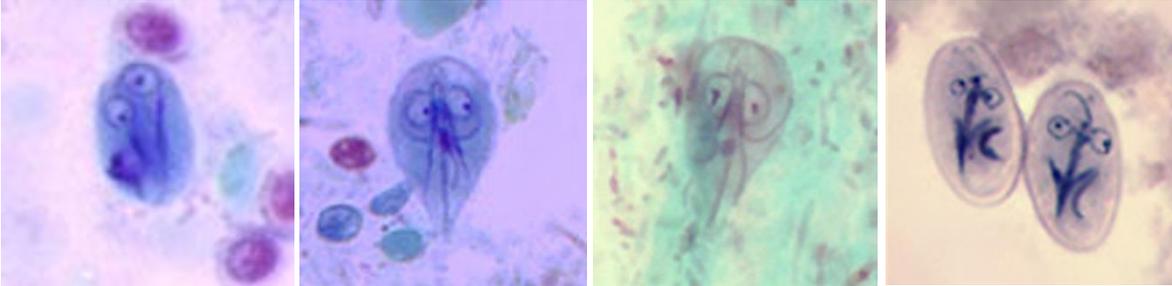


Parasitology Exam (Permanent Stain images)

Answers will be found at the end.

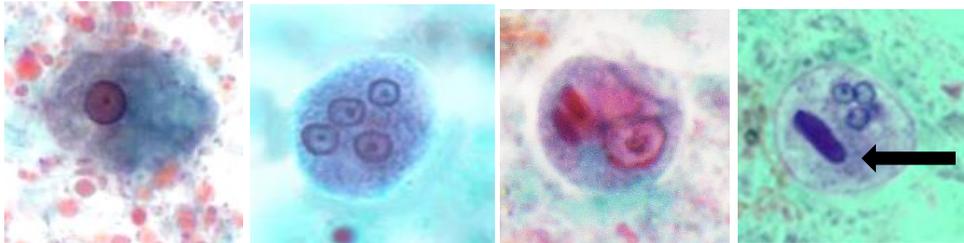
***Reminder: Slides and examination questions are copyrighted and cannot be copied for publication.

1. The following fecal permanent stain images are (oil immersion, 1000x):



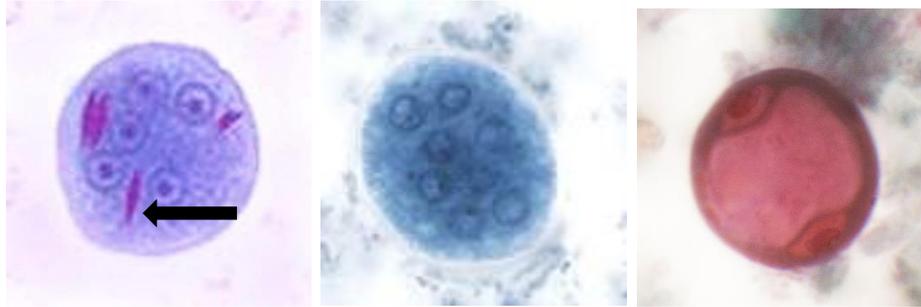
- A. *Chilomastix mesnili* trophozoites, cysts
- B. *Pentatrichomonas hominis* trophozoites
- C. *Giardia lamblia* (*duodenalis*, *intestinalis*) trophozoites, cysts
- D. *Trichomonas vaginalis* trophozoites

2. The following fecal permanent stain images are (oil immersion, 1000x):



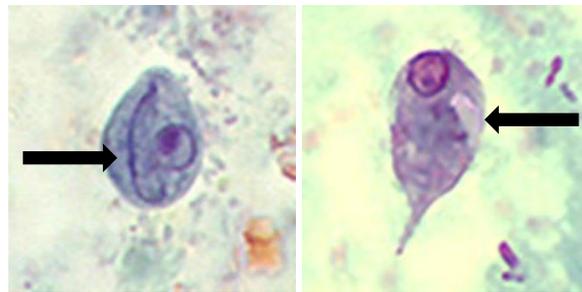
- A. *Entamoeba coli* cysts and trophozoite
- B. *Entamoeba hartmanni* cysts and trophozoite
- C. *Iodamoeba bütschlii* cysts and trophozoite
- D. *Entamoeba histolytica*/*E. dispar* cysts and trophozoite

