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and Control Project

Diagnostic Parasitology

Workshops in Swaziland

by

Everett L. Schiller, Sc.D.

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1901 North Fort Myer Drive • Suite 400 • Arlington, VA 22209 • (703) 527-6500 • Telefax: (703) 243-0013 • Telex: 248812 (MSCI UR)

Author

Everett L. Schiller, Sc.D, is Professor Emeritus, School of Hygiene and Public Health at the Johns Hopkins University in Baltimore, Maryland.

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I wish to take this opportunity to express my sincere gratitude to the individuals named below for their professional, efficient, thoughtful and willing assistance, which contributed so much to the success and enjoyment of these training programs in the beautiful country of Swaziland.

Miss Mary Pat Selvaggio
Miss Anita Henwood
Dr. Bill Hoadley
Mrs. Mary Huntington
Dr. Neil Nkanza
Ms. Sibongile Mthupha
Dr. Alan Foose
Dr. Jay Anderson
Dr. Edmund McGrath
Ms. Kara Hanson
Dr. Giulia Capati
Mr. Rudolph Maziya
Mr. Sammy Tsabedze
Mr. Kobus Le Grange
Mr. Aber Dlamini

To each of the students who participated in these courses, I would like to say, "Ukwente kahle."

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1. Introduction

The curricula and course of study for these workshops on diagnostic parasitology were based on the concept that accurate clinical diagnosis of a parasitic disease requires confirmation by laboratory procedures that involve the proper collection, demonstration and unequivocal identification of the etiological agent or its progeny. Without careful parasitological diagnosis, there is no rational basis for treatment. Reliable diagnostic information is also a prerequisite for planning and evaluating disease control and prevention programs. Quantitative measurements of prevalence, incidence and intensity of infection are needed to establish the public health significance of parasitism in the human population and to assess the efficacy of intervention.

All too often, scientific investigations of important tropical diseases concentrate solely on a particular pathogen without regard for other parasitic infections that coexist in the individuals under study. The problems of concomitant multiple infections and polyparasitism are important because they occur so frequently in many rural populations and affect the course of treatment. For these reasons, any investigation of a major parasitic disease should entail a holistic, quantitative diagnostic approach.

The workshops described were designed to improve the capability of Government of Swaziland (GOS) Ministry of Health (MOH) staff to diagnose parasitic diseases. They were taught in collaboration with a Swazi trainer, Ms. Mthupha, to train her to teach MOH courses in diagnostic parasitology in the future.

The first phase of this program was conducted in Manzini November 28 - December 10, 1988. The course focused on intestinal parasites and schistosomes. The report on the first phase recommended that future training should include life cycles, pathology, transmission, prevention, control and treatment of parasitic infections. It also recommended that the course should be extended to include training in the diagnosis of blood and tissue

parasites, especially malaria, to improve the support capabilities of the health centers and medical laboratories of the six main hospitals in Swaziland. The curriculum for the second phase of the program, which was conducted November 13 - 21, 1989 was designed in accordance with these recommendations.

2. Phase 1

The program consisted of 80 hours of formal training, including lectures and laboratory practice. The schedule involved eight hours of lab-class time per day for 10 days during the first two weeks (November 28 - December 10, 1988), with one day of follow-up training at each of the eight laboratories in the country during the week of December 12 - 16, 1988. The techniques and methodologies presented and practiced by the students in the laboratory are identified in the schedule in Appendix A-1.

Fecal and urine specimens collected by the staff of the Bilharzia Control Center were used for all procedures involving both fresh and preserved material as well as techniques involving the preparation of permanent microscope slides of stained specimens for future identification and reference collections. A series of permanent microscope slides selected from two sets of 100, each brought here from the instructor's private collection, were also studied in the laboratory. A list of these specimens is included (see Appendix B-4). Lectures were illustrated by 2 x 2 color transparencies. Those presented to the class by projection are indicated by a check mark on the list in Appendix B-5. Other visual documentary material consisted of a VHS video cassette entitled "The Fiery Serpent," which depicts the problem of Guinea worm disease as it occurs in Nigeria.

The small size of this class allowed for close personal supervision by the instructor. Some of the parasites found in the specimens collected locally included the following species: *Ascaris lumbricoides; Strongyloides stercoralis; Trichuris trichiuria; Enterobius vermicularis; Schistosoma mansoni; Schistosoma haematobium; Taenia sp; Hymenolepis nana; Fasciola hepatica; Dicrocoelium dendriticum; Entamoeba coli; Entamoeba histolytica; Endolimax nana; Iodamoeba butschli; Chilomatix mesnilli and Giardia lamblia.* One of our students was surprised to learn that he was infected with giardiasis, for which he sought treatment on the

following weekend. Many of the protozoan parasites had not been reported in a previous survey done in Swaziland. It is believed that the use of appropriate concentration techniques increases the accuracy of the diagnosis.

The workshop was conducted in collaboration with the GOS MOH training specialist so that the training program could be transferred to the MOH for future courses. This very responsible person is Ms. Sibongile Mthupha, who is in charge of the Bilharzia Control Center and its field programs. She not only cheerfully assisted with the arrangements for the course, but participated in it as a student. It is my opinion that she is well qualified to teach parasitology as learned in this workshop. She already has had field experience in schistosomiasis control in Swaziland and has received specialized training in schistosomiasis gained through a four-month course at the Danish Bilharziasis Laboratory in Copenhagen, Denmark.

Follow-up training was carried out in each of the trainee's individual laboratories according to the following schedule:

Follow-up Training

Monday, 12/12/88 - Pigg's Peak Hospital (Gracian Moloi)

Tuesday, am 12/13/88 - Mankayane Hospital (Sabelo Dlamini)

Tuesday, pm 12/13/88 - RFM Hospital (Maurice Magagula)

Wednesday, 12/14/88 - Good Shepherd Hospital (Joseph Ngcamphalala). We visited the Refugee Reception Center at Malindza on return to Manzini.

Thursday, am 12/15/88 - Bilharzia Control Unit (Sibongile Mthupha and Ellen Matsenjwa).

(Virginia Mazibuko).

In general, these laboratories were clean, well-organized, and exceptionally well-equipped with electronic instrumentation for procedures of analysis other than parasitological analysis. All had good quality binocular miscoscopes, which must be considered the most important of all the instruments for diagnostic parasitology.

Objectives

The main objectives of the follow-up training were:

1. To assess facilities available for diagnostic parasitology and determine needs.

2. To determine possibilities for obtaining supplies needed and intent to employ new techniques learned during the course for concentration and quantitation of parasites (e.g. Kato/Katz method nucleopore filtration, MIF & PVA fixation and preservation).

3. To evaluate procedures for reporting results of parasitological examinations and for keeping permanent records. Most labs did not record age, sex or locality of patient. Recommendations were made to do this for epidemiologic purposes relating to future treatment, control and prevention programs.

4. To assess safety procedures in handling fecal and urine specimens that may be contaminated with HIV and make recommendations for personnel safety. Most labs provide latex or plastic gloves but they are not always used.

5. To determine means of disposal of fecal and urine specimens after examination. This procedure varied from "open pit" dumping to incineration.

6. To emphasize the need for periodic surveillance as a quality control measure of the diagnostician's accuracy and reliability.

7. To make specific recommendations as they applied to each individual laboratory.

Course evaluations and certification

The students were asked to evaluate the course to make recommendations for future workshops on diagnostic parasitology. Copies of their comments are attached (Appendix A-6). In summary, these would seem to suggest that more time should be devoted to the workshop to allow students a better opportunity to grasp all of its objectives and procedures.

The instructor prepared a "draft" of a document to be presented to each student upon completion of the course, certifying his or her participation in it. The draft was submitted to Miss Anita Henwood for comments, amendments and subsequent approval by USAID/Mbabane. The amended form was approved and Ms. Henwood's office made arrangements for printing the document on high quality paper.

To provide the instructor and his counterpart, Ms. Sibongile Mthupha, an opportunity to summarize the activities of our program, a briefing was held in the office of Mr. Roger D. Carlson, Director, USAID/Mbadane, at 4:30 pm Thursday, December 15, 1988. Also present at this meeting were: Miss Mary Pat Selvaggio, Assistant Health Development Officer; Miss Anita Henwood and Dr. A.W. Hoadley, Rural Water Development Project, Swaziland. At the close of this most pleasant and interesting session, the certificates were signed by the instructor and Director Carlson. All but one of these documents are to be distributed to the participants as the occasion permits. The exception is that of Joseph Ngcamphalala, who, for reasons beyond his control, was compelled to withdraw from the course four days prior to its completion. Arrangements have been made for him to make up for this omission by special tutelage from Ms. Mthupha in her laboratory in Manzini. Upon satisfactory completion of the requirement and in accordance with her discretion, the certificate will then be awarded to him.

