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مشروع دعم قطاع مياه الشرب و الصرف الصحي

الشركة القابضة لمياه الشرب والصرف الصحى

الطفيليات فى مياه الشرب

تم إعداد هذا المستند بواسطة شركة كيمونكس إنترناشيونال ليقدم للوكالة الأمريكية للتنمية الدولية

الطفيليات فى مياه الشرب

مشروع دعم قطاع مياه الشرب و الصرف الصحي

ممول من الوكالة الأمريكية للتنمية الدولية

Contract No. EPP-I-00-04-00020-00, Order No. 3 T-2012.03.135

الديدان الطفيلية المعوية
في البيئة المائية

Intestinal Parasitic Helminths
in the Aquatic Environment

المحتويات

أولا: نظرة عامة على البرنامج التدريبي (الديدان الطفيلية المعوية في البيئة المائية)

١. الهدف العام للدورة التدريبية:

تجرى الاختبارات المعملية المختلفة (فيزيائية - كيميائية – بكتريولوجية - بيولوجية – طفيلية) لمياه الشرب ومصادرها قبل وبعد تنقيتها ومعالجتها بغرض الوقوف على مدى جودة المياه ومعرفة مكوناتها ومواصفاتها قبل وبعد تنقيتها وذلك للتحقق من صلاحية عملية المعالجة وتطابق نوعية المياه المنتجة مع المعايير والمواصفات القياسية المحلية والدولية لمياه الشرب.

لذا تهدف الدورة إلى رفع كفاءة العاملين بالمعامل المركزية والمعامل الفرعية التابعة لشركة مياه الشرب والصرف الصحي بشمال وجنوب سيناء وتزويدهم بالمعلومات النظرية والعملية الواجب إتباعها في إجراء التحاليل المعملية المتعلقة بالكشف عن الديدان الطفيلية في عينات المياه والتدريب على الاختبارات والطرق المختلفة والمعتمدة من الجهات المنوط بها وضع المواصفات القياسية لمياه الشرب.

٢. المجموعة المستهدفة

العاملون بالمعامل الرئيسية والمعامل الفرعية التابعة لشركة مياه الشرب والصرف الصحي بشمال وجنوب سيناء (إحدى الشركات التابعة للشركة القابضة لمياه الشرب والصرف الصحي) والمنوط بهم إجراء التحاليل الخاصة بمياه الشرب.

٣. عدد المتدربين

يقدر عدد المتدربين المرشحين لحضور دورة "الديدان الطفيلية المعوية في البيئة المائية" حوالي متدرب من معامل المحطات وتامعامل الفرعية التابعة.

٤. منهجية التدريب

تعتمد منهجية التدريب بالدورة على عدة أسس بحيث يكون الهدف الرئيسي منها توصيل المعلومة بسهولة ويسر للمتدرب , وكذلك ضمان المشاركة الفعالة من المتدربين أثناء جلسات التدريب والتأكد من الفهم الكامل لمحتويات وموضوعات الدورة مع التدريب العملي والشخصي على الموضوعات المتعلقة بمحتوى هذه الدورة.

هذا ويمكن تلخيص منهجية التدريب المتبعة في هذه الدورة فيما يلي:

- **التقييم الأولي:** حيث يقوم المدرب بعمل اختبار مبدئي للمتدربين لتحديد مستوى كل متدرب من حيث المعلومات المتعلقة بموضوع الدورة (الديدان الطفيلية) والخلفية العلمية لكل متدرب على حده.
- **المحاضرات:** والتي يقوم المدرب بتوصيل أحدث المعلومات النظرية والعملية المتعلقة بموضوع الدورة للمتدربين بما يؤدي إلى اكتساب معلومات صحيحة وموثقة عن الديدان الطفيلية وكيفية التعرف على الأطوار المعدية منها والتي قد تنتقل للانسان عن طريق المياه الملوثة بها .
- **الرسومات الإيضاحية:** التي يتم عرضها أثناء الشرح والخاصة بالديدان الطفيلية وذلك بغرض تسهيل وصول المعلومة وإبراز النقاط الرئيسية لكل موضوع في تسلسل منطقي لتثبيت المعلومة لدى المتدرب.
- **التجارب المعملية:** التي تجرى في المعمل لتطبيق الاختبارات المتعلقة بموضوع الدورة (الديدان الطفيلية) وذلك بطريقة صحيحة وبناءا على أسس علمية موثقة بحيث يستطيع المتدرب من تلاشي الأخطاء التي تؤثر في صحة النتائج المتحصل عليها وكيفية التعامل بأدق الوسائل للوصول إلى أفضل النتائج المرجوة.
- **المناقشات المفتوحة:** التي يديرها المدرب لإتاحة الفرصة لتبادل الآراء وتوجيه الأسئلة والحصول على معلومات جديدة يتم من خلالها نقل المعارف والخبرات العلمية والنظرية من المدرب إلى المتدربين وذلك بغرض إصلاح المفاهيم غير الصحيحة أو غير الحديثة لد المتدربين بخصوص الديدان الطفيلية.
- **المشاكل الواقعية المتعلقة بالمتدربين:** وذلك عن طريق مناقشة العقبات العلمية التي تواجه المتدربين أو المشكلات التي سوف يواجهونها والمتعلقة بموضوع الدورة (الديدان الطفيلية) وذلك بغرض الوصول إلى أنسب الطرث للتغلب عليها باستخدام الأسلوب العلمي الصحيح.
- **التدريب العملي:** حيث يتم إجراء الاختبارات والطرق القياسية الحديثة في المعمل بما يتيح لكل متدرب المشاركة في إجراء التجارب لضمان وصول المتدرب إلى مرحلة الفهم التام والتطبيق السليم للمعلومات والطرق العلمية التي تلقاها واكتسبها من المحاضرات .

- **المراجع العلمية:** يتم تزويد المتدرب بالمراجع العلمية المتعلقة بالديدان الطفيلية والتي يمكن الرجوع إليها للتأكد من صواب المعلومة والتعمق في اكتساب معلومات أخـرى متعلقة بنفس الموضوع.
- **التقييم النهائي:** حيث يتم عمل اختبار تحريري في نهاية الدورة يشتمل على أبرز ما تم دراسته خلال الدورة وذلك لتقييم المتدربين والوقوف على مدى الاستفادة الفرديـة لكل متدرب من حضور هذه الدورة.

٥. مساعدات التدريب

- جهاز عرض (Data show)
- شاشة عرض
- سبورة بيضاء وأقلام ملونة

٦. مكان التدريب وطريقة الجلوس بجلسات التدريب

يجلس المتدربون وفي مواجهتهم المحاضر في منتصف القاعة وعن يمينه جهاز الكمبيوتر لعرض ملفات الدورة على شاشة العرض , أما عن يساره توجد السبورة البيضاء والأقلام الملونة .ويكون وضع كل من شاشة العرض والسبورة البيضاء واضـح بحيـث يسـمح بسهولة الرؤية المتساوية لجميع المتدربين

تقدر مساحة قاعة التدريب المطلوبة بما لا يقل عن ٥ x ٧ متـرا لتسـتوعب المتـدربين وتسمح للمتدرب بسهولة الحركة والوصول لأماكن جلوس المتدربين.

يجب أن تتوفر بالقاعة إضاءة مناسبة وأجهزة صوتية واضحة ونظام تهوية ملائم.

ثانيا: خطة التدريس بالدورة التدريبية (الديدان الطفيلية المعوية في البيئة المائية)

المحاضر: ا.د./ أحمد زكريا سالم الهراوي

١. أهداف الدورة:

- التعرف على الأجناس والأنواع المختلفة للديدان الطفيلية.
- دراسة دورة حياه كل دودة لتحديد الطور المعدي لكل منها على حده.
- دراسة التركيب المورفولوجي للأطوار المعدية للديدان الطفيلية.
- المقارنة بين الأطوار المعدية للديدان وكيفية التفرقة بينها.
- تحديد طرق تلوث المياه بالطور المعدي للديدان.
- تأثير خطوات المعالجة الفيزيائية للمياه على إزالة الأطوار المعدية للديدان.
- تأثير خطوات المعالجة الكيميائية للمياه على إزالة الأطوار المعدية للديدان.
- تحديد طريقة دخول الأطوار المعدية للديدان الطفيلية إلى جسم العائل عن طريق المياه الملوثة بها.
- التدريب على طرق تركيز الطور المعدي للديدان في المياه.
- استخدام الميكروسكوب الضوئي لفحص الأطوار المعدية للديدان الطفيلية في المياه المركزة.
- تقييم النتائج المتحصل عليها من تحاليل المياه الخاصة بالديدان الطفيلية.
- كتابة تقارير التحاليل الطفيلية للمياه والتعليق على النتائج المتحصل عليها.

٢. موضوعات الدورة:

- نبذة عن الطفيليات وأنواعها وشرح الهدف من الدورة.
- تقسيم الطفيليات.
- مقدمة عن الديدان الطفيلية.
- الديدان الاسطوانية التي تصيب الإنسان.
- الديدان الشريطية التي تصيب الإنسان.
- الديدان الورقية التي تصيب الإنسان.
- الديدان الطفيلية المشتركة التي تنتقل من الحيوانات والطيور إلى الإنسان.
- تحديد العائل الأساسي والعائل الوسيط للديدان الطفيلية.
- الصفات المورفولوجية المميزة للأطوار المعدية للديدان الطفيلية.
- تلوث المياه بالطور المعدي للديدان الطفيلية.
- طرق تركيز الأطوار المعدية للديدان الطفيلية في المياه.
- استخدام الميكروسكوب الضوئي للتمييز بين الأطوار المعدية للديدان المختلفة.
- إعداد تقارير تحاليل الديدان الطفيلية والتعليق على النتائج.

٣. مدة الدورة:

تستغرق الدورة مدة خمسة أيام متواصلة حيث يبدأ العمل يوميا من الساعة التاسعة صباحا حتى الساعة الرابعة عصرا بواقع سبع ساعات يوميا تتخللها نصف ساعة لتناول المشروبات والغداء.

٤. البرنامج الزمني للدورة

الديدان الطفيلية في البيئة المائية

PARASITIC HELMINTHS IN THE AQUATIC ENVIRONMENT

DEFINITIONS

There is a glossary of water quality terminology on the Water Quality

AMOEBA: PROTOZOAN ORGANISMS THAT MOVE BY EXTENDING THE CELL MEMBRANE IN SOME DIRECTION AND FLOWING INTO THE EXTENSION.

AMOEBOID: ORGANISMS THAT RESEMBLE AMOEBA; MOVEMENT BY EXTENDING THE CELL MEMBRANE IN SOME DIRECTION AND FLOWING INTO THE EXTENSION.

CILIATES: ORGANISMS THAT MOVE BY WAVES OF BEATING BY MANY SMALL HAIRS WHICH COVER THEIR ENTIRE SURFACE OR ONLY CERTAIN AREAS OR ZONES ON THEIR SURFACE.

COMMENSAL: TWO ORGANISMS HAVE A COMMENSAL RELATIONSHIP WHEN ONE OR BOTH GET SOME BENEFIT FROM LIVING TOGETHER OR SHARING RESOURCES AND NEITHER IS HARMED.

CYST: A RESTING OR DORMANT STAGE OF A PROTOZOAN USUALLY FOUND IN THE ENVIRONMENT WHERE IT AWAITS INTRODUCTION INTO A NEW HOST.

DEFINITIVE HOST: THE FINAL AND NECESSARY HOST IN WHICH A PATHOGEN OR PARASITE COMPLETES ITS LIFE CYCLE, UNDERGOES SEXUAL REPRODUCTION AND SHEDS CYSTS TO START THE CYCLE AGAIN.

DISINFECTANT: A CHEMICAL, RADIATION, PHYSICAL OR OTHER TECHNIQUE THAT KILLS ORGANISMS.

ENCEPHALITIS: AN OFTEN FATAL DISEASE OR INFECTION OF THE SPINAL FLUID AND COLUMN, THE BRAIN AND THE NERVOUS SYSTEM OF AN ORGANISM.

ENDEMIC: AN ORGANISM THAT ORIGINATES AND IS, OR WAS PRIOR TO TRANSPORTATION BY MAN, RESTRICTED TO A SPECIFIC AREA AS OPPOSED TO AN ORGANISM WITH A WIDESPREAD DISTRIBUTION.

EUKARYOTIC: REFERS TO ORGANISMS THAT HAVE A DISCRETE NUCLEUS, MITOCHONDRIA AND VARIOUS ORGANELLES SUCH AS CHLOROPLASTS, INCLUDES ALL MULTICELLULAR ORGANISMS AND MANY UNICELLULAR ORGANISMS BUT NOT BACTERIA.

FLAGELLATES: ORGANISMS THAT MOVE BY BEATING SEVERAL LONG HAIRS THAT EMANATE FROM A SPECIFIC LOCATION ON THEIR SURFACE.

HERMAPHRODITE: REFERS TO ORGANISMS THAT HAVE MALE AND FEMALE SEX ORGANS IN THE SAME INDIVIDUAL.