3. The following fecal permanent stain images are (oil immersion, 1000x):



- A. *Entamoeba coli* cysts and precyst
- B. *Entamoeba hartmanni* cysts and precyst
- C. *Iodamoeba bütschlii* cysts and precyst
- D. *Entamoeba histolytica/E. dispar* cysts and precyst

4. The following fecal permanent stain images are (oil immersion, 1000x):



- A. *Pentatrichomonas hominis* cyst and trophozoite
- B. *Dientamoeba fragilis* cyst and trophozoite
- C. *Chilomastix mesnili* cyst and trophozoite
- D. *Retortamonas intestinalis* cyst and trophozoite

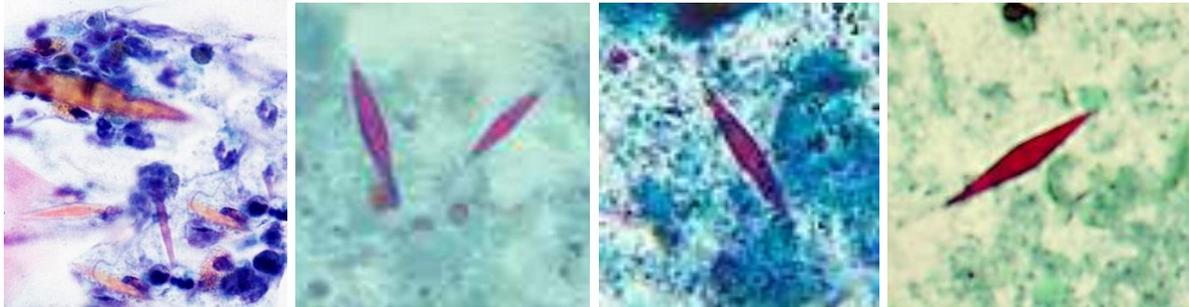
5. The following fecal permanent stain images are (oil immersion, 1000x):



- A. *Iodamoeba bütschlii* cysts
- B. *Entamoeba hartmanni* cysts

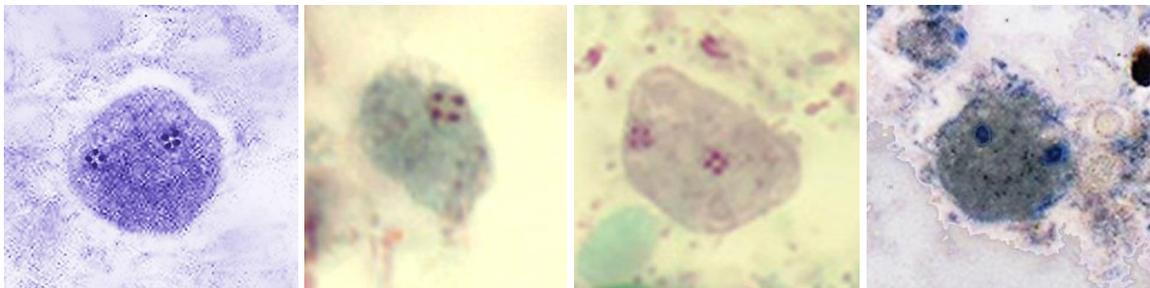
- C. *Dientamoeba fragilis* cysts
- D. *Entamoeba polecki* cysts