Recommendations

The following recommendations are made for future training in parasitology. A medical parasitologist is expected to be able to answer about six basic questions when his patient is told that he has a parasitic infection. These usually are:

1. What is it?
2. How did I get it?
3. What will it do to me?
4. Can I give it to someone else?
5. How can I get rid of it?
6. How can I keep getting it again?

It is evident that a course in diagnostic parasitology deals almost exclusively with the answers to the first question. Consequently, future training should include some exposure to the concepts of life cycles, pathology, transmission, prevention, control and treatment. It also is recommended that the course be extended to include training in the diagnoses of blood and tissue parasites. Health centers in the major provinces of Swaziland should have the capacity to diagnosing malaria and identify the species of *Plasmodium* involved. There should be no reason for the long delay currently incurred by the necessity of sending blood smears to a distant central laboratory for identification.

Such training would also enable the technician to recognize and identify the filarial parasites that cause elephantiasis and the blood stages of trypanosomiasis, which are diseases known to occur in Swaziland. Although onchocerciasis, or "river blindness," has not yet been reported to be endemic in this country, the potential for its introduction into Swaziland exists. Hyperendemic areas of this disease extend into the southern regions of Malawi, so onchocerciasis and other important tropical diseases could be brought into the country by immigrants. Certainly the vector for transmission of river blindness, the black fly *Simulium damnosum*, is abundant in the highveld of Swaziland.

Arrangements should be made through a central diagnostic laboratory for periodic inspection and supervision of the various outlying laboratories to ensure continued accurate and reliable parasitological diagnosis. This could be accomplished by submitting test "unknown" specimens to laboratories regularly.

Understandably beyond the scope of laboratories' responsibilities at the present time, but of importance for future planning, is the need to develop, at least in a central laboratory, an ability to identify the opportunistic parasites, such as *Pneumocystis carinii* pneumonia and *Cryptosporidia*, now so commonly associated with AIDS.

This course in diagnostic parasitology of intestinal organisms has proved an important step in upgrading the medical capabilities in Swaziland and those having the foresight to recognize this need are to be congratulated. Continuation of this type of training should be a basic objective of future programs.

3. Phase 2

The second workshop for GOS/MOH technical staff members was conducted at the Bilharzia Control Center in Manzini. It consisted of 64 hours of formal training, including lectures and laboratory practice. The schedule encompassed eight hours of lab-class time per day for eight days during the period of November 13 - 21, 1989. The main topics presented and the methodologies practiced by the students are shown in Appendices A-1 and A-2.

As in phase 1 of this program, the workshop was conducted in collaboration with the GOS/MOH training specialist for purposes of transferring the training program to MOH for future courses. In this second phase, Ms. Sigongile Mthupha served as the MOH training specialist. She is a very responsible person who currently is in charge of the Bilharzia Control Center and its field programs. She not only willingly assisted with the arrangements for the course, but again participated in it as a student. She has markedly improved her qualifications for teaching the practical aspects and newer methods applicable to diagnostic parasitology as learned in this workshop.

Six of the eight trainees who participated in phase 1 of this course in 1988 were participants in this advanced program. An additional seven students were enrolled, including four technicians from the Malaria Control Unit.

More than 400 permanent microscope slides of stained specimens of *Plasmodium, Leishmania, Trypanosoma, Wuchereria* and *Schistosoma,* from the instructor's private collection were brought to Manzini for the training program. Several representative specimens of the malaria and filarial parasites were donated to each student for inclusion in his or her reference collection. Fecal and urine specimens collected in country by the staff of the Bilharzia Control Unit served as the basis for the quantitative procedures in the measurements of schistosomiasis by the Kato and Nuclepore methods.

Lectures on each disease were illustrated by a wide range of 2 x 2 color transparencies. A list of these has been included in the appendix. Other visual materials included two VHS videotapes: "Conquest of Parasites" and "Life Cycle of the Malaria Parasite."

A large volume of reference literature, given to each student in the form of xerox copies, served as supplemental reading material. Other reference material consisted of:

Atlas of Medical Helminthology and Protozoology by H.C. Jeffrey and R.M. Leach, 2nd ed. Churchill Livingston, Edinburgh, London and New York. 1975.

A Colour Atlas of Clinical Parasitology, ed. Tomio Yamaguchi. 1981. Wolfe Medical Publications Ltd.

Manual for the Microscopical Diagnosis of Malaria in Man. U.S. Department of Health, Education and Welfare, Public Health Service. (This manual now is out of print, but the instructor brought the six copies remaining in his collection. These were given to six of the students and efforts are being made by VBC to locate seven additional to be mailed to other students in the near future).

Basic Clinical Parasitology by Brown and Neva.

Recent publications consisting of reports of WHO Expert Committees (Technical Report Series) on malaria, leishmaniasis, trypanosomiasis, filariasis and schistosomiasis also were available from the instructor's library. These have been donated to Ms. Sibongile Mthupha for her future training programs in parasitology.

Student's course evaluations

(As summarized by Sibongile Mthupha)

This course was excellent, beneficial and relevant to Swaziland, where one is likely to come across a wide range of parasites of man. Even though some parasites have not been reported in Swaziland, the current movement of people from one country to

another can introduce types of parasitic diseases that may not be detected without adequate skills in diagnosis. The students were all pleased with the quality and presentation of the course. They feel that the acquired skills will be properly implemented in their fields, i.e. hospitals and schistosomiasis/malaria control programs. Before, some of the students did not know more than spotting the malaria or bilharzia parasite stages. Now, with their newly acquired skills, the students have a wider capability for diagnosis.

The following recommendations were made by the students: initiation of a formal training institution, whereby they and others can upgrade their skills in laboratory technology and other relevant fields. They also recommended that such workshops be carried out annually as refresher courses and for learning new skills, as needed. Third, a situation analysis on prevalence of parasitic disease in Swaziland is needed, since little is known about this area. Practical field application of diagnostic parasitology and appropriate data management skills are necessary as a follow-up to this kind of training. Finally, good quality microscopes to be used in these courses should be provided by the MOH. All of the students expressed their appreciation to the MOH, USAID and VBC for the opportunity to participate in this program.

Certificate of accomplishment

As in the case of phase one of this program, a document certifying accomplishment of the requirements of the course was presented to each student (see appendix A-2). A simple, but impressive awards presentation ceremony was held in a conference room at the George Hotel in Manzini. Those officiating at this ceremony included Ms. Anita Henwood, USAID, who called the names of the recipients, and Dr. Neil K. Nkanza, Pathologist to the Kingdom of Swaziland and Director of Laboratory Services, MOH, who made the presentations. The occasion was followed by a dinner at the Hotel.

Debriefing USAID/Mbabane

A debriefing meeting held in the office of the Director, USAID/Mbabane, Wednesday, November 22, provided an opportunity for the instructor and his counterpart, Ms. Sibongile Mthupha, to summarize the activities of our program. Others present at this meeting were: Mrs. Mary Huntington, Deputy Director, USAID; Ms. Anita Henwood, Assistant Health Development Officer, USAID; Dr. Alan Foose, Department of Health, USAID; Dr. Jay Anderson, Assistant Regional Health and Population Development Officer, USAID; and Dr. Edmund McGrath, Deputy Director of Health Services, GOS/ MOH, Mbabane.

Following our discussion of the training program, attention was called to the institutional strengthening programs of the A.I.D. Bureau for Science and Technology. The VBC II's second five-year contract with A.I.D. includes an expanded component for training for medical support services in vector-borne infectious diseases. Accordingly, VBC II is prepared to respond to requests for assistance in establishing such centers in developing countries. Previously, this information was discussed at length with Dr. Nkanza (MOH) in Manzini and later with Dr. Edmund McGrath (MOH) in Mbabane. Both of these officials expressed a positive interest in pursuing this potential source of support.

Recommendations

With their advanced training in the diagnosis of blood and tissue parasites, these technicians should assume a greater responsibility in their respective laboratories for identifying malaria parasites. After phase 1 of this program, the participants began to recognize more and more intestinal parasites than they had seen before in the stool specimens they examined. One student, Joseph Ngcamphalala of the Good Shepherd Hospital, reported that now that he knew what to look for, he was finding that hookworm infection was highly prevalent in the area of Siteki. Similar observations of the students suggest that information on the occurrence,

distribution and intensity of intestinal parasitism in Swaziland is lacking. Therefore, it is recommended that parasitological surveys be undertaken by MOH to rectify this problem.

There is also a need for continuity and expansion of training programs in diagnostic and medical support services in the field of vector-borne infectious diseases. It is recommended that the MOH submit a request for VBC II for assistance in establishing such a training center in Swaziland.