IMMUNOCOMPETENT: REFERS TO A PERSON WHOSE IMMUNE SYSTEM IS FULLY FUNCTIONAL AND WHO IS ABLE TO PREVENT OR CONTROL INFECTIONS BY MANY PATHOGENS WITHOUT ANY MEDICAL HELP.

IMMUNODEFICIENT: REFERS TO A PERSON WHOSE IMMUNE SYSTEM IS NOT FULLY FUNCTIONAL AND WHO IS UNABLE TO PREVENT OR CONTROL INFECTIONS BY MANY PATHOGENS AND THUS IS AT RISK OF SERIOUS DISEASE OR DEATH FROM MANY PATHOGENS OR OTHER OPPORTUNISTIC ORGANISMS THAT DO NOT CAUSE A PROBLEM FOR IMMUNOCOMPETENT PEOPLE. AIDS, HIV AND ANTI-REJECTION DRUGS USED IN TRANSPLANT SURGERY ALL CAUSE AT LEAST PARTIALLY NON-FUNCTIONAL IMMUNE SYSTEMS.

IMMUNODEPRESSED: REFERS TO A PERSON WHOSE IMMUNE SYSTEM IS NOT FULLY FUNCTIONAL AND WHO IS UNABLE TO PREVENT OR CONTROL INFECTIONS BY MANY PATHOGENS AND THUS IS AT RISK OF SERIOUS DISEASE OR DEATH FROM MANY PATHOGENS OR OTHER OPPORTUNISTIC ORGANISMS THAT DO NOT CAUSE A PROBLEM FOR IMMUNOCOMPETENT PEOPLE. AIDS, HIV AND ANTI-REJECTION DRUGS USED IN TRANSPLANT SURGERY ALL CAUSE AT LEAST PARTIALLY NON-FUNCTIONAL IMMUNE SYSTEMS.

IMMUNOINCOMPETENT: REFERS TO A PERSON WHOSE IMMUNE SYSTEM IS NOT FULLY FUNCTIONAL AND WHO IS UNABLE TO PREVENT OR CONTROL INFECTIONS BY MANY PATHOGENS AND THUS IS AT RISK OF SERIOUS DISEASE OR DEATH FROM MANY PATHOGENS OR OTHER OPPORTUNISTIC ORGANISMS THAT DO NOT CAUSE A PROBLEM FOR IMMUNOCOMPETENT PEOPLE. AIDS, HIV AND ANTI-REJECTION DRUGS USED IN TRANSPLANT SURGERY ALL CAUSE AT LEAST PARTIALLY NON-FUNCTINAL IMMUNE SYSTEMS.

IMMUNOSUPPRESSED: REFERS TO A PERSON WHOSE IMMUNE SYSTEM IS NOT FULLY FUNCTIONAL AND WHO IS UNABLE TO PREVENT OR CONTROL INFECTIONS BY MANY PATHOGENS AND THUS IS AT RISK OF SERIOUS DISEASE OR DEATH FROM MANY PATHOGENS OR OTHER OPPORTUNISTIC ORGANISMS THAT DO NOT CAUSE A PROBLEM FOR IMMUNOCOMPETENT PEOPLE. AIDS, HIV AND ANTI-REJECTION DRUGS USED IN TRANSPLANT SURGERY ALL CAUSE AT LEAST PARTIALLY NON-FUNCTIONAL IMMUNE SYSTEMS.

MICRON: 1/1,000,000 OF A METRE; 1/1,000 OF A MILLIMETRE.

NEONATE: NEWBORN AND OFTEN STILL IMMUNOINCOMPETENT AND THUS SUSCEPTIBLE TO PATHOGENS.

NOSOCOMIAL: THIS REFERS TO DISEASES OR INFECTIONS THAT ARE ACQUIRED WHILE IN A HOSPITAL OR CARE FACILITY SETTING.

OPPORTUNISTIC: AN ORGANISM THAT IS NOT NORMALLY A PATHOGEN OR PARASITE BUT TAKES ADVANTAGE OF PEOPLE WITH NON-FUNCTIONAL IMMUNE SYSTEMS TO CAUSE DISEASE OR DAMAGE.

PARASITES: ORGANISMS THAT CAUSE HARM OR DEATH TO THEIR HOST ORGANISM WHEN THEY LIVE OR BREED ON OR WITHIN THE HOST ORGANISM.

PATENCY: THE LENGTH OF TIME EGGS OR OOCYCTS ARE VOIDED IN THE FECES. DURING THIS TIME, USUALLY MEASURED IN DAYS OR WEEKS, A PERSON IS INFECTIVE AND ACTIVELY SPREADING THE DISEASE.

PATHOGENIC: THESE ARE ORGANISMS WHICH CAUSE DISEASE OR DAMAGE IN ANOTHER ORGANISM.

POSTMORTEM: AFTER DEATH.

PREPATENT: THE TIME PERIOD BETWEEN THE FIRST INFECTION BY A PATHOGEN AND THE FIRST APPEARANCE OF EGGS OR OOCYCTS IN THE FECES. DURING THIS TIME, USUALLY MEASURED IN DAYS, A PERSON CAN BE AN UNKNOWN CARRIER OF A DISEASE AND TRAVEL ALL AROUND THE WORLD.

PROKARYOTIC: REFERS TO ORGANISMS THAT HAVE NO DISCRETE NUCLEUS, MITOCHONDRIA AND VARIOUS ORGANELLES SUCH AS CHLOROPLASTS, EXCLUDES ALL MULTICELLULAR ORGANISMS AND MANY UNICELLULAR ORGANISMS BUT INCLUDES BACTERIA, VIRUSES AND CYANOPHYTES.

PROTOZOAN: THESE ARE UNI-CELLULAR ORGANISMS SUCH AS AMOEBAS, CILIATES, MICROSPORIDIANS AND FLAGELLATES AS DISTINCT FROM MULTI-CELLULAR ORGANISMS.

TOXIN: A CHEMICAL SUBSTANCE PRODUCED BY AN ORGANISM FOR DEFENSE OR OFFENSE AGAINST OTHER ORGANISMS.

TROPHOZOITES: FREE-LIVING MOTILE STAGES OF PROTOZOAN PATHOGENS.

SEROPREVALENT: FOUND IN THE BLOOD SERUM, REFERS TO CIRCULATING ANTIBODIES BEING PRESENT INDICATING SOME PRIOR EXPOSURE TO THE PATHOGEN; SINCE THE BODY HAS ALREADY PREPARED ANTIBODIES THERE MUST HAVE BEEN A PREVIOUS EXPOSURE.

Helminth Worms

Ancylostoma braziliense
Ancylostoma ceylanicum
Ancylostoma duodenale
Ascaris lumbricoides
*Austrobilharzia variglandis**
Capillaria aerophila
Capillaria hepatica
Capillaria philippinensis
Clonorchis sinensis
Diphyllobothrium latum
Diphyllobothrium pacificum
Diphyllobothrium cordatum
Diphyllobothrium ursi
Diphyllobothrium dendriticum
Diphyllobothrium lanceolatum
Diphyllobothrium dalliae
Diphyllobothrium yonagoensis
Dipylidium caninum
Dracunculus medinensis
Echinococcus granulosus
Echinococcus multilocularis
Echinococcus vogeli
Echinococcus oligarthrus
Enterobius vermicularis
Fasciola gigantica
Fasciola hepatica

Fasciolopsis buski
*Gigantobilharzia**
*Heterobilharzia americanum**
Heterophyes heterophyes
Hymenolepis diminuta
Hymenolepis nana
Metagonimus yokogawai
*Microbilharzia**
Necator americanus
Opisthorchis viverrini
Opisthorchis felineus
Paragonimus westermani
Schistosoma haematobium
Schistosoma intercalatum
Schistosoma japonicum
Schistosoma mansoni
Schistosoma mekongi
*Schistosoma spindale**
*Schistosomatium douthitti**
Taenia saginata
Taenia solium
Toxocara canis
Toxocara cati
*Trichobilharzia ocellata**
*Trichobilharzia physella**
*Trichobilharzia stagnicolae**
Trichuris trichiura
Uncinaria stenocephala
* swimmer's itch

Location in the Body	**Helminth Organisms**
Central nervous system, Brain	*Taenia solium, Heterophyes heterophyes, Metagonimus yokogawai, Paragonimus westermani, Schistosoma haematobium*
Circulatory system, Heart	*Ancylostoma duodenale, Ascaris lumbricoides, Heterophyes heterophyes, Metagonimus yokogawai, Schistosoma mansoni, Schistosoma haematobium, Schistosoma japonicum, Schistosoma mekongi, Schistosoma intercalatum, Echinococcus granulosus, Echinococcus multilocularis, Echinococcus vogeli, Echinococcus oligarthrus*
Eye	*Toxocara cati, Toxocara canis*
Genito-urinary system	*Schistosoma haematobium*
Gut, Colon, Duodenum, Ileum, Small intestine, Lumen, Intestinal tract	*Diphyllobothrium latum, Dipylidium caninum, Hymenolepis diminuta, Hymenolepis nana, Taenia saginata, Taenia solium, Ancylostoma duodenale, Ascaris lumbricoides, Capillaria philippinensis, Enterobias vermicularis, Necator americanus, Trichuris trichiura, Clonorchis sinensis, Fasciola gigantica, Fasciola hepatica, Fasciolopsis buski, Heterophyes heterophyes, Metagonimus yokogawai, Opisthorchis viverrini, Opisthorchis felineus, Schistosoma mansoni, Schistosoma haematobium, Schistosoma japonicum, Schistosoma mekongi, Schistosoma intercalatum, Echinococcus granulosus, Echinococcus multilocularis, Echinococcus vogeli, Echinococcus oligarthrus*
Liver	*Taenia solium, Capillaria hepatica, Toxocara cati, Toxocara canis, Clonorchis sinensis, Fasciola gigantica, Fasciola hepatica, Opisthorchis viverrini, Opisthorchis felineus, Echinococcus granulosus, Echinococcus multilocularis, Echinococcus vogeli, Echinococcus oligarthrus*
Muscle	*Taenia saginata, Taenia solium, Ancylostoma*

	duodenale
Pancreas	*Opisthorchis viverrini, Opisthorchis felineus*
Respiratory tract, Lungs	*Ancylostoma duodenale, Ascaris lumbricoides, Capillaria aerophila, Necator americanus, Paragonimus westermani, Echinococcus vogeli*
Skin	*Ancylostoma ceylanicum, Ancylostoma duodenale, Ancylostoma braziliense, Uncinaria stenocephala, Necator americanus, Schistosoma mansoni, Schistosoma haematobium, Schistosoma japonicum, Schistosoma mekongi, Schistosoma intercalatum, Austrobilharzia variglandi, Gigantobilharzia, Heterobilharzia americanum, Microbilharzia, Schistosoma spindale, Schistosomatium douthitti, Trichobilharzia ocellata, Trichobilharzia physella, Trichobilharzia stagnicolae, Dracunculus medinensis*
Widespread	*Taenia solium, Paragonimus westermani, Echinococcus granulosus, Echinococcus vogeli*

INTESTINAL NEMATODES

Intestinal nematodes of importance to man are *Ascaris lumbricoides* (roundworm), *Trichinella spiralis* (trichinosis), *Trichuris trichiura* (whipworm), *Enterobius vermicularis* (pinworm), *Strongyloides stercoralis* (Cochin-china diarrhea), *Ancylostoma duodenale* and *Necator americanes* (hookworms) and *Dracunculus medinensis* (fiery serpents of the Israelites). *E. vermicularis* and *T. trichiura* are exclusively intestinal parasites. Other helminths listed above have both intestinal and tissue phases.

1-Ascaris lumbricoides (Large intestinal roundworm)

Epidemiology The annual global morbidity due to ascaris infections is estimated at 1 billion with a mortality of 20,000. Ascariasis can occur at all ages, but it is more prevalent in the 5 to 9 years age group. The incidence is higher in poor rural populations.

Morphology The average female worm measures 30 cm x 5 mm. The male is smaller.

Life cycle The infection occurs by ingestionof food contaminated with infective eggs which hatch in the upper small intestine. The larvae (250 x 15 micrometers) penetrate the intestinal wall and enter the venules or lymphatics. The larvae pass through the liver, heart and lung to reach alveoli in 1 to 7 days during which period they grow to 1.5 cm. They migrate up the bronchi, ascend the trachea to the glottis, and pass down the esophagus to the small intestine where they mature in 2 to 3 months. A female may live in the intestine for 12 to 18 months and has a capacity of producing 25 million eggs at an average daily output of 200,000 (figure 2). The eggs are excreted in feces, and under suitable conditions (21 to 30 degrees C, moist, aerated environment) infective larvae are formed within the egg. The eggs are resistant to chemical disinfectant and survive for months in sewage, but are killed by heat (40 degrees C for 15 hours). The infection is man to man. Auto infection can occur.

Symptoms are related to the worm burden. Ten to twenty worms may go unnoticed except in a routine stool examination. The commonest complaint is vague abdominal pain. In more severe cases, the patient may experience listlessness, weight loss, anorexia, distended abdomen, intermittent loose stool and occasional vomiting. During the pulmonary stage, there may be a brief period of cough, wheezing, dyspnea and sub-sternal discomfort. Most symptoms are due to the physical presence of the worm.

