EDITOR
David G. Baker

Flynn's Parasites of Laboratory Animals

SECOND EDITION

American College of Laboratory Animal Medicine

Blackwell Publishing

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First edition, ©1973 Iowa State University Press
Second edition, ©2007 Blackwell Publishing

Library of Congress Cataloging-in-Publication Data
Flynn's parasites of laboratory animals. – 2nd ed. / David G. Baker (editor-in-chief).
p. ; cm.
Rev. ed. of: Parasites of laboratory animals / Robert J. Flynn. 1st ed. 1973.
Includes bibliographical references and index.
ISBN-13: 978-0-8138-1202-1 (alk. paper)
ISBN-10: 0-8138-1202-X (alk. paper)

1. Laboratory animals–Parasites. I. Flynn, Robert J., 1923—. @CRTXS:II. Baker, David G., 1956—. III. Flynn, Robert J., 1923—. Parasites of laboratory animals. IV. American College of Laboratory Animal Medicine. V. Title: Parasites of laboratory animals. [DNLM: 1. Animals, Laboratory. 2. Parasitic Diseases, Animal. 3. Parasites. SF 996.5 F648 2007]

SF996.5.F59 2007
636.088'5–dc22 2006033056

The last digit is the print number: 9 8 7 6 5 4 3 2 1

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To Dr. Dale L. Brooks, mentor, colleague, and friend,
who envisioned this work fourteen years ago.

PREFACE TO THE FIRST EDITION

ALTHOUGH much is known about the parasites of laboratory animals, information is often lacking and what is available is scattered. It is the purpose of this book to gather what is known in this field so that it is readily accessible to those who need it, and to point out what is not known.

Some of the stated deficiencies in our knowledge are probably incorrect in that the information is available but either has been overlooked or has not been published. It is hoped that these incorrect statements will stimulate persons with contrary information to point out the error or to divulge previously unpublished data.

It is also recognized that in a work of this sort, other errors are likely. It would be appreciated if these are pointed out so that they can be corrected in future editions, should the reception of this book warrant future revisions.

Many people helped write this book. A draft of each chapter was first prepared by the appropriate collaborator and then rewritten by me. The rewriting was done primarily to emphasize laboratory animals and secondarily to provide uniformity of style. The rewritten chapter was then reviewed by the collaborator and, in some cases, by others. Thus, each chapter in the book represents a joint effort of at least two people and, in some cases, of several.

Many people, besides the collaborators, assisted in the preparation of this volume. These include persons who reviewed chapters or parts of chapters, furnished illustrations, made literature searches and helped or advised in various ways.

The parasites described are those that occur spontaneously. Experimentally induced conditions are mentioned only if they are of special significance. No attempt is made to include the parasites of all domestic and wild animals. As a general rule, those of the common laboratory animals (mouse, rat, hamster, guinea pig, rabbit, dog, cat, rhesus monkey, and chicken) are all included, but for the less common species (such as other rodents, other primates, reptiles, amphibians, and fishes), only the commonest parasites of the animal species most likely to be used in the laboratory are described. Agents that occur only in domestic animals of agricultural importance are not described, even though these animals are sometimes used in the laboratory, as this information is readily available elsewhere.

Except for a few rare or uncommon animals, the common name only is used in the text. Although this may appear unscientific, the repeated use, for example, of *Mesocricetus auratus*, when one means the usual laboratory hamster, and *Oryctolagus cuniculus*, when one means the laboratory rabbit, is undesirable. Also, scientific names sometimes change, but common names tend to remain the same. Great care was taken to ensure that the scientific name is given for every common name that appears in the text, and that the common name is specific. Authorities used to determine the appropriate names are cited.

It is my sincere hope that the usefulness of this book will justify the efforts of all who helped prepare it.

Robert J. Flynn

PREFACE TO THE SECOND EDITION

In the more than 30 years since publication of the first edition of this seminal text, dramatic changes have occurred in the fields of laboratory animal medicine and parasitology. Improvements in laboratory animal production, husbandry, transportation, veterinary care, diagnostics, and treatment, have resulted in dramatic declines in the prevalence of organisms causing parasitic diseases. Nowadays, commercially produced laboratory animals are free of nearly all unwanted organisms, including parasites. Modern facility design and husbandry practices preclude most infections or infestations. This is particularly true for parasites with indirect life cycles.

