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Advantages and limitations of diagnostic procedures for zoonotic helminth infections in feces

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Review Article

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Abstract

Helminthiasis is a common infection in both humans and other animal populations and negatively affects the health of the host, causing a range of morbidity and even mortality, especially in young people and those with weakened immune systems. A variety of diagnostic procedures with a number of modifications are available to identify the sources of infection and to assess the epidemiological situation, the effectiveness of parasite control programs, anthelmintic treatment, and the prevention of drug resistance. These can be simple and cheap methods – for example, stool smear examination – or modern highly sensitive methods – for example, PCR analysis. This review attempts to summarize the advantages and limitations of each of these frequently used methods.

Introduction

Adequate diagnostic techniques are necessary for the control, prevention, and treatment of common helminth infections in human and other animal populations (Crompton 2000; Utzinger and Keiser 2004). Fecal examination methods are one of the most accessible and simple techniques used for recovery of helminth eggs, larvae, and proglottids. The different fecal examination methods are broadly based on three principles such as floatation of helminth eggs in solutions with high specific gravity, sedimentation of fecal debris, and direct fecal examination. These techniques come with various modifications to increase the sensitivity of the assay such as centrifugation, a combination of different solutions, and straining (Crompton 2000). However, microscopy-based fecal examination tests have low sensitivity and low specificity due to the low concentration of helminth eggs and larvae in feces that can lead to false negative results (Thienpont *et al.* 1979). Moreover, some helminth eggs and larvae such as *Taenia* spp. eggs, Opisthorchiidae eggs, and some lungworm larvae cannot be differentiated by microscopy only (Annoscia *et al.* 2014; Jimenez *et al.* 2010; Rim 2005) Thus, alternative methods of detection of intestinal parasites must be developed. The detection of parasitic antigen in fecal samples and antibody in serum are highly sensitive and specific methods for the detection of individual parasitic infections, though both methods have their own limitations. Recently, a range of DNA-based methods for the detection of intestinal parasites in biological materials, such as feces, blood, and tissues, has been developed as a valuable diagnostic procedure for intestinal parasites. Single, duplex, and multiplex real-time PCR assays have been developed for the detection of many human and animal parasites (Frickmann *et al.* 2015; Ishida *et al.* 2003; Pilotte *et al.* 2016).

This review presents the advantages and limitations of fecal examination methods, ELISA, and PCR diagnostic methods utilized in the setting of helminthiasis detection.

Fecal examination methods

Fecal examination methods are the most simple and economical procedures for the detection of helminth infection. A variety of modifications are used, each with its own advantages and limitations (Thienpont *et al.* 1979). The most frequently applied procedures include direct fecal smear, floatation, and sedimentation methods. The selection of an appropriate method is highly dependent on the helminth egg's specific gravity. In most cases, this varies between 1.05 and 1.2 g/L . Roundworm and hookworm eggs have a lower specific gravity of around 1.05–1.1 g/L, whereas whipworm and tapeworm eggs are comparatively heavier and have a specific gravity of around 1.13–1.2 g/L. Fluke eggs have a specific gravity of more than 1.2 g/L and precipitate in

most flotation solutions, whose specific gravities usually fall between 1.18 and 2 g/L (David and Lindquist 1982; Harnnoi *et al.* 1998).

Direct fecal smear

Grassi and Parona first employed a direct fecal smear in 1878 as a diagnostic tool for the detection hookworm infection in humans (Rockefeller 1922). For many years, this method was used for the identification of different parasites with different modifications. For the simple direct fecal smear examination, a small sample of feces is placed on a glass slide, mixed with a drop or two of saline, spread thinly over the slide, and then covered with a glass coverslip. The smear needs to be thin enough to read newsprint through them. Later, Faust (1929) compared the sensitivity of the fecal smear technique without staining and with staining with hematoxylin. He observed that the staining of the fecal smear did not significantly improve diagnostic sensitivity compared to a direct fecal smear without staining. Thick smear technique was introduced by Kato and Miura in 1954 for fecal research (Kato and Miura 1954). They placed 60–70 mg fecal sample on the glass slide, covered it with a cellophane strip soaked with glycerol-malachite green, pressed it with a rubber stopper or finger, and kept it at a room temperature for about 30–60 min. Although the thick smear technique proved more sensitive than a direct fecal smear with a cover glass, it exhibited a low sensitivity for hookworm eggs, which are present in low density in fecal samples and often are destroyed during the long preparation period because hookworm eggs have a delicate consistency, quickly clear with glycerin, and after 30–60 minutes, they cannot be seen (Santos *et al.* 2005; https://iris.who.int/bitstream/handle/10665/324883/9789240014497-rus.pdf).

Katz adopted and modified this technique for use in routine epidemiological studies, resulting in its recognition by WHO for the quantitative and qualitative diagnosis of intestinal helminth infections such as *Ascaris lumbricoides*, hookworms, and *Schistosoma mansoni* (Katz *et al.* 1974; Santos *et al.* 2005). For the diagnosis of *S. mansoni*, this method may have low sensitivity. One of the main parameters affecting the sensitivity of the analysis is the minimum infection intensity, which depends on the egg output of one worm. One pair of *S. mansoni* produces about 100 eggs per day, so feces contain many eggs only in the case of intense invasion. Given the small amount of feces analyzed, detection of mild *S. mansoni* infections with only a few female worms is almost impossible (Barenbold *et al.* 2017).

However, fecal smear examination is the simplest and most economical procedure used in laboratories worldwide. The technique has been adopted for the detection of a wide range of parasites, including roundworms, whipworms, hookworms, flukes, and tapeworms. Among the numerous modifications of the direct fecal smear technique, Kato-Katz is recognized as a 'gold standard' and is widely used by both human and veterinary healthcare practitioners (Hong *et al.* 2003).

However, due to the small amount of stool being analyzed (41.7–70 mg), the sensitivity of the method suffers and can give false negative results due to low concentrations of helminth eggs in the stool sample or may appear highly clustered. Sensitivity can be increased by examining multiple Kato-Katz smears prepared from the same stool sample or from multiple samples from the same host or employing a combination of the Kato-Katz smear with a direct fecal smear (Glinz *et al.* 2010; Komiya and Kobayashi 1966). In addition, this method requires rapid sample processing – optimally within 20–30 minutes after slide preparation. The acceptable processing time for samples is within 24 hours. When stool samples are stored overnight, regardless of temperature, the number of parasite eggs decreases, which makes their detection difficult and does not allow reliably determining the intensity of infection (Bosh *et al.* 2021).

