic helminths such as Echinococcus, Toxocara, or Ancylostoma species. At the same time, there is a trend to reduce application of "chemicals," in this sense use of anthelmintic drugs. Thus, treatment decisions are quite frequently based on the outcome of regular coproscopical examinations. The present study was performed to supply validation data regarding sensitivity and efficiency of the combined zinc sulfate sedimentation-flotation method, McMaster method, and sedimentation method. Validation was performed for common parasites in domestic animals (dog, cat, horse, pig, and cattle) based on fecal samples spiked with different numbers of eggs per gram feces (egp).

Material and methods

Extraction of eggs

Eggs of Ancylostoma caninum, Uncinaria stenocephala, Cooperia oncophora, cyathostomins, Ascaris suum, Toxascaris leonina, Toxocara canis, Trichuris vulpis, Moniezia expansa, Anoplocephala perfoliata, and Fasciola hepatica were extracted for subsequent spiking of fecal samples. Feces containing eggs of cyathostomins and C. oncophora was taken from the rectum of experimentally infected animals. Eggs of A. caninum, U. stenocephala, T. canis, and T. leonina were obtained from infected animal feces not older than 24 h. Animal infection experiments were approved by the ethics commission of the German Lower Saxony State Office for Consumer Protection and Food Safety under reference numbers 33.9-42502-05-01A038 and 33.9-42502-05-06A395.

Feces with eggs of cyathostomins, *C. oncophora*, *A. caninum*, or *U. stenocephala* was mixed with saturated NaCl solution [specific gravity (SG) 1.2], poured through a hair sieve into a beaker, and allowed to float for 30 min. To wash out the salt solution, floated eggs were transferred into a beaker filled with tap water and allowed to sediment for 30 min. After decanting the supernatant, the beaker was filled with tap water again to repeat sedimentation at total of three times.

Feces containing *T. canis* and *T. leonina* was soaked in tap water for 1 h and subsequently sieved twice (mesh size 200 μ m, followed by 50 μ m). The second sieve retained the eggs, which were subsequently rinsed into a beaker. The beaker was filled up with saturated NaCl solution to float and subsequently sediment the eggs as described above.

To obtain *T. vulpis*, *M. expansa*, and *A. perfoliata* eggs, adult parasites were collected from intestines of a dog, sheep, or horse, respectively, sent to the Department of Pathology, University of Veterinary Medicine Hannover, for diagnostic necropsy. Parasites were grinded in a mortar and poured through a fine sieve into a beaker filled with tap water. Eggs were allowed to sediment overnight.

A. suum eggs were obtained from parasites collected from the intestine of freshly slaughtered pigs. The uterus content of female parasites was spread out into a petri dish, and subsequently sieved and transferred into a tap water-filled beaker. Again, eggs were allowed to sediment overnight.

Bile collected from fluke-infected freshly slaughtered cattle was used to obtain *F. hepatica* eggs. Ten milliliters was transferred into pointed plastic vessels and filled up with tap water. The supernatant was decanted after 30 min, and the washing was repeated until the supernatant became clear.

The number of eggs in the sediments was determined by applying six times 10 μ l on two slides (*n*=12), counting the eggs and calculating the average number of eggs per 10 μ l.

Coproscopical methods

Combined sedimentation-flotation method

To evaluate sensitivity of the combined sedimentationflotation method, feces of parasite-naïve animals was spiked with eggs of abovementioned parasite species at concentrations of 1, 5, 10, 20, 40, 60, and 80 eggs per gram (epg) and thoroughly mixed to distribute the spiked eggs as evenly as possible. Per parasite species and concentration, 15 fecal samples of 5 grams each were examined (n=105) with exception of A. caninum (n=155; 15 samples each for 5 and 80 epg, 25 samples each for 1, 10, 20, 40, and 60 epg) and T. canis (n=125; 15 samples each for 1, 5, 40, 60, and 80 epg, 25samples each for 10 and 20 epg). The fecal samples were given in a stainless steel tea strainer, and eggs were rinsed in a beaker with a strong water jet. The filtrate was left to stand for 30 min for sedimentation. The supernatant was decanted and the sediment was transferred into a 15-ml tube and filled up with zinc sulfate solution (ZnSO4, SG 1.30) followed by centrifugation at 450g for 5 min. The liquid surface was completely transferred onto a slide and covered with a coverslip. The sample was immediately examined microscopically at $\times 63$ or $\times 160$ magnification. If at least one egg per fecal sample was detected, the sample was classified as "positive". For each individual parasite species, the method's sensitivity (Se) at the different epg concentrations was calculated with the following formula:

