

**California Association  
for  
Medical Laboratory Technology**

**Distance Learning Program**

**CHALLENGES IN DIAGNOSING PARASITIC  
INFECTIONS IN CALIFORNIA**

**Course # DL-923**

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# CHALLENGES IN DIAGNOSING PARASITIC INFECTIONS IN CALIFORNIA

**INTRODUCTION:** At a time when there is increased possibility of parasitic infections in California due to foreign travel and immigration, there is decreased emphasis on diagnosis and identification. Parasitic infections may be under-diagnosed for a number of reasons:

- Minimal attention is paid to parasitic infections in medical school and physician training. Therefore physicians may not consider parasitic disease in the differential diagnosis.
- Lack of knowledge extends to the collection of specimens, both in preparation of the patient and when, how, and the number of specimens to collect.
- Lack of experience and practice among Clinical Laboratory Scientists (CLS) contributes to variability among laboratories.

The CLS can play a part in increasing the awareness of physicians as well as upgrading their own skills. This course will cover the reasons for increase in parasitic diseases in California, as well as important information the CLS should know to adequately evaluate fecal, blood, urine and tissue samples for protozoan and helminthic infectious agents. The quality of the sample submitted as well as the appropriate number of samples that should be examined to render a negative result reliable will be covered.

## OBJECTIVES:

At the end of this course the participant will be able to:

1. Define what is required for the correct and complete evaluation of a fecal sample for protozoan and helminthic parasites
2. Name the new techniques that are available to distinguish *Entamoeba histolytica* from *E. dispar* and its importance
3. Identify what is required to speciate *Plasmodium* organisms in a blood smear
4. List what samples to request from the patient when routine samples are not adequate
5. Discuss what information physicians should be told regarding samples being submitted
6. State what the limitations of fecal examinations are for the parasites discussed in the course and what alternatives may be used

## DISCUSSION

The number and variety of parasitic diseases seen in California has increased in recent years. A large number of immigrants from areas such as Southeast Asia, the Middle East, and Mexico where parasitic diseases are common have come to California. There is an increase in tourism including travel to remote areas. Twenty-five million people in the United States travel to other countries. Over 200,000 go to Africa each year. Travel medicine has become a specialty. In addition to the imported diseases there are endemic diseases such as giardiasis, amebiasis, taeniasis and others.

Frequently these parasitic diseases are misdiagnosed.

In light of the possibility of parasitic disease, every ulcerative disease of the large intestine should be evaluated for amebiasis. Every traveler who returns from a malarial area and presents with fever should be considered a candidate for malaria. Many physicians don't know what geographical areas need to be considered relevant to parasitic infections. For instance, the Galapagos have no Anopheline mosquitoes and therefore do not have human malaria. They may not realize that malaria may take a long time to appear. Also, the paucity of cases gives CLSs little opportunity to renew their skills.

This course will cover the important information the CLS should know to adequately evaluate fecal, blood, urine and tissue samples for protozoan and helminthic infectious agents.

The quality of the sample submitted as well as the appropriate number of samples that should be examined to render a negative result will be discussed.

While the morphological identification of certain protozoans and helminths is still the definitive method for diagnosis, an increasing number of immunologically based tests including monoclonal antibody antigen capture techniques, immunoblot strips and direct fluorescent monoclonal antibody tests are commercially available. Their use can improve the cost effectiveness of diagnosis as well as treatment.

Tissue impression smears, smears of lymph node aspirations and compression preparations of biopsy samples are highly valuable in the diagnosis of certain parasitic organisms. Though rarely provided directly to microbiology laboratories, the increased use of these samples can improve the diagnostic accuracy of the laboratory service as well as the cost effectiveness as will be illustrated.