Concentration method

Microscopic examination of the sedimented fecal matter was one of the first modifications of the fecal smear examination technique (Koutz 1941). Sedimentation relied on the concentration of helminth eggs, larvae, and protozoan cysts at the bottom of a tube to detect parasites or eggs occurring at low densities in feces.

Since 1948, various modifications and simplified improvements have been employed by a number of researchers in clinical laboratories (Blagg *et al.* 1955; Knight *et al.* 1976; Manser *et al.* 2016). The method uses several pieces of apparatus, which must be washed after use with each sample and includes several safety precautions since it involves the use of formalin (an irritant) and ether, which is flammable. These kits are tightly sealed to reduce the hazards of formalin. The major modification of sedimentation method is using the ethyl acetate, a more stable and less flammable alternative, rather than ether (Truant *et al.* 1981; Young *et al.* 1979).

The TF-Test® is another modification of the concentration method for use in routine parasitological surveys. This complex method combines multiple sampling (on three consecutive days), a fixative (SAF-sodium acetate-acetic acid formalin), a concentration method, and a permanent stain (Chlorazol Black dye) (Van Gool *et al.* 2003).

A highly competitive method for field studies is MIF (merthiolate–iodine–formalin). It is a concentration-based method that requires a centrifuge. This technique uses the MIF solution (50 mL formaldehyde at 37%, 10 mL glycerin at 87%, filled to 1 L with distilled water as stock solution I) as a preservative and staining (with 2 g potassium iodide in 10 mL distilled water as stock solution II). Ether is added to dissolve the fecal fats. The MIF method showed higher sensitivity for hookworms, while for *T. trichiura* and *A. lumbricoides*, Kato-Katz performed better. However, MIF detected *Strongyloides stercoralis* for which Kato-Katz method is not specific. Another advantage of this technique is that it allows for the preservation of fecal samples for a long time. Overall, the MIF method is simple and inexpensive, and is suitable for diagnosis and assessment of infection intensity in the field (Incani *et al.* 2016).

Flotation techniques

The principle of fecal flotation is based on the ability of a solution to allow less dense material (including parasites) to rise to the top (Ballweber *et al.* 2014; Dryden *et al.* 2010).

The specific gravity (SG) of the flotation solution must be higher than the SG of helminth eggs, so commonly used flotation solutions have an SG of around 1.18–1.33 g/L. Common flotation solutions are made by adding a measured amount of sugar (sucrose or dextrose) or salts such as sodium chloride, sodium nitrate, magnesium sulfate, or zinc sulfate to a specific amount of water to achieve the desired SG. Ideally, all helminth eggs would float without loss of morphological structures, while fecal debris would sink. Despite their low cost and simplicity, the flotation methods are highly influenced by several factors, such as

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floatation time, tube filling, and precise removal of the coverslip. Flotation methods have many modifications, ranging from simple passive flotation in solution with high SG, the addition of a centrifugation step, and using a chambered slide or special devices that utilize flat-bottomed vials in which the feces/flotation fluid mixture is placed (Ovassay Plus Fecalyzer) (Ballweber *et al.* 2014).

The presence of a large amount of fecal debris is a major obstacle for the fecal flotation technique. One modification to alleviate this problem was the addition of a centrifugation step to remove large floating debris. This technique was first introduced by Lane (1924) and later modified by several others (Dryden *et al.* 2010; Zajac *et al.* 2002). The centrifugation time varies from 5 to 20 min depending on the characteristics of the centrifuge used such as the size of the rotor and the relative centrifugation force. For example, Egwang and Slocombe (1982) showed that centrifuging for 4 or 5 min at 264 ×g provided statistically significantly better egg recoveries than shorter (1 min) or longer (20 min) durations. Dryden *et al.* (2010) also observed that a 5-min centrifugation time at 280 ×g showed a significantly higher fecal egg count when compared with the passive flotation method. Another modification of flotation techniques is a fecal egg count method based on microscopy of an aliquot of fecal suspension from a known volume of a fecal sample. This method allows the investigator to express the number of parasitic elements (eggs, larvae) in the fecal sample in terms of eggs per gram of feces and can be used to measure the distribution of infections for epidemiological surveys to detect the presence or build-up of anthelmintic resistance, and to quantify the efficacy of anthelmintic treatment (Dryden *et al.* 2010).

Gordon and Whitlock (1939) proposed the first egg count method using a chambered slide while working with sheep feces, which later came to be known as the McMaster method (Gordon and Whitlock 1939). This procedure used 2 g of feces mixed with 30 mL of flotation solution (Sheater's sugar or saturated sodium chloride), which was then shaken by hand to make a slurry. An aliquot of 1 mL was drawn from the center of the tube and added to three areas of the McMaster counting chamber. Since then, several modifications, including variations in the ratio of feces to fluid, centrifugation time, number of chambers counted, and area of slide counted, have been described. The sensitivity of this technique depends on the weight of the feces examined and the dilution ratio (g of feces/mL of water). In a study by Vadlejch *et al.* (2011), three modifications of the McMaster technique, like Wetzel, Zajíček, and concentration modification according to Roepstorff and Nansen, were compared; the concentration modification according to Roepstorff and Nansen used 4 g of the feces examined and determined that a low dilution ratio (1:14) was more sensitive than when a lower weight of feces (1–2 g) or a higher dilution ratio (1:30) was used (Blagg *et al.* 1955).

A modern modification of the McMaster Technique is FLOTAC®, introduced by Cringoli (2006), which incorporates a centrifugation-enhanced flotation method in a chambered device with a detection limit of 1 or 2 EPG (eggs per gramm) (Cringoli 2006; Utzinger *et al.* 2008). A further modification of FLOTAC® was proposed by Barda *et al.* (2013) called Mini-FLOTAC. In this method, a simple device Mini-FLOTAC apparatus comprises two physical components – namely, the base reading disc and two accessories, the key and the microscope adaptor (Barda *et al.* 2014). There are two 1-mL flotation chambers, designed for optimal examination of fecal sample suspensions in a total volume of 2 mL. A major advantage of this method is the lack of a centrifugation step.

