Ova and Parasites

Medical Parasitology for the Laboratory Technologist

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Preface

WHEN I JOINED the University of Hawaii's medical school faculty, the Dean made me an offer I couldn't refuse. In exchange for his good will, I would teach parasitology to medical technology students. At the time it seemed like a modest enough price for such a precious commodity. After all, I had taught parasitology to students of medicine and public health for more years than I liked to remember, and I foresaw no difficulty in making an instructional transfer. It required only a few lectures to disabuse me of this comfortable notion. It rapidly became evident that a course, new in content and organization, would have to be developed. For one thing, the emphasis would have to shift from clinical aspects and treatment to laboratory diagnosis. But it was also recognized that the instructional content should not consist entirely of laboratory methodology. As a professional member of the medical team, the laboratory worker should have a basic understanding of the biology, epidemiology, and pathologic effects of the pathogens he or she is charged to detect and identify. This book is the evolutionary product of that course as it finally came to be.

The book is primarily intended as the companion text for an undergraduate course in medical parasitology included in the clinical laboratory science curriculum. I hope it will also serve the student in his or her later professional career. The first four chapters introduce the student to the world of parasites: their taxonomic affinities, biologic behavior, complexity of life cycles, host-parasite relationships as they relate to infection, disease, and immune response, and the basic concepts of laboratory diagnosis.

The succeeding ten chapters deal with individual species of parasites. A difficult decision for the author of a parasitology textbook is how to organize the material in the most logical and effective format, since there are so many parasites with so many different life styles. Parasitology textbooks for zoology students group the parasites according to their taxonomic relationships, while most books for medical students have grouped the parasites according to the tissues which they invade. For students of clinical laboratory science, whose task it will ultimately be to make a laboratory diagnosis, we have found that the most practical instructional approach is to group the parasites by the specimen from which they are primarily diagnosed and then to subgroup them, within the "specimen block," by taxonomic affinities. This organizational format is followed in this book. The section for each "specimen group" is introduced by a chapter dealing with the nature of the specimen, how it is collected and then handled in the laboratory, and procedural techniques common to the diagnosis of a variety of parasites.

The chapters that follow give basic descriptions of the parasites and the geographic distribution and clinical aspects of infection. More attention is paid to special procedures applied in making a parasitologic diagnosis and the morphologic features of the parasites' "diagnostic stage" to be used for their identification and differentiation from any "look-alikes" of other species.

It should be noted that although a variety of techniques are described, this book is not a comprehensive formulary of parasitology's methodologies; rather, the techniques given are those considered appropriate for the general purpose clinical laboratory, in which most of the students will eventually work. Undoubtedly the instructor will offer his or her own input on this subject. Parasitologists are notoriously idiosyncratic regarding the methodologies they favor, somewhat like chefs in the art of cooking. Finally, to complete the diagnostic "package," serologic methods and laboratory findings for that infection are discussed.

The making of a parasitologic diagnosis is a particular responsibility of the laboratory service and it is a serious business. Nevertheless, we deal with rather spectacular beasts, and tracking them down always seems to provide a sense of satisfaction. To all those who are or will be engaged in that search—good hunting!

I am very grateful for the high quality of the text illustrations and wish to thank Larry Yamasato for his artistic skill.

ROBERT S. DESOWITZ
Honolulu, Hawaii

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1 Introduction: Parasites and the Laboratory Technologist

ON THE EXAMINATION request form submitted to a laboratory, there is an item labeled "ova and parasites." To the laboratory technologist working in the cooler regions of the world, the request for parasitologic examination may conjure images of romantic, steamy, and somewhat sinister lands. To the technologist in the tropics, requests for parasitologic examinations are so frequent and routine that they are almost a way of life. However, the "exotic" diseases are no longer rare curiosities in the temperate zones, and today's technologists find that the request for "ova and parasites" is being submitted with increasing frequency.