So, with all of these improvements, why is a new edition of this text warranted? Several reasons may be offered. First, in spite of the improvements in the components of animal care listed above, parasites continue to be found in and on laboratory animals. There are several possible reasons: infections or infestations were never completely eliminated from particular facilities; were inadvertently imported with incoming animals, either as a result of contamination during shipment or because parasitism was enzootic at the original location; entered the facility from feral animals in the local environment; or were carried in or on personnel and transferred to colony animals.

A second justification for revising the first edition is that animals in the wild are occasionally still collected and brought into the animal facility. While quarantine procedures should prevent transmission of parasites from wild to laboratory stock, transmission nevertheless occasionally occurs. Thirdly, the tremendous rise in the use of transgenic animals, some of which are immunologically compromised, provides opportunity for infections and/or infestations to take hold where such would not be the case with immunologically competent animals.

Finally, newer diagnostic and therapeutic approaches to controlling parasitism are available. These may facilitate discovery and elimination of unwanted pathogens. In addition to changes in the field of laboratory animal medicine, the field of parasitology has undergone radical changes. Here, changes have been most profound in the areas of diagnostics and treatment.

The stated purpose of the first edition was to gather into one source, what was known about the parasites of laboratory animals so that it was readily accessible to

those who needed it, and to point out gaps in our knowledge of parasites and the diseases they cause. The purpose of this second edition is essentially the same, with the additional significant task of updating information in a field that has advanced substantially, parasitology of laboratory animals.

As with the first edition, many people contributed to this monumental work. Foremost among them are the chapter authors. Their efforts are greatly appreciated. In addition, all chapters were subjected to peer review. On behalf of the authors, I offer thanks to the reviewers for their many valuable suggestions for improving early drafts. Others contributed illustrations, photographs, or conducted literature searches. These too are greatly appreciated. Lastly, we want to give special thanks to Drs. P. Coan, R. Ermel, S. Feldman, and D. McClure. They constituted an advisory committee charged with assisting the Editor-in-Chief in critically evaluating the first edition, in an effort to identify, if possible, areas in which the second edition could be even more valuable than the first.

The breadth and scope of the original edition has been retained, thereby ensuring continued usefulness to the widest possible readership, including bonafide parasitologists. Introductory chapters have been added, beginning with a chapter on modern diagnostic techniques. The next five chapters present overviews of parasite biology. These should help the reader to better understand information presented in the host-specific chapters. Most significantly, the text has been entirely reformatted, in an attempt to improve utility and readability. The informational content has been reorganized into chapters based on vertebrate host. Parasites are presented phylogenetically within chapters. In addition, information included in comprehensive tables from the first edition has been updated, organized by host body system, and reformatted to coincide with host chapters. Finally, a formulary of drugs, uses, dosages, routes, and mechanisms of action, has been added as an appendix. It is hoped that these changes will increase the usefulness of an already highly valuable reference text.

DAVID G. BAKER

ACKNOWLEDGEMENTS

THE authors wish to acknowledge those who contributed to the high quality of this revision through their thoughtful reviews and comments. These include Drs. Judy Bell, Valerie K. Bergdall, Cory F. Brayton, Patricia N. Coan, Philip S. Craig, Richard W. Ermel, Craig S. Frisk, Nina E. Hahn, Fred W. Knapp, Michael R. Lappin, James E. Miller, Edward J. Noga, Thomas J. Nolan, Kevin O'Hair, Glen M. Otto, Sarah L. Poynton, Philip J. Richter, Jr., Yehia Mo Saif, Peter M. Schantz, Mark St.Clair, C. Dayton Steelman, Steven J. Upton, Mark T. Whary, Michael J. Yabsley, Thomas A. Yazwinski, and Anne M. Zajac.

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FLYNN'S PARASITES OF LABORATORY ANIMALS

SECOND EDITION

CHAPTER

1

Collection, Preservation, and Diagnostic Methods

Pat H. Smith, BS; Seklau E. Wiles, MSc; John B. Malone, Jr., DVM, PhD; and Cliff M. Monahan, DVM, PhD

INTRODUCTION

As the scope of this book indicates, the term "laboratory animal" can encompass virtually any animal species used in research. The parasite fauna of such a wide spectrum of hosts seems unlimited. However, within phyla, parasites share many traits. The purpose of this chapter is to describe diagnostic methods useful for parasite phyla likely to be encountered in the research animal environment.