Immunological and molecular analyses

Development of immunoassays and PCR kits provides new highly sensitive and specific methods for the detection of parasitic infections. Coproantigen ELISA assays and PCR tests have been developed for the detection of major parasites of humans and animals in feces.

Coproantigen detection

Highly sensitive and specific immunological assays employing antibodies have been developed to identify helminth parasite antigens released in the host feces. The helminth coproantigens are detected by enzyme-linked immunosorbent assays (ELISAs). One major advantage of such coproantigen ELISAs over serum antibody assays is that this method indicates only the current infection and avoids handling of serum that may contain parasitic products from previous infestations. The first such assay using agar gel diffusion was developed for the detection of *Echinococcus granulosus* (Babos and Nemeth 1962). Other coproantigen assays have been developed for many human and animal cestode parasites to identify of major flatworm infections such as *Opisthorchis viverrini*, *Fasciola hepatica*, *F. gigantica*, and *Echinostoma caproni*i) (Abdel-Rahman *et al.* 1998; Estuningsih *et al.* 2004; Fraser and Craig 1997; Mezo *et al.* 2004; Watwiengkam *et al.* 2013).

Recently, an ELISA developed with monoclonal mouse IgG antibodies against *Clonorchis sinensis* was used for its detection in experimentally infected rats (Rahman *et al.* 2012). However, only a handful of coproantigen assays have been reported for nematodes, including assays for the detection of a number of species like *Haemonchus contortus* (Ellis *et al.* 1993), *Ascaris suum* (Schniering 1995), *Strongyloides ratti* (Nageswaran *et al.* 1994), and *Heligmosomoides polygyrus* (Johnson *et al.* 1996). These tests have been shown to be relatively sensitive and species-specific.

PCR based detection methods

The first polymerase chain reaction (PCR) based assay for the identification of parasite DNA from eggs in feces was demonstrated by Flisser *et al.* (1988, 1990) and then by Bretagne *et al.* (1993). Common techniques such as conventional PCR, multiplex PCR, and real-time PCR are used for detecting parasite DNA in host feces (Bergquist *et al.* 2009). Conventional PCR involves the amplification of a sequence of target DNA using a primer pair to detect foreign DNA belonging to any parasite stage in either tissue, blood, or urine. The conventional PCR technique is slightly modified to perform a multiplex PCR that allows amplification of multiple target DNA sequences simultaneously (Gordon *et al.* 2011; Toze 1999). Multiplex PCR can also be used for the detection of multiple parasites species from the same individual's fecal sample or blood.

The development of the real-time PCR introduced researchers to a powerful tool to study gene expression profiles as a function of the relative abundance of the target gene in the sample. Real-time or quantitative PCR (qPCR) does this by measuring the fluorescence released as a by-product of the PCR reaction and presenting it as a graph of fluorescence intensity relative to time. Thus, samples with a higher concentration of the target DNA show a peak in fluorescence at an earlier time point during the PCR (Frickmann *et al.* 2015; Heid *et al.* 1996). PCR, however, has a number of limitations, such as the risk of contamination, false positive results due to the presence of naked nucleic acids and non-viable microorganism, and difficulty in quantification in water and wastewater (Toze 1999).

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Modern diagnostic molecular-based techniques includes DNA sequencing, DNA barcoding and Loop-Mediated Isothermal Amplification (LAMP).

DNA barcoding was established as a rapid, powerful method for taxonomic research. DNA barcoding uses molecular data for identification and differentiation of species. PCR uses for amplification of short DNA fragments which compared to a pubilc DNA databases for the possible sequences matches. *Nuclear and mitochondrial genes, such as 18S rRNA, 16S rRNA, ITS regions, and* cox1*, are used for DNA barcoding of eukaryotic organisms.*

The cytochrome *c* oxidase (*cox1*) is the most slowly evolving gene, acknowledged as the 'gold standard' for DNA barcoding of eukaryotic organisms. For the determination of partial *cox1* sequences of Platyhelminthes, the primer set of JB3 (5'-TTT TTT GGG CAT CCT GAG GTT TAT-3') and JB4.5 (5'-TAA AGA AAG AAC ATA ATG AAA ATG-3') (Bowles *et al.* 1993) was widely used for investigating the inter- and intra-species variations of trematodes and cestodes. A PCR, followed by sequencing, on the mitochondrial genes *cox1* and *nad1* with primers published by Bowles and co-authors was the standard test at our laboratory for addressing both the *Taenia* sp. determination as the detection of *Echinococcus* sp. with subsequent species determination (Bowles *et al.* 1992; Bowles and McManus 1993; Gasser *et al.* 1999). The polymorphisms in these genes, which are so useful for typing, also interfere with the PCR. The annealing site for these primers is polymorphic as well. With several specimens of cestodes, the PCRs – most often the *nad1* PCR – failed to amplify one of the two targets. Many different PCR based approaches to detect and type various Cestodes have been described (Abbasi *et al.* 2003; Al-Sabi & Kapel 2011; Bart *et al.* 2004; Boubaker *et al.* 2013; Dinkel *et al.* 2004; Gonzalez *et al.* 2002; Jeon *et al.* 2009; Knapp *et al.* 2014; Maurelli *et al.* 2009; Mayta *et al.* 2008; Schneider *et al.* 2008; Siles-Lucas and Gottstein 2001; Stefanic *et al.* 2004; Trachsel *et al.* 2007; van der Giessen *et al.* 1999; von Nickisch-Rosenegk *et al.* 1999; Yamasaki *et al.* 2004). Most of these studies, however, use different PCRs for the detection of different cestodes. The PCRs are not specifically designed for human diagnostics. For routine diagnostics, a single test that is able to detect and type a range of cestodes is preferable. A widely published PCR target is the 12SrRNA gene, hereafter referred to as 12S. A number of different primers have been designed for the amplification of this gene because different research groups had different purposes (Boufana *et al.* 2008; Dinkel *et al.* 2004; Stefanic *et al.* 2004; Trachsel *et al.* 2007; van der Giessen *et al.* 1999). The primers on 12S were designed to amplify a broad range of cestodes. The reverse primer is essentially the same as one of the primers published by Trachsel and co-authors for *Echinococcus* sp. and *Taenia* sp. (Trachsel *et al.* 2007). In addition to the cestode 12S primers, the *Taenia* sp. specific primers designed by Trachsel and co-authors were also used.