Diagnosis is based on identification of eggs (40 to 70 micrometers by 35 to 50 micrometers - figure 2) in the stool.

Treatment and Prevention Mebendazole, 200 mg, for adults and 100 mg for children, for 3 days is effective. Good hygiene is the best preventive measure.

2-Trichinella spiralis (Trichinosis)

Epidemiology Trichinosis is related to the quality of pork and consumption of poorly cooked meat. Autopsy surveys indicate about 2 percent of the population is infected. The mortality rate is low.

Morphology The adult female measures 3.5 mm x 60 micrometers. The larvae in the tissue (100 micrometers x 5 micrometers) are coiled in a lemon-shaped capsule.

Life cycle Infection occurs by ingestion of larvae, in poorly cooked meat, which immediately invade intestinal mucosa and sexually differentiate within 18 to 24 hours. The female, after fertilization, burrows deeply in the small intestinal mucosa, whereas the male is dislodged (intestinal stage). On about the 5th day eggs begin to hatch in the female worm and young larvae are deposited in the mucosa from where they reach the lymphatics, lymph nodes and the blood stream (larval migration). Larval dispersion occurs 4 to 16 weeks after infection. The larvae are deposited in muscle fiber and, in striated muscle, they form a capsule which calcifies to form a cyst. In non-striated tissue, such as heart and brain, the larvae do not calcify; they die and disintegrate. The cyst may persist for several years. One female worm produces approximately 1500 larvae. Man is the terminal host. The reservoir includes most carnivorous and omnivorous animals

Symptoms Trichinosis symptoms depend on the severity of infection: mild infections may be asymptomatic. A larger bolus of infection produces symptoms according to the severity and stage of infection and organs involved

Pathology and Immunology Trichinella pathogenesis is due the presence of large numbers of larvae in vital muscles and host reaction to larval metabolites. The muscle fibers become enlarged edematous and deformed. The paralyzed muscles are infiltrated with neutrophil, eosinophils and lymphocytes. Splenomegaly is dependent on the degree of infection. The worm induces a strong IgE response which, in association with eosinophils, contributes to parasite death.

Diagnosis is based on symptoms, recent history of eating raw or undercooked meat and laboratory findings (eosinophilia, increased serum creatine phosphokinase and lactate dehydrogenase and antibodies to *T. spiralis*).

Treatment and Control Steroids are use for treatment of inflammatory symptoms and Mebendazole is used to eliminate worms. Elimination of parasite infection in hogs and adequate cooking of meat are the best ways of avoiding infection.

3-Trichuris trichiura (whipworm)

Epidemiology Trichuriasis is a tropical disease of children (5 to 15 yrs) in rural Asia (65% of the 500-700 million cases). It is, however, seen in the two Americas, mostly in the South and is concentrated in families and groups with poorer sanitary habits.

Morphology The female organism is 50 mm long with a slender anterior (100 micrometer dia,eter) and a thicker (500 micrometers diameter) posterior end. The male is smaller and has a coiled posterior end. The Trichuris eggs are lemon or football shaped and have terminal plugs at both ends.

Life cycle Infection occurs by ingestion of embryonated eggs in soil. The larva escapes the shell in the upper small intestine and penetrates the villus where it remains for 3 to 10 days. Upon reaching adolescence, the larvae pass to the cecum and embed in the mucosa. They reach the ovipositing age in 30 to 90 days from infection, produce 3000 to 10,000 eggs per day and may live as long as 5 to 6 years. Eggs passed in feces embryonate in moist soil within 2 to 3 weeks (Figure 5 and 6). The eggs are less resistant to desiccation, heat and cold than ascaris eggs. The embryo is killed under desiccation at 37 degrees C within 15 minutes. Temperatures of 52 degrees C and -9 degrees C are lethal.

Symptoms are determined largely by the worm burden: less than 10 worms are asymptomatic. Heavier infections (e.g., massive infantile trichuriasis) are characterized by chronic profuse mucus and bloody diarrhea with abdominal pains and edematous prolapsed rectum. The infection may result in malnutrition, weight loss and anemia and sometimes death.

Diagnosis is based on symptoms and the presence of eggs in feces (50 to 55 x 20 to 25 micrometers).

Treatment and Control Mebendazole, 200 mg, for adults and 100 mg for children, for 3 days is effective. Accompanying infections must be treated accordingly. Improved hygiene and sanitary eating habits are most effective in control.

4-Enterobius vermicularis (pinworm)

Epidemiology Enterobiasis is by far the commonest helminthic infection in the US (18 million cases at any given time). The worldwide infection is about 210 million. It is an urban disease of children in crowded environment (schools, day care centers, etc.). Adults may get it from their children. The incidence in whites is much higher than in blacks.

Morphology The female worm measures 8 mm x 0.5mm; the male is smaller. Eggs (60 micrometers x 27 micrometers) are ovoid but asymmetrically flat on one side.

Life cycle Infection occurs when embryonated eggs are ingested from the environment, with food or by hand to mouth contact. The embryonic larvae hatch in the duodenum and reach adolescence in jejunum and upper ilium. Adult worms descend into lower ilium, cecum and colon and live there for 7 to 8 weeks. The gravid females, containing more than 10,000 eggs migrate, at night, to the perianal region and deposit their eggs there. Eggs mature in an oxygenated, moist environment and are infectious 3 to 4 hours later. Man-to-man and auto infection are common (Figure 7 and 8). Man is the only host.

Symptoms Enterobiasis is relatively innocuous and rarely produces serious lesions. The most common symptom is perianal, perineal and vaginal irritation caused by the female migration. The itching results in insomnia and restlessness. In some cases gastrointestinal symptoms (pain, nausea, vomiting, etc.) may develop. The conscientious housewife's mental distress, guilt complex, and desire to conceal the infection from her friends and mother-in-law is perhaps the most important trauma of this persistent, pruritic parasite.

Diagnosis is made by finding the adult worm or eggs in the perianal area, particularly at night. Scotch tape or a pinworm paddle is used to obtain eggs.

Treatment and Control Two doses (10 mg/kg; maximum of 1g each) of Pyrental Pamoate two weeks apart gives a very high cure rate. Mebendazole is an alternative. The whole family should be treated, to avoid reinfection. Bedding and underclothing must be sanitized between the two treatment doses. Personal cleanliness provides the most effective in prevention.

5-Strongyloides stercoralis (Threadworm)

Epidemiology Threadworm infection, also known as Cochin-China diarrhea, estimated at 50 to 100 million cases worldwide, is an infection of the tropical and subtropical areas with poor sanitation. In the United States, it is prevalent in the South and among Puerto Ricans.

Morphology The size and shape of threadworm varies depending on whether it is parasitic or free-living. The parasitic female is larger (2.2 mm x 45 micrometers) than the free-living worm (1 mm x 60 micrometers) (figure 10). The eggs, when laid are 55 micrometers by 30 micrometers.

Life cycle The infective larvae of *S. stercoralis* penetrate the skin of man, enter the venous circulation and pass through the right heart to lungs, where they penetrate into the alveoli. From there, the adolescent parasites ascend to the glottis, are swallowed, and reach the upper part of the small intestine, where they develop into adults. Ovipositing females develop in 28 days from infection. The eggs in the intestinal mucosa, hatch and develop into rhabditiform larvae in man. These larvae can penetrate through the mucosa and cycle back into the blood circulation, lung, glottis and duodenum and jejunum; thus they continue the auto infection cycle. Alternatively, they are passed in the feces, develop into infective filariform larvae and enter another host to complete the direct cycle. If no suitable host is found, the larvae mature into free-living worm and lay eggs in the soil. The eggs hatch in the soil and produce rhabditiform larvae which develope into infective filariform larvae and enter a new host (indirect cycle), or mature into adult worms to repeat the free-living cycle.

Symptoms Light infections are asymptomatic. Skin penetration causes itching and red blotches. During migration, the organisms cause bronchial verminous pneumonia and, in the duodenum, they cause a burning mid-epigastric pain and tenderness accompanied by nausea and vomiting. Diarrhea and constipation may alternate. Heavy, chronic infections result in anemia, weight loss and chronic bloody dysentery. Secondary bacterial infection of damaged mucosa may produce serious complications.

Diagnosis The presence of free rhabditiform larvae (figure 10) in the feces is diagnostic. Culture of stool for 24 hours will produce filariform larvae.

Treatment and control Ivermectin or thiabendozole can be used effectively. Direct and indirect infections are controlled by improved hygiene and auto-infection is controlled by chemotherapy.

6-Necator americanes and *Ancylostoma duodenale* (Hookworms)

Epidemiology Hookworms parasitize more than 900 million people worldwide and cause daily blood loss of 7 million liters. Ancylostomiasis is the most prevalent hookworm infection and is second only to ascariasis in infections by parasitic worms. *N. americanes* (new world hookworm) is most common in the Americas, central and southern Africa, southern Asia, Indonesia, Australia and Pacific Islands. *A. duodenale* (old world hookworm) is the dominant species in the Mediterranean region and northern Asia.

Morphology Adult female hookworms are about 11 mm x 50 micrometers. Males are smaller. The anterior end of *N. americanes* is armed with a pair of curved cutting plates whereas *A. duodenale* is equipped with one or more pairs of teeth. Hookworm eggs are 60 micrometers x 35 micrometers.

Life cycle The life cycle of hookworms is identical to that of threadworms, except that hookworms are not capable of a free-living or auto-infectious cycle. Furthermore, *A. duodenale* can infect also by oral route.

Symptoms of hookworm infection depend on the site at which the worm is present (Table 2) and the burden of worms. Light infection may not be noticed.

Diagnosis is made by identification of hookworm eggs in fresh or preserved feces. Species of hookworms cannot be distinguished by egg morphology.

Treatment and control Mebendazole, 200 mg, for adults and 100 mg for children, for 3 days is effective. Sanitation is the chief method of control: sanitary disposal of fecal material and avoidance of contact with infected fecal material.

7-Dracunculus medinensis (Guinea worm; fiery serpent of the Israelites)

Epidemiology Guinea worm is estimated to infect about 50 million people in North, West and Central Africa, southwestern Asia, the West Indies and northeastern South America.

Morphology The adult female worm measures 50-120 cm by 1 mm and the male is half that size.

Life cycle The infection is caused by ingestion of water contaminated with

water fleas (Cyclops) infected with larvae. The rhabtidiform larvae penetrate the human digestive tract wall, lodge in the loose connective tissues and mature into the adult form in 10 to 12 weeks. In about a year, the gravid female migrates to the subcutaneous tissue of organs that normally come in contact with water and discharges its larvae into the water (figure 13). The larvae are picked up by Cyclops, in which they develop into infective form in 2 to 3 weeks.

Symptoms If the worm does not reach the skin, it dies and causes little reaction. In superficial tissue, it liberates a toxic substance that produces a local inflammatory reaction in the form of a sterile blister with serous exudation. The worm lies in a subcutaneous tunnel with its posterior end beneath the blister, which contains clear yellow fluid. The course of the tunnel is marked with induration and edema. Contamination of the blister produces abscesses, cellulitis, extensive ulceration and necrosis.

Diagnosis is made from the local blister, worm or larvae. The outline of the worm under the skin may be revealed by reflected light.

Treatment includes the extraction of the adult guinea worm by rolling it a few centimeters per day or preferably by multiple surgical incisions under local anaesthesia. Metronidazole is effective in killing the worm. Protection of drinking water from being contaminated with Cyclops and larvae are effective preventive measures.

8-Toxocara canis and *T. catti* (visceral larva migrans)

These are roundworms of dogs and cats but they can infect humans and cause damage of the visceral organs. Eggs from feces of infected animals are swallowed by man and hatch in the intestine. The larvae penetrate the mucosa, enter the circulation and are carried to liver, lungs, eyes and other organs where they cause inflammatory necrosis. Symptoms are due to the inflammatory reaction at the site of infection. The most serious consequence of infection may be loss of sight if the worm localizes in the eye. Treatment includes Mebendazole to eliminate the worm and prednisone for inflammatory symptoms. Avoidance of infected dogs and cats is the best prevention.

9-Ancylostoma braziliensis (cutaneous larva migrans, creeping eruption)
Creeping eruption is prevalent in many tropical and subtropical countries and in the US especially along the Gulf and southern Atlantic states. The organism is primarily a hookworm of dogs and cats but the filariform larvae in animal feces can infect man and cause skin eruptions. Since the larvae

have a tendency to move around, the eruption migrates in the skin around the site of infection. The symptoms last the duration of larval persistence which ranges from 2 to 10 weeks. Light infection can be treated by freezing the involved area. Heavier infections are treated with Mebendazole. Infection can be avoided by keeping away from water and soil contaminated with infected feces

CESTODES (TAPE WORMS)

Clinically important cestodes pathogenic to man are *Tenia solium* (pork tapeworm), *T. saginata* (beef tapeworm), *Diphyllobothrium lattum* (fish or broad tapeworm), *Hymenolepis nana* (dwarf tapeworm) and *Echinococcus granulosus* and *E. multilocularis* (hydatid).