Most laboratory animal facilities should be capable of performing most of the fundamental techniques outlined in this chapter. Performing any of these techniques correctly and reliably requires expertise developed through repetition. For uncommon techniques or obscure parasites, it is often more expedient to send samples to a laboratory with more extensive diagnostic capabilities. Several resources are available for more complete treatment of diagnostic techniques[1–3].

SAMPLE COLLECTION AND PRESERVATION

Feces

Number of Samples to Collect

The number of samples to be collected depends on several factors, including the source and health status of the animals, available financial resources, and the parasite phyla likely to be encountered. For routine screening of an asymptomatic animal, a single sample should suffice. For newly arrived animals with potential parasite exposure or questionable health history, or for symptomatic animals within the colony, sequential fecal examinations are warranted. These are typically performed over three days.

Most nematode infections are easily identified with a single fecal examination because the female worms pass hundreds to thousands of eggs per day. In contrast, low level trematode, cestode, or protozoal infections may not be detected with a single examination because eggs or oocysts may not be passed continuously or daily, or in great number. In these cases, collecting fecal specimens passed on three sequential days will increase diagnostic power. To assess the parasite status of a group of animals, 30 animals or 10% of the group, whichever is greater, should provide adequate sampling coverage.

Sample Collection

Proper collection and preservation methods are critical for finding fecal parasites. A fresh fecal sample, collected rectally or just dropped, is optimal. When feces must be collected from the ground, the specimen should be taken from the middle of the dropping. This will minimize contamination with organisms from the environment. When sampling a group of animals, individual samples should be collected and tested separately. Mixing samples may mask or underestimate the true extent of infection, because parasites are not evenly distributed within host populations. Collected specimens should be placed in clean, wide-mouth plastic containers with screw-top lids, or in sealable plastic bags. Using a permanent marker, specimens should be properly identified with animal identification, date of collection, and species of animal. Specimens should be refrigerated as soon as possible, unless direct smears are to be prepared for the detection of motile protozoa. If collections are made in the field, specimens may be placed among refrigeration packs.

Sample Preservation

Specimens which will not be immediately processed should be immersed in a suitable fixative. The choice of fixative depends on the tests to be performed (Table 1.1). Often, an initial fecal examination is performed on a fresh sample. Positive test results then direct the diagnostician to the appropriate fixation medium for additional testing of the remainder of the sample.

TABLE 1.1 Common fixatives and applications

Fixative	Applications
Formalin	2% in distilled water for modified Knott's recovery of microfilariae 5–10% for concentration techniques (formalin-ethyl acetate; flotations and centrifugations) *Cryptosporidium* and *Giardia* antigen tests Not useful for making permanent mounts of most staining procedures
Schaudin's fluid	Permanent mounts of protozoa stained with trichrome or iron hematoxylin
Polyvinyl alcohol (PVA)	Permanent mounts of protozoa stained with trichrome or iron hematoxylin
Sodium acetate-acetic acid-formalin (SAF)	Concentration techniques (formalin-ethyl acetate; flotations and centrifugations) Permanent mounts of protozoa stained with trichrome or iron hematoxylin
Merthiolate-iodine-formalin	Wet mounts or direct smears Formalin-ethyl acetate sedimentation Limited use for staining of permanent mounts

Adapted from Ash and Orihel (1991) and Garcia (2001).

When sending samples to a commercial diagnostic laboratory, the protocol for preserving and shipping samples should be obtained prior to collection of samples. By adhering to these guidelines, the likelihood of an accurate diagnosis is maximized, and regulatory standards for shipping potential pathogens can be met. Pre-measured fixative vials are available for all of the fixatives described below, and simplify sample processing.

Regardless of the fixation method to be used, sample quality can be improved with centrifugation, or sieving followed by sedimentation. These methods remove water-soluble pigments and debris, and concentrate parasite forms. Diarrheic samples will benefit most by concentration. Ethyl acetate extraction is also useful for removing excess lipid. Once washed or cleaned, droplets of the unfixed sediment can be placed on slides for immediate examination or dried for staining and the remainder of the pellet fixed for shipment to a reference laboratory if necessary. Regardless of the fixative used, samples must be well mixed to ensure complete and uniform fixation of the specimen.