6. The following fecal permanent stain images are (oil immersion, 1000x):



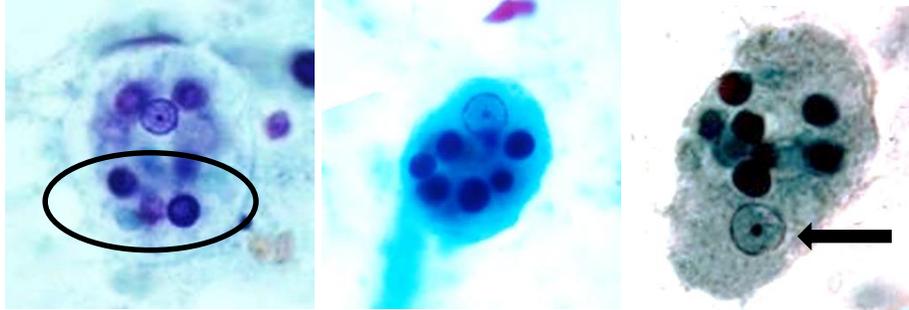
- A. Algae
- B. Plant spines
- C. Charcot-Leyden crystals
- D. String artifacts

7. The following fecal permanent stain images are (oil immersion, 1000x):



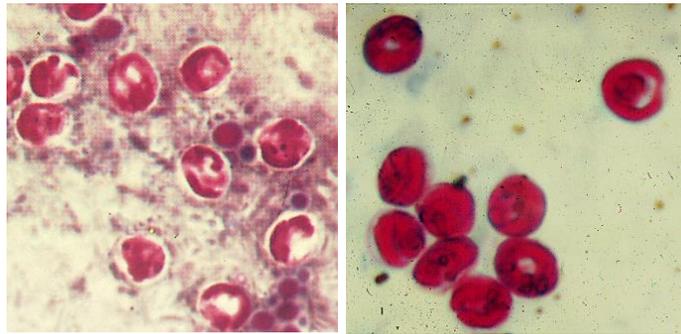
- A. *Dientamoeba fragilis*
- B. *Endolimax nana*
- C. *Pentatrichomonas hominis*
- D. *Retortamonas intestinalis*

8. The following fecal permanent stain images are (oil immersion, 1000x):



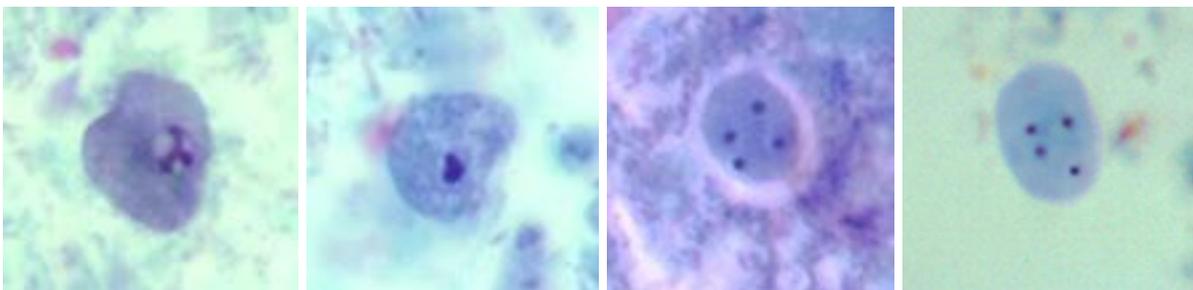
- A. *Entamoeba polecki*
- B. *Entamoeba histolytica*
- C. *Entamoeba coli*
- D. *Entamoeba hartmanni*

9. The following fecal permanent stain images are (4-6 microns) (oil immersion, 1000x):



- A. *Cyclospora cayetanensis* oocysts
- B. *Isospora belli* oocysts
- C. *Cryptosporidium* spp. oocysts
- D. *Enterocytozoon bieneusi* spores