Appendix A-1. Workshop Schedule

Day 1	Introduction Course objectives Adjustment of microscopes Video: "Conquest of Parasites" Video: "Life Cycle of the Malaria Parasite"
Day 2	Malaria: the parasites Progressive stages of development Demonstration of distinct differences between the developmental stages of *Plasmodium falciparum, P. malariae, P. vivax* and *P. ovale*
Day 3	Malaria: the disease Sources of infection Relationships between symptoms and parasite development Introduction to *P. falciparum* in thick films Comparative microscopic study of *P. falciparum, P. vivax* and *P. malariae* in thick and thin blood films
Day 4	Malaria: Methods of preparation of thick and thin blood films Methods of staining blood films Microscopic study and review of all four species Practical test of ability to differentiate malaria species
Day 5	Leishmaniases: the parasites Life cycle of the *Leishmania* Diagnostic characteristics of the parasites Introduction to species parasitic in man Microscopic study of the parasites in stained blood films

Day 6

Trypanosomiases: the parasites
Life cycle of *Trypanosoma*
Diagnostic characteristics of the parasites
Species parasitic in man
Microscopic study of the parasites in stain blood films
Microscopic study of the parasites in stained tissue sections

Day 7

Filariases: the parasites
Life cycle of the filarial worms
Diagnostic characteristics of the parasites
Introduction to the species parasitic in man
Microscopic study of the parasites in stained blood films
Microscopic study of the parasites in stained tissue sections

Day 8

Schistosomiasis: the parasites
Life cycles of the *Schistosoma*
Diagnostic characteristics of the schistosomes
Review of the species parasitic in man
Microscopic study of the stained species
Practice in preparation of specimens for examination
Student preparation of specimens by the Kato and Nuclepore filtration techniques
Practice in methods of quantitation
Final practical examination

Appendix A-2. Class Participants and Certificate Awarded

The names, titles and places of employment of these individuals are listed below:

Garcian Moloi	Lab. Asst., Pigg's Peak Hospital
Sabelo V. Dlamini	Lab. Asst., Mankayane Hospital
Maurice M. Magagula	Lab. Asst., R.F.M. Hospital
Joseph Ngcamphalala	Lab. Asst., Good Shepherd Hospital
Mirriam B. Mofokeng	Lab. Asst., Hlathikhulu Hospital
Sibongile Mthupha	Hlth. Insp., Bilharzia Control Ctr.
Simon Simelane	Lab. Asst., Malaria Control Unit
Monica D. Lukhele	Hlth. Asst., Bilharzia Control Unit
Thoko Diamini	Hlth. Asst., Bilharzia Control Unit
Magnificent Khumalo	Hlth. Asst., Bilharzia Control Unit
Elias M. Dlamini	Lab. Asst., Malaria Control Unit
Michael Sukati	Hlth. Asst., Malaria Control Unit
William Gama	Hlth. Insp., Bilharzia Control Unit

Vector Biology and Control Project

Certificate of Participation

This is to certify that

Sibongile Mthupha

has successfully completed a course of training in the diagnosis of parasites that occur in the blood and tissues of humans.

This workshop was held at the Bilharzia Control Centre in Manzini, Swaziland, from 8 to 21 November 1989.

The course consisted of a series of lectures, demonstrations, applied methodologies and laboratory exercises involving the practical diagnosis of malaria, leishmaniasis, trypanosomiasis, filariasis and schistosomiasis.

This workshop was sponsored by the United States Agency for International Development/Mbabane and organized by the Vector Biology and Control Project under contract to Medical Service Corporation International, Arlington, Virginia, U.S.A., for the Agency for International Development, Office of Health, Bureau for Science and Technology.

Dr. Everett Schiller
Professor Emerius
The Johns Hopkins University
Baltimore, Maryland, USA
VBC Project Instructor

Roger D. Carlson
Director
United States Agency for
International Development
Swaziland

Appendix A-3. Course Objectives

The objectives of this course are to provide answers to the following questions as they pertain to certain parasitic diseases of human. These questions may be considered from the point of view of the patient when told by the doctor that he or she has a parasitic infection:

1. What is it?

   (identification, classification, diagnosis)

2. What will it do to me?

   (symptomatology, clinical disease, pathology)

3. How did I get it?

   (life cycles)

4. Can I give it to someone else?

   (mode of transmission)

5. How can I get rid of it?

   (treatment, chemotherapy)

6. How can I keep getting it again?

   (immunology, prevention, control)

Emphasis will be placed on five major diseases: malaria, leishmaniasis, trypanosomiasis; filariasis and schistosomiasis.

Appendix A-4. Malaria

Lecture outline

I. Historical considerations

   A. Antiquity of malarial parasites
   B. Discovery of the method of transmission

II. Developmental stages of the parasite

   A. Human cycle

      1. Pre-erythrocytic schizogony
      2. Erythrocytic schizogony
      3. Gametogony
      4. Exo-erythrocytic schizogony

   B. Mosquito cycle

      1. Completion of gametogony
      2. Sporogony

III. Species parasitic in man

   A. *Plasmodium falciparum*, causative agent of malignant tertian malaria
   B. *Plasmodium malariae*, causative agent of quartan malaria.
   C. *Plasmodium vivax*, causative agent of tertian malaria.
   D. *Plasmodium ovale*, causative agent of ovale malaria.

IV. The disease

   A. Sources of infection
   B. Vectors
   C. Types of endemicity

V. Prevention and control.

Transparencies shown

1. Life cycle of the malaria parasite.
2. Sporozoites in saliva of mosquito.
3. Schizonts in human liver.
4. *Plasmodium falciparum* in red blood cells.
5. *P. falciparum* gametocytes in blood.
6. Male gametocyte.
7. Fertilized gamete.
8. Oocysts in *Anopheles*.
9. Oocysts on midgut wall.
10. *P. falciparum* young trophozoites.
11. *P. falciparum* erythrocytic schizont.
12. *P. vivax* advanced trophozoite.
13. *P. vivax* mature schizont.
14. *P. vivax* macrogametocyte.
15. *P. vivax* microgametocyte.
16. *P. ovale* tropozoite.
17. *P. ovale* schizont with 8 nuclei.
18. *P. ovale* developing schizont.
19. *P. malariae* young trophozoite.
20. *P. malariae* mature schizont.
21. *P. malariae* female gametocyte.
22. Temperature chart of infected patient.
23. Relapses in vivax malaria.
24. Malaria patient.
25. Palpation of spleen for diagnosis of malaria.
26. Nigerian children with enlarged spleens.
27. Diagnosis by blood films.
28. Making thin blood films.
29. Romanowsky staining.
30. Liver pathology.
31. Brain from fatal case of falciparum malaria.
32. Brain smear with *P. falciparum* in red blood cells.
33. Developing forms of *P. falciparum* in intervillous spaces of placenta.
34. Blackwater fever is a complication of falciparum malaria.
35. Malarial edema.
36. Enlarged spleen due to *P. malariae*.
37. Distribution of malaria.
38. Eggs of *Anopheles*.

39. Larva of *Anopheles*.
40. Pupae of *Anopheles*.
41. *Anopheles*, bloodfed female.
42. Collecting larvae of *Anopheles*.
43. Biting catches of adult mosquitoes.
44. *A. gambiae* resting on straw mats after blood meal.
45. Catching adult mosquitoes in test tube.
46. African mud hut.
47. Collecting dead mosquitoes for dissection.
48. Collecting *A. gambiae* in pit-trap.
49. Mud puddles containing *A. gambiae* larvae.
50. Cattle watering places where *A. gambiae* breed.
51. Spraying of rural dewellings in Cameroon.
52. *A. funestus* larvae found in clear streams.
53. *A. pharoensis* breeding site place in Egypt.
54. *A. hyrcanus* breeding site in Afghanistan rice field.
55. *A. culifacies* breeding site in Nepal.
56. *A. stephensi* breeding site in domestic water reservoirs.
57. *A. punctulatus* may be found in small collections of water.
58. *A. farauti* is adaptable in its breeding sites.
59. Some vectors breed in specialized places.
60. Spraying team in malaria eradication program.
61. Typical compound in rural India.
62. Walls sprayed after replastering.
63. Spray team in New Guinea.
64. Rural hut in New Guinea.
65. Nomads often reintroduce malaria.
66. Residual spraying is short-lived in Brazil.
67. Man-made "borrow pits" create breeding habitats of mosquitoes.
68. Rice fields are important breeding sits of *Anopheles*.
69. Concrete drain for removal of surface water.
70. Concrete drain useful as method of malaria control.
71. Drag line used in drainage of *Anopheles* breeding areas.
72. Knapsack type of sprayer.
73. Ultra-low-volume application of synthetic pyrethroid insecticide.
74. Helicopter used in spraying marshes.
75. Mosquito nets.
76. Spraying team in Nigeria.
77. Spraying walls.