Formalin

Formalin is a readily available fixative that rapidly kills most pathogens, thus decreasing the zoonotic concerns of handling fecal samples. Formalin is not suitable for identifying whole helminths because it makes worms brittle and may interfere with special stains. Formalin fixation also may change the density of parasite structures such that recovery with flotation solutions is decreased. Flotation solutions of higher specific gravity (1.23–1.25) provide optimal recovery of formalin-fixed helminth eggs. Many fecal antigen tests are designed for use with formalin-fixed specimens, but this is not universal and must be verified before use. Also, formalin fixation results in cross-linking of many proteins associated with DNA. This may preclude using formalin-fixed specimens in polymerase chain reaction (PCR)-based assays. For fixation of fecal samples, 5% to 10% neutral buffered formalin solutions (NBF) are most commonly used.

Schaudin's fluid

Schaudin's fluid or fixative is used in-house and for fixing specimens in preparation for shipment. Droplets of a mixture of fresh feces and Schaudin's fluid can be applied directly to microscope slides for drying, then staining. Schaudin-fixed samples are not used in concentration procedures. Specimens can be fixed when passed, or can be prewashed as described below. The latter concentrates parasite forms. Schaudin's fixative provides excellent morphological preservation of trophozoites and amoebic cysts. Schaudin-fixed samples do not adhere well to glass slides, and so must be handled gently. Also, Schaudin's fixative contains mercury and therefore must be handled with caution. Newer preparations are available that employ zinc or copper as a substitute. While there may be a slight decline in the preservation of protozoal morphology, such as the chromatin pattern of amoebic cysts, handling and disposal of reagents with zinc or copper are less problematic than for reagents containing mercury.

Polyvinyl alcohol

Polyvinyl alcohol (PVA) was developed to overcome specimen adherence problems of Schaudin's fixative. While PVA fixation optimizes staining of some parasites, particularly intestinal protozoa, other fixatives are preferred for concentration procedures. Because PVA is carcinogenic, it must be handled with caution.

Merthiolate-iodine-formalin

Merthiolate-iodine-formalin (MIF) is commonly used for fecal specimens to be examined as direct wet mounts or following concentration techniques. It is not useful for preparing permanent mounts or for fixing specimens prior to staining. This fixative will also inactivate most pathogens.

Sodium acetate-acetic acid-formalin

Sodium acetate-acetic acid-formalin (SAF) is a good compromise fixative for shipment of samples destined to be processed either as permanent stains or concentration procedures. There may be a slight decline in protozoal integrity compared to the use of Schaudin's or PVA, but SAF does not contain mercury. Samples fixed with SAF can be stained with iron hematoxylin or trichrome stains.

Blood

Blood-borne parasites include the protozoan hemoparasites and the microfilariae (MF) of filarid nematodes, both of which benefit from collection of blood with an anticoagulant. Blood samples are also collected in tubes lacking anticoagulant, for use for antigen and antibody tests. Protozoan hemoparasites are typically identified by microscopic examination of stained blood smears. Thin films can be prepared immediately or from preserved whole blood. Most staining procedures can be performed on films that have been fixed with methanol. Although MF can often be found on blood films, adequate visualization

is difficult for identification to genus or species. Samples of blood with an anticoagulant are necessary because the MF cannot be removed from a clot for staining.

Collecting adequate blood from small animals can be problematic. Following venipuncture, blood can be drawn into a single hematocrit tube from which a blood smear can immediately be made. The remainder can be centrifuged for determination of packed cell volume. The tube can then be scored and broken at the buffy coat for recovery of MF, and the small quantity of serum or plasma can be harvested for serology.

Urine

Urine samples can be collected and centrifuged to concentrate helminth eggs or microsporidia. These can be stored in saline and refrigerated for days if they cannot be examined immediately. For longer periods, fixation with 10% NBF or 70% ethanol and 5% glycerin are useful preservatives.

Tracheal Lavage Samples

Tracheal lavage samples should be collected from deep within the respiratory tract, using sterile saline. Lavage samples can be viscous in nature, and high viscosity can interfere with sample processing. Viscous samples should be mixed with a solution of 3% sodium hydroxide in saline, then centrifuged to concentrate parasite forms. Very thick mucus plugs can be subjected to ethyl acetate sedimentation as described for fatty fecal samples. Following centrifugation, samples can be preserved in 10% NBF, 70% ethanol (for helminths), or PVA fixative (for protozoa).