## CHALLENGES IN DIAGNOSING PARASITIC INFECTIONS IN CALIFORNIA

### A. Quality of sample received at the laboratory

Information that should be available on specimens or explained to the patient and to the physician:

1. Date and time of collection, number and type of specimens

The laboratory should have a set of instructions to physicians outlining the number, time, type of specimens and procedure for collecting

Examples:

- a. Unpreserved sample for: copro-antigen tests in amebiasis and for evaluation of presence of live schistosomes. (Unpreserved samples need special handling. The sample bottles should be wide-mouthed, allowing for better specimen collection by the patient and for better sampling of the specimen by the laboratory)
- b. Peri-anal swabs with scotch tape slides for suspected *Enterobius vermicularis*.
2. Drugs or procedures performed on patient prior to sample collection  
Lomotil, antibiotics, antacids, barium studies all interfere with quality of fecal samples
3. Preserved samples
  - a. 10% formalin - too concentrated for some copro-antigen tests for taeniasis and amoebiasis. Will kill miracidium of schistosomes.
  - b. Polyvinyl alcohol (PVA) - (now usually mercuric chloride free). Sample should not be refrigerated after mixing. Must mix well in order to ensure fixation is effective. PVA does not penetrate formed stool well.
  - c. Blood smears, thick and thin, time of collection in relation to fever, if any. Speciation of *Plasmodium* requires developmental forms beyond young trophozoites to be present if possible, e.g., *P. falciparum* - usually only early trophozoites or gametocytes present in peripheral blood. *P. vivax*, *P. malariae*, *P. ovale* have all stages in peripheral blood.
4. Antigen identification kits
  - a. valuable adjuncts to diagnosis but kits are expensive and some have short shelf life
  - b. may require special sample collection

### B. Specific Parasitic agents and the diagnostic challenges they can present

1. Fecal borne parasites

a. *Giardia lamblia*

Cyst excretion by symptomatic individuals is **NOT** uniform. High cyst excretors, low cyst excretors and cyclical high-low patterns are seen. It takes 8-15 stool samples over a 4-5 week period to accept a negative diagnosis. The "3 O & P" (three ova and parasites) over 7 days is not adequate to rule out giardiasis if all are negative.

Monoclonal antibody copro-antigen test is very specific and sensitive. It provides a reliable alternative to trichrome stained smears. The ELISA based test relies on detection of a specific antigen, present in the stool of infected individuals, by a

monoclonal antibody. This antigen is both stable to 10% formalin and host GI digestion. In a study of 232 stool samples the ELISA test had a sensitivity of 96% vs. 74% for microscopic examination<sup>1,2</sup>. The test is commercially available through Alexon Biomedical, Inc., Rolling Meadow, IL and is called Prospect/Giardia™ test.\*

b. Taeniasis due to *Taenia saginata* or *Taenia solium*

In California we are beginning to find *T. solium* especially in immigrants from Central and South America and S.E. Asia.

*Taenia* tapeworms have all stages, immature, mature, and gravid proglottides. The laboratory needs to recover gravid proglottides in order to speciate. Speciation is done by counting the number of lateral branches on one side of the main uterine stem of the gravid proglottid. *T. saginata* has over 13; *T. solium* has fewer than 12.

The cyclophyllidean tapeworms lack a uterine pore and the ova are not consistently expelled into the fecal stream by the gravid proglottides. Even when the gravid segments detach from the main strobila, ova may not spill into the lumen of the bowel from the broken edges. Because of this some stool samples from humans carrying *Taenia* species will not contain ova. There is a commercially available ELISA test based upon a copro-antigen capture method using a polyclonal antibody. This technique cannot speciate the infection, so *T. saginata* and *T. solium* infections cannot be distinguished but its sensitivity is superior to stool exam, particularly with *T. solium* taeniasis.

c. Cysticercosis due to *T. solium* (pork tapeworm)