The speed and extent of travel in our modern age have conveyed millions of tourists, students, business people, and military and government servants to the farthest corners of the tropical regions. Travel may be broadening, but it is not without its medical hazards. Travelers diarrhea (familiar to the afflicted as Montezuma's revenge, Delhi belly, and other geographic metaphors) is a common aspect of travel not described in the tourist brochure. The unwary and unlucky may not escape easily; they bring back with them not only the usual souvenirs but also malign mementos, such as malaria, amebiasis, sleeping sickness, and a remarkable assortment of intestinal worms. The ancient infectious diseases of the tropics are, for the most part, still firmly entrenched in their ancestral homes. Indeed, the activities and enterprises of man have caused many of these infections to broaden their range and intensify in endemicity. Malaria parasites, for example, which, 20 years ago, were pronounced to be joining the dodo as extinct species, are currently making a lively and disastrous comeback.

Parasitic infections are not confined to the lands of mangos, mañana, and malaria. Although parasites do flourish in the tropics, some species are indigenous to the temperate and subarctic regions, including the countries of North America. Our containerized foods, gleaming toilets, and generally sanitized lives have not afforded complete protection. Amebiasis and giardiasis occur in the American gut with chronic persistence as well as with an occasional acute outbreak. In a kind of class warfare, the arthropod parasitic mite *Sarcoptes scabiei*, the cause of scabies, has come to afflict the economically more affluent. Pinworms, *Trichomonas vaginalis*, and *Toxoplasma gondii*, in the true spirit of American egalitarianism, infect citizens without discrimination as to race, creed, or economic class.

Paradoxically, some forms of medical treatment have caused certain parasites that are often of a benign character to become highly virulent pathogens. A quiescent infection of *Toxoplasma gondii*, for example, can become explosively lethal in patients given steroids and other immunosuppressive drugs for the treatment of cancer and autoimmune diseases or for the facilitation of organ transplantation. The infectious disease problems of immunologically compromised patients are currently causing considerable concern to the medical community.

There are few laboratories specializing in parasitologic diagnosis. The majority of patients—the traveler, the immigrant, the infected "normal" American, and the immunologically compromised—are customarily diagnosed through the professional skills of the technologist working in the general clinical laboratory, where automated methodologies are the accepted standard. To a large extent, electronic wizardry has made the laboratory technologist an attendant to the machine. Samples are fed into the apparatus and after a few whirrs and clicks, the hematologic and clinical chemistry values appear neatly printed from its innards. This is not true in parasitologic diagnosis; in comparison, the needed skills may seem conspicuously old-fashioned. The electronic brain has yet to be made that can identify and diagnose the ameba or worm egg in the fecal specimen, the malaria parasite in the stained-blood film, or the trypanosome swimming in the fresh blood sample. Parasites of humans must still be diagnosed by humans, and it is the skill and perseverance of the technologist that make this possible. The diagnosis of most noninfectious diseases involves the deductive process of the physician from the indirect clues afforded by the patient's hematologic and biochemical picture. However, with few exceptions, diagnosis of parasitologic infections is made by actually *seeing* the causative organism, and it is the technologist who usually does the seeing.

Parasitologic diagnosis requires the technologist's expertise not only for identifying the parasite, but also for selecting the most efficient diagnostic method. In the succeeding chapters of this book, a multitude of parasites will be described, and it will be noted that there is a procedure appropriate for the diagnosis of each parasite.

In most instances, the technologist, not the physician, makes the decisions regarding methodology. This necessitates a closer relationship between physician and technologist than is customary in other branches of medicine. It is not good enough for the physician to simply check "ova and parasites" on the request form. The physician may be on a "fishing expedition" to determine whether any parasites are present in a patient who comes from or has been to an endemic area. If this is the case, it should be stated on the form so that the technologist can apply a battery of methods. If a particular parasite is suspected, this too should be stated so that the specimen can be processed by the appropriate techniques.

It is also useful for the physician to inform the laboratory of the patient's travel history, in order to give some clue regarding which parasites might be present. For example, a person arriving from Africa would not have clonorchiasis, or a patient from Thailand, African sleeping sickness; therefore, effort should not be wasted in searching for these parasites. Also, the technologist, as an integral member of the medical team acting for the patient's benefit, should remember that when there is inadequate information from the physician, he should be bold enough to seek it. This is particularly important for the diagnosis of parasitic infections.