1-Tenia solium or *T. saginata* (Teniasis)

Epidemiology These cestodes have a worldwide distribution but incidence is higher in developing countries. Infection rate is as low as 1 per 1000 in most of North America and as high as 10% in the third world. Pork tapeworm shows a higher incidence but this is dependent on dietary habits.

Morphology *T. saginata* can be up to 4 to 6 meters long and 12 mm broad; it has a pear-shaped head (scolex) with four suckers but no hooks or neck. It has a long flat body with several hundred segments (proglottids). Each segment is about 18 x 6 mm with a branched uterus (15-30 branches). The egg is 35 x 45 micrometers, roundish and yellow-brown. It has peripheral radial striations and contains an embryo with 3 hooklets (figure 2).

T. solium is slightly smaller than *T. saginata*. It has a globular scolex with four suckers and a circular row of hooks (rostellum) that gives it a solar appearance. There is a neck and it has a long flat body (0.1 meter in length). The proglottids are 5 x 10 mm with a 7-12 branch uterus. The eggs of *T. solium* and *T. saginata* are indistinguishable.

Life cycle A tapeworm larval cyst (cysticercus) is ingested with poorly cooked infected meat; the larva escapes the cyst and passes to the small intestine where it attaches to the mucosa by the scolex suckers. The proglottids develop as the worm matures in 3 to 4 months. The adult may live in the small intestine as long as 25 years and pass gravid proglottids with the feces. Eggs extruded from the

proglottid contaminate and persist on vegetation for several days and are consumed by cattle or pigs in which they hatch and form cysticerci (Figure 1).

Symptoms Light infections remain asymptomatic, but heavier infections may produce abdominal discomfort, epigastric pain, vomiting and diarrhea.

Cysticercosis *T. solium* eggs can also infect humans and cause cysticercosis (larval cysts in lung, liver, eye and brain) resulting in blindness and neurological disorders. The incidence of cerebral cysticercosis can be as high 1 per 1000 population and may account for up to 20% of neurological case in some countries (e.g., Mexico); cysticercosis ocular involvement occurs in about 2.5% of patients and muscular involvement is as high as 10% (India).

Pathology and Immunology Gastrointestinal symptoms are due to the presence of the tape worm. Cysticercosis symptoms are a result of inflammatory/immune responses. Antibodies are produced in cysticercosis and are useful epidemiological tools.

Diagnosis is based on the recovery of eggs or proglottids in stool or from the perianal area. Cysticercosis is confirmed by the presence of antibodies.

Treatment and control Praziquantel is the drug of choice. Expulsion of scolex must be assured to assume a satisfactory treatment. A thorough inspection of beef and pork, adequate cooking or freezing of meat are effective precautions, since cysticerci do not survive temperatures below -10^{o} C and above 50^{o} C.

2-Diphyllobothrium latum (fish or broad tapeworm)

Epidemiology Fish tapeworm infection is distributed worldwide, in the subarctic and temperate regions; it is associated with eating of raw or improperly cooked fresh water fish.

Morphology This is the longest tapeworm found in man, ranging from 3-10 meters with more than 3000 proglottids. The scolex resembles two almond-shaped leaves and the proglottids are broader than they are long, a morphology reflected in the organism's name. Eggs are 30 x 50 micrometers in size and contain an embryo with 3 pairs of hooklets (figure 4).

Life cycle Man and other animals are infected by eating uncooked fish that contains plerocercoid larvae (15 x 2 mm) which attach to the small intestinal wall and mature into adult worms in 3 to 5 weeks. Eggs

discharged from gravid proglottids in the small intestine are passed in the feces. The egg hatches in fresh water to produce a ciliated coracidium which needs to be ingested by a water flea (Cyclops) where it develops into a procercoid larva. When infected Cyclops are ingested by the freshwater fish, the procercoid larva penetrates the intestinal wall and develops into a plerocercoid larva, infectious to man (figure 3).

Symptoms Clinical symptoms may be mild, depending on the number of worms. They include abdominal discomfort, loss of weight, loss of appetite and some malnutrition. Anemia and neurological problems associated with vitamin B12 deficiency are seen in heavily infected individuals.

Diagnosis is based on finding many typical eggs and empty proglottids in feces (Figure 3). A history of raw fish consumption and residence in an endemic locality is helpful.

Treatment and control Praziquantel is the drug of choice. Freezing for 24 hours, thorough cooking or pickling of fish kills the larvae. Fish reservoirs should be kept free of raw sewage.

3-Hymenolepis nana (dwarf tapeworm)

This is a small tapeworm (20 x 0.7 mm) which infects children. Rodents are the reservoir. Infection is by the oro-fecal mode and, hence, cross infection and auto infection by eggs in feces in normal (figure 6). The worm develops from ingested eggs into an adult in the small intestine and resides there for several weeks (figure 5). Light infections produce vague abdominal disturbances but heavier infections may cause enteritis. Diagnosis is based on finding eggs in the feces. Nicolsamide is the drug of choice. Hygiene is the best control.

Echinococcosis (hydatid)

Echinococcus granulosus and *E. multilocularis* are causative agents of hydatid cysts.

4-Echinococcus granulosus

Epidemiology The organism is common in Asia, Australia, Eastern Africa, southern Spain, southern parts of South America and northern parts of North America. The incidence of human infection about 1 to 2 per 1000 population and may be higher in rural areas of affected regions.

Morphology This is the smallest of all tapeworms (3 to 9 mm long) with only 3 proglottids.

Life cycle The adult worm lives in domestic and wild carnivorous animals. Eggs, passed by infected animals, are ingested by the grazing farm animals or man, localize in different organs and develop into hydatid cysts containing many larvae (proto-scolices or hydatid sand) (Figure 8). When other animals consume infected organs of these animals, proto-scolices escape the cyst, enter the small intestine and develop into adult worms (Figure 7). Echinococcus eggs, when swallowed by man, produce embryos that penetrate the small intestine, enter the circulation and form cysts in liver, lung, bones, and sometimes, brain. The cyst is round and measures 1 to 7 cm in diameter, although it may grow to be 30 cm. The cyst consists of an outer anuclear hyaline cuticula and an inner nucleated germinal layer containing clear yellow fluid. Daughter cysts attach to the germinal layer, although some cysts, known as brood cysts, may have only larvae (hydatid sand). Man is a dead end host.

Symptoms comparable to those of a slowly growing tumor, depend upon the location of the cyst. Large abdominal cysts produce increasing discomfort. Liver cysts cause obstructive jaundice. Peribronchial cysts may produce pulmonary abscesses. Brain cysts produce intracranial pressure and Jacksonian epilepsy. Kidney cysts cause renal dysfunction. The contents of a cyst may produce anaphylactic responses.

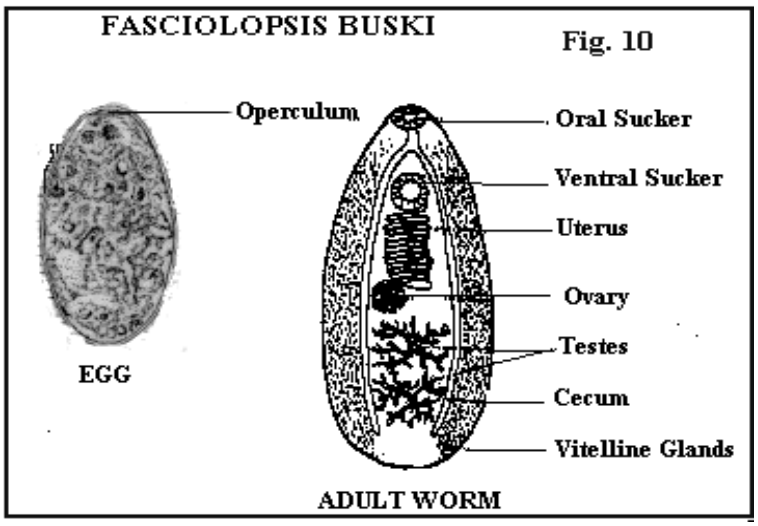
Diagnosis Clinical symptoms of a slow-growing tumor accompanied by eosinophilia are suggestive. Intradermal (Casoni) test with hydatid fluid is useful. Pulmonary cysts and calcified cysts can be visualized using x-rays. Antibodies against hydatid fluid antigens have been detected in a sizable population of infected individuals by ELISA or indirect hemagglutination test.

Treatment and control Treatment involves surgical removal of cyst or inactivation of hydatid sand by injecting the cyst with 10% formalin and its removal within five minutes. It has been claimed that a high dose of Mebendazole results in some success. Preventive measures involve avoiding contact with infected dogs and cats and elimination of their infection.

5-E. multilocularis

This is a tapeworm, similar to *E. granulosus*, that also causes hydatid in northern parts of Asia and North America. It has a very similar morphology and life cycle except that rodents are its intermediate host. Humans, when infected with this worm, also develop hydatid cysts which produce symptoms similar to those caused by *E. granulosus*. However, the cysts are multilocular (many chambers). The organism is resistant to praziquantel; high doses of Albendazole has some anti-parasitic effect. Surgery is the means of removing the cyst. Rodent control is the means of prevention.

INTESTINAL TREMATODES



In comparison with Trematodes of liver and lungs, intestinal Trematodes are less well known, with limited distribution. Human infection with these parasites have been reported from a larger number of countries, and much higher number of species are infecting human and they have wider range of intermediate hosts.

Intestinal Trematode infecting man can be divided according to their methods of transmission into 6 groups which are; fish-borne; snail-borne, mollusc and crustacea-borne; plant-borne; amphibian-borne; insect-borne; and food-borne intestinal Trematodes (WHO, 1994). There are about 70 species of intestinal flukes infecting human. Of those, Twenty-three species belonging to seven families are mostly found in Southeastern Asian countries. The majority of species belongs to the Heterophyidae and Echinostomatidae families (Waikagul, 1991).

The intestinal Trematode which infect human, can be classified based on their morphology into the following 13 families:

1- Family Fascioplidae
2- Family Paramphistomatidae
3- Family Echinostomatidae
4- Family Plagiorchiidae
5- Family Troglotrematidae
6- Family Heterophyidae
7- Family Diplostomidae
8- Family Gastrodiscidae
9- Family Gymnophallidae
10- Family Lecithodendriidae
11- Family Microphallidae
12- Family Strigeidae
13- Family Brachylaimidae

1- **Family Fasciolidae:** The main species is *Fasciolopsis buski*.

1.1-Fasciolopsis buski (LANKESTER 1857) ODHNER 1902

The synonyms of *F.buski* are *Distomum crassum* Busk, 1859; *D.rathouisi* Porier, 1887; Ward, 1903; *F. fulleborni* Rodenwaldt, 1909; *F.yoddardi* Ward, 1910. The giant intestinal fluke *Fasciolopsis buski*, a typical parasite in East Asia, is one of the largest species of Trematodes found in man. It is chiefly found in India, Thailand, China and Formosa Its occurrence is determined by the presence of certain species of snails, which act as its intermediate hosts, and by aquatic plants, to which the cercariae attach themselves and develop into the infective metacercariae.

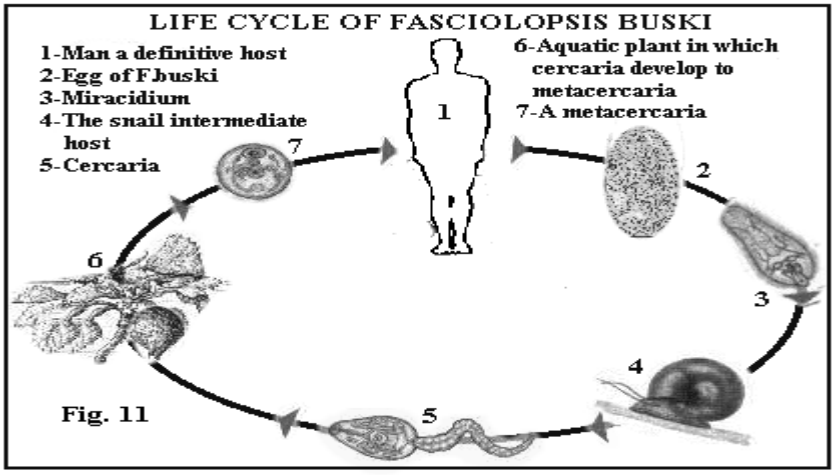


Fig. 11

Morphology: This elongate-oval intestinal fluke is 20 to 75mm in length by eight to 20 mm wide. The cephalic con is absent.

Its surface spines arranged in transverse rows, which are especially dense in the region of the larger ventral sucker. The digestive tract includes pharynx, esophagus and the cecum, which is not branched. Testes are located one behind the other in the posterior half the worm.

The ootype, which is surrounded by Mehlis' glands, is located in the middle of the body of the worm (Fig. 10).