Se (%) = (number of positive samples / total number of samples) \times 100

Furthermore, the method's egg recovery rate, the socalled efficiency (Ef), was calculated for each individual parasite species. Ef was calculated using the following formula:

 $Ef(\%) = (number of rediscovered eggs / number of total eggs in the sample) \times 100$

In addition to Se and Ef calculations at individual parasite level, the method's mean Se and Ef including all examined parasite species were calculated at different epg concentrations (n=165-185 per concentration). Furthermore, Se and Ef were calculated for parasite orders (Strongylida, Ascaridida, Enoplida, and Anoplocephalida).

McMaster method

For evaluation of the McMaster method, fecal samples of parasite-naïve animals were spiked with 1, 30, 50, 80, 100, 500, and 1000 epg of abovementioned parasite species and thoroughly mixed. Per parasite species and concentration, 15 fecal samples of 4 g each were examined

(n=105) except *A. perfoliata* that was not tested at the concentration of 1000 epg (n=90). Each sample was mixed with 10-ml saturated NaCl solution (SG 1.20) except *T. vulpis* which was mixed with ZnSO4 solution (SG 1.30). The mixture was sieved through a stainless steel tea strainer into a 100-ml cylindrical stand, filled up to 60 ml with NaCl solution or ZnSO4, respectively, and mixed thoroughly. The suspension was then transferred into a flask with a ground-glass stopper. Following thorough mixing again, three McMaster counting grids were filled with the suspension, left 3 min for egg flotation and were subsequently screened (×25 or ×63 magnification). Two counting grid sidelines (left and below) were included in the counting. The epg was calculated as follows:

epg = number of counted eggs out of three counting grids $\times 100 / 3$

Individual parasite-specific, order-specific, and mean Se and Ef including all examined parasite species at the different epg concentrations (n=165 per concentration) were determined as described above.

Sedimentation method

Fecal samples of 5 g each were spiked with *F. hepatica* eggs at concentrations of 1, 5, 10, 15, 20, 25, and 30 epg to evaluate the sedimentation method. Per epg concentration, 15 fecal samples were examined, resulting in a total of 105 samples. Samples were sieved through a stainless steel tea strainer with a strong water jet until a 250-ml beaker was completely filled. After 30 min of sedimentation, the supernatant was decanted and the sediment was transferred into a 500-ml pointed plastic vessel. After filling up with tap water and 3 min of sedimentation, the supernatant filled up with tap water until the solution became clear. One drop methylene blue solution (1 %) was given on the sediment before it

was examined for eggs with $\times 63$ magnification. Se and Ef for *F. hepatica* eggs were calculated by the formulas given above.

Results

Combined sedimentation-flotation method

At a concentration of 1 epg, 6.6 % of cyathostomin samples were positive, whereas all other parasite samples were detected negative. At concentrations as of 10 epg, positive samples were occasionally identified in each included parasite with Se ranging from 12.0 to 86.7 %. All samples of all parasites were correctly identified as positive (100 % Se) at 80 epg. Over all concentration levels, the order Strongylida revealed an average Se of 59.9 %, Ascaridida of 48.1 %, Enoplida of 52.4 %, and Anoplocephalida of 56.8 %. Individual values for each examined parasite species as well as mean Se at each epg level are listed in Table 1. The Ef of the combined sedimentation-

Table 1 Sensitivities (in %) of the combined sedimentation-flotation, McMaster, and sedimentation method