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2 The Lives of Parasites and the Parasitized

WHAT IS A PARASITE?

Parasitology is the study of parasites, and the popular image seemingly provides clear definition of the organisms falling under the umbrella of this discipline. The plasmodium that causes malaria is a parasite. The trypanosome that causes sleeping sickness is patently a parasite, as is the hookworm clamped to the intestine, industriously sucking blood from its host. The implication is, therefore, that parasites must cause harm, that they are many species of many genera of virtually all phyla adapted to a life of crime in which they live in close intimacy with (and off) other living animals called **hosts.** The host gives shelter and shops for food, and, indeed, for the parasite, the host *is* the supermarket. This dietary allusion may not be overly strained when we consider the Greek origin of the word *parasite:* "one who eats at the table of another." Curiously, in ancient Greece, the assistants in religious rites who dined with the priests after a sacrifice were known as *parasitos*.

However, it is the concept of inevitable pathogenicity that troubles the common definition. Actually there are many kinds of host-parasite relationships, ranging from those which produce fatal harm to a few examples in the biologic curiosity cabinet in which the parasite actually confers some benefit to the host. Consider, for example, the case of *Trypanosoma rhodesiense*, the cause of Rhodesian sleeping sickness. In humans, this parasite produces a fulminating infection which kills in a few short weeks or months if left untreated. However, *Trypanosoma rhodesiense* also infects the wild antelope of Africa, and in these animals it has struck an adaptive harmony producing no detectable pathogenic effect. In other cases, with other species of parasites, some humans may be afflicted while others remain well.

Disease, therefore, may not be the inevitable companion of infection. The underlying mechanisms that moderate and modulate these relationships are still imperfectly understood, but it is believed that the nature of the host's immune response plays an important role.

A ZOOLOGIC PRIMER OF PARASITOLOGY

While virtually every phylum of the animal kingdom has representative species that have adopted a parasitic mode of life, the majority of parasites within humans belong to one of three phyla: *Protozoa*, *Platyhelminthes*, and *Nemathelminthes* (or *Nematoda*). The latter two phyla are referred to collectively as the "worms" or, more elegantly, as helminths.

PROTOZOA

Protozoa are single-celled animals. More appropriately they should be considered as acellular organisms because within their life unit are performed all the functions carried out by the concerted effort of the specialized tissues of the pluralistic society that is the metazoan animal. The electron microscope reveals a marvelous complex of organelles that make up the protozoan. However, the technologist's light microscope shows only the basic structural features: the limiting membrane enclosing the organism and its internal cytoplasm, the nucleus, and any organelles of locomotion. The four taxonomic divisions of the Protozoa are

1. **Mastigophora** (flagellates). These are protozoa which possess one or more whiplike filamentous structures (**flagellum;** pl., **flagella**) at some or all stages of their life cycle. The motion of the flagella propels the organism in the particular body fluid of its habitat. The parasitic Mastigophora have adapted to a variety of body fluid compartments. The trypanosomes dwell in the blood, lymph, and cerebrospinal fluid. Other flagellates inhabit the intestinal tract, and one important parasite, *Trichomonas vaginalis*, causes an infectious disease of the urogenital tract.
2. **Ciliata** (ciliates). These are protozoa whose external surfaces bear short, hairlike structures, the **cilia.** The cilia move in unison, "rowing" the organisms about in the sea of body fluid.

There is only one ciliate parasite affecting humans, *Balantidium coli*, which lives in the large intestine and has the potential of invading the submucosal tissue layer.

3. **Amebae** (Rhizopoda). Amebae have no specialized organelles of locomotion and are characterized by their ability to change body shape to allow the flowing ameboid movement. The cytoplasm of the ameba flows to form footlike extensions called **pseudopodia.** The most important pathogen in this group is *Entamoeba histolytica*, the cause of amebiasis (amebic dysentery and amebic liver abscess).
4. **Sporozoa** are parasitic protozoa with a complex life cycle involving an asexual reproductive phase **(schizogony)** and a sexual reproductive phase **(sporogony).** At some period in its life cycle, it develops into a sporelike body containing the infective form, the **sporozoite.** Some sporozoa, such as the eminently important malaria parasites (*Plasmodium*), use an invertebrate intermediate host as a vector in the course of their life cycles. The malaria parasites of humans develop in and are transmitted by various species of *Anopheles* mosquitoes.