The relatively large eggs (130 -140 microns) are always quite numerous and they are easily found in the stool. They are almost identical with the eggs of *F.hepatica.*

Life cycle: The eggs if are discharge in the water, will develop into miracidium in 3 to 7 weeks. Ciliated larvae (miracidium) emerge from the egg and penetrate into snails of the genera Planorbis, Segmantina, Hippeutis sp. and Gyraulus through the body surface and in these develop into sporocysts. Inside these arise rediae and daughter rediae, which develop into cercariae, which become free and attach themselves to freshwater aquatic plants of the genera Trapa and *Eliocharis tuberosa* and develop into metacercariae. When the fruits of these aquatic plants are shelled out with the teeth, the metacercariae succeed in entering the gastrointestinal tract of man (Fig. 11).

Clinical manifestations: Numerous flukes are often found in a single patient which result in the intensity of the infection. Clinical symptoms appear 1 to 3 months after infection.

Pathological features of infection are inflammation at the sites of attachment of adult worms to the duodenal and jejunal mucosa and resulting ulceration of the mucosa with occasional submucosal and external hemorrhage. Heavy worm burden may cause intestinal obstruction.

Symptoms of intoxication and the sensitization due to metabolic products of the adult worm may develop in severe infections. Most of the infections are asymptomatic. In heavier infections the first symptoms are diarrhea with epigastric pains, simulating a peptic ulcer. Toxic and allergic symptoms are, edema, particularly of the face, abdominal wall, and lower limbs. Blood test shows anaemia and moderate leucocytosis with eosinophilia. Heavy infections due to profound intoxication resulted in mortality among children

in Thailand, India and China (Sadun & Maiphoon, 1953; Beaver et al., 1984).

Diagnosis is made by finding eggs of the helminth in the stool. The peripheral blood count shows slight macrocytic anaemia and moderate leucocytosis with eosinophilia.

Treatment: is by using praziquantel with dose of 40 mg/kg body weight for one or two days.

Epidemiology and geographical distribution: The mode of transmission is closely related to the nutritional customs (feeding habits) of the people of East Asia, who have a liking for the fruits of the water nut which are infested with metacercariae. In addition to pigs, which are important reservoir hosts, dogs and rabbits can be infected; they do not however, play an important part in the transmission of the infection. Different species of snails are served as the intermediate host in various parts of the world. For example, in China and other part of the Far East include Segmantina and Hemiphaerula. Human infection was reported from India (22.4% to 70.1%); Bangladesh (4.7% to 39.2%); China (8.5% to 69.6%); Thailand (10.4% to 17%); Indonesia (23.3% to 27%) and Laos 3.8%. Infection rates as high as 70% are reported from parts of China (Yuan, 1992).

2- **Family Paramphistomaidae Flukes:**

Two helminth species in this group infect human which are: *Watsonius watsoni* and *Fischoederius elongates*

2-1 *Watsonius watsoni* (Conyngham, 1904)

Human infection with this parasite was reported only once at the autopsy of a West African man who died of a severe diarrhea. Several worms were recovered from the intestine, some were free in the lumen of the colon and some attached to the duodenal and jejunal wall. Various species of primates in eastern Asia and Africa are natural hosts of this parasite. The source of the infection is probably vegetation on which the metacercariae have encysted.

2-2 *Fischoederius elongatus* (Poirier, 1883)

The first human infection was reported from Guangdong, China (Huang et al., 1992). The main symptom in the 35-year-old infected women was epigastric pain, which disappeared after she vomited a worm. The worm was identified as Fischoederius elongatus. This parasite of ruminants is probably acquired by ingesting aquatic plants with the encysted metacercariae.

3- Family Echinostomatidae (Poche, 1926):

Echinostome trematodes are mainly intestinal parasites of birds and mammals. More than 12 species of the family were reported infecting human in Asia and Western Pacific. Although no human infection with member of this family was reported from Africa and Europe, an outbreak of infection with echinostoma (echinostomiasis) was reported among 20 American tourists returning from Kenya and Tanzania (Poland et al, 1985). They had abdominal cramps or mild abdominal complaints.
Stool examination of these tourists revealed the presence of eggs resembling those of *Echinostoma sp* in 18 of them. Treatment with praziquantel resulted in parasitological and clinical cure. Several freshwater snails are the first intermediate hosts such as Lymnaea. Radix, Gyraulus, Hippeutis, and Cipangopaludina. The second intermediate hosts of most of the species are freshwater fish, particularly loach (Misgurnus sp.). In a few species, infection is transmitted through snails, and some amphibians.
Clinical manifestations: The main pathology of echinostomiasis or infection with species of Echinostoma is due to attachment of flukes to the mucosa of the small intestine causing inflammatory lesions. Heavy infections may produce sever focal necrosis in the intestinal mucosa. The sever lesion may cause destruction of villi and loss of mucosal integrity (Chai & Lee, 1990). Although morbidity due to infections is limited, and most persons are asymptomatic, in heavy infections there are clinical manifestations such as flatulence, intestinal colic, and loose bowel movements. Other symptoms reported were diarrhea, abdominal pain, anaemia, and oedema, which are more sever in children. Some fatal cases were reported either due to extremely heavy infection, particularly in children, or due to ectopic localization of the parasites in various developmental stages in the important organs, such as brain and heart. Members of this family can be divided according to the method of transmission into two groups. Members of the first group are fish-born and second group are snail, mollusc and crustacea-borne intestinal Trematodes. Members of this family which infect human by ingestion of raw or not properly cooked fish are as follows:

3-1 *Echinostoma hortense* Asada, 1926:

Human infection with this species was reported from Korea (Hong et al, 1994; Chai & Lee, 1990) and China (Fan & Sun, 1989). The infection rate was 22.4%, found among residents of one area in southern Korea. Also in northeast China, 6 out of 10 hospitalized hepatitis patients who had eaten raw loach fish were found infected by *Echinostoma hortense* (Chen et al, 1993). The parasite was found postmortem at autopsy in rural areas of Java, Indonesia. The second intermediate hosts are fish (especially *Misgurnus anguillicaudatus*) and freshwater snails of the Radix, Physa, Planorbis and Lymnaea sp (Chai & Lee, 1990). Dog and *Rattus norvegicus* are the other definitive hosts.

3-2 *Echinostoma cinetorchis* Ando and Ozaki, 1923

This parasite was reported from people in Japan, Taiwan and Korea (Seo et al, 1980a). Its first intermediate host snails are *Hippeutis cantori*, and *Segmentina nitidella*; and two species of Cipangopaludina snails have been reported to serve as its second intermediate host in Korea (Chung and Jung 1999).

3-3 *Echinoparyphium paraulum* Dietz, 1909

This Trematode is Probably a synonym of *Echinostoma revolutum* is a natural parasite of ducks, geese, swans, and doves. The first human infection was described in the former USSR. Human infections were also found in Yunnan, China (Shen, 1979) .

3-4 *Episthmium caninum* (Verma, 1935) Yamaguti, 1958

Three human cases were reported from northeast Thailand. Definitive host is dog. The sources of infection are freshwater fish (Radomyos et al, 1991).

3-5 *Echinochasmus perfoliatus* (Ratz, 1908) Dietz, 1910

It is a common parasite of the small intestine of dogs and cats in Hungary, Italy, Romania,former USSR, and the Far East. Human infection was reported from parts of China and about 14 000 worms were found in autopsy of a child who died from the infection. The infection rate in this area was 1.84% (Anonymous, 1979; Wang et al., 1979).

3-6 *Echinochasmus Japonicus* Tanabe, 1926

Human infections with this parasite have been reported from some provinces in China and in Korea (Lin et al., 1985; Chai & Lee, 1990). The human source of infection includes 18 species of fish when eaten raw or improperly cooked fish. The rate of infection in one study in China was 4.9% among people, 39.7% among dogs and 9.5% among cats (Lin et al., 1985).

3-7 *Echinochasmus Iiliputanus* (Looss, 1896) Odhner, 1910

Natural hosts are dog, cat, fox and badger (Xiao et al., 1992). Human infection with this worm was first reported in 1991 from Hexian, Anhui province of China. The prevalence among 2426 persons was 13.4%. In a nationwide survey on parasitic infection in China during 1988-1992, human infection with *Echinochasmus liliputanus* for the first time was reported (Yu et al, 1994).

3-8 *Echinochasmus jiufoensis* sp. nov.

This new species which is closely allied to *Echinochasmus beleocephalus* Dietz 1909 was discovered from the intestine of a six month old girl who died from pneumonia and dehydration in China (Liang & Ke, 1988).

3-9 *Echinochasmus fujianensis* sp. nov.

Dogs, cat, pig, and Rattus species are natural definitive hosts (Cheng et al., 1993).
This new species of Trematode was reported from inhabitants in 5 cities of southern Fujian Province, of China. The average prevalence among inhabitants was 3.2% mostly in the 3-15 year age group. In a nationwide survey on parasitic infection in China during 1988-1992, human infection with *Echinochasmus fujianensis* was for the first time reported (Yu et al, 1994).

3-10 *Echinostoma angustitestis* sp nov.

Two human infections with this parasite were reported in Fujian, China (Wang, 1977; Cheng et al, 1992). In a nationwide survey on parasitic infection in China during 1988-1992, human infection with Echinostoma angustitestis was for the first time reported (Yu et al, 1994). Members of the family Echinostomatidae, which are among the smallest intestinal trematodes of man, are transmitted by snail, mollusc and crustacea:

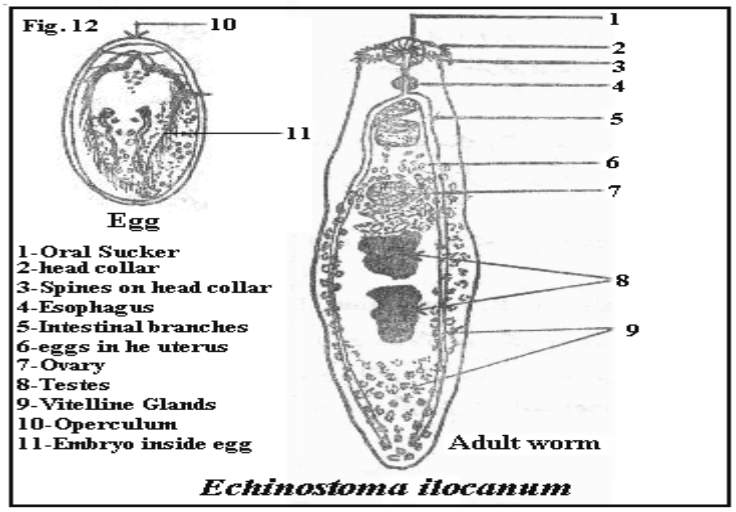
Morphology: These flukes live in the small intestine and are covered with numerous small spines, which are arranged in transverse rows and begin immediately behind the so-called head-collar. In the middle of this is the oral sucker; the larger ventral sucker is located shortly behind the bifurcation of the intestine.

Life Cycle: The eggs (about 95x65 microns) pass out in the faeces. Within 6-15 days a miracidium develops in the egg and this emerges into the water and actively penetrates into the first intermediate host, which is a snail of the genus Gyraulus. In the region of the digestive gland of the snail, it develops into a sporocyst, then into rediae and finally into cercariae. The cercariae become free and seek for snail hosts, especially those of the species of Pila and bivalves of the genus Corbicula, which is the daily food of the native population in some areas. Inside these snails, the cercariae become metacercariae. In the intestine of the final host such as man, pigs, dogs, cats and monkeys, the metacercariae develop into sexually mature flukes, which are found mostly in the region of the jejunum.
Clinical symptoms: Most of the infected people are asymptomatic: Attachment of the flukes to the mucosa of the small intestine, results in inflammatory lesions. Heavy infections may cause focal necrosis. Main symptoms are mild abdominal pain, diarrhea and also signs of a general intoxication, such as, headaches and anaemia. These symptoms appear specially when massive infections are present and mostly among children. High eosinophilia (up to 38%)often appears. A Chinese parasitologist who voluntarily swallowed 113 metacercariae of *E.japonicus*, developed abdominal pain, intestinal gurgling and diarrhea about ten days after infection (Lin et al., 1985).

Treatment: A single dose of 150mg of Levamisole has produced cure among patients.

Epidemiology: Infection with Echinostoma occurs only by eating the flesh of uncooked snails or bivalves that contains the metacercariae. The native population often eats these mollusks prepared as a kind of salad dressing.

Dogs, cats and rats are natural reservoir hosts. The10 species of Trematodes in this group that infect human are as follows.



3-11 *Echinostoma ilocanum* (GARRISON 1908)

Synonyms: *Fascioletra ilocanum* and *Euparyphium ilocanum*.
Human infection was first reported from Luzon, Mindanao and Leyte in the Philippines.The prevalence found during surveys made between 1967 and 1972, ranged from 1% to 44%(Cross & Basaca-Sevilla, 1981).
The adult worm is 3 to 6.5 mm long and is covered with spines in most parts of the cuticular body (Fig. 12A). The operculated eggs which are about 90 microns, pass outside in the feces.