Cysticercosis is a reportable disease, while taeniasis is not. Cysticercosis is infection by the larval form of *T. solium*. It is acquired by ingesting ova from proglottides passed by humans carrying the adult tapeworm. Humans are the only definitive host (it is not a zoonosis). Cysticercosis can be a devastating disease. Immigrants as well as native residents are now involved. In Mexico City 25% of patients who go to surgery for brain tumors have cysticercosis. When the organism lodges in the spinal cord or brain the cysticercus can survive 5 to 7 years. Disease is produced only when the cysticercus dies. The patient usually presents with grand mal or petit mal seizures. Diagnosis involves the use of two procedures: CT or MRI scan and serological evidence of specific antibody to cysticerci of *T. solium*.

d. *Echinococcus granulosus* (Hydatid disease)

Hydatid disease is enzootic in California especially in the San Joaquin Valley. Basque shepherders keep dogs as sheep dogs and as family pets. If dogs eat dead sheep they may become infected with the adult tapeworm. Humans acquire the larval stage by ingesting ova from proglottides passed in dog feces. The cysts grow slowly so humans may acquire the infection in childhood but the cysts may not be detected until the second or third decade of life. Most cysts lodge in the liver. The next most commonly involved organ is the lung. A cyst in the liver or lung should never be punctured percutaneously until hydatid disease has been ruled out. Release of the fluid may cause an anaphylactic reaction in the patient and spilled scolices can result in secondary hydatidosis.

An Immune Transfer Blot (IBT) serological test for hydatid infection is available. This test is 100% specific and with liver cysts about 80% sensitive. It is less sensitive with lung cysts only.

e. *Entamoeba histolytica* vs. *Entamoeba dispar*

Amebiasis is endemic in the United States with a 1-5% infection, particularly in Southeastern US.

In the 1970s metropolitan areas such as Chicago, New York, Los Angeles and San Francisco reported a large number of cases of amebiasis with no invasive disease. The incidence peaked in 1981 or 1982. This was transmitted by anal-genital sexual

intercourse. The organism was morphologically indistinguishable from *E. histolytica*. In 1919 Brumpt had proposed the existence of two organisms, one causing invasive disease the other non-invasive, but at the time there was no way to prove they were different. Although these two amoebas are morphologically indistinguishable they behave entirely differently in the gut of humans.

*E. dispar* is non-invasive and is about 10 times more common, based upon isoenzyme analysis of 3,000 examined clones, than is *E. histolytica* which is invasive and produces both ulcerations in the colon, rectum, cecum, and appendix and abscesses in the liver in up to 20% of the people with large bowel invasion. *E. histolytica* invades the gut in specific sites where stasis occurs, the hepatic flexure, sigmoid colon, and appendix. If an ulcer is seen on a sigmoidoscopy the physician can get a specimen. A direct smear may show trophozoites.

Since the non-invasive *E. dispar* and the invasive *E. histolytica* cannot be distinguished morphologically if only microscopic evaluation of trichome stained smears is available, the lab must report positive slides as *E. histolytica/E. dispar* and the clinician has little choice but to treat all such cases. This means 10 times the number of cases will be treated than is actually necessary. There is genetic evidence that justifies the recognition of *E. dispar* as a distinct amoeba from *E. histolytica*, as follows:

- Restriction fragment length polymorphism between the two
- Sequences of single copy genes are different between the two
- Small subunit ribosomal RNA sequences are all different between the two

The distinction between *E. histolytica* and *E. dispar* may be made by the diagnostic lab using an ELISA copro-antigen capture test via a monoclonal antibody that is specific for surface lectin unique to *E. histolytica*. The fecal sample however must be submitted unpreserved and tested within 24 hours of collection.

f. Schistosomiasis due to *Schistosoma haematobium*, *S. mansoni*, *S. japonicum*, *S. mekongi*

Immigrants from endemic areas as well as travelers to such areas may harbor organisms for many years and may have ova in tissues for even longer. Fecal samples, up to 5, should be processed for *S. japonicum*, *S. mansoni* or *S. mekongi*. Urine sample (24 hour collection without formalin since this kills the miracidia) is processed for *S. haematobium*.

Use sedimentation since ova are too dense to float.

