Field Parasitology Techniques for Use with Mammals

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Appendix 6

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Introduction

Obtaining parasites from mammals that are collected during surveys or bioinventories is time-consuming, and in the past such collections have rarely been made. Parasites and other symbions are important components of the biology of the host, however, and must be sampled for a complete picture of its ecology and other aspects of its life history. For example, work on parasite diversity and biogeography of mammals in Bolivia (Gardner and Duszynski 1990; Gardner 1991; Gardner et al. 1991; Gardner and Campbell 1992a, 1992b) could not have been accomplished without proper collections of the parasite and symbiont fauna of the hosts.

Many researchers are reluctant to assign one or two persons from a field crew to process parasites from mammals when the specimens and associated data are considered “auxiliary” or “collateral.” The most efficient and cost-effective method of obtaining data on the parasite fauna of a host group, however, is to collect the parasites when mammals are being processed in the field. Examples of the types of data that may be obtained when a host is collected are prevalence, intensity of infection, and distribution of parasites in or on individuals and through populations of hosts.

Studies of the systematics and ecological characteristics of hosts and parasites require proper identification of both groups. If the parasites associated with a host are not collected and preserved properly, species-level diagnostic characters (i.e., morphological characters) will almost certainly be destroyed; in addition, improper preservation of parasite material will severely limit studies based on intact DNA molecules of those parasites.

Problems with identification of correct hosts may occur when parasites accidentally transfer from one...
host to another at the time of collection. Anthropogenic host-transfer can occur anywhere and is difficult to avoid in the field. For example, ectoparasites on one host may remain in a trap after the animal is removed. Those parasites may then transfer to another host of a different species at the same or a different collecting locality. Anthropogenic host-transfers occur even more frequently when hosts are placed in a killing jar that has not been cleaned completely after each use.

In this appendix, I outline methods for collecting parasites that maximize the amount of morphological and molecular information available from each parasite obtained. The procedures are designed to minimize the likelihood of mistakes in recording data due to anthropogenic host-transfers. My collaborators—Dr. Robert L. Rausch, Ms. Virginia Rausch, Dr. Terry L. Yates, and Dr. Sydney Anderson—and I have been collecting parasites in the field for more than 25 years. The protocols I present here have evolved considerably over that time and are based on our collective experience. Additional information on collecting techniques is presented in Anderson (1965) and Pritchard and Kruse (1982).

Data are collected in the field laboratory in assembly-line fashion, with different individual researchers in the field crew assigned to different tasks. Every part of every animal that is collected is processed in some way. Because of the possibility of contracting various viral diseases from handling wild-caught mammals, I recommend that surgical gloves and lab coats be worn during all laboratory procedures.

Procedures for collecting ectoparasites and ectosymbionts

1. Each live host is placed in a new bag made of thin plastic (1.5–2 mil). Dead hosts that are removed from traps should be placed immediately in new plastic bags to prevent loss of ectoparasites and cross-contamination of hosts. A 200 × 350-mm bag is a good, all-purpose bag for most small mammals.

2. A bag with a host is placed into a glass or plastic jar containing cotton moistened with chloroform or ether. Such chemicals should be used in well-ventilated areas and away from any open flame. Carbon dioxide introduced into the jar kills ectoparasites too slowly to be effective. Dead hosts also must be treated with chloroform or ether to ensure that their ectoparasites are killed.

3. After the ectoparasites die, while the animal is still in the bag, the investigator shakes and brushes the dead ectoparasites from the fur into the bottom of the bag. She or he also examines the pinnæ, ears, and body of the mammal for ticks and trombiculid mites, which are removed and stored. The host is then passed to the next team member for other processing (see “Processing Procedures,” Appendix 3). A labeled tag (Fig. 58) is placed in the plastic bag with the ectoparasites; the bag is tied shut and sealed. The label on the tag is written in permanent India ink and includes the host identification number or the field collection number of the collector. The investigator repeats the procedure with the next animal until all animals have been processed.