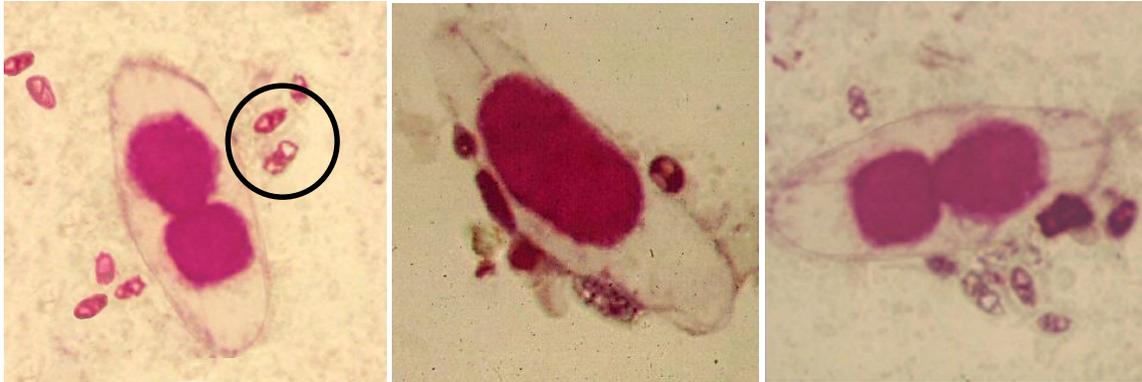
10. The following fecal permanent stain images are (oil immersion, 1000x):



- A. *Endolimax nana* trophozoites, cysts
- B. *Iodamoeba bütschlii* trophozoites, cysts
- C. *Entamoeba hartmanni* trophozoites, cysts

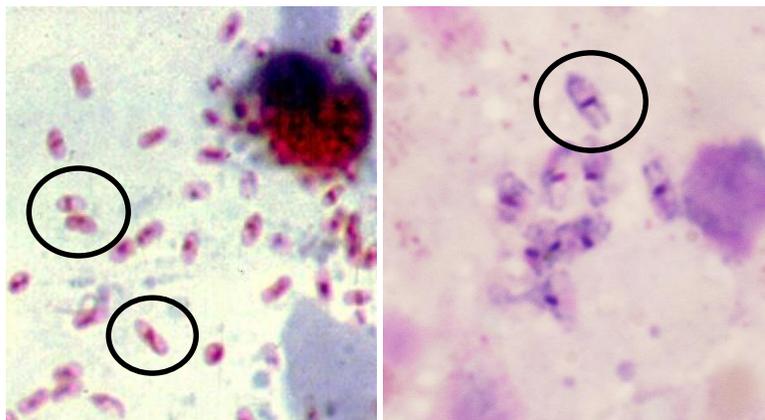
D. *Entamoeba histolytica*/*E. dispar* trophozoites, cysts

11. The following fecal permanent stain images are (oil immersion, 1000x):



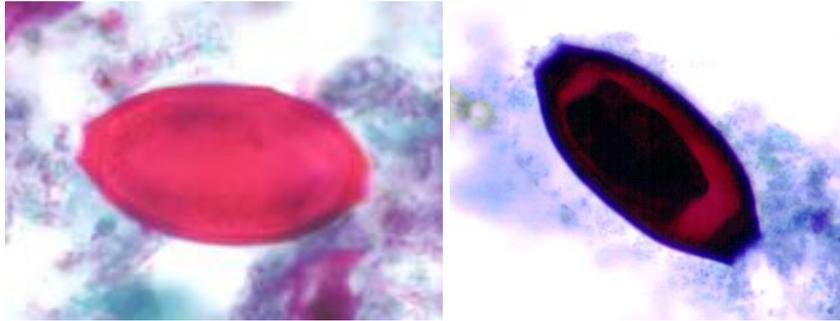
- A. *Isospora belli* oocysts and microsporidian spores
- B. *Sarcocystis* oocysts and microsporidian spores
- C. *Cryptosporidium* oocysts and microsporidian spores
- D. None of the above

12. The following fecal permanent stain images are (oil immersion, 1000x):



- A. *Leishmania* amastigotes
- B. Yeast cells
- C. Plant spores
- D. Microsporidian spores