PARASITE COLLECTION AND PRESERVATION

Helminths

Helminths collected during necropsy examinations or passed directly by animals should be placed immediately into a container of 70% ethanol heated to 60°C to 63°C. This treatment will cause the helminths to straighten. Also, adult cestodes and acanthocephalans will protrude the rostellum or proboscis, respectively. Worms can then be transferred to 70% ethyl alcohol and 5% glycerin for long-term storage.

Arthropods

Macroscopically visible arthropods should be placed into 70%–90% ethanol. Formalin should not be used because fixation in NBF renders arthropods brittle. Skin scrapings can be collected directly onto microscope slides bearing a drop of mineral oil. However, initial processing with 10% potassium hydroxide (KOH) will facilitate visualization of arthropods by rendering the keratin more transparent. External parasites may frequently be recovered on clear adhesive tape that is brushed across the animal's fur, then adhered to a microscope slide.

FECAL EXAMINATION TECHNIQUES

Direct Smear Method

The direct smear is used only with samples in which motile trophozoites are suspected. The small quantity of sample employed is inadequate for other diagnostic procedures. The fecal sample should be either loose stool or diarrhea. Formed feces are unlikely sources of trophozoites, since under such conditions trophozoites either dehydrate and become distorted or form cysts during normal intestinal transit. Specimens must be examined immediately, before low external temperatures decrease trophozoite motility. Refrigeration of fecal samples renders trophozoites nonmotile and should not be used prior to preparing direct smears.

Materials

- Microscope slide and coverslip
- Saline
- Fecal loop or applicator stick
- Lugol's iodine

Method

1. Place a drop of saline on one end of a microscope slide and a drop of Lugol's iodine on the other.
2. Add a small quantity of fresh fecal specimen first to the saline drop and mix thoroughly, then transfer a small amount of the specimen to the Lugol's iodine drop.
3. Place a coverslip over each mixture.
4. Examine the saline/sample side first, with the light adjusted for ample contrast. Do not mistake Brownian motion for motility. Examine the entire coverslip using the 10 × objective, then 20 fields using the 40 × objective.
5. Examine the drop with Lugol's iodine for comparison.

Interpretation

The direct smear is a method for finding motile trophozoites. The quantity of sample used is so small that this method is not likely to accurately reflect the range of parasites which may be discovered using a concentration technique. Even when a direct smear is found to be positive, a concentration technique is still warranted to detect additional parasite forms. Not all protozoa observed in direct smears are parasitic, and therefore responsible for the clinical signs observed. During bouts of loose stool or diarrhea, intestinal or cecal protozoa can be expelled that are not normally seen during fecal examinations of asymptomatic animals. This is particularly true with herbivores, including reptiles and amphibians, because several ciliates and flagellates participate in digestion. Unwarranted treatment of these protozoa may alter the normal intestinal flora and prolong the symptoms.

Fecal Concentration Methods

The recovery of fecal parasites is enhanced by concentration procedures. These include flotation and sedimentation techniques, both of which depend on differences in specific gravity (sg) between the parasite form and the surrounding solution. Flotation techniques concentrate parasites by employing hypertonic solutions so that parasite forms rise to the surface of the flotation solution, while most debris fall (Table 1.2).

Sedimentation techniques employ solutions less dense than the parasites, so that parasite forms concentrate at the bottom of the collection vessel. Sedimentation methods generally allow for the recovery of more parasites than do flotation methods. With sedimentation, everything can be recovered, whereas with flotation techniques only those items of lower specific gravity than the flotation medium are recovered. Sedimentation techniques also are more easily performed in the field. In contrast, sedimentation has the disadvantage of greater debris, which can complicate examination. Furthermore, when examining sediment, one must focus through multiple focal planes because parasite forms will drift at different levels within the solution between the slide and the coverslip. This results in longer examination time, versus flotations.

TABLE 1.2 Common flotation solutions.

Solution	Specific Gravity	Ingredients/1 L H_2O
Sodium chloride	1.20	311 g sodium chloride
Sodium nitrate	1.20	338 g sodium nitrate
Sodium nitrate	1.30	616 g sodium nitrate
Sugar	1.20	1170 g sugar*
Sheather's sugar	1.27–1.30	1563 g sugar*
Zinc sulfate	1.20	493 g zinc sulfate

*Requires refrigeration of stock solution or addition of 9 ml phenol as preservative

Passive Flotation

Passive flotation relies solely on gravity to separate parasites and debris, and is therefore much less sensitive than centrifugal flotation (discussed below). The densities of many parasite forms are too similar to those of the common flotation media to be recovered without the added force provided by centrifugation.