4. The specimen preparator should inspect the mammal carefully for small ectoparasites. Generally, the external pinnæ and the fur from the skin to the tips of the hairs are examined using a dissecting microscope. The fur is parted with jeweler's
forceps and dissecting needles. Examination begins at low power (2×-40×). The magnification is increased (50×-70×) to see if a suspicious object has legs. Several types of mites live in the subcutaneous connective tissue of mammals. These are often found by pulling the skin off the host and examining the underside of the skin. Larvae of flies (botflies or warbles) may also be found in the subcutaneous layer. A reliable identification can be obtained for these larvae only if they are reared to adulthood in an artificial brood chamber. Larval botflies cannot be identified at the present time. Live botfly larvae are removed from the host and placed head-up in a small, highly porous box. Ammonium cartridge case boxes (about .45 caliber or 10–12 mm in diameter) made of styrofoam work especially well for this purpose; several larvae can be placed in the box, stored, and transported at one time. Parasites and other symbionts can be removed with forceps, a swab, or a needle dipped in ethanol. If a tick or mite is embedded in the skin of the host, it is carefully dipped from the skin, rather than scraped, to ensure that mouthparts are included. Small mites that are attached to a hair or are embedded in a follicle are collected by removing the complete hair and preserving both. The locations of all ectoparasites collected are also recorded in the field notebook of the collector (Fig. 59). Recording the numbers of ectoparasites collected from each body region of the host and the type of vial or other container used to store them facilitates locating the organisms during later laboratory analyses. Ectoparasites and other ectosymbionts are preserved in 70% ethanol (EtOH). Parasites from each host individual should be stored in a separate vial.

5. A small amount of 70% EtOH (2–6 ml) is squirited into each plastic bag used to hold a host mammal. The contents are washed down the sides and into a corner of the bag. The bag is held over a Whirl-Pak (plastic bag with integral wire twist-ties), the corner is cut from the bag holding the ectoparasites and ethanol and the contents drain into the smaller Whirl-Pak bag. This method produces an uncontaminated sample of ectoparasites and ectosymbionts with minimal time expenditure. To avoid contamination, bags should never be reused. The contents of the Whirl-Pak bags should be transferred to vials as soon as possible after returning to the laboratory.

Procedures for collecting endoparasites and endosymbionts

The following procedures are appropriate for collecting protozoa (coccidia), acanthocephala (spiny-headed worms), cestoda (tapeworms), trematoda (flukes), and nemata (nematodes).

1. The researcher collects endoparasites from a host specimen after its femur has been removed to obtain tissue for karyotyping but before its organs have been removed and preserved for genetic analyses (see "Processing Procedures," Appendix 3). Scissors, forceps, and probes are always rinsed in distilled water or 70% EtOH and dried with a tissue between animals to avoid contamination with blood or other sources of foreign DNA.

2. If the animal is still warm and fresh (i.e., the blood has not coagulated), the investigator makes a blood smear. He or she places a drop of blood (approximately 250 µl) in the middle of a slide using a disposable plastic pipet. The edge of another slide held at an angle is placed on the slide with the blood so as to contact the blood drop. The inclined slide is pushed evenly and rapidly away from the blood drop, drawing the blood out into a thin (one cell layer–thick) smear (Fig. 60). The slide is labeled with the field identification number of the host using a diamond-point pencil and allowed to dry. Depending on the humidity, a slide should dry for from 10 to 30 minutes. New blood smears are fixed at the end of each day, or sooner if the temperature and humidity are high. Dry blood smears are fixed in 100% methanol (MeOH) for from 7 to 5 minutes. Blood smears that are well formed and of even thickness are important for documenting and detecting protozoa and microfilariae (juvenile nematodes).

3. To obtain endoparasites from the digestive system, the investigator cuts across the esophagus just above the stomach and across the colon just anterior to the rectum. Care must be taken not to perforate the organs during this procedure or parasites may transfer from one organ to another and invalidate information on the distribution of parasites within an individual host. The digestive tract is removed intact and placed in a clean petri dish with a tag bearing the field identification number of the host and a small amount of water. If the or-
Bolivia; La Paz, 11.5 Km W. San Andres de Machaca; 16°57'47"S, 69°03'31"W; 3800 m.