78. Numbering houses.
79. Making blood slide.
80. Examination of blood for malaria parasites.
81. Patient with relapsing malaria parasites.
82. Medicated salt project in Iran.
83. Distribution center for antimalarial drugs on Amazon River.
84. Printed literature of the national malaria control program in India.
85. Malaria life cycle.
86. Malaria life cycle.
87. *P. falciparum* thick smear.
88. *P. falciparum* Maurer's dots.
89. *P. falciparum* fatal case.
90. *P. falciparum* phagocytes containing pigment and parasites.
91. *P. falciparum* female gametocyte.
92. *P. falciparum* male gametocyte.
93. *P. vivax* oökinete
94. *P. cynomolgi* exoerythrocytic cycle.
95. *P. cynomolgi* exoerythrocytic cycle.

Appendix A-5. Leishmaniases

Lecture outline

I. Life cycle of the *Leishmaniases*

II. Clinical aspects of the disease.

A. Visceral leishmaniasis (kala azar) due to *L. donovani*.
B. Cutaneous leishmaniasis (oriental sore) due to *L. tropica*.

1. *Leishmania recidiva*
2. Diffuse cutaneous leishmaniasis

C. Mucocutaneous leishmaniasis (espundia) due to *L. braziliensis*.

III. Diagnosis

A. Direct microscopic examination
B. Culture
C. Immunodiagnosis
D. Genetics

IV. Treatment and prevention

A. Chemotherapy
B. Vaccination

Transparencies shown

1. Life cycle of *Leishmania*.
2. Amastigotes inside a macrophage.
3. Amastigote showing enclosed flagellum.
4. Longitudinal section through infected sandfly.
5. Promastigotes from stomach of infected sandfly.
6. Infant infected with *L. infantum*.
7. Adolescent infected with *L. donovani*.
8. Kala-azar is characterized by prolonged fever.
9. Sandfly bites produce an itching red papule.
10. Crater-like edge of papule contains infected macrophages.
11. Crust forming on ulcer.
12. Reddening of infected area is common.
13. Man infected with *L. major*.
14. Invasion of nasal mucosa and septum by *L. major*.
15. Serious and disfiguring lesions from infection with *L. major*.
16. Severe ulceration by *L. major*.
17. Single ulcer infected with *L. mexicana*.
18. Multiple ulceration may be caused by *L. mexicana* or *L. braziliensis*.
19. Multiple but discrete ulcers caused by *L. mexicana*.
20. Man infected with *L. braziliensis*.
21. Lymphatic spread and secondary ulceration.
22. Ulceration of penis and scrotum resembles that of syphilis.
23. Severe erosion of cartilage of the ear caused by *L. mexicana*.
24. Lymphatic spread and secondary ulceration.
25. Spread onto mucosal surfaces by *L. braziliensis*.
26. Destruction of the nasal septal cartilage.
27. Man with "Tapir nose."
28. Man with hole in nasal septum.
29. Spread to the hard and soft palate.
30. Gross tissue destruction and disfigurement.
31. "Diffuse cutaneous leishmaniasis" due to *L. aethiopica*.
32. Woman with "post kala-azar dermal leishmanoid."
33. PKDL usually responds to a change of antileishmanial drug.
34. *Leishmania* amastigotes found in smears from bone marrow or spleen tissue.
35. Amastigotes are seen as clusters in macrophages.
36. Positive splenic material from patient with kala-azar.
37. "Aldehyde test" is a crude indicator of a high IgG level.

38. Visceral leishmaniasis is associated with increase in immunoglobulins.
39. Flourescent test for antileishmanial antibodies.
41. Aspirated material cultured on NNN medium.
42. Hamster infected with *L. infantum*.
43. Parasites occur in swollen growing edge of ulcer.
44. Structure of a dental probe.
45. *Leishmania* amastigotes in macrophages or as individual parasites.
46. Inoculation into foot of hamster.
47. Immunity to reinfection demonstrated by positive skin test.
48. Skin "punch" used to remove a piece of tissue.
49. Large accumulation of phagocytic cells in subcutaneous tissue.
50. Large numbers of amastigotes forming "nest cells."
51. Distribution of cutaneous and visceral leishmaniasis.
52. Distribution of cutaneous and visceral leishmaniasis.
53. Distribution of cutaneous and visceral leishmaniasis.
54. Typical shape of larva of *Phlebotomus* and *Lutzomyia*.
55. Sandfly larvae transform to pupae.
56. Female sandfly.
57. Male sandfly feeds only on nectar.
58. Examination of children.
59. Survey of village environment.
60. Collecting *P. papatasii*.
61. Simple light traps attract flies.
62. Search for sandflies in mud-walled village.
63. Dog being bitten by *P. arias* and other species.
64. Paper trap at entrance to a gerbil burrow.
65. Greater gerbil is a common reservoir host of *L. major*.
66. Rodent-baited trap used to collect *Lutzomyia*.
67. Flies resting inside mosque (*P. sergenti*).
68. People sleeping outside are exposed to bites.
69. *Rhombomys opimus* is main reservoir of *L. major* in N. Afghanistan.
70. Entrance to burrow of *Psammomys obesus*.
71. Captured *Psammomys*.
72. Ulcerated ears of this rodent contained amastigotes of *L. major*.
73. Smears from rodent made directly onto microscope slide.

74. *Lutzomyia* may be collected resting in buttresses of shaded trees.
75. Various species of *Prochimys* are reservoirs of *L. mexicana*.
76. Opossum infected with *Leishmania*.
77. Various species of sloth are reservoirs of leishmaniasis.
78. Termite hills house rodents that are reservoirs of *L. major*.
79. Dogs are reservoirs of *L. infantum*.
80. Dog infected with *L. infantum*.
81. Jackals, foxes, hyenas, wolves may serve as reservoirs of *L. infantum*.
82. Moat to protect farm from gerbils.
83. Spraying with residual insecticides reduces sandflies.
84. Infection with *Leishmania* trested with Pentostan.
85. Post kala-azar dermal leishmanoid.
86. Post kala-azar dermal leishmanoid.
87. *Leishmania donovani* on impression smear.
88. *Leishmania tropica*.
89. Promastigote of *L. donovani*.
90. *Leishmania* life cycle.
91. *Leishmania donovani* in vitro.
92. *Leishmania donovani* in vitro.
93. *Leishmania donovani* in vitro.
94. *Leishmania donovani* in vitro.
95. *Leishmania donovani* in vitro.
96. *Leishmania donovani* in vitro.
97. *Leishmania donovani* in vitro.
98. *Leishmania donovani* in vitro.
99. *Leishmania donovani* in vitro.
100. *Leishmania donovani* in vitro.

Appendix A-6. Trypanosomiasis

Lecture outline

I. West-African sleeping sickness

   A. The causative agent, *Trypanosoma gambiense*
   B. Insect vectors, *Glossina palpalis* and *G. tachinoides*.
   C. Life cycle

   D. Pathogenic lesions

      1. Incubation period
      2. Febrile stage
      3. Sleeping sickness stage

   E. Diagnosis

II. East-African sleeping sickness.

   A. The causative agent, *Trypanosoma rhodesiense*
   B. Transmission
   C. Pathogenesis

III. Laboratory practice

   A. Study of parasites in stained blood films
   B. Study of parasites in stained tissue sections

Transparencies shown

1. Life cycles of *Trypanosoma rhodesiense* and *T. gambiense*.
2. Blood stream forms of *T. rhodesiense* and *T. gambiense*.
3. Intermediate forms of *T. gambiense* in tissue fluid from lymph gland.
4. Parasites from midgut of the tsetse fly *Glossina*.
5. Parasites in salivary glands of *Glossina*.
6. Metacyclic trypanosomes in a salivary probe after staining with Giemsa.
7. "Antigenic variants" are produced during the infection.
8. Electron micrograph of bloodstream form of a trypanosome.
9. Trypanosomal chancre.
10. Section of trypanosomal chancre.
11. Fever charts of two trypanosomiasis patients showing intermitent fever.
12. Circinate rash of trypanosomiasis over and below left clavicle.
13. Early "sleeping sickness" show enlarged lymph glands.
14. Myxoedema a typical feature of advanced sleeping sickness.
15. Myxoedema in a patient with advanced sleeping sickness.
16. Comatose patient in late stages of disease.
17. Palpation of glands in surveys for *T. gambiense*.
18. Gland puncture.
19. Lumbar puncture.
20. Collection of cerebrospinal fluid.
21. Chest x-ray in toxic myocarditis in *T. gambiense* infection.
22. Examination of buffy coat in diagnosis.
23. Blood testing for antibodies to trypanosomes.
24. Fluorescing parasites in presence of antibodies in patient's serum.
25. Card agglutination test.
26. Animal inoculation for diagnosis.
27. Effect of parasites in man.
28. Morula cell of Mott.
29. Perivascular cuffing of artery in brain in sleeping sickness.
30. Section of myocardium and pericardium in fatal human case.
31. Thickening of basal membrane indicative of glomerulonephitis.
32. Distribution of human trypanosomiases in Africa.
33. *Glossina* in typical posture.