13. The following fecal permanent stain images are (high dry, 400x):



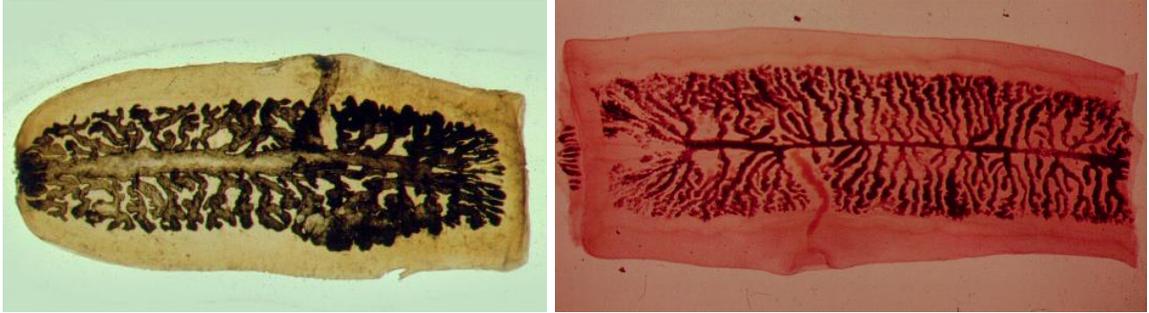
- A. *Paragonimus* spp. eggs
- B. *Trichuris trichiura* eggs
- C. Pollen grains
- D. *Baylisascaris procyonis* eggs

14. The following fecal permanent stain image is (high dry, 400x): In order to identify this structure, your next step should be:



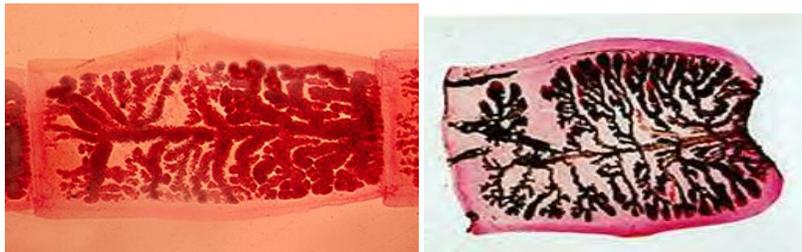
- A. Examine a wet mount
- B. Assume it is an artifact
- C. Report no parasites seen
- D. Try another stain option

15. The following fecal permanent stain images are (very low power, 2x):



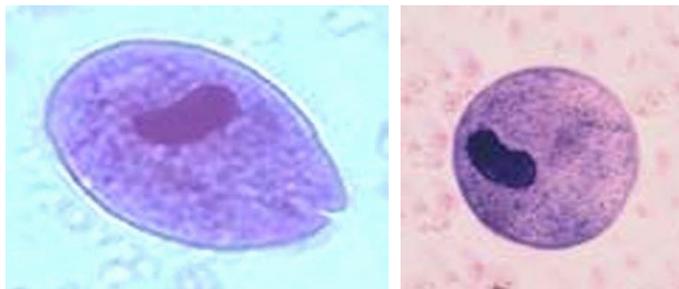
- A. *Taenia solium* gravid proglottid
- B. *Taenia* spp. gravid proglottid
- C. *Taenia saginata* gravid proglottid
- D. *Dipylidium caninum* gravid proglottid

16. The following fecal permanent stain images are (very low power, 2x):



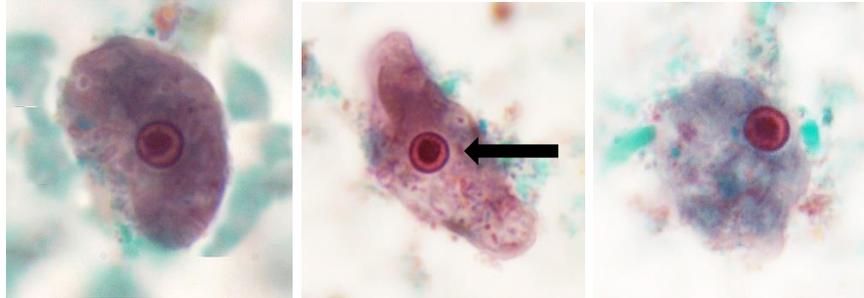
- A. *Taenia solium* gravid proglottid
- B. *Taenia* spp. gravid proglottid
- C. *Taenia saginata* gravid proglottid
- D. *Dipylidium caninum* gravid proglottid