4 August 1993

SLG156-93  NK30699  Ceratomyra leucodon
Feces, Blood Smear.
Stomach ( ); Saved contents in Snap Cap.
Small intestine, 5 gastodes in duodenum.
(4 → 10% Formalin, 15 ml Vial).
(1 → 75% EtOH) dram vial.
Many trichostongylids in duodenum.
Approx. 10 pres. in liquid N2.
Rest in 10% Formalin. Ex. in GAA → 10% Formalin (dram vial).

Rectum and large intestine: whiteworms →
Trichurus, 10 in 10% Formalin; 2 in GAA → 70% EtOH (dram vial)

Ectos: 10 small mites from perianal skin (dram vial, 70% EtOH).
Estimated 500 small lice in nape region of neck; 10 collected (dram vial) 70% EtOH.

4 August 1993

SLG157-93  NK30699  Galea musculoides
Feces, Blood Smear.
Stomach ( ); Small intestine ( );
Rectum + Large intestine ( ); No ectos.
1. Move back to contact blood drop

2. Move forward drawing blood behind top slide

ANGLE DETERMINES SMEAR THICKNESS

Etch label on slide with diamond tip pen, include host number and name

Example of slide with smear finished and with label added.

Figure 60. Preparation of a blood smear. A smear must be made from fresh, uncoagulated blood. The angle of the upper slide determines the thickness of the smear; a steep angle creates a thin smear; a shallow angle provides a thicker smear. A 45° angle is good for general purposes.

gans are large, buckets and porcelain pans are used in place of petri dishes. If organs are very large (for example, those of a moose, whale, or elephant), they can be subsampled.

4. At this point, the researcher examines the body cavity, liver, kidneys, and lungs for helminth cysts or filarial worms. All organs to be removed from the host for future molecular or biochemical-genetic work must be carefully examined for parasites. Filarial nematodes may be encountered in the heart, aorta, pleural cavity, mesenteries, or subcutaneous tissues. Larval or juvenile acanthocephalans, pentastomes, nematodes, cestodes, and trematodes may be found in the liver, mesenteries, or any other tissues.

If cestode cysts are encountered, some are fixed in situ in the host tissue. Part of the organ is removed with the cyst intact and preserved in 10% formalin (assume for fixation purposes that 37% formaldehyde = 100% formalin for the 10%

Figure 59. Sample field notebook entries for parasites. The collecting locality is written at the top of every page or more frequently if the locality changes. The date is included with every record. The field collector number is next, followed by the field identification number and field identification of the host. Above: Parasite examination record for a specimen of Ctenocephalides licuodon. The collection of feces and blood or organ smears is recorded. The present entry indicates that the stomach had no parasites but that contents were saved for later analysis of diet. Five cestodes were recovered from the duodenum of the small intestine; 4 were fixed in 10% formalin in a 15-mL vial, and 1 cestode was preserved in 95% ethanol in a 1-dram vial. Small trichstrongilid nematodes were also found in the duodenum; approximately 20 were preserved in a cryotube in liquid nitrogen; the rest were preserved without counting in 10% formalin in a 1-dram screw-cap vial. The cecum and large intestine contained whipworms (genus Trichuris); 10 were preserved in 10% formalin and 2 in 70% ethanol in 1-dram vials. Ectoparasites were preserved in 70% ethanol in two 1-dram vials. Below: Parasite examination record for a specimen of Ginea monticola. Feces and a blood smear were taken. No parasites were found. Negative data are always recorded to ensure accurate estimations of prevalence, intensity of infection, and other ecological parameters.
dilution). If more than 10 cestode cysts are encountered in one host, a few cysts are removed and carefully cut open; the cestode strobila is relaxed in distilled water and fixed in 10% formalin. Additional cysts are stored in 95% EtOH, and some are frozen in liquid nitrogen. These various methods of preservation ensure that adequate material will be available for future investigations of both morphology and genetic attributes.