Although both zinc sulfate or sodium nitrate solutions can be used, zinc sulfate is preferable to sodium nitrate because the latter is more caustic and will degrade many helminth eggs, as well as protozoan cysts. Additionally, sodium nitrate solutions crystallize more quickly than zinc sulfate, and crystallization can distort parasitic structures.

Common mistakes in performing passive flotation include setting up multiple samples at one time and reading each sample as time permits. This results in nonuniformity in flotation time, and greater potential for crystallization to render slides unreadable. To minimize crystallization, slides may remain in place on top of the flotation apparatus until they are ready to be read. However, exceeding the recommended 15-minute flotation may result in salt solutions equilibrating with the internal milieu of the egg or oocyst, either by passive diffusion or by extraction of water into the hypertonic float solution through osmotic forces. As a result, eggs or oocysts will become distorted and no longer buoyant, and may fall away from the microscope slide. False negative results are more often obtained with the last slides to be read.

If zinc sulfate solution is used, all of the slides could be removed and coverslips applied at the 15 minute time point. Slides should then be placed on a rack in a simple humidified chamber to decrease the rate of crystal formation (Figure 1.1). These slides can be removed from the chamber and read as soon as possible, or the chamber placed in a refrigerator to be read later in the day. All salt solutions will crystallize, thus the timing of microscopy is very important.

Materials

- Pill vial or sputum jar
- Small petri dish or watch glass

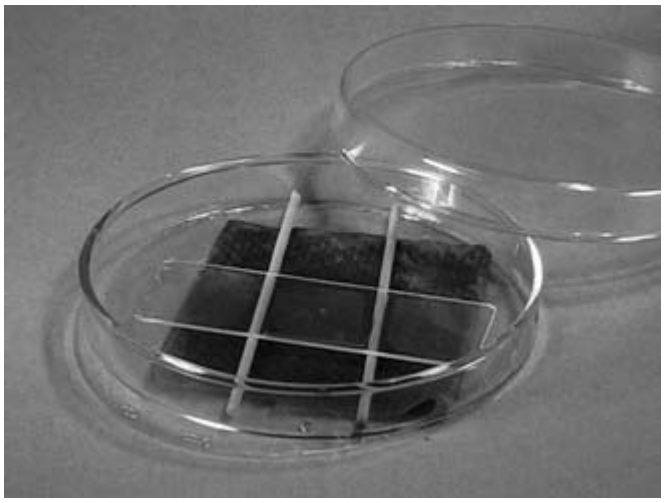

Fig. 1.1 A simple humidified chamber can be assembled to decrease the rate of crystal formation of flotation solutions.

- Disposable cup
- Applicator sticks
- Flotation medium (1.20 sg)
- Microscope slides and coverslips
- Tea strainer

Method

1. Place 2 to 3 g of fecal sample in the disposable plastic cup using the applicator sticks.
2. Add a small quantity of flotation medium and mix into a slurry.
3. Continue adding flotation medium, stirring to mix thoroughly.
4. Place the pill vial in the small petri dish as a guard against overflow.
5. Pour the mixture through the tea strainer into the pill vial, stirring with the applicator sticks to facilitate flow through the strainer.
6. Add drops of the float medium until a slight, bulging meniscus forms above the rim of the vial.
7. Place the microscope slide on top of the meniscus.
8. Allow 15 minutes for parasite forms to rise to the surface.
9. Gently lift the slide from the pill vial, invert the slide, and place a coverslip on the droplets of sample adhering to the slide.
10. Examine the entire coverslip using the 10 × objective, followed by 20 fields using the 40 × objective.

Interpretation

Passive flotation can be used effectively when the technician understands the limitations of the technique. Only a small subset of parasite forms will be recovered even when the technique is performed optimally. Strongyle-type eggs and coccidian oocysts are often passed in sufficient numbers that the poor sensitivity of passive flotation is overcome during routine fecal screening. Other parasite forms may not be sufficiently recovered. For this reason, passive flotation is not the diagnostic method of choice where accuracy is required.

Centrifugal Flotation

Centrifugal flotation is more sensitive than passive flotation because it magnifies gravitational forces, thereby accelerating the downward movement of more dense debris and the upward movement of less dense parasite forms.