Combined sedimentation-flotation method											
epg	A. caninum	U. stenocephala	cyathostomins	C. oncophora	A. suum	T. canis	T. leonina	T. vulpis	A. perfoliata	M. expansa	Mean
1	0.0	0.0	6.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.7
5	6.7	6.7	46.7	6.7	0.0	26.7	13.3	26.8	26.7	13.3	17.4
10	44.0	26.7	86.7	35.3	40.0	12.0	13.3	26.8	35.3	46.7	36.7
20	84.0	33.3	86.7	80.0	60.0	64.0	20.0	33.3	60.0	60.0	58.1
40	92.0	60.0	100	93.3	66.6	66.7	53.3	80.0	80.0	93.3	78.5
60	96.0	86.7	100	100	86.7	93.3	93.3	100	86.7	93.3	93.6
80	100	100	100	100	100	100	100	100	100	100	100.0
McMa	aster method										
epg	A. caninum	U. stenocephala	cyathostomins	C. oncophora	A. suum	T. canis	T. leonina	T. vulpis	A. perfoliata	M. expansa	Mean
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
30	40.0	46.7	13.3	40.0	26.7	0.0	40.0	26.7	13.3	12.0	25.9
50	60.0	53.3	60.0	46.7	53.3	26.7	46.7	66.7	26.7	33.3	47.3
80	60.0	46.7	60.0	60.0	53.3	53.3	73.3	53.3	60.0	46.7	56.7
100	53.3	93.3	80.0	73.3	53.3	53.3	80.0	80.0	80.0	60.0	70.7
500	100	100	100	100	100	100	100	100	100	100	100.0
1000	100	100	100	100	100	100	100	100	100	100	100.0
Sedim	entation meth	iod									
epg	F. hepatica										
1	0.0										
5	26.6										
10	73.3										
15	93.3										
20	100										
25	100										
30	100										

flotation method was constantly in the single-digit range as shown in Table 2. Average Ef was 1.4 % for the Strongylida, 0.6 % for the Ascaridida, 0.7 % for the Enoplida, and 1.0 % for the Anoplocephalida.

McMaster method

At concentrations as of 30 epg, positive samples were identified in each parasite species except *T. canis* with Se values between 12.0 and 46.7 %; mean Se at 30 epg was 27.2 %. *T. canis* turned positive at 50 epg with 26.7 % Se. As of 500 epg, all samples were detected positive (Table 1). For the order Strongylida, Se was 60.2 %, for Ascaridida 55.2 %, for Enoplida 61.0 %, and for Anoplocephalida 52.3 %. Species-specific average Efs in samples detected positive were lowest for *A. perfoliata* and highest for *T. leonina*. Mean Ef including all species remained within a relatively narrow range of 41.6-51.5% among different positively detected epg levels (Table 2). On parasite order level, Strongylida revealed an average Ef of 41.8 %, Ascaridida of 36.0 %, Enoplida of 47.9 %, and Anoplocephalida of 27.1 %.

Sedimentation method

F. hepatica eggs were detected as of 5 epg (Se=26.6 %), whereas as of 20 epg, sensitivity was constantly 100 % with Efs between 18.4 and 19.7 %. Detailed sensitivity and efficiency values are given in Tables 1 and 2.

Discussion

The validity of a coproscopical method can be assessed by two parameters, which are the number of false negative samples [sensitivity (Se)] and the proportion of rediscovered eggs [efficiency (Ef)] (Egwang and Slocombe 1981). The combined sedimentation-flotation method focuses particularly on recognizing positive samples with maximum reliability. Thus, Se is of particular importance. To obtain reliable data regarding the limits of Se, the present study was performed with low numbers of eggs per gram fecal sample (1–80 epg). According to the study's results, no false negative results (100 % Se) can be expected by use of ZnSO₄ (SG 1.3) as flotation solution as Table 2 Efficiencies in % rediscovered eggs of the combined sedimentation-flotation, McMaster, and sedimentation method