PLATYHELMINTHES

Platyhelminthes include the flukes **(Trematoda)** and the tapeworms **(Cestoda).**

Trematoda (flukes) are a class of invertebrates flattened dorsoventrally to give a leaflike appearance. The compact body is filled with cells and lacks a body cavity and an anal opening from the digestive tract. Most trematodes are hemaphroditic, each adult worm having both male and female sex organs. An exception is the schistosome blood fluke, in which the sexes are separate.

All trematodes parasitizing humans employ snails as intermediate hosts. Prodigious asexual reproduction takes place within the body of the snail, culminating in the escape of the **cercaria,** the infective stage, into the water. The schistosome cercariae penetrate directly through the skin of the human host, but all other parasitic trematodes in humans undergo another stage of development **(metacercaria)** in another host which may be a fish, a crab, or a plant, depending on the life cycle of the species. Man becomes infected by ingesting the metacercaria in the second intermediate host.

Cestoda are tapeworms, parasitic animals composed of a joined chain **(strobila)** of segments **(proglottids).** Each proglottid contains a complete set of male and female reproductive organs. Tapeworms lack a digestive tract and absorb nutrients through their integument. The most anterior segment, the **scolex,** is modified as a hold-fast organ with hooks or suckers or both. The scolex is also the germinal center from which other segments arise. Maturation of the segments proceeds as they progress toward the posterior with segments containing functional testes and ovaries (mature proglottids) located toward the middle of the chain. The posterior segments become, essentially, sacs full of eggs (gravid proglottids) which detach from the strobila and are shed in the stool.

With the exception of one tapeworm, *Hymenolepis nana*, all cestodes parasitizing humans have complicated life cycles involving an intermediate host. The larvae, usually cystlike forms, develop in intermediate hosts. For some species, humans can also serve as an intermediate host and the presence of the larval stage may produce serious disease.

NEMATHELMINTHES (NEMATODA)

The **nematode** parasites are cylindrical-shaped, "wormy" creatures, having a mouth and anal opening to the digestive tract, a type of body cavity (pseudocoelom), and separate sexes. Nematodes are the most ubiquitous parasites found in humans.

Some nematodes, such as the insect-transmitted filarial worms, utilize an intermediate host during their life cycle; other species do not. All, however, develop to maturity in similar fashion. Their eggs hatch to release a minute wormlike larva. The larvae increase in size by successive molts (usually three), shedding the cuticle at each molt. The infective stage larva (**filariform** larva) is produced by the second molt. The filariform then molts once again within the vertebrate host to become the juvenile adult.

ZOONOTIC INFECTIONS AND HOST SPECIFICITY

When, under natural conditions, a parasite infects wild or domestic animals as well as humans, such as has been described for *Trypanosoma rhodesiense*, the phenomenon is known as a **zoonosis.** The animals that serve as potential sources of infection to humans are called **reservoir hosts.** Whether or not a parasite infection is a zoonosis depends upon its host **specificity**, that is, the range of hosts that the organism is capable of infecting. For some species, the bonds of their relationship to the host may be so exquisitely restrictive that the parasite can only develop and survive in that host. The adult filarial worm, *Wuchereria bancrofti*, for example, parasitizes only humans, and even under experimental conditions cannot be made to infect laboratory animals.

Obviously, the host specificity of any particular parasite greatly influences factors associated with transmission and compounds the problems of control. In instances in which humans are the sole host of a parasite, or any stage of its development, eradication or effective control could be brought about (theoretically at least) by mass administration of antiparasitic drugs to the population at risk or by modification of human behavior or conditions so that the parasite can no longer be acquired. However, if the infection is a zoonosis, these measures can be frustrated by the existence of the reservoir in animals, which poses a constant threat of reinfection to the treated people.