5. The collector next examines the intestinal tract after freeing it from attached mesentery and straightening it. The stomach, small intestine, cecum, and large intestine are cut apart and placed in separate petri dishes, each with a tag bearing the field identification number of the host. Petri dishes must always be cleaned and dried between uses. Each organ is opened (the intestine is cut lengthwise) and examined for parasites. It is important to use scissors with blunt ends because scissors with sharp points perforate the organ while cutting, making it difficult to open the organ quickly. For small specimens, iris scissors are appropriate. An enterotome is appropriate for opening the intestines of larger animals. If water is abundant, intestinal contents can be washed in a soil screen and then placed in a petri dish and searched. If the water supply is limited, one can take advantage of the fact that worms sink and other materials float. The intestinal contents are covered with water and gently stirred, and the lighter materials (plant parts and other food items), which float or do not settle quickly, can be decanted. This procedure is repeated several times. The remaining material is searched for helminths with either a dissecting microscope or a 10x jeweler’s magnifying visor. If many helminth specimens are encountered, the intestinal contents should be preserved in 10% formalin so that accurate counts of the numbers of parasites can be made in the laboratory.

Organs such as skin, eyes, urinary bladder, lungs, and gallbladder must also be examined, preferably with a dissecting microscope. Small nematodes commonly occur in the bile ducts and gallbladder of the liver and in the mesenteric veins; small nematodes can be found in the urinary bladder.

6. Cestodes, trematodes, and acanthocephalans are relaxed and killed by placing them in distilled water, although tap water or filtered river water can also be used. Osmotic imbalance causes water to move into the body cavity of the worm, leading to osmotic shock and death. The increasing pressure within the body cavity also causes the scolex or proboscis to extend and the strobila of a cestode to relax; it is especially important to leave a specimen in water long enough for excision and relaxation to occur. The relaxation process can take from 10 minutes to more than an hour, depending on the size of the worm and the species. The relaxed, dead helminths are fixed in 10% formalin and placed in vials with both the field identification number and the location of the parasite in the host written on the tag. If abbreviations are used (e.g., SI for small intestine, C for cecum), they must be fully defined in the collector’s field notebook. Formalin can be pre-mixed, or specimens can be placed in a vial 90% full of water and enough 100% formalin added to make a 10% solution. Parasites from each organ must be preserved separately in their own vials.

7. Saline is never used to kill cestodes or other platyhelminths because it prevents both osmotic imbalance and subsequent death. Nematodes, however, are never placed in distilled water, because the resulting osmotic imbalance causes them to burst, and in many cases the specimens are destroyed. During the dissection of the host, nematodes can be placed temporarily in saline and then transferred directly to a vial 90% full of very hot (not boiling) water. A 10% formalin solution is made by filling the vial with 100% formalin. Alternatively, nematodes can be placed in glacial acetic acid (GAA) for a few minutes before being transferred to either 10% formalin or 70% ethanol for storage. The GAA treatment causes the nematodes to uncoil. Such specimens are much easier to identify than those fixed without straightening, because the morphological characters are more readily seen. Specimens to be saved for molecular analyses should be washed in saline, placed in a cryotube, labeled, and stored in liquid nitrogen or placed in a vial containing 95% ethanol.

8. If sufficient numbers of helminths are available, representative individuals should be preserved in liquid nitrogen for future allozyme and DNA studies. Because GAA and formalin destroy DNA, individuals preserved for future genetic analysis should not be treated with these chemicals before freezing or preservation in EtOH. Investigators should preserve parasites using many different methods, thus ensuring availability of adequate material for future studies.
9. Fecal material should be preserved for later collection and study of coccidian parasites. A fecal pellet or some material from the cecum is placed in a vial half full of a 20% solution of potassium dichromate (K₂Cr₂O₇), along with a tag bearing the field identification number and the generic name of the host. Wheaton snap-cap vials (15 ml) are best for this purpose, because one vial half full of 20% K₂Cr₂O₇ contains a sufficient quantity of oxygen to keep the coccidia alive. The vials can be used many times, and they rarely leak.

10. If many conspecific hosts are available, from two to five entire gastrointestinal tracts should be preserved individually in 10% formalin. This approach will allow for future examination of the morphological characteristics of the intestines and any associated worms in situ.