Additionally, the primers for the *nad5* gene specifically for amplification of *Echinococcus* sp. were designed. Both the 12S and *nad5* genes are mitochondrial, which ensures high sensitivity. The genes are also genetically highly variable, which is especially useful for species determination within the genus *Echinococcus* sp. We investigated the sensitivity and specificity of the PCRs on these genes. Furthermore, material from suspected echinococcosis patients was tested with *cox1*, *nad1*, 12S, and *nad5* PCRs to compare sensitivity in clinical samples. To compare the targets for their use in species determination, we tested a variety of different *Echinococcus* sp. samples.

DNA barcoding has emerged as a powerful, rapid, molecular-based method with significant contributions to both taxonomic and biodiversity research (Hajibabaei *et al.* 2007; Hebert *et al.* 2003; Pereira *et al.* 2013; Weigt *et al.* 2012). Organisms can be accurately identified at the species-level by this process, which uses polymerase chain reaction (PCR) to amplify short DNA fragments corresponding to standardized regions of the genome with associated discriminatory sequence variations that are then subject to DNA sequencing. These sequences are then compared to a public DNA database of all the possible sequence matches or to a defined reference library populated with DNA barcoding sequences of voucher specimens (e.g., BOLD Systems v3; http://www.boldsystems.org/).

Nuclear (e.g., 16S rRNA, 18S rRNA, ITS regions), mitochondrial (e.g., cytochrome *b*, mitochondrial control regions), and some chloroplastic genes have all been used in barcoding eukaryotic organisms (Patwardhan *et al.* 2014). The more slowly evolving gene of the mitochondrial genome, cytochrome *c* oxidase I (*cox1*), is acknowledged as the 'gold standard' for species identification and DNA barcoding of animals (Hebert *et al.* 2003). However, for parasite identification, discovery, and diversity research, the nuclear 18S small subunit (SSU) rRNA gene has been more commonly used (e.g., Harris & Rogers 2011; Maia *et al.* 2012; Netherlands *et al.* 2014; Perkins & Keller 2001; Wozniak *et al.* 1994).

Notomi and co-authors, in 2000, described a new method of DNA amplification called Loop-Mediated Isothermal Amplification (LAMP) that has since been used for identification of major human and animal parasites and demonstrated comparable sensitivity and specificity to that of qPCR (Notomi *et al.* 2000).

LAMP employs a DNA polymerase with high strand displacement activity and a set of four primers that recognize six distinct sequences of the target DNA, giving it very high specificity. Although relatively new in the category of diagnostic techniques, the LAMP has distinct advantages over both conventional PCR and qPCR, such as high specificity of DNA amplification in the presence of non-target DNA, cost-effective amplification at isothermal conditions since no thermal cycler is necessary, and simple detection of amplification products using fluorescent dyes. However, the lack of data with this technique hinders the development of LAMP kits and their use in mass screening of helminth infections (Biswal 2016).

Which method is the best?

The sensitivity and specificity of diagnostic tests significantly influence the results of epidemiological surveys for helminth infections and the particular efficacy of anthelmintic treatment or drug resistance. The sensitivity of the diagnostic tool used is also important in helminth control programs; a low sensitivity leads to false negative results that in turn may lead to premature treatment cessation. Among the different diagnostic techniques described above, the fecal smear examination methods are the most frequently used in epidemiological surveys. The World Health Organization recommends the Kato-Katz method of duplicate slides for the detection of soil-transmitted helminth infections (*Ascaris lumbricoides*, *Trichuris trichiura*, *Ancylostoma duodenale*). The Faust method with zinc sulfate solution is one of the most common methods used in veterinary studies (Faust *et al.* 1929). Other methods such as sedimentation, McMaster, and FLOTAC are also used in parasitological studies (Biswal 2016). Comparative data on fecal examination methods are controversial since each procedure varies due to helminth species and study methodology. Moreover, the similar examination methods can have different sensitivities for the same parasite species in different studies. However, a summary of recently published data on the comparison of different fecal

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examination methods FLOTAC and mini-FLOTAC shows higher sensitivity for most parasites when compared with the gold-standard Kato-Katz method, McMaster egg count method, and sedimentation techniques. PCR-based methods have a higher sensitivity than traditional coproscopical examination techniques, ranging from 78.9% to 100% (Knopp *et al.* 2009; Taniuchi *et al.* 2011) for different parasitic species. Coproantigen ELISA tests are also highly sensitive methods, especially for identification of tapeworm infections – for example, sensitivity of the coproantigen test for the detection of *Taenia pisiformis* is 87.5% (Schär *et al.* 2013), and recently developed coproantigen assays for detection of fluke worm infections such as *Opisthorchis* and *Clonorchis* range in sensitivity from 93.3% to 100% (Craig *et al.* 1995; Teimoori *et al.* 2017).

In terms of specificity, coproantigen tests rank lower than PCR tests, ranging from 54.2% to 100% (Rahman 2012; Watwiengkam 2013). In a study by Nageswaran *et al.* (1994), the coproantigen test for detection of *S.ratti* had cross-reactions with *Necator americanus* and *S. muris* (Nageswaran *et al.* 1994).

One major advantage of PCR analyses over ELISAs is a significant decrease in the detection limits. For example, positive results have been obtained with a concentration 0.11–0.35 ng of parasite DNA (Ai *et al.* 2010), whereas ELISA coproantigen tests typically require around 0.3–0.6 ng of parasite antigens (Mezo *et al.* 2004).

Coproscopical examination methods are still favored by diagnostic clinics owing to their simple and economical set-ups. Without the use of costly equipment like thermal cyclers or spectrophotometers, coproscopic examinations can be performed in small laboratories or field studies. In order to circumvent the possibility of false negative results due to low concentrations of helminth eggs, low detection limit techniques like the Wisconsin and FLOTAC methods (detection limit 1 EPG), the McMaster method (10 EPG), and Kato-Katz (24 EPG) are recommended (Glinz *et al.* 2010; Knopp *et al.* 2009; Levecke *et al.* 2012). The sensitivity can be augmented by multiple stool examinations (Bogoch *et al.* 2013).