Miracidium is released in the water and will continue its development inside the first snail host of the species Gyraulus to cercaria which penetrate into large snail of Pila species, which its consumption by man results in human infection (Fig. 12 B). Most of the infections are asymptomatic but in few cases abdominal discomfort, diarrhea and high blood eosinophilia occurs. Diagnosis is made by finding eggs in the faeces. Treatment of cases among residents of an area in northeast Thailand with praziquantel for opisthorchiasis resulted in high cure rate (Radomyos et al, 1982).

Human infections have also been reported in Yunnan, China, the Celebes, Java, and Indonesia. The first intermediate hosts were planorbid snails, Gyraulus or Hippeutis sp and the second hosts are different freshwater mollusks such as *Pila conica*, *Viviparus javanicus* and *Lymnaea rubiginosa* brevis. Human infection is acquired by eating rawsnails harboring encysted metacercariae

3-12 *Echinostoma lindoense* Sandground and Bonne, 1940

This species is morphologically resembles *E.revolutum.* Heavy infection with this worm was found among the ethnic groups in the central Celebes, Indonesia, between 1937 and 1956. The prevalence as high as 96% reported in some villages (Harinasuta, 1974). Since1970s, due to changes in eating habits, and almost extinction of Corbicula, a mussel that is the main source of infection, human infection has disappeared(Carney et al, 1974 and Carney et al, 1980).

3-13 *Paryphostomum sufrartyfex* (Lane, 1872) Bhalerao, 1931

Synonyms: *Artvfechinostomum sufrartyfex* Lane, 1915; *Euparyphium malayanum* Leiper, 1911; Scanning electron microscopy of the tegumental surface of this worm the revealed the presence of double rows of spines in the collar (Roy and Tandon 1996).

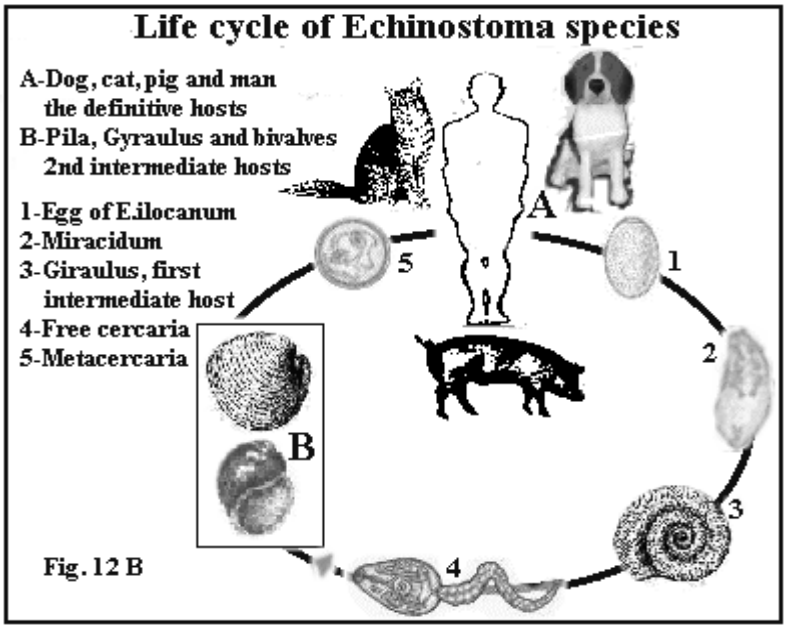


Fig. 12 B

3-14 *Echinostoma sufrartyfex* Lane, 1915.

Infection with this parasite was first observed in an Assamese girl, then found in pigs in India (Beaver et al, 1984). According to Lie Kian Joe (1963), this species is synonymous with *Echinostoma malayanum*. The source of infection is *Digoniostoma pulchella* (Harinasuta et al., 1987).

3-15 *Echinostoma malayanum* Leiper, 1911

Human infections were reported from Singapore and Kuala Lumpur in 1911, Chiangmai, Thailand in 1915, then from Sumatra, Indonesia. It is endemic in northeast and northern Thailand, northern Luzon of the Philippines. In 1993, Maji et al reported the first human infection with *Echinostoma malayanum* in India.

3-16 *Echinostoma macrorchis* Ando and Ozaki, 1923

Originally reported from Japan, it was described together with E. cinetorchis by Beaver (1984). Snail host are *Cipangopaludina malleata, C. japonica, Segmentina nitidella*, and *Viviparus malleatus.*

3-17 *Echinostoma revolutum* (Froelish, 1802)

Looss, 1899 Synonym : *Echinostoma echinatum* Zeder, 1803 .

The first human case with this parasite was reported from a Taiwanese woman. The infection rate found in Taiwan estimated to be between 2.8% and 6.55%. Human infections were also reported from Yunnan and Guangdong, of China, in northeast Thailand and Indonesia (Beaver et al., 1984; Shen, 1979). The first intermediate snail hosts are: species of *Lymnaea*, *Physa*, *Heliosoma*, *Segmentina* and *Paludina.* Second hosts, which are the source of infection, are mollusc (Physa and *Lymnaea* species) or tadpole and clam (Corbicula producta in Taiwan).

3-18 *Hypoderaeum conoideum* (Block, 1872) Dietz, 1909

This is a common human parasite in northeast Thailand, where 55% of 254 persons examined were found to be infected (Harinasuta et al., 1987). It is usually a parasite of birds including duck, goose, and fowl.

3-19 *Artyfechinostomum mehrail*

which is a probable synonym of *Paryphostomum sufrartyfex*.

Human infection with this parasite was reported twice from India. Several hundred worms were found upon autopsy of the first case who died from malnutrition and anemia caused by the infection. The source of infection is ingestion of raw snails *Indoplanorbis exustus*.

3-20 *Himasthla muehlensi* Vogel, 1933

The adult worms were obtained from a German who had lived in Colombia for 6 years but believed he acquired the infection from eating raw clams in New York City (WHO, 1994).

4-FAMILY PLAGIORCHIIDAE

The second intermediate-hosts in this group of Trematodes are insects and cercariae emerging from snails must go through insects to become infective. Four species in this group i.e. *Plagiorchis philippinensis, P.muris, P.javensis* and *P.harinasutai* were found in human. Species of Plagiorchis occur in the intestine of many species of vertebrate hosts.

4-1 *Plagiorchis philippinensis* Sandground, 1940

Adult worm of this Trematode was recovered during autopsy of a local resident from Ilocano region of the Philippines. The main definitive hosts are birds and rats.

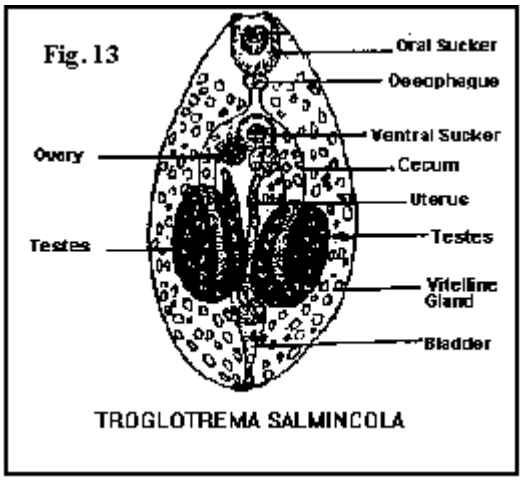
4-2 *Plagiorchis muris* Tanabe, 1921

This parasite was reported in a Japanese patient who was under treatment for heavy *M. yokogawai* infection. Another case was reported from Republic of Korea (Hong et al, 1966). The freshwater snail, Lymnaea sp. is both the first and second intermediate host; certain insects (*Chironomus dorsalis* and *Anisogam marus annandalei*) also serve as second intermediate hosts, and birds, dog, and rat as reservoirs.

Life cycle of the parasite has been experimentally established by giving metacercariae obtained from naturally infected insect albino rats. Mature flukes were found in the lower part of the small intestine on day 5 after infection with the peak of egg production occurring on day one (Hong et al, 1998).

4-3 *Plagiorchis javensis* Sandground,1940

Human infection was reported from Indonesia. The worm was recovered at postmortem of the patient who was also heavily infected with *E. ilocanum*. Later, two other cases were reported from Indonesia.



4-4 *Plagiorchis harinasutai* n. sp.

The first human infection with *Plagiorchis harinasutai* was found in Thailand. The parasite was recovered from four patients who were treated for opisthorchiasis using praziquantel (Radomyos et al, 1989). In one study in Thailand the infection rate for *Plagiorchis harinasutai* was 0.7% (Radomyos et al, 1994).

5- FAMILY TROGLOTREMATIDAE (NANOPHYETIDAE)

There are one species and one subspecies in this family that infect human. These species are synonyms of nanophyes species of family Nanophyetidae. Human infection occurs by ingestion of the metacercariae in raw salmon (Salmo sp, Oncorhynchus sp, Brachymystax sp, Coregonus sp,) and non-salmonid fishes.

5-1 *Troglotrema salmincola* (CHAPIN, 1926)WITENBERG 1932

This species is synonyms of *Nanophyes salmincola* Chapin, 1926 (of the family Heterophyidae) Beaver (1984) believed that *N. salmincola* was a synonym of *Troglotrema salmincola* (Fig. 13). A new species of Nanophyetus was described by Skrjabin and Podjapolskaja (1931), named *Nanophyetus schikhobalowi* recovered from residents of far eastern Siberia. This species was later relegated by Gebhardt et al (1966) and Filimonova (1966, 1968) to subspecies status, *Nanophyetus salmincola schikhobalowi* (cited from Milleman & Knapp, 1970). This helminth lives in the intestine of dog and fox. it is one to 2 by 0.3 to 0.5 mm. Its first host is snail and the second species of fish. Human infection has been reported from Khabarovsk in Russia with rate of infection reaching up to 60% in some areas (Iarotski and Pirokovskaya, 1993). The prevalence of infection was 16% and 5% in north Sakhalin in 1970, and the population at risk was estimated to be more than 4 millions (larotsky & Pirogovskaya, 1993). Twenty human cases were reported in the United States from 1974 to 1986 (Eastburn et al, 1987).

Nanophyetus salmincola infection (nanophyetiasis) a zoonotic disease was found again among 10 cases in the coastal US Pacific Northwest and considered endemic in North America (Fritsche et al, 1989). Of ten cases found from the same geographic area, 9 had histories of ingestion of not properly cooked fish. The main symptoms were, histories of gastrointestinal complaints in 5 and high eosinophilia in another 5 patients. Treatment of 9 patients with praziquantel resulted in resolution of symptoms and absence of eggs of Nanophyetus (Fritsche et al, 1989). A case of human infection with *Nanophyetus salmincola* (nanophyetiasis) has occurred as the result of handling of salmon (*Oncorhynchus kisutch*) highly infected with metacercariae of the parasite.

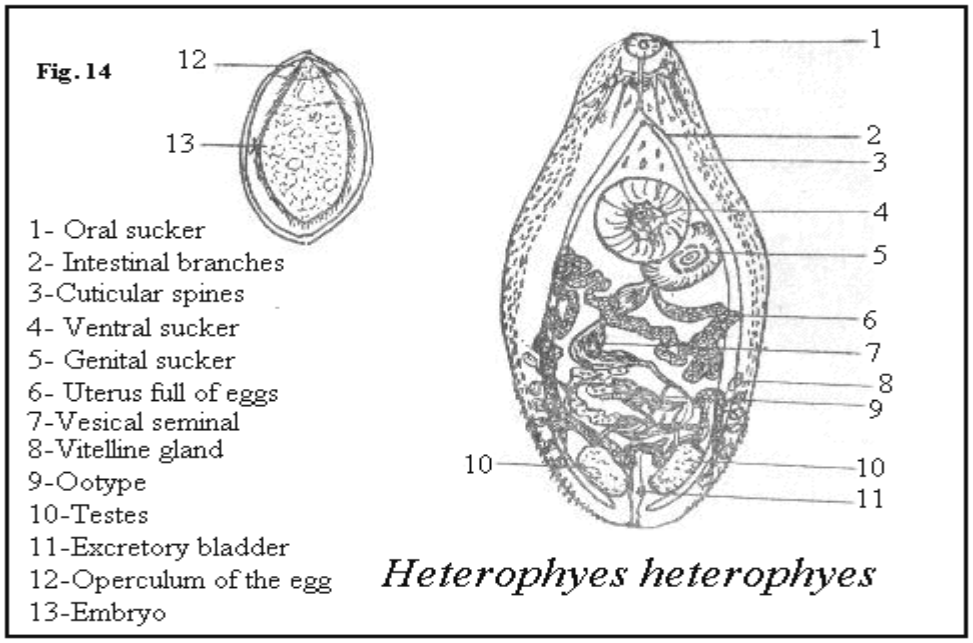
Symptoms were; abdominal discomfort, chronic diarrhea, nausea, and a blood eosinophilia of 43%. Infection was confirmed by finding eggs of the parasite in a stool specimen (Harrell and Deardorff 1990).