Pathology is due to ova in tissues but therapeutic drugs are available to treat only adult worms that reside in blood vessels. It takes about 15 days for ova to work their way through tissues if the miracidia are alive. To determine viability the active “flame cell” in the miracidium must be seen.

If only ova with dead miracidia are recovered, live worms are unlikely to be present. Get an unfixed sample to detect live miracidia. Dilute 1-10 with water, put in graduated cylinder covered with black paper. Miracidia swim to the top. Aspirate the top and examine microscopically.

To demonstrate ova, a compression slide may be made from a biopsy of a pseudotubercle from the gut or urinary bladder. The morphology of ova from slides made in this manner is retained rather than being fragmented, which occurs when histological sections are made.

g. *Enterobius vermicularis* (pinworm)

The specimen is peri-anal swab, **NOT** a fecal sample

Dogs and cats are **NOT** a source of pinworms to humans.

7 consecutive first morning swabs are required to test for pinworms because female worms do not migrate out of the anus every night to lay ova.

## 2. Sexually transmitted Protozoa

- *Trichomonas vaginalis*

Very common venereal transmitted agent. Males are more often asymptomatic than females and frequently don't get diagnosed or receive therapy.

Organism can be grown in broth culture media or on McCoy cells but results may require several days.

Preputial (under the foreskin) washings from males and urine or cervical smears from women can contain organisms. Examination in wet mounts has low sensitivity. Organisms round up, stop moving and may be easily missed.

The use of a monoclonal antibody with a fluorescent tag can improve recognition of *Trichomonas* from urethral samples or vaginal swabs.

Microscopic examination of wet mounts from the patient have about a 60% sensitivity. If the sample has first been cultured in appropriate media (37° for 72 hours) and then wet mounts examined, the sensitivity improves to about 85-95%. However, this requires a 3 day period to elapse for culture to be done, and there are limitations to the culture and microscopic method of diagnosis. This has led to other methods of demonstrating the organism via histological stains of one type or another. The Papanicolaou stain, used in routine PAP smears, can stain *Trichomonas*, however a large percentage of false positives as well as false negatives have been reported where the PAP smear was used as the sole criterion for diagnosis of *T. vaginalis*.

A monoclonal antibody antigen capture test that utilizes several monoclonal antibodies to detect various *T. vaginalis* structures is commercially available. It has been reported to be as sensitive as culture methods and requires 60 minutes to run.

## 3. Vector borne Protozoa

- *Plasmodium* species in humans.

There are around 300 cases of malaria diagnosed in California each year. Usually these cases have been acquired outside the United States, and patients have not taken prophylaxis while in the endemic area of malaria or have not been given a 14 day course of primaquine after leaving the endemic area. California, however, does have 2 species of *Anopheles* capable of transmitting *P. vivax*. Ambient temperatures in California are sufficiently high to allow development in the mosquito from August to October.

Inadequate management of malarial infections can be attributed to 3 sources of effort:

- 1. Failure to include malaria in the list of differential diagnostic considerations in patients with fever, myalgia, headache and a history of travel to an endemic area.**

Thick and thin blood smears should always be made from patients with a history of travel to an endemic malaria area when they present with a febrile disease.

- 2. Failure to recognize the organisms in blood smears submitted for diagnosis.**

Parasitemias with some Plasmodia may be low (*P. vivax*, *P. ovale* and *P. malariae*). Careful and thorough examination of blood smears is essential as is reinforcement of the morphological features of the Plasmodia that infect humans.

Centrifugation of the venous blood sample followed by examination of stained smears from the red cells just beneath the buffy coat can increase

the concentration of infected cells since such cells are lighter and will rise to the top of the red cell column. Likewise, when the smear is made the infected cells are carried to the outer margins of the smear. Examining this portion of the slide on the edges of the smear can increase the chances of finding infected erythrocytes.