The choice of an appropriate examination method is also dependent on the study objectives, including host species, estimation of overall parasite prevalence or individual parasite infection, the intensity of parasite infection, and efficacy of anthelmintic treatment or drug resistance. Moreover, recently published reports claim an absence of the so-called 'gold standard' of helminth infections diagnostics (Nikolay *et al.* 2014; Tarafder *et al.* 2010). Thus, different methods can be employed in parasitological studies.

Conclusions

For the detection of helminth infestations, different methods, based on the relative ease of use, the purpose of the study, the availability of laboratory equipment, and the practitioner's qualifications, must be tested. Attaining an adequate accuracy using each methodology should be the goal of selecting a particular examination method.

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References

Abbasi I, Branzburg A, Campos-Ponce M, Abdel Hafez SK, Raoul F, Craig PS and Hamburger J (2003) Copro-diagnosis of Echinococcus granulosus infection in dogs by amplification of a newly identified repeated DNA sequence. *American Journal of Tropical Medicine and Hygiene* **69**, 324–330.

Abdel-Rahman SM, O'Reilly KL and Malon JB (1998) Evaluation of a diagnostic monoclonal antibody-based capture enzyme-linked immunosorbent assay for detection of a 26-28-Kd *F. hepatica* coproantigen in cattle. *American Journal of Veterinary Research* **59**(**5**), 533–537.

Ai L, Dong SJ, Zhang WY, Elsheikha HM, Mahmmod YS, Lin RQ, Yuan ZG, Shi YL, Huang WY and Zhu XQ (2010) Specific PCR-based assays for the identification of *Fasciola* species: Their development, evaluation and potential usefulness in prevalence surveys. *Annals of Tropical Medicine & Parasitology* **104**(**1**), 65–72.

Al-Sabi MNS and Kapel CMO (2011) Multiplex PCR identification of *Taenia* spp. in rodents and carnivores. *Parasitology Research* **109**(**5**), 1293–1298.

Annoscia G, Latrofa MS, Campbell BE, Giannelli A, Ramos RAN, Dantas-Torres F and Otranto D (2014) Simultaneous detection of the feline lungworms *Troglostrongylus brevior* and *Aelurostrongylus abstrusus* by a newly developed duplex-PCR. *Veterinary Parasitology* **199**(**3–4**), 172–178.

Babos S and Nemeth I (1962) Az echinococcosis szerodiagnosztikijinak kerdesehez. *Magyar Allatorvosok Lapja* **17**, 58–60.

Ballweber LR, Beugne, F, Marchiondo AA and Payne PA (2014) American Association of Veterinary Parasitologists' review of veterinary fecal flotation methods and factors influencing their accuracy and use—Is there really one best technique? *Veterinary Parasitology* **204**(**1**), 73–80.

Barda B, Cajal P, Villagran E, Cimino R, Juarez M, Krolewiecki A, Rinaldi L, Crindoli G, Burioni R and Albonico M (2014) Mini-FLOTAC, Kato-Katz and McMaster: Three methods, one goal; highlights from north Argentina. *Parasite Vectors* **7**, 271.

Barenbold O, Raso G, Coulibaly JT, N'Goran EK, Utzinger J and Vountasou P (2017) Estimating sensitivity of Kato-Katz technique for the diagnosis of *Shistosoma mansoni* and hookworm in relation to infection intensity. *PLoS Neglected Tropical Diseases* **11**(**10**): e0005953.

Bart JM, Bardonnet K, Benchikh-Elfegoun MC, Dumon H, Dia L, Vuitton DA and Piarroux R (2004) *Echinococcus granulosus* strain typing in North Africa: Comparison of eight nuclear and mitochondrial DNA fragments. *Parasitology* **128**(**2**), 229–234.

Bergquist R, Johansen MV and Utzinger J (2009) Diagnostic dilemmas in helminthology: What tools to use and when? *Trends in Parasitology* **25**(**4**), 151–156.

Biswal DK, Debnath M, Kharumnuid G, Thongnibah W and Tandon V (2016) Northeast India Helminth Parasite Information Database (NEIHPID): Knowledge base for helminth parasites. *PLoS One* **11**(**6**), e0157459

Blagg W, Mansour NS, Schloegel EL and Khalaf GI (1955) A new concentration technic for the demonstration of protozoa and helminth eggs in feces. *American Journal of Tropical Medicine and Hygiene* **4**(**1**), 23–28.

Bogoch II, Andrews JR, Speich B, Utzinger J, Ame SM, Ali SM and Keiser J (2013) Mobile phone microscopy for the diagnosis of soil-transmitted helminth infections: A proof-of-concept study. *American Journal of Tropical Medicine and Hygiene* **88**(**4**), 626–629.

Bosh F, Palmeirim MS, Ali SM, Ame SM, Hattendorf J and Keiser J (2021) Diagnosis of soil-transmitted helmints using the Kato-Katz technique: What is the influence of stirring, storage time and storage temperature on stool sample egg counts? *PLOS Neglected Tropical Diseases* **15**(**1**), e0009032.

Boufana BS, Campos-Ponce M, Naidich A, Buishi I, Lahmar S, Zeyhle E, Jenkins DJ, Combes B, Wen H, Xiao N, Nakao M, Ito A, Qiu J and Craig PS (2008) Evaluation of three PCR assays for the identification of the sheep strain (Genotype 1) of *Echinococcus granulosus* in Canid feces and parasite tissues. *American Journal of Tropical Medicine and Hygiene* **78**(**5**), 777–783.

Bowles J, Blair D and McManus DP (1992) Genetic variants within the genus *Echinococcus* identified by mitochondrial DNA sequencing. *Molecular and Biochemical Parasitology* **54**(**2**), 165–173.

Bowles J, Hope M, Tiu WU, Liu SX and McManus DP (1993) Nuclear and mitochondrial genetic markers highly conserved between Chinese and Philippine *Schistosoma japonicum*. *Acta Tropica* **55**(**4**), 217–229.

https://doi.org/10.1017/S0022149X24000798 Published online by Cambridge University Press

Bowles J and McManus DP (1993) Molecular variation in *Echinococcus*. *Acta Tropica* **53**(**3–4**), 291–305.

Boubaker G, **Macchiaroli N**, **Prada L**, **Cucher MA**, **Rosenzvit MC**, **Ziadinov I**, **Deplazes P**, **Saarma U**, **Babba H**, **Gottstein B and Spiliotis M** (2013). A Multiplex PCR for the simultaneous detection and genotyping of the *Echinococcus granulosus* complex. *PLOS Neglected Tropical Diseases* **7**(**1**), e2017.