The basic process of preparing a fecal sample for centrifugation is identical regardless of the flotation medium to be used. The sample should first be centrifuged with water to remove water-soluble pigments, free lipids, and other small debris.

Flotation solutions range from 1.20–1.30 sg (Table 1.2). The preferred salt solution for examination of fecal samples from carnivores is zinc sulfate at 1.20 sg. Zinc sulfate is sufficiently gentle to protozoal cysts that it enhances their recovery without distortion. Zinc sulfate at 1.20 is less effective at recovery of very dense parasite forms, such as *Physaloptera* eggs. For improved visualization of *Giardia* cysts, drops of Lugol's iodine can be added to the fecal pellet and mixed thoroughly for 30 seconds prior to addition of the zinc sulfate.

In general, sugar solutions are less sensitive than zinc sulfate. Sugar solutions are more viscous than salt solutions, and therefore are not very useful for passive flotation. Sugar solutions should be prepared with a preservative (e.g. formalin) to retard bacterial or yeast growth, since digestion of the sugar molecules will lower the specific gravity. Sheather's sugar is a more concentrated or super-saturated solution (1.30 sg) that is particularly suited for recovery of *Cryptosporidium* sp. oocysts.

Sugar solutions are superior to salt solutions in many ways. Sugar solutions are less expensive to make, do not distort eggs or oocysts to the same degree as salt solutions, and will not crystallize rapidly. The latter advantages mean that prepared slides may be refrigerated for days prior to

examination, without loss of parasite structural integrity. Sugar solutions are particularly useful for processing herbivore fecal samples. Flotation solutions should be compared through side-by-side preparations using known positive samples.

Centrifuges with swinging bucket rotors are preferred because they allow each tube to be filled more than is possible with fixed-head rotors. Many diagnosticians prefer to place the coverslip on the sample tube during the centrifugation steps. This is not possible with fixed-head rotors. Because small vibrations can cause a coverslip to be lost during centrifugation, many laboratories perform the centrifugations with the fluid level in the tube at the maximum possible, then transfer the tube into a stationary rack before placing the coverslip on the sample to allow parasite stages to adhere to the coverslip. Sensitivities are equivalent for the two variations, and the difference in time required is negligible.

Materials

- Disposable plastic cups
- Applicator sticks
- Water or saline for washing
- Plastic centrifuge tubes and screens
- Centrifuge; swinging-bucket preferred, but fixed-head is also possible
- Test tube rack
- Flotation solution

Method

1. Place 2 to 3 g of feces in a disposable plastic cup. Mix very well with a small quantity of water and when mixed thoroughly, increase quantity of water to create a loose slurry. The quantity of water used should be approximately the volume of the centrifuge tube being used (approximately 15 ml).
2. Pour this mixture through a screen into a centrifuge tube and assist the passage through the screen by agitating with the applicator sticks.
3. Bring the volume of water in the sample tube to the top of the centrifuge tube, and equal to the volume in a second (balance) tube.
4. Centrifuge at 400 g for 3–5 minutes.
5. Remove sample tube from centrifuge and decant supernatant. If it is difficult to visualize the pellet apart from the supernatant, repeat this washing step by mixing the pellet thoroughly with water or saline a second or third time until the supernatant is clear.
6. Place a small drop of the washed pellet onto a microscope slide and examine as a sediment, or dry for staining.
7. Mix the remainder of the pellet thoroughly with a small volume of the flotation solution of choice, until a loose paste is achieved.
8. Bring the volume of the flotation solution to within millimeters of the rim of the centrifuge tube. Return the tube to the centrifuge. Place a balance tube opposite the sample tube. The specific gravity of water is only 1.00, thus a separate balance tube for flotation solutions is necessary.
9. Centrifuge for 5 minutes; 10 minutes if anticipating *Cryptosporidium* oocysts.
10. Transfer the tubes from the centrifuge to a test tube rack.
11. Add drops of the flotation solution to the top of the tube until a slightly bulging meniscus is formed. Do not overfill the tube because the floating parasite stages will be lost.
12. Place a coverslip on the slightly bulging meniscus and allow to stand 10 additional minutes.
13. Remove the coverslip to a microscope slide for examination.