34. Characteristic wing pattern in genus *Glossina*.
35. Blood engorged *Glossina*.
36. Pupae of *Glossina*.
37. Northern and southern limits of distribution of *Glossina*.
38. Riverine forest fringe habitat of *G. tachinoides* and *G. palpalis*.
39. Drier savannas are typified by narrow forest.
40. *G. palpalis* habitat in forest zone of West Africa.
41. Proximity of dense vegetation habitate of *G. fuscipes* to huts.
42. Dense thicket type habitat for *G. pallpidipes*.
43. Savana woodland habitat of *G. morsitans*.
44. Swamp region endemic focus of *T. rhodesiense*.
45. Tsetse found in damp, shady places.
46. Collecting *Glossina*.
47. Suspended bushbuck skin used to catch *Glossina*.
48. Tsetse fly trap.
49. Biconical trap used to catch riverine species of *Glossina*.
50. Sticky substance on board to capture *Glossina*.
51. Riverine vegetation habitat of *Glossina*.
52. Gathering water brings man into contact with *Glossina* vectors.
53. Daily activities associated with vector contact.
54. Village in close proximity to vegetation providing habitat for tsetse.
55. Deserted villages are evidence of depopulation due to the disease.
56. Disease dissemination through population movement.
57. Sacred groves provide habitats for riverine *Glossina*.
58. Fishing brings people in contact with riverine *Glossina*.
59. Honey gathering puts people in contact with riverine *Glossina*.
60. Hunters can become infected with *T. rhodesiense*.
61. Tourists have become infected.
62. Domestic African pig may be reservoir of *T. rhodesiense*.
63. Bushbuck has been found infected with *T. rhodesiense*.
64. Hartebeest are reservoir hosts of *T. rhodesiense*.
65. Hyena have been found infected with trypanosomes infective to man.
66. Zebu cattle are reservoir hosts of *T. rhodesiense*.
67. Zymogram of trypanosome extracts.
68. Health authorities communicate with village elder.

69. Organization of local population for gland palpation.
70. Accurate records important.
71. Patients given record cards.
72. Team of microscopists on survey.
73. Treatment requires hospitalization for about 30 days.
74. Exfoliative dermatitis may follow treatment with Melarsoprol.
75. Severe fetal reaction to treatment with Melarsoprol.
76. Insecticide being applied to vegetation by hand-held lance.
77. Knapsack motorized mist blowers.
78. Vehicle-mounted spraying.
79. Extensive bush clearing to reduce tsetse fly habitat.
80. Bush clearing has been used in tsetse control.
81. Spraying insecticide over *G. pallidipes* thicket.
82. Helicopter and the service vehicle that accompanies it.
83. Helicopter prepared for the application of insecticide.
84. Biologists sampling fish population to detect insecticide residue.

Appendix A-7. Filariases

Lecture outline

I. Tissue parasites requiring intermediate hosts.

   A. *Wuchereria bancrofti*
   B. *Brugia malayi*
   C. *Onchocerca volvulus*
   D. *Loa loa*
   E. *Dipetalonema perstans*
   F. *Mansonella ozzardi*
   G. *Dracunculus medinensis*

II. Life cycles

   A. Location of adult and larval stages in humans
   B. Reproduction of worms in the definitive host

      1. Sheathed forms
      2. Unsheathed forms

   C. Development in the insect host
   D. Reservoir hosts

III. Pathogenesis

IV. Diagnosis

V. Prevention and control

Transparencies shown

1. *Wuchereria bancrofti* microfilaria.
2. *W. bancrofti* tail of microfilaria.
3. *Brugia malayi* microfilaria.
4. *Loa loa* microfilaria.
5. *Loa loa* til of microfilaria.
6. *Tetrapetalonema perstans.*
7. *Mansonella ozzardi* microfilaria.
8. *Onchocerca volvulus* microfilaria.
9. *T. streptocerca* microfilaria.
10. Distribution of *W. bancrofti.*
11. W. bancrofti life cycle.
12. W. bancrofti larva developing in mosquito.
13. Mosquito taking blood meal.
14. Examining blood for microfilaria.
15. Drop of blood placed on microscope slide.
16. Thick blood smear.
17. Counting chamber.
18. Membrane filter technique.
19. Blood passed through filter.
20. Microfilariae of *W. bancrofti* and *M. ozzardi.*
21. *W. bancrofti* adults in dilated lymphatics.
22. Male with hydrocoel.
23. Left: milky urine sample; right: fatty chyle dissolved in ether.
24. Patients with elephantiasis.
25. Man with elephantiasis of scrotum.
26. Calcification of inguinal lymph nodes and ducts.
27. *Anopheles* and *Aedes* species are important vectors.
28. *Culex pipiens quinquefasciatus.*
29. Typical habitat of urban filariasis.
30. *Aedes polynesiensis* can breed in small bodies of water.
31. Residual house spraying in DDT.
32. Septic tank spraying to destroy larvae of *C. pipiens.*
33. Malayan filariasis is confined to Asia.
34. Elephantiasis in a woman with Malayan filariasis.
35. *Mansonia* species
36. *Mansonia* larvae and pupae breath using water plants.
37. Microfilarial survey of dogs.
38. Chronically enlarged superficial inguinal node *B. timori.*

39. Distribution of *Loa loa.*
40. *Loa loa* life cycle.
41. Man with fugitive Calabar swelling caused by *Loa loa.*
42. Section of dermis containing dying male of *Loa.*
43. *Loa* adult crossing the eye beneath the conjunctiva.
44. Surgical removal of *Loa* from the eye.
45. Shaded streams of forest areas provided habitat for larval *Chrysops.*
46. *Chrysops dimidiata* adult.
47. *Culicoides* is vector of *T. perstans, T. streptocerca,* and *M. ozzardi.*
48. Subcutaneous tissues with adult female of *T. streptocerca.*
49. *Onchocerca volvulus* geographical distribution.
50. *Onchocerca volvulus* life cycle.
51. Onchocercoma with mass of adult worms.
52. Section of skin with microfilariae of *Onchocerca.*
53. Section of thoracic muscles of vector *Simulium* with larvae of *Onchocerca.*
54. Skin snips.
55. Corneoscleral punch.
56. Motile microfilariae emerge from skin.
57. Girl with characteristic onchocercal nodules in hip region.
58. Nodules on head of boy.
59. Section of nodule with adult female worm.
60. Rash consisting of raised papules.
61. "Sowda."
62. Mottled depigmentation.
63. "Hanging groin."
64. Elephantiasis of leg.
65. Blind farmer with onchocerciasis being led by child.
66. Three blind children.
67. Early eye lesions.
68. Punctuate keratitis with shifting opacities.
69. Advanced sclerosing keratitis has led to complete blindness.
70. Atrophy of optic nerve.
71. *Simulium* larvae.
72. *Simulium* associated with large fast-growing rivers in Africa.
73. Freshwater crab
74. Deserted village.
75. *Simulim* species in C.A. usually breed in small streams.
76. Control by treating rivers with larvicide.

77. Dam on Nile River where insecticide control undertaken.
78. Surgical removal of nodules.
79. Chemotherapy is unsatisfactory.
80. *Dirofilaria immitis* in dog heart.
81. *Dirofilaria immitis* in dog heart.
82. *Dirofilaria immitis* male and female specimens.
83. *Onchocerca volvulus* section of skin (Republic of Chad).
84. *O. volvulus* section of female worm.
85. *O. volvulus* section.
86. *O. volvulus* section.
87. *O. volvulus* section.
88. *O. volvulus* section.
89. *O. volvulus* section.
90. *O. volvulus* section.
91. *O. volvulus* section.
92. *O. volvulus* section.
94. *O. volvulus section.*
95. *O. volvulus* excised nodule (Guatemala).
96. *W. bancrofti* infective larvae emerging from mouth parts of mosquito.
97. *W. bancrofti* microfilaria.
98. *W. bancrofti* microfilaria.
99. *W. bancrofti* section of lymph node.
100. *W. bancrofti* section of lymph vessel.
101. Life cycles of filarial worms.