LIFE CYCLES AND TRANSMISSION

The alterations which parasites undergo as they progress through their developmental life cycles may be a marvel to the parasitologist, but admittedly a plague to the student who has to remember the name and character of the different stages of each species of parasite. In this respect, parasitology differs from most other microbiologic disciplines. Generally, a bacterium is a bacterium throughout its existence, whereas a trematode, for example, is successively a miracidium, sporocyst, redia, cercaria, and adult. Of course, descriptive names are given to the growth stages of other plants and animals. A human, after all, is known progressively as an infant, adolescent, and adult. The main difference between human development and parasite development is that, throughout the growth process, the human is clearly recognizable as a human, while the trematode assumes a markedly different form at each growth stage. It is even more remarkable that the nature of the trematode's metabolic processes as well as its antigenic character can also differ radically between growth stages. The African trypanosome, the cause of sleeping sickness, can be used as a model for this phenomenon. While it is in the stage found in the bloodstream of its warm-blooded host, the trypanosome has an insatiable "sweet tooth," consuming its own weight of glucose every hour to fuel its energy needs. In contrast, the developmental forms in the tsetse fly consume about one-tenth the oxygen and sugar of that of the bloodstream form.

A knowledge of the parasite's life cycle is important for two practical reasons. First, **transmission**, the passage of the parasite from host to new host, is a function of the developmental cycle. Thus, a rational means of preventing parasitic infections must be based upon understanding of the life cycle. The other reason, especially important to the laboratory technologist, is that parasitologic diagnosis is usually made only from certain developmental stages: the eggs of some helminths and the larvae from others, the motile forms of some protozoa and the resting cyst stage of others.

Parasites employ a variety of maneuvers to effect transmission. There are species incapable of surviving the hostile conditions of the external environment that travel from host to host by such means as sexual intercourse (venereal transmission) or by means of ingestion of infected tissues of another host. Other species of parasites have a sojourn in the outside world, in soil or water or on plants. For hookworms and some other species of nematodes, the larvae are adapted to live in the soil. For other helminths, the strategy is to have their larvae live under protective cover such as that of *Ascaris lumbricoides*, whose thick-shelled egg protects the larvae within.

The examples of transmission described so far have been relatively simple and direct in nature. There is, however, a group of parasites obligated to undergo a much more complicated life cycle in their passage between hosts in which they become sexually mature **(definitive hosts).** Developmental stages of these parasites are present in one or more **intermediate hosts.** There are parasites, certain trematodes, for example, that require two intermediate hosts while others such as the malaria parasite employ a single intermediate host.

The "way of parasitism" is as hazardous for parasites as for their hosts and yet parasites are remarkably successful animals, as the many hundreds of millions of people harboring zoologic gardens of parasites within their bodies can testify. The complex life cycles of many parasites seem to make their survival as a species highly precarious. Consider the perils of *Paragonimus*, the lung fluke. Consider the possibility that a person with paragonimiasis defecates in a river or stream. There is a further possibility that there is a susceptible species of snail in the area and, later, a susceptible species of fresh water crab near the snail when it discharges the infective larvae (cercariae). Finally, there is the possibility that another human may eat an uncooked crab, and thus become infected.

In fact, it is not biologic Russian roulette. Evolution and nature are coconspirators that over the millenia have assembled and selected the various hosts in the life cycle. The behavior of the hosts, driven by culture (humans) or genetic program (intermediate hosts), ensures the intimate associations facilitating transmission. Obviously, humans, because of their intelligence, have a latitude for behavioral change that can break the chain of transmission. However, to the despair of public health workers, culturally directed human behavior often seems to be as resolutely fixed as that of the unthinking, genetically directed invertebrate vector.

EPIDEMIOLOGIC-ECOLOGIC INTERACTIONS

Parasites and their vectors are not isolated organisms indifferent to and independent of the ecosystem. Unlike a human (a remarkable animal, able to adapt to a wide variety of physical environments—deserts, tundras, tropical rain forests, polar ice caps, and cities), parasitic creatures are usually restricted to highly specialized environments inside and outside their hosts. The hookworm larva must have the proper moisture, warmth, shade, and soil type. The snail vector of the schistosome blood fluke must have water