Combined sedimentation-flotation method											
epg	A. caninum	U. stenocephala	cyathostomins	C. oncophora	A. suum	T. canis	T. leonina	T. vulpis	A. perfoliata	M. expansa	Mean
1	0.00	0.00	1.20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.12
5	0.27	0.24	2.12	0.52	0.00	1.32	0.52	1.04	1.60	0.52	0.82
10	1.28	0.52	3.46	1.60	1.06	0.32	0.40	0.80	1.32	1.60	1.24
20	1.36	0.30	2.00	1.73	0.60	0.84	0.26	0.60	0.80	0.87	0.94
40	0.74	0.43	4.13	2.70	0.83	0.83	0.20	0.80	0.90	1.43	1.30
60	0.87	0.75	2.22	2.75	1.06	1.04	0.96	1.13	0.91	1.37	1.31
80	1.50	0.78	3.51	3.00	1.28	1.17	0.71	0.62	1.00	1.40	1.50
McMa	aster method										
epg	A. caninum	U. stenocephala	cyathostomins	C. oncophora	A. suum	T. canis	T. leonina	T. vulpis	A. perfoliata	M. expansa	Mean
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.00
30	59.2	74.0	22.2	59.2	44.4	0.0	74.0	37.0	22.2	44.4	43.66
50	57.7	44.4	44.4	39.9	48.8	17.8	48.8	62.2	26.6	35.5	42.61
80	38.9	33.3	61.0	61.0	41.6	27.7	52.7	36.0	30.5	33.3	41.60
100	39.9	37.7	37.7	48.9	22.2	35.35	59.9	79.9	39.9	35.7	43.72
500	45.3	75.9	43.1	57.8	38.2	36.9	49.3	43.3	14.9	29.7	43.44
1000	43.8	40.7	51.1	53.1	34.4	33.1	90.7	76.7	not tested	39.8	51.49
Sedim	nentation meth	od									
epg	F. hepatica										
1	0.0										
5	6.6										
10	12.0										
15	16.4										
20	19.7										
25	18.9										
30	18.4										

of 80 epg for all examined parasite species. Below this concentration, the rates of false negative samples varied depending on the species examined. Best sensitivity was reached for cyathostomine eggs, which were reliably detected (100 % Se) as of 40 epg, and even at 1 epg, one out of the 15 examined samples was found positive (6.66 % Se). By contrast, the rather poorly detectable T. leonina eggs reached only 53.33 % Se and 0.00 % Se at these epg concentrations. These differences probably result from differences in specific egg weights of various parasites. Significantly higher Se values were obtained by Egwang and Slocombe (1981) by using Cornell Direct Centrifugal Flotation (DCF) and Wisconsin-DCF, which are concentration methods including flotation and centrifugation as well. With these methods, 100 % Se was found at Haemonchus contortus concentration levels of 7, 30, and 60 epg. Results are not directly comparable to the present study as methods differ in the flotation solution, and with Haemonchus contortus, another parasite species was used for evaluation of the method. Comprehensive studies comparing different solutions were performed by Mayerhofer (1985), Schragner (1986), and Hinaidy et al. (1988), who determined concentrated sugar solution (SG 1.26) to be the most effective flotation medium for eggs of different parasites in different host species. However, these studies used a significantly lower concentrated ZnSO₄ solution (SG 1.24) than the present study (SG 1.30). Zajac et al. (2002) also compared sugar solution (SG 1.25) against ZnSO₄ (SG 1.18), determining higher numbers of positive samples with the latter. According to their results, ZnSO₄ is an effective flotation solution when used with centrifugation procedure, offering the best balance between reliable flotation of eggs and cysts of different parasites and less distortion of sensitive protozoal cysts. Another advantage of $ZnSO_4$ is the possibility of stock-keeping, whereas sugar solution is quickly inhabited by fungi and leads to sticky contaminations of the workplace. Interestingly, the two hookworm species investigated, A. caninum and U. stenocephala, show distinct differences in sensitivity values for the combined sedimentationflotation method, where A. caninum shows higher values than U. stenocephala. This is also reflected in their efficiency

values for this method, but not reproduced by the McMaster method as another technically comparable method (flotation), however, with a different solution.

As the McMaster method is a quantitative method, Ef is of crucial importance besides Se. By use of NaCl as flotation medium, an overall Se of 100 % was achieved only as of 500 epg. But, as this method has been invented to process samples with high numbers of eggs with measureable quantity (Gibson 1965), this characteristic seems to be acceptable. Except 1 epg, the mean Ef of the McMaster method varied only slightly between different epg concentrations and was 44.4 % on average. Increasing the epg - at least in the examined range up to 1000 epg - showed no clear tendency toward improving the Ef. Different authors investigated the various parameters that influence McMaster results. Cringoli et al. (2004) showed a significant influence of flotation solutions on the quantity of discovered eggs and recommended sucrose-based solution (SG 1.20-1.35) as suitable for strongyle eggs. The present study showed that the used saturated NaCl solution (SG 1.20) is suitable for the examined parasite eggs. As T. vulpis eggs are known for their higher specific weight, they were floated with $ZnSO_4$ solution (SG 1.30), resulting in 76.7 % Ef at 1000 epg.