Appendix A-8. Schistosomiasis

Lecture outline

I. The schistosomes of humans

A. *Schistosoma haematobium*
B. *Schistosoma mansoni*
C. *Schistosoma japonicum*
D. *Schistosoma intercalatum*

II. Life cycles and development

A. Oviposition
B. Release of eggs from body of the host
C. Hatching of eggs
D. Penetration of the snail host
E. Development within the snail host
F. Cercarial emergence
G. Migration within the definitive host
H. Development to maturity

III. Pathogenesis

A. The invasion period
B. The migration period
C. The maturation period
D. The oviposition period

1. Pre-, early and late egg deposition stages
2. Cirrhotic stage
3. Cachexial stage

IV. Diagnosis

A. Physican examination and symptoms
B. Specific identification of the parasite

1. Direct examination of feces and urine
2. Rectal biopsy

3. Cystoscopy and sigmoidoscopy
4. Serological tests

Transparencies shown

1. Life cycle of schistosomes
2. Daily life where infection with *Schistosoma* exists.
3. Daily life.
4. Daily life.
5. Daily life.
6. Daily life.
7. Daily life.
8. Daily life.
9. Daily life.
10 Daily life.
11. Daily life.
12. Daily life.
13. Daily life.
14. Water carried to homes may be source of infection.
15. Snail vector in its natural habitat.
16. Distribution of *S. mansoni*.
17. Egg of *S. mansoni*.
18. Miracidium of *S. mansoni* leaving egg.
19. Iodine stained miracidium of *S. mansoni*.
20. *Biomphalaria glabrata* intermediate host of *S. mansoni*.
21. Developing *S. mansoni* in snail.
22. Snail shedding hundrds of cercariae.
23. Mature cercariae of *Schistosoma*.
24. Cercaria in human subcutaneous tissue.
25. Pair of adult schistosomes.
26. Section of adult schistosomes in blood vessel of liver.
27. Adult schistosomes in mesenteric vessels.
28. *S. mansoni* within uterus of adult female worm.
29. Mesenteric vessel filled with schistosome eggs.
30. Areas affected by *S. mansoni*.
31. Granuloma formation in intestine caused by schistosome eggs.
32. Rectal and colonic polyps due to infection with *S. mansoni*.
33. Granulomata formed in liver around schistosome eggs.

34. Granuloma formation and fibrosis in the liver.
35. Symmer's fibrosis.
36. Fibrosis due to *S. mansoni* or *S. japonicum*.
37. Portal hypertension may ensue as consequence of periportal fibrosis.
38. Young boys with severe schistosomiasis due to *S. mansoni*.
39. Women with schistosomiasis with masive ascites.
40. Man with schistosomiasis with masive ascites.
41. Granulomata within the lungs.
42. Clubbing of fingers a clinical sign of cor pulmonale.
43. Chest x-ray of person with cor pulmonale.
44. Distribution of schistosomiasis due to *S. japonicum*.
45. Egg of *S. japonicum*.
46. *Oncomelania* snails are intermediate hosts of *S. japonicum*.
47. Dense population of *Oncomelania* snails.
48. Clinical and pathological changes due to *S. japonicum*.
49. Portal venous hypertension and enlarged splenic vein due to *S. japonicum*.
50. Boy infected with *S. japonicum*.
51. Distribution of schistosomiasis due to *S. haematobium*.
52. Schistosomiasis caused by *S. haematobium*.
53. Miracidium of *S. haematobium*.
54. Snails of genus *Bulinus* are intermediate hosts of *S. haematobium*.
55. *Bulinus* shedding cercariae.
56. *S. haematobium* adult.
57. Section of adult of *S. haematobium*.
58. Eggs of *S. haematobium*.
59. Egg of *S. haematobium* in bladder wall.
60. Haematuria.
61. Pathological effects of *S. haematobium* on urinary tract.
62. Bladder calculi.
63. Intravenous pyelogram.
64. Calcification of bladder rim.
65. Massive calcification of bladder in *S. haematobium* infection.
66. Microscopic examination of fecal sediment.
67. Egg of *S. mansoni* in sediment.
68. Diagnosis of infection with *S. haematobium* by sedimentation of urine.
69. Egg of *S. haematobium*.
70. Rectal biopsy for diagnosis of schistosomiasis.

71. Chemotherapy for schistosomiasis control.
72. Swimming pool to discourage swimming in infected waters.
73. Construction of laundry to discourage washing in infested water.
74. Canal.
75. Sluice rate for regulation of stream.
76. Irrigation canal.
77. Health education.
78. Equipment for measuring concentration of molluscicides.
79. Field health team.
80. Focal mollusciciding.
81. Automatic administration of molluscicides.
82. Spraying molluscicides on cleared irrigation canals.
83. Snail traps in irrigation canals.
84. Floating containers used to distribute molluscicides.
85. Life cycles of schistosomes.
86. *S. mansoni* adult male.
87. Central nervous system of *S. mansoni*.
88. Reproductive system of female *S. mansoni*.
89. Reproductive system of female *S. mansoni*.
90. Eggs of *S. mansoni*.
91. Egg of *S. mansoni*.
92. Miracidium in egg of *S. mansoni*.
93. Schistosomule of *S. mansoni* in rat skin.
94. "Pipestem fibrosis" in human liver due to *S. mansoni*.
95. Adults of *S. mansoni* in mesenteric vein.
96. *S. mansoni* adult male in mesoappendical vein.
97. *S. mansoni* adults in mesenteric vein.
98. *S. mansoni* ova in adenocarcinoma of rectal mucosa.
99. *S. mansoni* ovum in pancreas.
100. *S. mansoni* ova in nerve plexus of neck.
101. *S. mansoni* adult male in mesoappendiceal vein.
102. *S. mansoni* ova in bowel.
103. *S. mansoni* rectal biopsy (egg-shells acid fast).
104. *S. mansoni* granulomata (Masson's collagen stain).
105. *S. mansoni* granulomata.
106. *S. mansoni* granulomata.
107. *S. mansoni* granulomata.
108. *S. mansoni* granulomata.
109. *S. mansoni* granulomata.
110. *S. mansoni* granulomata.

111. *S. mansoni* granulomata.
112. *S. mansoni* granulomata.
113. *S. mansoni* granulomata.
114. *S. haematobium* in bladder.
115. *S. haematobium* in bladder.
116. *S. haematobium* ova in bladder.
117. *S. haematobium* ova in bladder.
118. *S. haematobium* ova in bowel.
119. *S. haematobium* ova in bladder.
120. *S. haematobium* polyp formation and squamous cell metaplasia in bladder.
121. Pair of adult *S. japonicum*.
122. Miracidia of *S. japonicum*.
123. *S. japonicum* adults in mesenteric vein.
124. *S. japonicum* rectal biopsy.
125. *S. japonicum* adults in mesenteric vein.
126. *S. japonicum* ova in intestinal tissue.
127. *S. japonicum* ovum in monkey lung.
128. *S. japonicum* ova in rabbit intestine (experimental).
129. *S. japonicum* infiltration around ovum (experimental).

Appendix B-1. Course Schedule

Day 1

Introduction.
Calibration of microscope.
Practice with the ocular micrometer.

Day 2

Collection and handling of unpreserved fecal specimens.
Preservation of fecal specimens (formalin and PVA fixatives).

Microscopic examination of fecal specimens.
Preparation of wet mounts for microscopy.

Day 3

Wet mounts with saline and iodine solutions.
Merthiolate-iodine-formaldehyde (MIF) staining.
Microscopic examination of fecal specimens collected locally.

Identification of helminths and protozoa.

Day 4

Concentration techniques.
Flotation procedures (zinc, sulfate, centrifugal flotation; brine flotation).

Sedimentation procedures (simple gravity; formalin ethyl acetate concentration).

Microscopic examination of local fecal specimens prepared by flotation and sedimentation methods.

Identification of helminths and protozoa.

Day 5

Preparation of permanently stained fecal specimens (Heidenhain's iron hematoxylin stain technique).

Preparation and microscopic examination of fecal specimens collected locally.

Identification of "unknown" specimens (helminths and protozoa).

Day 6

Microscopic examination and identification of stained permanent preparations of various helminths and protozoa (slides provided by the instructor).

Preparation of charcoal cultures for recovery of helminth larvae.

Day 7

Techniques for diagnosing enterobiasis.
Microscopic examination of cellulose-tape slides for pinworms.
Identification of "unknown" specimens (helminths and protozoa).

Day 8

Kato thick-smear technique for fecal material.
Microscopic examination of specimens prepared by the Kato method.

Quantitative procedures for estimating worm burdens.

Day 9

Nucleopore filter method for quantitating eggs for *Schistosoma haematobium* in urine.

Practical application.

Day 10	Compression technique for demonstrating trichina larvae. Microscopic review of stained permanent Preparations of various intestinal helminths and protozoa. Quality control, reference collections, reporting procedures and record keeping.
Days 11-15	Follow-up training at each of the eight labs in country.