### **3. Failure to speciate the Plasmodium correctly.**

Because the strains of *P. falciparum* are resistant to chloroquine, fansidar and mefloquine in various parts of the world, and chloroquine resistant *P. vivax* has been reported in New Guinea and parts of South America, the treating physician needs to be apprised of the actual species of *Plasmodium* found in specimens from the patient. At least, the laboratory should be able to tell the physician whether the organism is *P. falciparum* or not at the time the laboratory diagnosis of “Malaria” is sent out.

If the initial blood smear does not contain stages in the erythrocytes with morphological features sufficient to speciate the *Plasmodium*, request that thin smears be made from a finger stick blood every 4 hours over the next 8-12 hours. Even if malaria therapy has been started, development of the early trophozoites will proceed.

Characteristics for speciation include morphology of the organism, variety of developmental forms seen in the peripheral blood smear, as well as changes in the erythrocyte envelope. For instance, *P. falciparum* usually lacks intermediate forms in peripheral blood. In order to improve the detection of such changes as stippling, smears should be hand stained in Romanowsky type stains (Wrights, Leishman, Giemsa), stains composed of methylene blue and eosin. The pH of the distilled water should be between 6.7 and 7.21 depending upon the stain being used. Automated slide staining machines should not be used since the times of staining and the pH of the stain cannot be controlled. Even the surface of the slide on which the thick and thin smears are made is critical. The slides should be dust and oil free and pre-cleaned by the laboratory in a solution of 100 gm potassium bichromate and 250 ml sulphuric acid made up to one liter with distilled water. The slides to be used for malaria smears should be soaked in this solution for 12 hours, then rinsed in distilled water, dried with a lint-free cloth and stored in a closed slide box until use. Thick blood films will float off a slide while staining if even a trace of oil is on the slide. “Pre-washed” slides out of the manufacturer’s box often contain a residue because the glass cutters are cooled by a thin film of oil, some of which gets on the slides. Few, if any drawing centers or laboratories go to the effort to prepare their slides correctly. Correct staining technique, more careful examination of correctly stained smears and periodic review of positive malaria smears to reinforce visual recognition of infected cells will improve laboratory diagnosis and correct speciation of *Plasmodium* infections.

Serological, antigen capture, and PCR procedures are replacing the microscopic examination of specimens from patients in the diagnosis of a wide variety of parasitic infections. A list of these techniques and the names of manufacturers may be found in Table 5, pg 1915 of the 8<sup>th</sup> edition of the Manual of Clinical Microbiology.

## CONCLUSION

Problems in diagnosing parasitic diseases in California include lack of physician training, lack of knowledge in specimen collection, and lack of experience and practice among laboratory personnel.

In this course we have addressed these problems by discussing how the laboratory and the Clinical Laboratory Scientist can implement procedures that will improve the identification of parasitic diseases which may occur in California.

## PARASITOLOGY REFERENCES

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## Review Questions Course DL-923

Choose the **one** best answer

1. PVA for processing fecal samples
  - a. is not used at present because it contains mercuric chloride
  - b. is layered over the fecal specimen
  - c. penetrates the stool sample easily
  - d. should be kept at room temperature after mixing with the sample.
2. 10% formalin preservative may be used for which of the following?
  - a. miracidium of schistosomes
  - b. copro-antigen test for *Giardia lamblia*
  - c. copro-antigen test for *Entamoeba histolytica*
  - d. copro-antigen test for *Taenia*
3. Malaria should not be suspected in travelers returning from the Galapagos Islands because
  - a. they are too remote to harbor Plasmodia
  - b. the climate is too cold
  - c. there are no Anopheline mosquitoes there
  - d. The large turtles eat all the mosquito larvae
4. All but which of the following are fecal borne parasites?
  - a. *Taenia solium*
  - b. *Giardia lamblia*
  - c. *Entamoeba histolytica*
  - d. *Trichomonas vaginalis*
5. Antigen identification kits were mentioned for all but which of the following
  - a. *Schistosoma*
  - b. *Giardia lamblia*
  - c. *Entamoeba histolytica*
  - d. *Taenia*
6. In order to rule out *Giardia lamblia* it is necessary to obtain
  - a. three O & P over 7 days
  - b. stool specimens passed first in the morning
  - c. 8-15 stool samples over 4-5 weeks
  - d. areas of stool specimens showing blood
7. *Taenia solium* can be distinguished from *Taenia saginata* by
  - a. examination of gravid proglottides
  - b. examination of ova
  - c. use of ELISA based copro-antigen kit
  - d. examination of immature proglottides
8. Cysticercosis disease
  - a. is caused by the larva moving around in the brain
  - b. is acquired by ingesting undercooked pork
  - c. may be due to either *T. solium* or *T. saginata*