Recording data

Each researcher should maintain a field notebook (Fig. 59) for recording data on the collections and the preparations that are made. The notebook should contain 100% cotton rag paper. Only permanent India ink should be used for recording data. For each record, the field collection number of the host should be recorded as well as the individual collection number. The host collection number is necessary to allow accurate cross-referencing with the voucher or symbiotype specimen (Frey et al. 1992).

Collectors should number their specimens sequentially, beginning with 1 and continuing indefinitely, rather than beginning the sequence anew with each collecting trip. The collector records the number with his or her initials so that he or she can be identified at a later date. The date and geographic locality of the collection must be provided for each specimen. The species name of the host should be entered, acknowledging the provisional nature of field identifications. The location of a parasite in a host must be noted, as should negative searches. The collector should also record the general kinds of parasites encountered in each organ.

Materials

In this section I list the equipment, disposable supplies, and reagents needed to collect endo- and ecto-

<table>
<thead>
<tr>
<th>Table 19. Estimated Quantities of Disposable Supplies Required for Collecting Parasites from 100 Mammals in the Field</th>
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</thead>
<tbody>
<tr>
<td>144 (1 gross) 15-ml Wheaton snap-cap vials</td>
</tr>
<tr>
<td>144 20-ml Wheaton snap-cap vials</td>
</tr>
<tr>
<td>144 1-dram vials with Teflon-lined screw caps</td>
</tr>
<tr>
<td>2 boxes of standard precleaned microscope slides (not frosted)</td>
</tr>
<tr>
<td>10 boxes of tissues or Kim-Wipes for cleaning equipment</td>
</tr>
<tr>
<td>100 cryotubes with brown-colored caps for parasites</td>
</tr>
<tr>
<td>2 rolls of paper towels</td>
</tr>
<tr>
<td>200 disposable plastic pipes</td>
</tr>
<tr>
<td>500 ml of 100% formalin</td>
</tr>
<tr>
<td>500 ml of 95% ethanol</td>
</tr>
<tr>
<td>1,000 ml of 70% ethanol</td>
</tr>
<tr>
<td>500 ml of 100% methanol</td>
</tr>
<tr>
<td>200 rectangular plastic bags, ca. 200 mm × 350 mm, and 1.5–2 mm thick</td>
</tr>
<tr>
<td>400 Whirl-Pak or other plastic bags with twist tie closures</td>
</tr>
<tr>
<td>200 sheets of 100% cotton-rag field-notebook paper</td>
</tr>
</tbody>
</table>

For general collecting, investigators should plan on using approximately 1 large plastic bag, 2 Whirl-Pak bags, 2 1 dram vials, and 2 15 ml snap-cap vials per host (actual use may be less, because not all hosts are infected with parasites).

parasites from mammals in the field. An estimate of the quantities of disposable materials required for collection of parasites from 100 small mammals is provided in Table 19.

Equipment

Durable equipment needed for collecting parasites includes the following items: dissecting microscope with 0.5×–30× magnification; bright light source (headlamps [e.g., Justirite] that use four size-D batteries work well); two pairs of jeweler’s forceps (100 mm); two pairs of gross dissection forceps with blunt tips (120 mm and 140 mm); scissors of differ-
Expendable Supplies and Reagents

The following materials are expendable and will need to be replenished before each field trip: microscope slides; small, medium-size, and large plastic petri dishes; small insect pin probes, dissection probes and needles; many disposable plastic pipets; 15- or 20-ml snap-cap Wheaton vials; 1-ml screw-cap vials with Teflon cap inserts; 15-ml screw-cap vials with Teflon cap inserts; cryotubes with brown lid inserts to indicate parasite samples; paper for labels placed inside vials (100% cotton-rag notebook paper or museum-quality label stock); 100% cotton-rag field-notebook paper; 100% MeOH; 95% EtOH for making 70% EtOH; and 100% formalin (37% formaldehyde).

Acknowledgements. I thank the many people who have contributed to our studies of mammalian-parasite biodiversity. Special thanks go to Mariel Campbell and Bill Gannon for input on various methods of collecting and maintaining data and specimens.