The results of the present study do not show apparent regularities regarding detection of floated eggs of different parasites. Se of the McMaster method was 100 % for all parasites as of 500 epg, whereas Ef varied among the different parasite species at this and the other epg levels. The combined sedimentation-flotation method showed the most reliable detectability (both Se and Ef) for herbivore gastrointestinal nematodes (C. oncophora and Cyathostominae). Strongyloidea as well as Anoplocephalidae differed from Ascarididae, which were distinctly lesser detectable. Sawitz (1942) showed that density of A. lumbricoides eggs varies even within the unicell stadium, as well as between fertile and infertile eggs, and Sasaki's investigations (1927) of fertile Ascaris eggs revealed that less developed eggs were heavier than mature eggs. Bailenger (1979) considered varying flotation behaviors of different eggs as a result of electrostatic surface characteristics, and Sawitz et al. (1939) observed different flotation behaviors of Trichuris eggs in various flotation media with the same specific gravity and attributed this to interactions of eggs surface ions with those of the medium.

The sedimentation method revealed 93.3 % Se at a *F. hepatica* concentration of 15 epg and 100 % Se as of 20 epg. Related Ef was 16 and 20 %. Happich and Boray (1969) achieved rather better results with only 3 g of spiked fecal samples. They found 100 % Se as of 10 epg, and Ef was 25.4, 29.6, and 32.1 % in 10, 100, and 1000 epg. Conceicao et al. (2002) achieved 33.3 % Se from 0.5 to 1.5 epg and 100 % Se as of 2 epg by use of 10-g spiked fecal samples. Ef was 39.2 % at 0.5–1.5 epg, 69.2 % at 2–8 epg, and 84.7 % at 12–100 epg. The use of 30-g feces increased Se to 83.3 %

and Ef to 50.6 % at 0.5–1.5 epg. These values are significantly higher than those of the present study and show clearly that a higher sample weight entails increase of Se and Ef.

In conclusion, it can be postulated that positive fecal samples can be reliably identified with the combined sedimentation-flotation method as of 80 epg and a sample amount of 5 g when ZnSO₄ is used as flotation medium (SG 1.30). Fasciola positive fecal samples of 5 gram were reliably detected with the sedimentation method as of 20 epg. Even though lower detection limits would be desirable, the epg levels turning a fecal sample reliably positive can be considered acceptable. The McMaster method based on saturated NaCl reliably tested samples positive as of 500 egp and 4-g feces. However, as a quantitative method, the recovery rate or efficiency, respectively, is an equally important parameter and was found to be about 44.4 %, meaning that the McMaster method underestimated the epg concentrations averagely by a good half. Overall, the present study again points out that results obtained for one parasite species cannot be readily extrapolated to another species, not even within the same order. Thus, coproscopical methods need to be validated for each species separately. The fact that a negative coproscopical result does not necessarily tell that the animal does not harbor parasites or does not pass eggs into the environment is of particular importance for companion animals when the pet owner intends to prevent any zoonotic infection ("zero tolerance"), e.g., due to immunocompromised individuals, babies, or toddlers in the household. Even though coproscopical results from composite fecal samples (taken each day for 3 days) are more reliable than those of singleday samples, it has to be considered that the combined zinc sulfate sedimentation-flotation method (SG 1.30) reaches 100 % sensitivity and thus diagnostic certainty only as of 80 epg. By use of saturated NaCl (SG 1.20) or other solutions with a lower SG than 1.30 as flotation medium or a lower fecal sample weight, the reliably detectable epg value might be even higher. Therefore, monthly anthelminthic treatments instead of fecal examinations should be advised if any zoonotic human infection risk should be excluded as far as possible.

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