- d. is caused by death of the larval form in tissues
9. Hydatid disease is best diagnosed by
- a. percutaneous puncture of a cyst and examination for scolices
  - b. examination of the stool for ova
  - c. use of western blot for detection of antibody to hydatid cyst
  - d. examination of pet dog feces for proglottides of *E. granulosus*
10. *E. histolytica* may be distinguished from *E. dispar* by
- a. morphological examination of trophozoites
  - b. ELISA copro-antigen tests
  - c. morphological examination of cysts
  - d. identification of an antibody in the patient to *E. histolytica*
11. Urine samples may be used for diagnosis of
- a. *Schistosoma haematobium*
  - b. *S. mansoni*
  - c. *S. japonicum*
  - d. *S. mekongi*
12. Indication that live schistosome worms are present in blood vessels include
- a. recovery of schistosome ova from the patient's feces
  - b. looking for miracidia by aspirating from the bottom of the cylinder containing the diluted specimen
  - c. observing the active flame cell in miracidia
  - d. observing any miracidia, live or dead, in ova
13. Fecal samples are used to detect
- a. *E. vermicularis*
  - b. *S. haematobium*
  - c. *G. lamblia*
  - d. *E. granulosus*
14. Concerning *P. vivax* in California, which statement is not true?
- a. cannot be transmitted because there are no Anopheline mosquitoes here
  - b. should be tested for in travelers from malarial areas who present with fever
  - c. may be missed on blood smear because of low parasitemia
  - d. most of the 300 diagnosed annual cases are from travelers infected in other countries.
15. To increase the chances of finding and speciating *Plasmodium* infected erythrocytes
- a. centrifuge the blood and aspirate the RBC below the buffy coat to make the smear
  - b. examine the central portion of the blood smear
  - c. use automatic blood staining machines because they have well controlled pH
  - d. use the thick smear because the RBC changes are most evident
16. The *Plasmodium* species which usually does not have all developmental forms in peripheral blood is
- a. *P. vivax*
  - b. *P. malariae*
  - c. *P. ovale*

- d. *P. falciparum*
17. The most sensitive method of diagnosing *Trichomonas vaginalis* in a timely manner is by
- examination of wet mounts from cervical smears
  - use of a monoclonal antibody to *T. vaginalis* antigens
  - use of Papanicolaou stain on specimens
  - culture of the organisms in broth culture or McCoy cells
18. Information that should be available on specimens includes all but which of the following?
- what drugs the patient has taken
  - when specimen was obtained
  - whether specimen is preserved
  - how many bowel movements patient has per day
19. Which of the following is not endemic in California?
- Giardia lamblia*
  - Schistosoma mansoni*
  - Taenia saginata*
  - Entamoeba histolytica*
20. Which of the following was not mentioned as good sampling technique?
- use of laboratory cleaned slides for *Plasmodium* examination
  - use of Lomotil by the patient to decrease the amount of diarrhea
  - use of wide mouth jars for fecal specimen sampling
  - use of preputial samples for *Trichomonas* examination

Please circle the one best answer for each question.

COURSE NAME: CHALLENGES IN DIAGNOSING PARASITIC INFECTIONS IN CALIFORNIA COURSE # DL-923

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