Craig PS, **Gasser RB**, **Parada L**, **Cabrera P**, **Parietti S**, **Borgues C**, **Acuttis A**, **Agulla J**, **Snowden K and Paolillo E** (1995) Diagnosis of canine echinococcosis: comparison of coproantigen and serum antibody tests with arecoline purgation in Uruguay. *Veterinary Parasitology* **56**(**4**), 293–301.

Cringoli G (2006) FLOTAC, a novel apparatus for a multivalent faecal egg count technique. *Parassitologia* **48**(**3**), 381–384.

Crompton DWT (2000) The public health importance of hookworm disease. *Parasitology* **121**, S39–S50.

David ED and Lindquist WD (1982) Determination of the specific gravity of certain helminth eggs using sucrose density gradient centrifugation. *Journal of Parasitology* **68**(**5**), 916.

Dinkel A, **Njorogeb EM**, **Zimmermanna A**, **Walza M**, **Zeyhleb E**, **Elmahdic IE**, **Mackenstedta U and Romiga T** (2004) A PCR system for detection of species and genotypes of the *Echinococcus granulosus*-complex, with reference to the epidemiological situation in eastern Africa. *International Journal for Parasitology* **34**(**5**), 645–653.

Dryden MW, **Payne PA**, **Ridley R and Smith V** (2010) Comparison of common fecal flotation techniques for the recovery of parasite eggs and oocysts. *Veterinary Therapeutics* **6**(**1**), 15–28.

Egwang TG, **Slocombe JO and Can J** (1982) Evaluation of the Cornell-Wisconsin centrifugal flotation technique for recovering trichostrongylid eggs from bovine feces. *Canadian Journal of Comparative Medicine* **46**(**2**), 133–137.

Ellis TM, **Gregory A**, **Turnor R**, **Kalkhoven M and Wroth RH** (1993) Detection of *Haemonchus contortus* surface antigen in faeces from infected sheep. *Veterinary Parasitology* **51**, 85–97.

Estuningsih SE, **Widjayanti S**, **Adiwinata G and Piedrafita D** (2004) Detection of coproantigens by sandwich ELISA in sheep experimentally infected with *Fasciola gigantica*. *Tropical Biomedicine* **21**(**2**), 51–56.

Faust EC, **Sawitz W**, **Tobie J**, **Odom V**, **Peres C and Lincicome DR** (1929) Comparative efficiency of various technics for the diagnosis of protozoa and helminths in feces. *Journal of Parasitology* **25**(**3**), 241–262.

Fraser A and Craig PS (1997) Detection of gastrointestinal helminth infections using coproantigen and molecular diagnostic. *Journal of Helminthology* **71** (**2**), 103–107.

Frickmann H, **Schwarz NG**, **Rakotozandrindrainy R**, **May J and Hagen RM** (2015) PCR for enteric pathogens in high-prevalence settings. What does a positive signal tell us? *Infection Diseases* **47**(**7**), 491–498.

Gasser RB, **Woods WG**, **Huffman MA**, **Blotkamp J and Polderman AM** (1999) Molecular separation of *Oesophagostomum stephanostomum* and *Oesophagostomum bifurcum* (Nematoda: Strongyloidea) from non-human primates. *International Journal for Parasitology* **29**(**7**), 1087–1091.

Glinz D, **Silué KD**, **Knopp S**, **Lohourignon LK**, **Yao KP**, **Steinmann P**, **Rinaldi L**, **Crindoli G**, **N'Goran K, and Utzinger J** (2010) Comparing diagnostic accuracy of Kato-Katz, Koga agar plate, ether-concentration, and FLOTAC for *Schistosoma mansoni* and soil-transmitted helminths. *PLOS Neglected Tropical Diseases* **4**(**7**), e754.

Gonzalez LM, **Monteroa E**, **Puenteb S**, **Lopez-Velezc R**, **Hernandez M**, **Sciutto E**, **Harrisone LJS**, **Parkhouse MRE and Garate T** (2002) PCR tools for the differential diagnosis of *Taenia saginata* and *Taenia solium* taeniasis/cysticercosis from different geographical locations. *Diagnostic Microbiology and Infection Diseases* **42**(**4**), 243–249.

Gordon HM and Whitlock HV (1939) A new technique for counting nematode eggs in sheep faeces. *Journal of the CSIR* **12**, 50–52.

Gordon CA, **Gray DJ**, **Gobert GN and McManus DP** (2011) DNA amplification approaches for the diagnosis of key parasitic helminth infections of humans. *Molecular and Cellular Probes* **25**, 143–152.

Hajibabaei M, **Singer GA**, **Hebert PD and Hickey DA** (2007) DNA barcoding: How it complements taxonomy, molecular phylogenetics and population genetics. *Trends Genetics* **23**, 167–172.

Harnnoi T, **Wijit A**, **Morakote N**, **Pipitgool V and Maleewong W** (1998) Specific gravity of *Opisthorchis viverrini* eggs. *Journal of Helminthology* **72**, 359.

Harris LB and Rogers SO (2011) Evolution of small putative group I introns in the SSU rRNA gene locus of *Phialophora* species. *BMC Research Notes* **4**, 258.

Hebert PDN, **Cywinska A**, **Ball SL and deWaard JR** (2003) Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London B* **270**, 313–321.

Hong ST, **Choi MH**, **Kim CH**, **Chung BS and Ji Z** (2003) The Kato-Katz method is reliable for diagnosis of *Clonorchis sinensis* infection. Diagnostic Microbiology *and* Infectious Disease **47**, 345–347.

Incani RN, **Homan T**, **Pinelli E**, **Mughini-Gras L**, **Guevara H and Jesus J** (2016) Comparison between mertiolate-iodine-formalin and Kato-Katz methods for the diagnosis of human helminth infections in resourse-limited settings. *Journal of Helminthology* **91**, 1–8.

Ishida MMI, **Rubinsky-Elefant G**, **Ferreira AW**, **Hoshino-Shimizu S and Vaz AJ** (2003) Helminth antigens (*Taenia solium*, *Taenia crassiceps*, *Toxocara canis*, *Schistosoma mansoni* and *Echinococcus granulosus*) and cross-reactivities in human infections and immunized animals. *Acta Tropica* **89**, 73–84.