Although infection with this fluke itself is probably not clinically important, its role in the transmission of rickettsial organism, *Neorickettsia helminthoeca*, which cause a serious and often fatal systemic infection known as "salmon poisoning" in d dogs, foxes and coyotes, make it an important parasite. This pathogen has not been associated with human disease. Subspecies *N. salmincola schokhobalowi* apparently is not a vector for the rickettsial organism

(Milleman & Knapp, 1970).

6- FAMILY HETEROPHYIDAE

The human infections have been reported with 30 species of these flukes, but the two main species in this group which infect man are: *Heterophyes heterophyes* and *Metagonimus yokogawai*. Members of this family are small or minute intestinal flukes usually parasites of birds and mammals. Pathological changes due to presence of these species are atrophy of villi and crypt hyperplasia in the middle section of the small intestine. The inflammatory cell infiltration may cause shallow ulcers and mild irritation. Clinical manifestations are dyspepsia, mild, intermittent and mucous diarrhea, abdominal pain, colicky pain, high eosinophilia, and lethargy. Factors contributing to severity of the infection are; worm burden, depth of their penetration into the wall, and susceptibility or resistance of the patient. However, in some cases high number of worms was tolerated without any sever symptom. For example, a Korean man who harbored as many as 63,587 worms at one time, complained only of minor symptoms such as epigastric pain, and mild indigestion (Chai & Lee, 1990). More sever symptoms may be manifested if eggs are infiltrated in vital organs such as the intestinal capillaries and Iymphatic, the myocardium, brain, and spinal cord. These types of pathological changes were described for *H.heterophyes* and *M.yokogawai* (Tantachamrun & Kliks, 1978).
Eggs of *Heterophye*s species were discovered in cardiac lesions of a patient who died of cardiac failure. Eggs of Haplorchis pumilio found in sections of the spinal cord and lesions caused were responsible for the loss of motor and sensory function (WHO, 1994). In the Philippines, workers believe that infiltration of eggs of the parasite through the intestinal wall, mesenteric lymphatics, and eventually into the cardiac valves and myocardium, cause myocarditis leading to cardiac failure, or cerebral lesions. They estimated that up to 15% of fatal heart disease in the Philippines might result from heterophyid myocarditis. Adult *H. heterophyes* and their encapsulated eggs have also been demonstrated or in the brain causing neurological manifestations (Zhang & Fan, 1990).



6-1-*Heterophyes heterophyes* (Von Sibold,1851)

Stiles & Hassal 1900 Synonyms: *Distoma heterophyes* v. Siebold, 1852; *Heterophyes aegyptiaca* Cobbold, 1866; *Heterophyes nocens* Onji and Nishio, 1915.

Infection with this parasite, which was first discovered by Bilharz in 1851 at autopsy of an Egyptian in Cairo, is found in the Far East and in Tunisia, Egypt, Turkey, Iran, and India. Adult worm is 1 to 1.7 mm by 0.3 0.4 mm to and is covered by fine spines. The ventral sucker is 230 microns much larger than the oral sucker, which is about 90 microns. Eggs are 20 to 30 by15 to 70 microns and have mature miracidium when are found in the feces of the host (Fig. 14). The main character of this worm is the presence of a genital sucker in addition to two other suckers.

Life cycle: Miracidium emerges from the eggs in water and penetrate into snails of the genera Pirenella and Cerithidae into which it develops to metacercaria which infect fish, and man become infected by ingestion of uncooked flesh of infected fish. The snail intermediate host are. *Pironella conica* in Egypt and *Cerithida cingulata* in Japan. The main fish hosts are: *Mugil cephalus, Tilapia nilotica, Aphanius fasciatus* and *Acanthogobius sp*. In addition to the human infection, heterophyes infects a number of other mammals.

Clinical manifestations: Adult worm in the jejunum of the host causes inflammation and ulcer, resulting in abdominal pain and diarrhea. In a few cases penetration of the worm into the tissue of the intestine results in liberation of the eggs in lymph nodes from where they may migrate to the heart and brain causing sever cardiac and nervous symptoms.

Diagnosis: is made by finding eggs in the feaces.

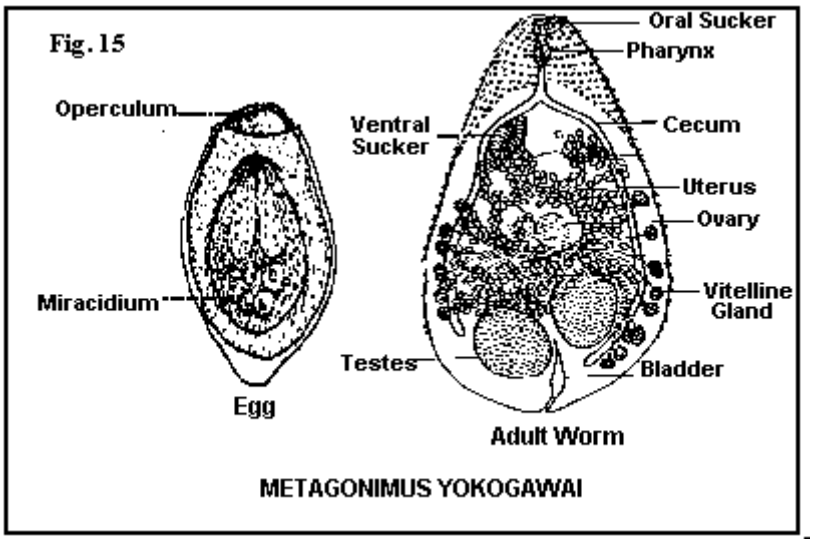
Treatment Praziquantel is the drug of choice for this parasite. Prevention is possible by avoiding consumption of not properly cooked fish.

Epidemiology: The life span of the worm is normally short and is about 6 to 8 weeks. metacercaria can live in the body of salted fish as long as seven days. The number of Heterophyes worm is very high in the body of the host reaching sometimes to 4000. Infection rate is also high in some infested areas such as in Port Said in Egypt where 88% of school children were previously infected (Watson, 1960). Lower infection rates were reported among residents of 5 governorates in Egypt. In Dakahlia Governorate of Egypt the disease is common in both urban and rural localities due to the habit of consuming recently salted or insufficiently baked infected fish (Sheir & Aboul-Enein, 1970). In Iran, Massoud et al (1981) found heterophyid infections in residents of villages in Khuzestan. The mean prevalence in the villages was 8% (range 2-24%). Infection with these flukes were also found in 33.3% of foxes, 14.2% of jackals, 2.5% of dogs (Massoud et al, 1981). In Asia, several foci of infection have been reported in Korea, Thailand, Japan, and southern China including Taiwan. Human infections were reported from Korea, the Philippines, and Indonesia. The third human infection by Heterophyopsis continua was reported in Korea in 1996 (Hong et al, 1996). In Korea, Chai et al (1998a) reported presence of two endemic foci of human infection in western coastal areas. Human infections have also been reported from the provinces of Guangdong, Hubei, Beijing and Taiwan, China (Xu & Li, 1979). While infection with this parasite was found in dogs in India (Beaver et al., 1984; Harinasuta et al., 1987),
two cases of human infection with heterophydes was also reported from Assam, India (Mahanta Mahanta et al, 1995).

6-2-*Metagonimus yokogawai* (KATSURADA, 1913)

Synonyms: *Losotrema ovatum* Kobayashi, 1912; *Metagonimus ovatus* Yokogawa, 1913; *Loossia romanica* Ciurea, 1915. Human infection with this helminth is found in the Far East, parts of former Soviet Union, Siberia, in Spain and Israel.

Morphology: The larvae of *Metagonimus yokogawai* was for the first time isolated by Yokogawa in 1911 from experimentally infected dogs and the adult worm from a Taiwanese man. Adult worm is 1.5 by 0.6 mm. Eggs are 27 by 16 microns.



It is very similar to *H.heterphyes* except that in Metagonimus ventral sucker is deviated to the right side of the body (Fig. 15). Eggs are 27 by 16 microns and has a distinct operculum.

Life Cycle is similar to that of *H.heterophyes*. The snail hosts are Thiara and Semisulcospira species. The miracidium in the body of snails will develop to a sporocyst and then redia and Then become free cercariae. Cercariae enters the body of fish and develop into metacerceriae . The source of human is eating raw or undercooked freshwater fish (*Plecoglossus altivelis, Tribolodon taczanowskii, Odontobutis obscurus*, etc). Most other fish-eating mammals including dog, cat, pig are reservoir hosts of the infection. The main clinical symptoms are abdominal pain and diarrhea and lethargy. The extend of severity of symptoms depend on the number of worm present. But in Korea where the infection is widespread, in one case although 63587 worms were found, the only complain were mild indigestion and gastric pain (Chai and Lee, 1990). Treatment: praziquantel with a dose of 25mg/Kg is very effective on the infection.
Epidemiology: Human infection with this parasite is probably the most common intestinal fluke infecting man in the Far East. In the Republic of Korea, metagonimiasis is endemic in southern coastal areas. The infection rates in infested villages were between 10 and 20% or higher (Chai & Lee, 1990). Human infection has also been reported from some provinces in China (Xu & Li, 1979). It is also endemic in Japan, and Indonesia. In one

study in Thailand, the infection rate for *H. yokogawai* was 2.9% (Radomyos et al, 1994). In the Russian Federation, the prevalence of infection was between 1 and 2 % in the endemic areas of the Amur and Ussuri valleys of Khabarovsk territory.
In the northeast of the territory, the prevalence in the ethnic minority groups was between 20% and 70%. Metagonimiasis is also endemic In the north of Sakhalin Island. The population at risk is estimated to be 859 000, or 14.7% of the total population (Iarotsky & Pirogovskaya, 1993). In addition to species of heterophyid flukes described, there are several other species of the family, which infect human. Included are 3 other species of genus Heterophyes; 6 species of genus Centrocestus; 6 species of genus Haplorchis; 3 species of Dichrochiterma; 2 species of Metagonimus; and 2 species of Procerovum.

6-3 *Heterophyes nocens* Onji and Nishio, 1916

Some parasitologists consider this species as a subspecies named *H.heterophyes nocens*. The second intermediate hosts are fish (Chaenogobius sp, Acanthogobius sp, and *Mugil cephalus*). Dogs and cats are the mammalian reservoirs. The prevalence of human infection in one Korean island was 43%. It has also been reported in Japan (Seo et al., 1980b; Chai & Lee, 1990). In a small village in Korea Chai et al (1997) found75.0% infected with heterophyid mostly with *Heterophyes nocens*. Based on finding a high endemic focus of the infection in coastal areas of Korea, Chai et al (1994) speculated that *H.nocens* might be widely distributed along the southwestern coastal areas where the brackish water fish hosts are popularly eaten raw. In another study in Korea, clinical symptoms found were gastrointestinal disorders such as epigastric pain, indigestion, severe diarrhea, abdominal pain, and vomiting (Ryang et al, 1999). The prevalence of infection was 17.6% and 18.8% in these foci respectively. *Heterophyes nocens* was collected from all of the 22 patients treated in one village (Chai et al, 1998b).

6-4 *Heterophyes dispar* Looss, 1902

It was first isolated from dogs and cats in Egypt. Other carnivorous mammals including foxes and wolves in north Africa and the Eastern Mediterranean were also found infected. Human infections were reported in Korea and Thailand. The second intermediate hosts are fish; Tilapia, Mugil, Epinephelus, Barbus and Lichia (Chai & Lee, 1990; Kaewkes et al., 1992).

6-5 *Heterophyes katsuradai* Ozaki et Asada, 1926

Human infection was reported from Japan (Cross, 1974). Dog is the definitive host.

6-6 *Centrocestus armatus* Tanabe, 1922

Human infection is reported from Korea. Source of infection is fish and reservoirs of the parasite are dog, cat, and rabbit (Chai & Lee, 1990).

6-7 *Centrocestus formosanus* Nishigori, 1924

Studies on the morphology of *Centrocestus formosanus* using scanning electron microscopy, have indicated the followings: The surfaces of the metacercariae and adult worms were closely similar. The body surface was covered with scale-like spines of varying sizes. The oral sucker was surrounded by a circumoral expansion with two rows of 32 spines (Srisawangwong et al, 1997). Human infection: In a nationwide survey on parasitic infection in China during 1988-1992, human infection with *Centrocestus formosanus* was for the first time reported (Yu et al, 1994). Infection was also reported from Taiwan and the Philippines. Source of infection: freshwater fish and frogs and reservoirs hosts are rat, cat, dog, chicken, duck (Harinasuta et al., 1987; Yamaguti, 1958).

6-8 *Centrocestus cuspidatus* Looss, 1896

Human infection was reported from Egypt and Taiwan. The source of infection was freshwater fish.

6-9 *Centrocestus caninus* Leiper, 1912

Human infection of *Centrocestus caninus* is found in Thailand and Taiwan (Waikagul et al, 1997). The worms had 26-30 spines arranging in two rows around the oral sucker resembling *C. caninus* (Waikagul et al, 1997). Human reservoirs are dog, cat and rat, and the source of infection are fish (*Channa formosana, Cyprinus carpio,* and *C.auratus*) and frog.

6-10 *Centrocestus kurokawai* Kurokawa, 1935.

Human infection was reported from Japan. Source of infection: freshwater fish.

