Banding and bird blood

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Virtually all banders are aware of current attempts to maximize the use and amount of data collected from each bird captured. Stephen Russell recently summarized efforts to computerize and centrally locate information not currently kept by the Bird Banding Laboratory (Russell 1980). Most of us who band regularly record obvious injuries, molt condition, ectoparasites, etc. That may or may not be of future use to others or to ourselves. One area largely ignored by banders but of tremendous potenial for cooperative or individual investigation is the collection of internal or endoparasites, particularly blood parasites. We at California State University, Fresno, have an intradepartmental cooperative project that has been operational for 2 years. In my recent studies of birds in the Rocky Mountains and the Sierra, I have noted rates of parasitism in some bird populations as high as 90%. Some individuals harbor multiple parasite infections with up to 4 different species of parasites being fairly common.

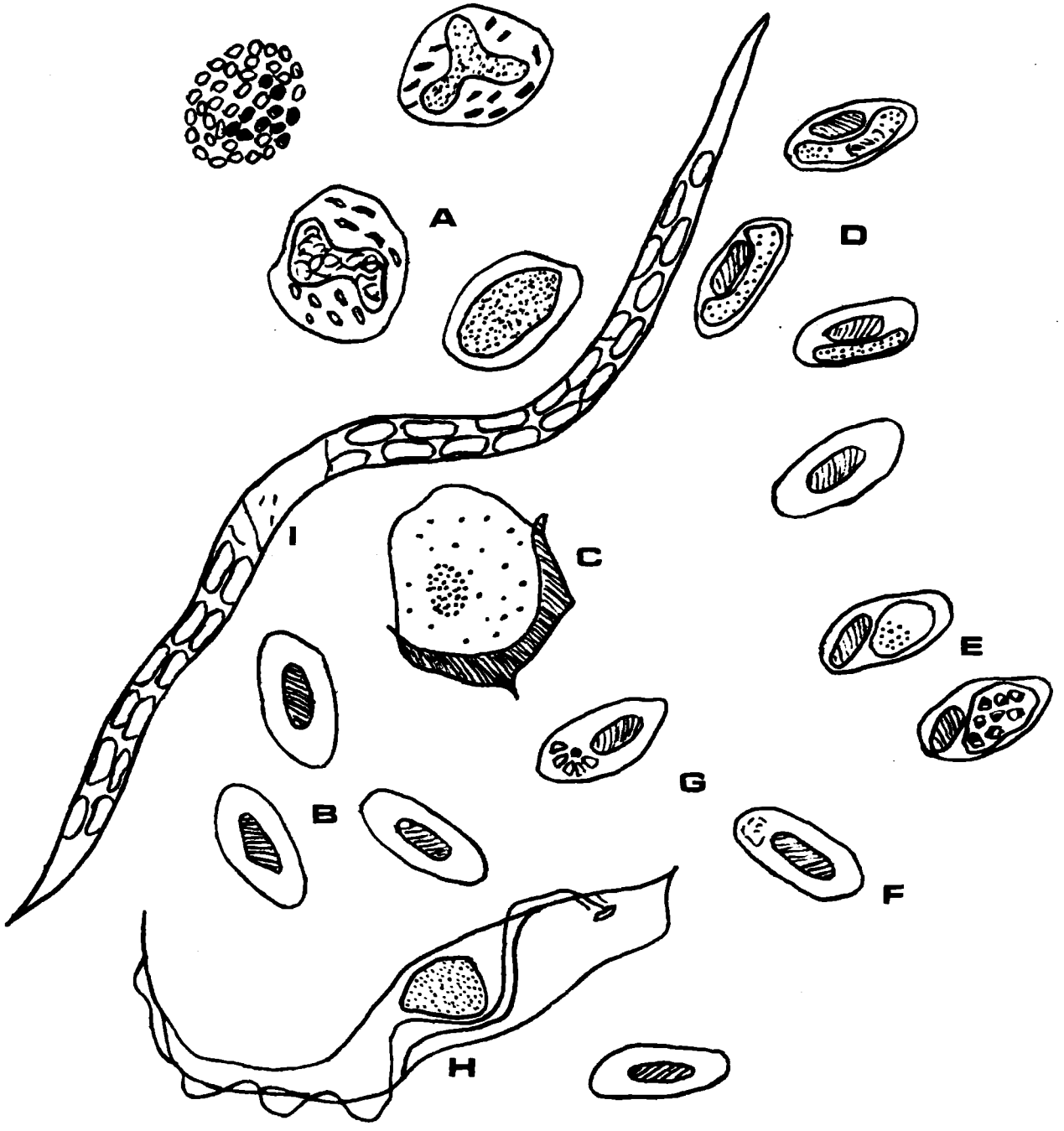
The vast majority of parasites encountered are sporozoan protozoans, including many kinds of avian malaria. These protozoans are transmitted from bird to bird by vectors in the form of mosquitoes or other biting arthropods. Life cycles of the parasites vary, as do the types of cell each species infects; some infect red blood cells, others white blood cells, in addition to infecting other organ tissues. The most commonly encountered groups and the cells they attack are the avian malarias in the genus *Plasmodium* (red cells); *Haemoproteus*, a malaria-like parasite (red cells); and *Leucocytozoon* (white cells). In many cases, particularly in the genus *Plasmodium*, the vector or carrier of the infection is unknown. Species are sometimes difficult to identify, and a great deal of taxonomic controversy exists. Good sources of information on life cycles are Garnham (1966) and Kudo (1966). Life cycles are also depicted in many parasitology texts.