17. The following fecal permanent stain images are (oil immersion, 1000x):



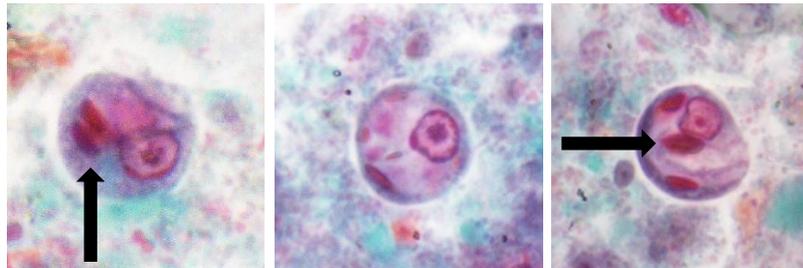
- A. *Schistosoma japonicum*
- B. *Balantidium coli*
- C. *Trichomonas vaginalis*
- D. *Diphyllobothrium latum*

18. The following fecal permanent stain images are (oil immersion, 1000x):



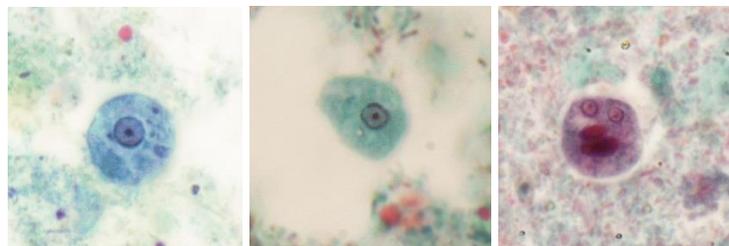
- A. *Entamoeba histolytica* trophozoites
- B. *Entamoeba coli* trophozoites
- C. *Iodamoeba bütschlii* trophozoites
- D. *Endolimax nana* trophozoites

19. The following fecal permanent stain images are (oil immersion, 1000x) (they measure >13 microns):



- A. *Entamoeba histolytica*/*E. dispar* precysts
- B. *Entamoeba coli* precysts
- C. *Entamoeba polecki* precysts
- D. *Iodamoeba bütschlii* precysts

20. The following fecal permanent stain images are (oil immersion, 1000x) (they measure <10 microns):



- A. *Entamoeba histolytica*/*E. dispar* trophozoites and cyst
- B. *Entamoeba hartmanni* trophozoites and cyst
- C. *Entamoeba coli* trophozoites and cyst
- D. *Iodamoeba bütschlii* trophozoites and cyst

ANSWERS:

ANSWER. 1. C. The flagellate trophozoites and cysts seen would be identified as *Giardia lamblia* (*duodenalis*, *intestinalis*) trophozoites, cysts. Note the teardrop shape of the trophozoite and multiple inclusions in the cysts (multiple nuclei, curved median bodies and linear axonemes). Note: the trophozoites stain lighter than the cyst forms.

ANSWER. 2. D. Based on the organisms seen, the correct identification would be *Entamoeba histolytica*/*E. dispar*. There are no visible RBCs within the trophozoite; thus the identification of *Entamoeba histolytica*, the true pathogen, would be incorrect. Note the chromatoidal bar with rounded ends (far right image - arrow) and the four nuclei in the cysts. Also, note the precyst (third from left) with the very large single nucleus and chromatoidal bars.

ANSWER 3. A. The amebic cysts seen would be identified as *Entamoeba coli* cysts (5 or more nuclei and chromatoidal bars with sharp, pointed ends - arrow). Note the precyst at the far right contains the two typical large nuclei, one at each side of the precyst.