Jeon J-K, **Chai J-Y**, **Kong Y**, **Waikagul J**, **Insisiengmay B**, **Rim HJ and Eom KS** (2009) Differential diagnosis of Taenia asiatica using multiplex PCR. *Experimental Parasitology* **121**, 151–156.

Jimenez JA, **Rodriguez S**, **Moyano LM**, **Castillo Y and García HH** (2010) Differentiating *Taenia* eggs found in human stools: Does Ziehl-Neelsen staining help? *Tropical Medicine & International Health* **15**, 1077–1081.

Johnson MJ, **Behnke JM and Coles GC** (1996) Detection of gastrointestinal nematodes by a coproantigen capture ELISA. *Research in Veterinary Science* **60**, 7–12.

Kato K and Miura M (1954) Comparative examinations. *Japanese Journal of Parasitology* **3**, **35** (in Japanese).

Katz N, **Chaves A and Pellegrino J** (1974) A simple device for quantitative stool thick-smear technique in schistosomiasis mansoni. Revista do Instituto Medicina Tropical de *São Paulo* **14**, 397–400.

Knight WB, **Hiatt RA**, **Cline BL and Ritchie LS** (1976) A modification of the formol-ether concentration technique for increased sensitivity in detecting Schistosoma mansoni eggs. *American Journal of Tropical Medicine and Hygiene* **25**, 818–823.

Knopp S, **Glinz D**, **Rinaldi L**, **Mohammed KA**, **N'Goran E.K**, **Stothard JR**, **Marti H**, **Grindoli G**, **Rollinson D and Utzinger J** (2009) FLOTAC: A promising technique for detecting helminth eggs in human faeces. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **103**, 1190–1194.

Komiya Y and Kobayashi A (1966) Evaluation of Kato's thick smear technic with a cellophane cover for helminth eggs in feces. *Japanese Journal of Medical Science and Biology* **19**, 59–64.

Koutz FR (1941) A comparison of flotation solutions in the detection of parasite ova in feces. *American Journal of Veterinary Research* **2**, 95–100.

Knapp J, **Millon L**, **Mouzon L**, **Umhang G**, **Raoul F**, **Ali Z S**, **Combes B**, **Comte S**, **Gbaguidi-Haore H**, **Grenouillet F and Giraudoux, P** (2014). Real time PCR to detect the environmental faecal contamination by *Echinococcus multilocularis* from red fox stools. *Veterinary Parasitology* **201**, 40-47.

Levecke B, **Rinaldi L**, **Charlier J**, **Maurelli M P**, **Bosco A**, **Vercruysse J and Cringoli G** (2012) The bias, accuracy and precision of faecal egg count reduction test results in cattle using McMaster, Cornell-Wisconsin and FLOTAC egg counting methods. *Veterinary Parasitology* **188**, 194–199.

Maia JP, **Gómez-Díaz E and Harris DJ** (2012) Apicomplexa primers amplify Proteromonas (Stramenopiles, Slopalinida, Proteromonadidae) in tissue and blood samples from lizards. *Acta Parasitologica* **57**, 337–341.

Manser MM, **Saez ACS and Chiodini PL** (2016) Faecal parasitology: Concentration methodology needs to be better standardised. *PLoS Neglected Tropical Diseases* **10**, e0004579.

Maurelli MP, **Rinaldi L**, **Capuano F**, **Perugini AG and Cringoli G** (2009) Development of a real-time PCR for the differentiation of the G1 and G2/G3 genotypes of *Echinococcus granulosus*. *Parasitology* **1**, 255–259.

Mayta H, **Gilman RH**, **Prendergast E**, **Castillo JP**, **Tinoco YO**, **Garcia HH**, **Gonzalez AE and Sterling CR** (2008) Nested PCR for specific diagnosis of *Taenia solium* taeniasis. *Journal of Clinical Microbiology* **46**, 286–289.

Mezo M, **González-Warleta M**, **Carro C and Ubeira FM** (2004) An ultrasensitive capture ELISA for detection of *Fasciola hepatica* coproantigens in sheep and cattle using a new monoclonal antibody (MM3). *Journal of Parasitology* **90**, 845–852.

https://doi.org/10.1017/S0022149X24000798 Published online by Cambridge University Press

Nageswaran C, Craig PS and Devaney E (1994) Coproantigen detection in rats experimentally infected with *Strongyloides ratti*. *Parasitology* **108**, 335–342.

Netherlands EC, Cook CA and Smith NJ (2014) Hepatozoon species (Adeleorina: Hepatozoidae) of African bufonids, with morphological description and molecular diagnosis of *Hepatozoon ixoxo* sp. nov. parasiting three Amietophrynus species (Anura: Bufonidae). *Parasites & Vectors* **7**, 552.

Nikolay B, Brooker SJ and Pullan RL (2014) Sensitivity of diagnostic tests for human soil-transmitted helminth infections: A meta-analysis in the absence of a true gold standard. *International Journal for Parasitology* **44**, 765–774.

Notomi T, Okayama H, Masubuchi H, Yonekawa T, Watanabe K, Amino N and Hase T (2000) Loop-mediated isothermal amplification of DNA. *Nucleic Acids Research* **28**, e63.

Patwardhan A, Ray S and Roy S (2014) Molecular markers in phylogenetic studies – a review. *Journal of Phylogenetics & Evolutionary Biology* **2**, 131.

Pereira LH, Hanner R, Foresti F and Oliveira C (2013) Can DNA barcoding accurately discriminate megadiverse Neotropical freshwater fish fauna? *BMC Genetics* **14**, 20.

Perkins SL and Keller AK (2001) Phylogeny of nuclear small subunit rRna genes of hemogregarines amplified with specific primers. *Journal of Parasitology* **87**, 870–876.

Pilotte N, Papaiakovou M, Grant JR, Bierwert LA, Llewellyn S, McCarthy JS and Williams SA (2016) Improved PCR-based detection of soil transmitted helminth infections using a next-generation sequencing approach to assay design. *PLoS Neglected Tropical Diseases* **10**, e0004578.

Rahman SM, Choi MH, Bae YM and Hong ST (2012) Coproantigen capture ELISA for detection of *Clonorchis sinensis* infection in experimentally infected rats. *Parasitology International* **61**, 203–207.