In addition to the sporozoa, one occasionally encounters trypanosomes (flagellated protozoans) and microfilaria (larval nematode worms). These organisms are larger than the blood cells. They are far from numerous. Both are presumably transmitted by arthropods and are infrequently encountered. Very little is known of their life cycles.

The technique involved in obtaining samples from birds is simple, quick, and inexpensive. A tiny drop of blood is obtained by needle puncture of the tarsal vein on the inside of the foot. Humeral veins can be used in large birds. Only one tiny drop is needed — just enough to make a standard blood smear on a microscope slide. Instructions for blood smears can be found in most medical hematology texts. Slides are available from all bio-

Figure 1. Some examples of avian blood parasites (approx. 2000X)

A. Leucocytes (normal).
B. Erythrocytes (normal).
C. Leucocyte infected by *Leucocytozoon* sp. sexual stage (gametocyte). The nucleus of the leucocyte has been pushed to the cell membrane to form the crescent shape. The nucleus of the parasite may be visible in good preparations. Sexes of the parasite are separable by color (males pink, females blue).
D. Erythrocytes with *Haemoproteus* sp. sexual stages. Sexes of the parasite are separable by color as in *Leucocytozoon* sp. In females, pigment granules are more uniformly distributed through the parasite. In males, the nucleus is large and the pigment granules restricted to the "ends" of the parasite cytoplasm.
E. *Plasmodium* sp. of the subgenus *Haemamoeba*. Sexual stage (top) and asexual stage (bottom) in erythrocytes.
F. Early stage in a *Plasmodium* sp. infection. Only a small amount of undifferentiated cytoplasm is visible.
G. *Plasmodium* sp. of the *Novyella* subgenus. Four to eight nuclei of merozoites clustered around one or more pigment granules typify the asexual stages of these forms. Merozoites will eventually produce more merozoites or sexual stages in other erythrocytes. Sexual stages in the subgenus *Novyella* resemble those in *Haemoproteus*.
H. *Trypanosoma avium*.
I. Microfilaria.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logical supply houses. Local hospital laboratories may be of help in obtaining materials or in demonstrating how to make smears. Some birds seem to be bleeders, so we usually keep tissues handy to assist in clotting. We make 2 smears from each bird. Making a good smear requires practice, so we let our students practice first on domestic pigeons. The procedure does not harm the bird; little or no indication of bleeding or a wound is visible on the foot as little as an hour later. Each bird so treated automatically becomes a status code 6 (experimental) bird in the banding record.

After the smear has dried, it is fixed to the slide immediately by spraying with absolute methanol. The slide can then be stored indefinitely until staining can proceed, or mailed or shipped elsewhere. We label smears with a diamond pencil and include species (4-letter code), date, age, sex, band number, and location. We stain our slides with Giemsa stain at a dilution of 40:1 for 45 to 60 minutes. Tap water can be used as the diluent; however, we get the most consistent quality by using a buffer solution (sodium phosphate) at pH 7. After staining, slides are rinsed in tap water and allowed to air dry.

Examination of slides requires a microscope equipped with an oil immersion lens and preferably phase contrast. Oil is placed directly on the smear (no cover slip) and the slide scanned for at least 10 minutes. Artifacts and dust are commonly encountered, so suspect objects (potential parasites) are best examined under phase to determine the position relative to the surface of the cell. Parasites will focus in the same plane as the nucleus (remember, birds have nucleated red cells). A scan of the slide under low power should be made to look for larger parasites, the trypanosomes and microfilaria. Slides are cleaned of oil with lens paper and xylene. The lens paper is placed on the oil, xylene added, and the paper drawn off gently sideways.

Recognizing parasites and specifically identifying them can be difficult. Line drawings included here (Figure 1) provide only a rough guide, as not all possible parasites are illustrated, and as there is a great deal of variation both within and between species. Good additional references are available, however. For normal cells and artifacts, Lucas and Jamroz (1961) is a good source of illustrations. Garnham (1966) and Kudo (1966) have several types of drawings and photographs. Greiner et al. (1975) and Greiner and Bennett (1975) are collections of color photographs of parasites on microfilm that are helpful. (Available from Wildl. Dis. Assn., P.O. Box 886, Ames, Iowa 50010.) A handy bibliography is Herman et al. (1976).

As with banding itself, parasite work *should not be done without purpose*. You might wish to contact your friendly local parasitologist for advice, or ask if he or she has an interest in cooperating with you. We will be happy to consult with you or assist you where possible. We at California State University, Fresno, are interested in obtaining certain types of material for our research and will supply a more detailed list of interests on request.

There is an international depository for avian blood parasites at Memorial University of Newfoundland. Dr. Gordon Bennett of that institution has informed me that reference specimens in certain categories are needed. Of particular interest are species from the southwestern U.S. and from unusual or infrequently visited areas worldwide. Specimens from infrequently captured passerines, seabirds, shorebirds, and raptors are of interest. All specimens for the center should be from residents or migrants established in the area of collection. Birds in migration are less likely to provide useful information. The address of the center is: International Reference Centre for Avian Haematozoa, Memorial University of Newfoundland, St. John's, Newfoundland, Canada A1C 5S7.

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