ANSWER. 4. C. These organisms are a *Chilomastix mesnili* cyst and a trophozoite; note the large nucleus and the curved fibril in the pear-shaped cyst (Shepherd's Crook; image on the left – arrow). Also, you can see the clear feeding groove in the trophozoite on the right (arrow). For proficiency testing specimens, *Chilomastix mesnili* identification is based on the cyst form in the permanent stained fecal smear.

ANSWER. 5. A. These organisms are *Iodamoeba bütschlii* cysts with the large single nuclear karyosome and large glycogen vacuole (may stain intensely with iodine in a wet mount, but is clear on the permanent stained smear).

ANSWER. 6. C. Charcot-Leyden (C-L) crystals are formed from the breakdown products of eosinophils. These crystals are an indication of an immune response, which may or may not be linked to a parasitic infection. Also, there may or may not be eosinophils present in the stool. C-L crystals tend to stain somewhat intensely with iodine, but are easily visible without iodine. There is a large size range of crystals in a single fecal specimen as seen in these permanent stain images.

ANSWER. 7. A. These organisms are the protozoan flagellate (internal flagella – look more like the amebae) *Dientamoeba fragilis*, often causing an infection that may be as common as giardiasis. These are trophozoites and can have a single nucleus or two nuclei. Note that the nuclei tend to fragment into four to five chromatin granules. Recently a cyst form has been confirmed; however, they are quite rare in clinical specimens and somewhat difficult to identify. This organism

often causes symptoms and should be reported as a potential pathogen in both children and adults.

ANSWER. 8. B. These images represent *Entamoeba histolytica*, the true pathogen and cause of amebiasis. The nucleus is typical with evenly arranged chromatin and a central, compact karyosome (arrow). The key feature for the identification is the presence of ingested RBCs within the trophozoite cytoplasm (oval); the RBCs are round (ingested yeast will tend to remain oval). When identifying RBCs in the cytoplasm, make sure that RBCs are also seen in the background of the stained smear. Although the RBCs may have unusual shapes in the background, once they are ingested, they become round as seen in these images.

ANSWER. 9. C. These images represent *Cryptosporidium* spp. oocysts. Although there are two or more species that can infect humans, the two most common are *C. parvum* and *C. hominis*; they look the same. These oocysts measure 4-6 microns, while *Cyclospora cayetanensis* measures 8-10 microns. Regardless of the stool type (formed, soft, diarrheic), *Cryptosporidium* oocysts are mature and immediately infectious when passed; note the sporozoites within the oocyst wall in the left image while they are not as clear in the image on the right (modified acid-fast stain for both images).

ANSWER 10. A. These images are *Endolimax nana* trophozoites and cysts. Note the tremendous nuclear variation seen in the trophozoites (left images), while the cyst nuclei appear as four dots within the round to oval cysts (right images). Because *E. nana* and *Iodamoeba bütschlii* trophozoites look almost identical, cysts would need to be present to confirm the identification of *E. nana*.

ANSWER. 11. A. These images are from a dual stain (modified acid-fast stain for coccidia and modified trichrome for the microsporidia) showing *Isospora belli* oocysts and microsporidian spores. The central *Isospora* image is much less mature than the oocysts on the far left and far right. Also in some of the microsporidian spores, one can see the cross line (polar tubule – circle).

ANSWER 12. D. These images represent microsporidian spores. Individual spores showing the cross line (polar tubule) are seen within the circles in both images. These spores cannot be differentiated to genus/species on the basis of morphology; the spores vary in size from 1.5 to 2.5 microns, but look very much the same. They would be reported as: Microsporidia (or microsporidian spores) present. Additional information for the physician could be provided as a report comment.

Comment to be used for reporting microsporidian spores:

The report would indicate “Microsporidian spores present”

Probably *Enterocytozoon bieneusi* or *Encephalitozoon intestinalis* or both; these tend to disseminate from the GI tract to the kidneys. Identification to the genus/species level not possible from stained smears.