The workshop for GOS MOH technical staff member was conducted at the Bilharzia Control Center in Manzini.

Appendix B-2. Course Participants

Garcian Moloi	Lab. Asst., Piggs Peak Hospital
Sabelo V. Dlamini	Lab. Asst., Mankayane Hospital
Maurice M. Magagula	Lab. Asst., R.F.M. Hospital
Joseph Ngcamphalala	Lab. Asst., Good Shepherd Hospital
Ellen M. Matsenjwa	Hlth. Insp., Bilharzia Control Ctr.
Virginia L. Mazibuko	Lab. Asst., Central Public Hlth. Lib.
Mirriam B. Mofokeng	Lab. Asst., Hlathikulu Hospital

Appendix B-3. Student Course Evaluations
(as summarized by Sibongile Mthupha)

This course was excellent, beneficial, and relevant to Swaziland where one is likely to come across a wide range of parasites of man. Even though some parasites have not been reported in Swaziland, the current movement of people from one country to another can introduce new types of parasitic diseases which may not be detected unless adequate skills in their diagnosis is acquired. The students were all pleased about the quality and presentation of the course. They feel that the acquired skills will be properly implemented in their field, i.e. hospitals, and schistosomiasis/malaria control programs. Before, some of the students did not know more than spotting the malaria or bilharzia parasite stages. Now, with their newly acquired skills, the students have a wider capability of diagnosis. The following recommendations were made by the students. Initiation of a formal training institution, whereby they and others can upgrade their skills in laboratory technology and other relevant fields. Also recommended, was that such workshops be carried out annually as refresher courses and for learning new skills, as needed. Thirdly, the situation analysis on prevalence of parasitic diseases in Swaziland is needed, since little is known about this area. Practical field application of diagnostic parasitology and appropriate data management skills are necessary as a follow-up to this kind of training. Finally, good quality microscopes to be used in these courses, should be provided by the MOH. All of the students expressed their appreciation to the MOH/USAID/VBC for the opportunity to participate in this program.

Appendix B-4
List of Supplies for Course in Diagnostic Parasitology

Specimen containers, half-pint waxed cardboard cartons with overlapping lids, #100.
Clear cellulose tape, 3/4 inch wide (clear scotch with dispenser) #2
Tongue depressors, wood, #100
Surgical cotton sterile, #1
Applicator sticks, #200
Blood lancets, disposable, #100
Cotton swabs, sterile, #1 box
Facial tissue, #6 units
Gauze squares, 2x2 inch, sterile, or bolt of sterile gauze
Rubber bulbs for capillary pipettes, #24
Test tubes, 100 x 13, #36
Microscope slides, 3 x 1 inch, #2 boxes; 3 x 2 inch, 2 boxes
Glass syringes, 10 ml, #36; 25 ml, #36
Coverslips, 22 mm square, no. 1 thickness, #500
Wash (squeeze) bottles, 250 ml, #12
Dropping bottles, 250 ml, #12
Pipettes, serologic, 1 ml, 5 ml, 10 ml, #12 each
Pipettes, capillary (Pasteur), 5 3/4 inches long, disposable, #500
Screw cap vials, wide mouth, 10 ml, #144
Petri dishes, with covers, 90 mm diameter, #36
Petri dishes, with covers, 140 mm diameter, #24
Funnels, 4 oz, conical #6
Xylene (carbon), #1 pint
Malachite green, #10 grams
Ethyl alcohol (absolute) #1 pint
Methyl alcohol (absolute) #1 pint
Ethyl acetate, #1 pint
Formalin (full strength) #1 pint
Glacial acetic acid, #1 pint
Glycerine, 250 ml
Glycerine Jelly, 1/4 lb
Iodine crystals, #10 grams

Sodium chloride, reagent grade, #1 lb
Potassium iodide, #10 grams
Permount, #1 pint
Schaudinn's fixative, #1/2 pint
Giemsa or Wright's stain, #250 ml
Zinc sulphate, #1 lb
Collodion, #1 pint
Terpineol, Alpha, #1 pint
Mercuric chloride, #10 grams
Charcoal (fine granular, wood or bone - not powdered) #1 lb
Immersion oil, Type A, #6 1-fluid oz. bottles
Vaseline #25 grams
Paraffin #1/4 lb
PVA fixative #500 ml (commercially available through: Meridian Diagnostics, Inc.,
P.O. Box 44216, Cincinnati, OH 45244; Marion Scientific Corporation, 9233 Wark Parkway, Kansas City, Missouri 64114: or Medi-Chem, Box 445, Santa Monica, California 90404
Cellophane (wettable), about 40 microns thick - no. 124 PD, E.I. Dupont de Nemours, Inc., Film Department, Wilmington Delaware, # 12 sheets
Stainless steel bolting cloth, 105 - mesh (W.S. Tyler Co., Cleveland, Ohio. #360 sw. inches
Nucleopore filters, 8-micron pore size, 13 mm diameter (Nucleopore Corp., Pleasanton, California, 94566) #100
Path Urinary Schisto Diagnostic Kit (Sample Kit, 100 filters @ 30.00 per kit per telephone call on 9/9/99 (206-285-3500) can supply on rush order, # 2 kits
Compression device (trichinoscope) Swinney adapters for Nucleopore filter technique, 13 mm diameter, # 6
Eyepiece Micrometer discs, 19 mm diameter, mountable inside standard eyepiece, subdivision 0.01 in, # 6
Stage micrometer, 25 x 75 mm, glass slide with calibration scale ruled to 0.01 mm, #2
Forceps, medium pointed, serrated tips, 4 1/2 inches long, common #6
Scissors, dissecting, stainless steel, blunt/sharp, 4 1/2 inches long, common # 6
Labeling pens, permanent-ink marking, Fischerbrand*, black, # 6
Magic markers, black, #6

Lens paper, 4 x 6 in., 50 sheets per booklet, # pack of 12 booklets
Slide trays, cardboard for 20 slides, #12
Microscope slide lables, rectangular plain white, self-adhesive, 7/8 x 7/8" #pack of 500
Weighing paper, 4 x 4 inch, # 1 pack of 500 sheets
Wire inoculating loop, platinum-iridium, 3 mm i.d., #2
Footlocker, standard size for shipping supplies, # as needed

Equipment expected to be available on location

Microscopes, compound with illuminators
Gram balance
Centrifuge, for 15 ml tubes (horizontal table model)
Refrigerator
Hot plate
Magnetic stirrer (optional)
Incubator, 37 C (optional)
Test tube racks

Lab Manuals

Laboratory Procedures for the Diagnosis of Intestinal Parasites, U.S. Dept. of Health and Human Services, Public Health Service, CDC. Dorothy M. Melvin and Marion M. Brooke. 3rd Ed., 1982 (CDC Laboratory Improvement Program Office, Lab Training and Consultation Division, Atlanta, Ga. 30333. HHS Publication No. (CDC) 82-8282 #6

Atlas of Medical Helminthology and Protozoology by H.C. Jeffrey and R.M. Leach, 2nd ed. Churchill Livingston, Edinburgh, London and New York. 1975. There may be a new edition by now. Library of Congress Catalogue Card Number 74-81190. #6

The Color Atlas of Intestinal Parasites by F. A. Spencer and Lee S. Monroe. Fifth Printing, 1973. Charles C. Thomas (Bannerstone House, 301327 East Lawrence Avenue, Springfield, Ill. $11.50. (I have one copy.) #6

A Colour Atlas of Clinical Parasitology ed. by Tomio Yamaguchi. 1981. Wolfe Medical Publications Ltd. $98.00. Optional. (I have one copy, which I took along.)