Rim HJ (2005) Clonorchiasis: An update. *Journal of Helminthology* **79**, 269–281.

Rockefeller Foundation International Health Board (1922) *Bibliography of Hookworm Disease*. New-York City Baltimore: impr. Waverley Press, the Williams & Wilkins Company.

Santos FLN, Cerqueira EJL and Soares NM (2005) Comparison of the thick smear and Kato-Katz techniques for diagnosis of intestinal helminth infections. *Revista da Sociedade Brasileira de Medicina Tropical* **38**, 196–198.

Schär F, Odermatt P, Khieu V, Panning M, Duong S, Muth S, Marti H and Kramme S (2013) Evaluation of real-time PCR for *Strongyloides stercoralis* and hookworm as diagnostic tool in asymptomatic schoolchildren in Cambodia. *Acta Tropica* **126**, 89–92.

Schneider R, Gollackner B, Edel B, Schmid K, Wrba F, Tucek G, Walochnik J and Auer H (2008) Development of a new PCR protocol for the detection of species and genotypes (strains) of *Echinococcus* in formalin-fixed, paraffin-embedded tissues. *International Journal for Parasitology* **38**, 1065–1071.

Schniering A (1995) Untersuchungen zum Koproantigen-Nachweis des Intestinalparasiten *Ascaris suum* unter Verwendung von aviären vitellinen Antikörpern. PhD dissertation, University of Berlin.

Siles-Lucas MM and Gottstein BB (2001) Review: Molecular tools for the diagnosis of cystic and alveolar echinococcosis. *Tropical Medicine & International Health* **6**, 463–475.

Štefanić S, Shaikenov BS, Deplazes P, Dinkel A, Torgerson PR and Mathis A (2004) Polymerase chain reaction for detection of patent infections of Echinococcus granulosus ("sheep strain") in naturally infected dogs. *Parasitology Research* **92**, 347–351.

Taniuchi M, Verweij JJ, Noor Z, Sobuz SU, van Lieshout L, Petri JrWA, Haque R and Houpt ER (2011) High throughput multiplex PCR and probe-based detection with Luminex beads for seven intestinal parasites. *American Journal of Tropical Medicine and Hygiene* **84**, 332–337.

Teimoori S, Arimatsu Y, Laha T, Kaewkes S, Sereerak P, Sripa M, Tangkawattana S, Brindley PJ and Sripa B (2017) Chicken IgY-based coproantigen capture ELISA for diagnosis of human opisthorchiasis. *Parasitology International* **66**, 443–447.

Thienpont D, Rochette F and Vanparijs OFJ (1979) Diagnosing helmithiasis through coprological examination. Janssen Research Foundation.

Toze S (1999) PCR and the detection of microbial pathogens in water and wastewater. *Water Research* **33**, 3545–3556.

Trachsel D, Deplazes P and Mathis A (2007) Identification of taeniid eggs in the faeces from carnivores based on multiplex PCR using targets in mitochondrial DNA. *Parasitilogy* **134**, 911–920.

Truant AL, Elliott SH, Kelly MT and Smith JH (1981) Comparison of formalin-ethyl ether sedimentation, formalin-ethyl acetate sedimentation, and zinc sulfate flotation techniques for detection of intestinal parasites. *Journal of Clinical Microbiology* **13**, 882–884.

Utzinger J and Keiser J (2004) Schistosomiasis and soil-transmitted helminthiasis: Common drugs for treatment and control. *Expert Opinion on Pharmacotherapy* **5**, 263–285.

Utzinger J, Rinaldi L, Lohourignon LK, Rohner F, Zimmermann MB, Tschannen AB, N'Goran EK and Cringoli G (2008) FLOTAC: A new sensitive technique for the diagnosis of hookworm infections in humans. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **102**, 84–90.

Vadlejch J, Petrtýl M, Zaichenko I, Čadková Z, Jankovská I, Langrová I and Moravec M (2011) Which McMaster egg counting technique is the most reliable? *Parasitology Research* **109**, 1387–1394.

van der Giessen JWB, Rombout YB, Franchimont JH, Limper LP and Homan WL (1999) Detection of *Echinococcus multilocularis* in foxes in the Netherlands. *Veterinary Parasitology* **82**, 49–57.

van Gool T, Weijts R, Lommerse E and Mank TG (2003) Triple faeces test: An effective tool for detection of intestinal parasites in routine clinical practice. *European Journal of Clinical Microbiology & Infectious Diseases* **22**, 284–290.

von Nickisch-Rosenegk M (1999) Contributions to the phylogeny of the Cyclophyllidea (Cestoda) inferred from mitochondrial 12S rDNA. *Journal of Molecular Evolution* **48**, 586–596.

Watwiengkam N, Sithithaworn J, Duenngai K, Sripa B, Laha T, Johansen MV and Sithithaworn P (2013) Improved performance and quantitative detection of copro-antigens by a monoclonal antibody based ELISA to diagnose human opisthorchiasis. *Acta Tropica* **128**, 659–665.

Weigt LA, Driskell AC, Baldwin CC and Ormos A (2012) DNA barcoding fishes. *Methods in Molecular Biology* **858**, 109–126.

Wozniak EJ, Telford SR Jr, **Mclaughlin GL** (1994) Employment of the polymerase chain reaction in the molecular differentiation of reptilian hemogregarines and its application to preventative zoological medicine. *Journal of Zoo and Wildlife Medicine* **25**, 538–547.

Young KH, Bullock SL, Melvin DM and Spruill CL (1979) Ethyl acetate as a substitute for diethyl ether in the formalin-ether sedimentation technique. *Journal of clinical microbiology*. Journal of Clinical Microbiology **10**, 852–853.

Yamasaki H, Allan J C, Sato M O, Nakao M, Sako Y, Nakaya K, Qiu D, Mamuti W, Craig P S and Ito A. (2004). DNA Differential Diagnosis of Taeniasis and Cysticercosis by Multiplex PCR. *Journal of Clinical Microbiology* **42**, 548-553.

Zajac AM, Johnson J and King SE (2002) Evaluation of importance of centrifugation as a component of zinc sulfate fecal flotation examinations. *Journal of the American Animal Hospital Association* **38**, 221–224. http://www.boldsystems.org/ https://iris.who.int/bitstream/handle/10665/324883/9789240014497-rus.pdf (in Russian)

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