Interpretation

Common mistakes in the performance of centrifugal flotation, which result in false negative results include:

1. Failure to thoroughly mix the sample with water prior to passage through the screen into the centrifuge tube, resulting in failure of parasite forms to pass through the screen. Often, too much water is added initially, so that the fecal sample drifts about without breaking apart.
2. Failure to stir or agitate the fecal slurry as it passes through the screen, rather than allowing it to simply drip through the tube, resulting in the buildup of debris on the screen that traps the suspended eggs or oocysts. This mat must be disrupted by stirring with the applicator sticks.
3. Failure to mix the pellet formed after centrifugation with a small quantity of flotation medium before filling the tube. The pellet is difficult to mix when the tube is too full with solution. Failure to mix adequately will trap any eggs or oocysts within the pellet, reducing sensitivity.
4. Overfilling the tube so that instead of forming a meniscus, parasite forms spill out of the tube and are lost.

Baermann Sedimentation

The Baermann technique uses simple gravity sedimentation to recover nematode larvae, either from a fecal culture or from tissue digests that liberate any larvae that may be present. The sample is placed into a funnel with warm water to facilitate nematode motility. Pulmonary tissues may be homogenized in a blender to recover lungworms, and diaphragm or other muscle tissues may be homogenized and placed in a Baermann apparatus for recovery of *Trichinella spiralis* larvae.

Materials

- Fine screen mesh or sieve, nylon coffee filter, or cheesecloth
- Funnel with latex tubing attached, with clamp
- Ring stand to hold funnel
- Collection tube
- Dish to collect spillage
- Petri plate for microscopic examination of the collected sediment
- Warm water to fill the Baermann apparatus

Method

1. Place clamp on latex tubing in open position and attach one end of the tubing to the funnel.
2. Insert collecting tube into the other end.
3. Place funnel assembly into a ring stand.
4. Add warm water to fill latex tubing and collecting tube until the funnel is half full.
5. Loosely wrap fecal or tissue sample in cheesecloth or place into sieve or coffee filter.
6. Place the sample into the funnel and gently fill with warm water until the sample is covered.
7. Leave the sample in the funnel for 12 to 18 hours.
8. Clamp the latex tube to prevent excess water from draining when the collecting tube is removed from the latex.
9. Decant the collected volume into a petri plate and examine this sediment for larvae.

Interpretation

The Baermann sedimentation is a technique often requested inappropriately due to a misunderstanding of its strengths and weaknesses. Historically, the Baermann has been used to recover cattle lungworm and strongylid larvae from feces. These larvae are very active and will swim free of the fecal sample. With parasitic infections that pass eggs or oocysts, or less active larvae, the Baermann sedimentation is far less sensitive than centrifugal flotation techniques. The first-stage larvae of most Metastrongyloidea are not active enough to free themselves from the feces in which they were passed, since these nematodes use gastropods as intermediate hosts. Gastropods are drawn to feces for the nitrogenous meal that feces can provide, thus active larvae that leave the feces are less likely to be consumed by gastropods. This feature favors larvae that remain with the feces. In contrast, cattle lungworms and larvae of strongylid nematodes develop directly on pasture without an intermediate host. Larvae of these nematodes more actively extricate themselves from the fecal sample.

Simple Gravity Sedimentation

Simple gravity sedimentation can be performed without a centrifuge and is intended to collect parasite eggs too dense to recover with common flotation media, such as eggs of *Fasciola hepatica.* It also cleans some debris and water-soluble pigments in the process of decanting. The process involves a two-step sedimentation and decanting method whereby the first step follows a brief sedimentation that removes the densest debris while the parasite forms remain in the water column that is decanted into a second vessel for the second, longer sedimentation step. A pilsner glass or funnel-shaped vessel provides an advantage over a flat-bottom beaker in that the sediment is concentrated into the narrow bottom of the pilsner glass.

Materials

- Fecal sample and mixing container
- Water or saline
- Pilsner glasses or conical, round-bottomed vessels, approximately 250 ml capacity
- Petri dish for microscopic examination
- Methylene blue as an optional stain

Method

1. Mix the fecal sample in a container using water or saline of the approximate volume of the pilsner glass or other vessel.
2. Suspend the sample well and pour into the pilsner glass.
3. Allow the heaviest debris to sediment for about 2 minutes.
4. Decant the suspended sample into the second pilsner glass and allow this to sediment for at least 2 hours.
5. Decant the supernatant carefully so as to leave the sediment undisturbed.
6. Pour aliquots of the sediment into a petri dish and examine with a dissecting microscope. Several drops of methylene blue can add contrast to aid in visualization.