Appendix B-5
Medical Parasitology Microscope Slides

1. *Ascaris*, male x-section
2. *Ascaris*, female x-section
3. *Ascaris*, x-section from pig
4. *Ascaris*, larvae from guinea pig
5. *Ascaris*, larvae in liver of mouse
6. *Enterobius vermicularis*, adult
7. *Enterobius vermicularis*, ova, scotch tape
8. *Trichuris trichiura*, adult
9. *Ancylostoma duodenale*, adult
10. *Ancylostoma braziliense*, adult
11. *Ancylostoma caninum*, adult
12. *Necator americanus*, adult
13. *Ancylostoma caninum*, dog intestine
14. *Strongyloides stercoralis*, adult
15. *Strongloides stercoralis*, dog intestine
16. *Trichinella spiralis*, rat diaphragm
17. *Trichinella spiralis*, rat tongue
18. *Wuchereria bancrofti*, microfilaria
19. *Brugia malayi*
20. *Acanthocheilonema perstans*, microfilaria
21. *Onchocerca volvulus*, nodule
22. *Onchocerca volvulus*, smear from nodule
23. *Dirofilaria immitus*,, dog heart
24. *Hymenolepis diminuta*, scolex
25. *Hymenolepis nana*, scolex
26. *Taenia saginata*, mature proglottid
27. *Taenia solium*, gravid proglottid
28. *Taenia solium*, scolex
29. *Taenia solium*, mature proglottid
30. *Taenia solium*, gravid proglottid
31. *Cysticercus cellulosae*
32. *Taenia* ova
33. *Coenuris serialis*, muscle of rabbit
34. *Diphyllobothrium latum* (*Dibothriocehalus latus*) scolex
35. *Diphyllobothrium latum*, proglottid
36. *Diphyllobothrium latum*, ova and coracidia

37. *Echinococcus granulosus*, adult
38. *Echinococcus granulosus*, section
39. *Echinococcus multilocularis*, adult
40. *Echinococcus multilocularis*, liver section
41. Hydatid sand
42. *Clonorchis sinensis*, adult
43. *Clonorchis snensis*, liver of man
44. *Fasciola hepatica*, adult
45. *Fasciolopsis buski*, adult
46. *Heterophyes heterophyes*, adult
47. *Heterophyes heterophyes*, eggs in liver of man
48. *Paragonimus westermani*, adult
49. *Paragonimus westermani*, eggs in tissue
50. *Paragonimus*, ova
51. *Metagonimus yokogawi*, adult
52. *Schistosoma japonicum*, adult
53. *Schistosoma mansoni*, adult
54. *Schistosoma mansoni*, rat liver
55. *Schistosoma mansoni*, mouse intestine
56. *Schistosoma mansoni*, rectum
57. *Schistosoma japonicum*, rabbit tissue
58. *Schistosoma haematobium*, rabbit tissue
59. *Schistosoma* rediae & cercariae from snail liver
60. *Leishmania mexicana*, cutaneous nodule
61. *Leishmania donovani*, bone marrow smear
62. *Leishmania donovani*, smear from culture
63. *Leishmania tropica*
64. *Phytomonas elmassiana*, from *Asclepias*
65. *Trypanosoma lewisi*
66. *Trypanosoma brucei*, from guinea pig
67. *Trypanosoma equiperdum*, from guinea pig
68. *Trypanosoma gambiense*
69. *Trypanosoma rhodesiense*
70. *Trypanosoma cruzi*
71. *Trypanosoma cruzi*, rat heart
72. *Trypanosoma cruzi*, dog heart muscle
73. *Toxoplasma gondii*, mouse, smear
74. *Toxoplasma gondii*, mouse brain section
75. *Sarcocystis miescheriana*
76. *Trichomonas hominis*

77. *Trichomonas vaginalis*
78. *Giardia lamblia*, cysts
79. *Giardia lamblia*, vegetative stage
80. *Giardia*, intestinal section
81. *Chilomastix mesnili*, trophozoites
82. *Chilomastix mesnili*, trophs & cysts
83. *Balantidium coli*, trophs & cysts, monkey
84. *Balantidium coli*, monkey tissue section
85. *Entamoeba coli*, trophozoites
86. *Entamoeba coli*, cysts
87. *Entamoeba hystolytica*, trophozoites
88. *Entamoeba hystolytica*, cysts
89. *Entamoeba hystolytica*, section human intestine
90. *Entamoeba hystolytica*, liver abscess
91. *Endolimax nana*, trophozoites
92. *Endolimas nana*, cysts
93. *Iodamoeba butschlii*
94. *Plasmodium vivax*, Schuffner's dots
95. *Plasmodium vivax*, thick and thin smear
96. *Plasmodium falciparum*, rings and crescents
97. *Plasmodium falciparum*, thick and thin smear
98. *Plasmodium malariae*
99. *Plasmodium ovale*
100. *Plasmodium cynomolgi*, exflagellation

Appendix B-6. Geohelminths

1. *Trichuris trichiura* life cycle.
2. *T. trichiura* egg.
3. *T. trichiura* unembryonated egg.
4. *Ascaris lumbricoides* life cycle.
5. *A. lumbricoides* fertile eggs.
6. *A. lumbricoides* non-fertile egg.
7. *A. lumbricoides* fertile eggs.
8. *A. lubricoides* larvae from sputum.
9. Life cycles of *N. americanus* and *A. duodenale.*
10. Hookworm eggs recovered from stools.
11. Hookworm eggs recovered from stools.
12. Hatching larvae of hookworms.
13. Rhabditiform larva of hookworm.
14. Hookworm larva.
15. Mouthparts of *N. americanus.*
16. Mouthparts of *A. duodenale.*
17. *S. stercoralis* life cycle
18. *S. stercoralis* rhabditiform larva in stool.
19. *S. stercoralis* filariform larva.
20. Eggs of *A. braziliense.*
21. Typical wandering track of animal hookworm larvae.
22. Eggs of *Trichostrongylus* and hookworm.
23. *Toscara canis* egg.

Appendix B-7. Other Helminths

1. Comparative shapes and sizes of some helminth eggs.
2. *Clonorchis sinensis.*
3. *Clonorchis sinensis* life cycle.
4. *Clonorchis* egg.
5. *Fasciola hepatica.*
6. *Paragonimus westemani.*
7. Egg of *Paragonimus.*
8. *Fasciolopsis buski.*
9. Egg of *Fasciolopsis.*
10. Life cycles of *Taenia solium* and *T. saginata.*
11. Scolex of *T. solium.*
12. Gravid segment of *T. solium.*
13. Eggs of *T. solium.*
14. Scolex of *T. saginata.*
15. Gravid segment of *T. saginata* containing numerous eggs.
16. Scoles and immature segments of *Diphyllobothrium latum.*
17. Mature segment of *D. latum.*
18. Eggs of *D. latum* in fecal smear.
19. Scolex of *Hymenolepis nana.*
20. Egg of *H. nana.*
21. Section of cysticercus of *T. solium* in human muscle.
22. Muscle biopsy containing one encysted larva of *Trichinella.*
23. Egg of *C. philippinensis.*
24. *Enterobius vermicularis* female.
25. Scotch tape technique for diagnosis of pinworm infection.
26. Egg of *Enterobius vermicularis.*
27. *Strongyloides* ovum (rarely seen).
28. *S. stercoralis* larva in human sputum.
29. *E. vermicularis* egg hatching.
30. *Echinococcus granulosus* upper; *E. multilocularis* lower.
31. *E. granulosus* section of hydatid cyst.
32. *E. multilocularis* in vole liver.
33. *H. diminuta* eggs in vitro.
34. Scolex of *Hymenolepis nana.*
35. Scolex of *Taenia solium.*
36. Scolex of *Taenia saginata.*
37. Gravid proglotid of *Taenia saginata.*

38. Scolex of *Diypyllobothrium latum.*
39. Gravid proglottids of *D. latum.*
40. Section through gravid ragion of *D. latum.*
41. Egg of *D. latum.*
42. Scolex of *Dipylidium caninum.*
43. *Schistosoma mansoni* adult male.
44. Miracidium in egg of *S. mansoni.*
45. Egg of *S. haematobium.*
46. *Necator americanus.*
47. *Ancylostoma baziliense.*
48. Hookworm ovum from human.
49. Egg of *Enterobius vermicularis.*
50. *Fasiola hepatica.*
51. *F. hepatica.*
52. *Fasciolopsis buski.*

Appendix B-8. Additional Parasitic Protozoa

1. *Giardia lamblia* cyst.
2. *Giardia lamblia* trophozoites with bacteria.
3. *Giardia lamblia* trophozoites.
4. *Trichomonas hominis.*
5. *Trichomonas hominis.*
6. *Balantidium coli* trophozoite in human intestine.
7. *Balantidium coli* trophozoite in human intestine.
8. *Balantidium coli* cyst.
9. *Endolimax nana.*
10. *Endolimax nana* cyst.
11. *Iodamoeba butschli* cyst.
12. *Entamoeba gingivalis* trophozoite.
13. Various protozoa, diagramatic.
14. *Entamoeba histolytica* trophozoite in human colon.
15. *Entamoeba histolytica* trophozoite in intestine.
16. *Entamoeba histolytica* precystic stage.
17. *Entamoeba histolytica* cyst, note chromatoid body.
18. *Entamoeba coli* trophozoite.
19. *Entamoeba coli* trophozoite.
20. *Entamoeba coli* cyst.
21. *Toxoplasma gondii* crescents.
22. *Toxoplasma gondii* crescents.
23. *Entamoeba histolytica* life cycle.
24. Malaria life cycle.
25. *Plasmodium falciparum* Maurer's dots.
26. *Plasmodium falciparum* fatal case.