ANSWER. 13. B. These structures are *Trichuris trichiura* eggs. Note the typical oval shape with the two polar plugs (these are not opercula), one at each end of the egg. The overall shell is somewhat thick; however, identification is generally based on the typical shape and presence of the clear polar plugs seen in a wet mount. Note that when stained, the typical characteristics are much more difficult to see; these stained eggs can often be confused with debris and/or artifacts.

ANSWER. 14. A. Based on the image, it is clear that a nematode larva is seen; however, the stain obscures any internal morphology that would be required for identification to genus/species. Examination of a concentration wet mount of the same specimen would be required to find additional larvae in which one could see if there is a packet of genital cells present and/or the short buccal capsule (mouth). With more careful examination of a wet mount, characteristics could be seen to confirm the identification as a *Strongyloides stercoralis* rhabditiform larva. Proficiency testing specimens for *Strongyloides stercoralis* are sent out as wet preparations.

ANSWER 15. C. These structures are *Taenia saginata* gravid proglottids. By counting the side branches as they come off the central uterine branch, the number will confirm *Taenia saginata*, the beef tapeworm (count one side only – usually more than 12, often 18 branches). For ink injection of the proglottids, they need to be handled very carefully to avoid contamination with the eggs, although these eggs are not infective for humans.

ANSWER. 16. A. These structures are *Taenia solium* gravid proglottids. By counting the side branches as they come off the central uterine branch, the number will confirm *Taenia solium*, the pork tapeworm (count one side only – usually less than 12, often 8 branches). For ink injection of the proglottids, they need to be handled very carefully to avoid contamination with the eggs.

Accidental ingestion of *T. solium* eggs can lead to a serious illness – cysticercosis.

ANSWER. 17. B. These images represent a *Balantidium coli* trophozoite on the left and a *B. coli* cyst on the right. Note the large bean-shaped macronucleus can be seen in both forms. The micronucleus is quite small and is difficult to see in either the trophozoite or cyst. Often the cilia can be seen around the trophozoite, but cilia is very difficult to see within the cyst wall. If these organisms are overstained, they are so large they may resemble helminth eggs. For proficiency testing, this organism is sent as a wet specimen rather than a permanent stained smear.

ANSWER. 18. B. These images represent *Entamoeba coli* trophozoites. Note the typical nucleus with a very large, dense blot-like karyosome (arrow). This is very unlike that seen with *Entamoeba histolytica/E. dispar* that has a delicate, compact, dot-like karyosome. The position of the karyosome (central vs eccentric) is much less important than the overall morphology of the karyosome; even or uneven nuclear peripheral chromatin also varies tremendously and is less important than the karyosome appearance. Also, the trophozoite cytoplasm of *E. coli* tends to contain more debris and appears more “messy” than the delicate/clean cytoplasm of *Entamoeba histolytica/E. dispar*.

ANSWER. 19. A. These images represent precysts of *Entamoeba histolytica/E. dispar* with a very large, single nucleus, and chromatoidal bars with smooth, rounded ends (arrows). This morphology separates *Entamoeba histolytica/E. dispar* precysts from *E. coli* precysts (contains two large nuclei, one at each side of the precyst). However, the morphology of the precyst does not allow differentiation between *Entamoeba histolytica/E. dispar* and the true pathogen, *Entamoeba histolytica*.

ANSWER. 20. B. These images represent *Entamoeba hartmanni* trophozoites and a cyst. The size is very important in making this identification, since the overall morphology is so similar to *Entamoeba histolytica/E. dispar*. Note: it is common for the cyst to contain only 2 nuclei and often multiple chromatoidal bars with smooth, rounded ends (image on the right). Although rarely seen, the mature cyst will contain 4 nuclei.

REFERENCES

1. Garcia, L.S. 2016. *Diagnostic Medical Parasitology*, 6th Ed., ASM Press, Washington, D.C.