6-11 *Centrocestus longus* Onji and Nishio, 1916

Probably a synonym of *C.caninus*. Human infection is found in Taiwan.

6-12 *Haplorchis pumilio* Looss, 1896

Synonym: *Monorchotrema taihokui* Nishigori, 1924; Haplorchis taihokui Nishigori, 1924.

Morphology of the worm was described in a study using scanning electron microscopy (Srisawangwonk et al, 1989). Human infection was reported from the Philippines, China, Taiwan and Egypt. In Thailand in 1983 *H.pumilio* adults were found in the faeces of 12 cases of 411 patients who were treated for opisthorchiasis with praziquantel (Radomyos et al., 1983; Xu & Li, 1979). In another study in Thailand the infection rate for *H. pumilio* was 6.2%(Radomyos et al, 1994). Sources of infection are freshwater fish and reservoirs hosts are dog, cat and night heron.

6-13 *Haplorchis yokogawai* Katsuta, 1932

Human infection is found in Thailand, Indonesia, the Philippines, and south China including Taiwan. Source of infection are mullet and shrimp, and reservoirs are dog and cat.

6-14 *Haplorchis pleurolophocerca* Sonsino, 1896

Human infection was reported from Egypt. Source of infection: Gambosia affinis. Reservoir: cat.

6-15 *Haplorchis taichui Nishigori,* 1924

Human infection was found in Bangladesh, Thailand, the Philippines, Laos, and Taiwan. Source of infection are freshwater fish. Reservoirs: dog, cat (Kuntz, 1960; Harinasuta et al., 1987; Giboda et al., 1991). In another study in Thailand the infection rate for *Haplorchis taichui* was 7.8% (Radomyos et al, 1994). By using scanning and transmission electron microscopy, Scholz et al (1991) found differences in
the body surface of 2 heterophyid flukes, i.e. *Haplorchis yokogawai* and *H.taichui*.

6-16 *Haplorchis microrchis* Matsuda, 1932

Human infection was reported from Japan.

6-17 *Haplorchis vanissimus* Africa, 1938

Human infection found in the Philippines.

6-18 *Diorchitrema formosanum* Katsuta, 1932

is considered a synonym of Stellantchasmus (Yamaguti , 1958). Human infection: Taiwan. Source of infection: Mugil sp. Reservoirs: cat and rat.

6-19 *Diorchitrema amplicaecale* Katsuta, 1932

Synonym: *Stellantchasmus amplicaecale*. Human infection was reported from Taiwan. Source of infection are Mugil sp. Reservoirsare dog, cat and rat.

6-20 *Diorchitrema pseudocirratum* Witenberg, 1929

Human infections reported from Hawaii and the Philippines. Reservoirs are dog and cat.

6-21 *Heterophyopsis continua* (Onji and Nishio, 1916)

Yamaguti, 1958 Synonym: probably *Heterophyopsis expectans* Africa and Garcia, 1935, Human infection is reported from the Philippines, Korea, Japan, and China. Source of infection are *Mugil cephalus, Mugil affinis, Larus argentatus,* and *Cyprinus carpio*. Reservoirs: cat, experimentally dog and chick (Chai & Lee, 1990; Yamaguti, 1958).

6-22 *Metagonimus takahashii* Suzuki, 1930

Human infections were reported from Korea (Hong et al, 1996) Furthermore, the presence of Human infection with *M.miyata* in two coastal villages of Korea was confirmed (Chai et al, 1998). The Source of infection: *Cyprinus carpio, Carassius carassius*, and *C.auratus*.

6-23 *Metagonimus minutus* Katsuta, 1932

Human infection: Taiwan. Source of infection: mullet. Reservoirs: cat.

6-24 *Stellantchasmus falcatus* Onji and Nishio, 1916

Human infection is found in the Philippines, Hawaii, Japan, Thailand and Korea (Tantachamrun & Kliks, 1978; Radomyos et al., 1990; Chai & Lee, 1990). In addition, *Stellantchasmus falcatus* has been reported from a 33 year old male residing in Seoul Korea. This is the 4th human case of infection with this worm in Korea (Sohu et al, 1989). In one study in Thailand the infection rate for *Stellantchasmus falcatus* was 0.3% (Radomyos et al, 1994). Source of infection: Mugil cephalus. Reservoirs: cat, dog, birds.

6-25 *Stictodora fuscatum* (Onji and Nishio, 1916)

Yamaguti, 1958 Human infection was reported from Korea. The first case was a young Korean, subsequently 13 more cases were found in a seashore village of Korea (Chai & Lee, 1990). More cases were later reported by Chai et al (1997). Source of infection: Metacercariae were found in *Pseudorasbora parva*.

6-26 *Procerovum varium* Onji and Nishio, 1916

Human infection reported from Japan..

6-27 *Procerovum calderoni* Africa and Garcia, 1935

Human infection: Philippines, China, Africa (Cross, 1974; Harinasuta et al., 1987). Source of infection: *Ophiocephalus striatus, Glossogobius giurus* and Creisson sp, Mugil sp. Reservoirs: cat, dog.

6- 28 *Pygidiopsis summa* Onji and Nishio, 1916

Human infection was reported from Japan and Korea (seashore villages) (Seo et al., 1981; Chai & Lee, 1990). Source of infection are *Mugil cephalus, Liza menada*, and *Acanthogobius flavimanus*. Reservoirs: cat naturally, rat and mouse experimentally. In a survey for parasitic infections new cases of *Pygidiopsis summa* was detected (Chai et al, 1997).

6-29 Phagicola sp.

Morphology of *Phagicola* species has been recently redescribed on the basis of examination of type specimens from *Pithecophaga jeffreyi*, from the Philippines (Scholz, 1999). The genus Phagicola is the parasite of fish-eating birds or mammals. First report of human infection with phagicola sp. in Brazil was reported by Chieffi et al in 1990. Patient was a 31 year-old woman who, in 1987, stayed several months in the municipality of Cananeia in Brazil. Her main complain was intestinal pain and was successfully treated by praziquantel (Chieffi et al, 1990a). In 1988, nine cases of human parasitism by *Phagicola s*p. were diagnosed in the municipality of Registro (Sao Paulo State, Brazil) by stool examinations, in patients who ate raw mullet (Mugil sp.). Clinical symptoms were; flatulence, diarrhoeal episodes; and slight eosinophilia (Chieffi et al, 1992). Eggs of the parasite were found in only one out of sixty-one dog examined. The source of infection is freshwater fish (Mugil sp.).

6-30 *Appophalus donicus* (Skrj. and Lindtrop, 1919)

Price, 1931 Synonyms: *Rossicotrema donicus* Skrj. and Lindtrop, *Cotylophallus venustus* Ransom, 1920, and *C.similis* Ransom, 1920. Human infection was reported from USA. Source of infection are fish and reservoirs of infection are dog, cat, rat, fox and rabbit (Harinasuta et al., 1987; Yamaguti, 1958).

6-31 *Cryptocotyle lingua* (Crepl., 1825)

Fischoeder, 1903 Human infection: Greenland. Source of infection: Gobius ruthensparri, Labrus bergylta. Reservoirs are, cat, dog and rat (Harinasuta et al., 1987; Yamaguti, 1958).

7- FAMILY DIPLOSTOMIDAE

The adult worms of the Diplostomidae are parasites of the intestine of birds and mammals. The two species of Alaria americana and Fibricola seoulensis are reported to infect humans. Morphological features of cercariae of the genus Diplostomum of this family were studied and described by Shigin in 1996.

7-1 *Alaria americana* Hall et Wigdor, 1918

The adult species of Alaria resides in the intestine of wild carnivores such as wolves, foxes and raccoons. Miracidia, after emerging from eggs in the water, penetrate the body of freshwater snails of the genus Helisoma and develop to cercariae, which infect frog tadpoles. The first human infection with Alaria spp. was reported under the name of mesocercaria (non-encysted free parasite forms in the tissue) in 1966 in United States. Patient, a 38-year-old man developed bronchospasms shortly after returning from a hunting trip. One year later, examination of an excised subcutaneous nodule demonstrated infection with a mesocercaria and the worm was identified as Alaria spp. or Strigea spp. most probably, source of infection was eating undercooked wild goose (Kramer et al, 1996).
Two cases of human intraocular infection with mesocercariae of Alaria were found in two Asian men in California. Both patients had unilateral decreased vision and pigmentary tracks in the retina. The worm in one of

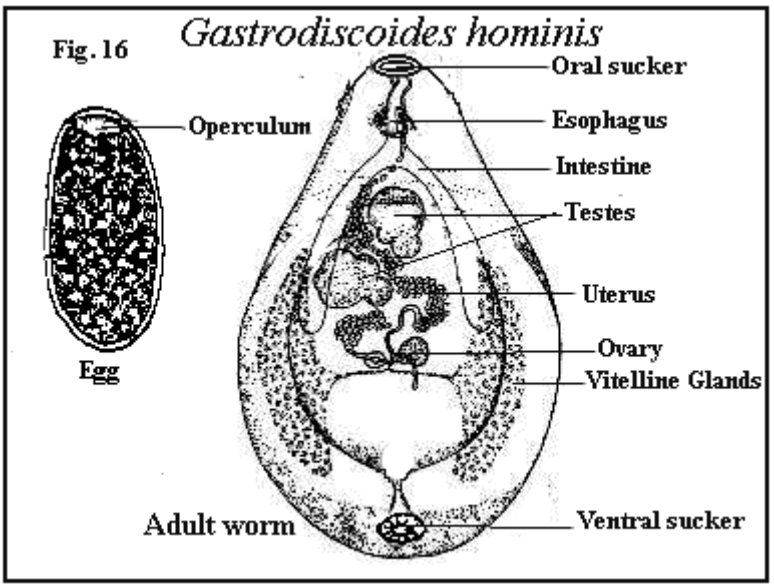
the patients was diagnosed as an Alaria mesocercaria based on its shape, size (500 x 150 microns), and its movement. It was successfully killed with laser. Both patients use to eat undercooked or raw oriental dishes, including frogs legs, which were considered to be the likely source of infection (McDonald et al, 1994). Another human infection with mesocercariae of *A. americana* was reported from Ontario, Canada. Presence of several thousand mesocercariae in the peritoneal cavity, bronchial aspirate, brain, heart, kidney, liver, lung, lymph node, pancreas, spinal cord, spleen, and stomach resulted severe respiratory failure due to extensive pulmonary hemorrhage and death of this patient. Infection, is assumed to have resulted from eating inadequately cooked frogs' legs (Freeman et al, 1976). Another case of infection with mesocercaria was reported from Louisiana. Two larval Trematode about 0.5 mm in length each were removed from two areas of intradermal swelling in the upper thigh and iliac crest of a 43-year-old man. Based on morphology reconstructed from serial sections, the two worms were identified as a species belonging to the subfamily Alariinae (Beaver et al, 1977).
Mesocercaria of Alaria sp., a Trematode from frogs was found in intra-retinal part of the eye of one Canadian woman in 1977 (Walters et al, 1977).

7-2 *Fibricola seoulensis* Seo, Rim and Lee, 1964

This is the only diplostomatid fluke which develop into the adult stage in man (Seo et al., 1982). First described in Korea, and is widely prevalent among house rats in this inland. The second intermediate host is frog and its tadpole and several kinds of terrestrial snakes (*Rhabdophis tigrina*) are regarded as the paratenic host. Eggs of *F.seoulensis* were found in the feces 15 out of 244 Korean males who had ingested the snakes or frogs (Hong et al., 1984). Only one of this infected cases had clinical symptoms including epigastric discomfort or pain, diarrhea, fever and eosinophilia. Another case of human infection with *Fibricola seoulensis* was reported from Korea in 1991 (Chai and Lee 1991).

8- Family Gastrodiscidae:

Infection with Gastrodiscoides species (gastrodiscoidiasis) is prevalent in India, Bangladesh, Kazakestan and the Far East (Harinasuta et al, 1987).The main species in this family, which cause human infection, is *Gastrodiscoides hominis.*



8-1 *Gastrodiscoides hominis* (Lewis and McConnell, 1876; Leiper, 1913)

Synonyms: *Amphistoma hominis* and *Gastrodiscus hominis* (Lewis and McConnell, 1876) Fischoeder, 1902.

This parasite is a representative of the family Gastrodiscidae and also considered as a member of Amphistomate.

Morphology: *Gastrodiscoides hominis* is the only human Trematode that its ventral sucker is located at the posterior end of the worm. The worm is red in color, 5-8 mm long and 35 mm wide. Eggs are 152x68 u with a small yellow operculum and are found in the stool (Fig. 16). Adult worms live in colon and cecum and cause pathological changes such as inflammation of the mucosa of the caecum and the ascending colon causing mild to sever diarrhoea. Praziquantel is used for its treatment. In India the planorbid snail, *Helicorbis coenosus*, has been found experimentally to serve as an intermediate host (Beaver et al., 1984). The pig, field rat (*Rattus brevicaudatus*) and the rhesus monkey are reservoir hosts.
The parasite is acquired by ingestion of metacerceriae encysted on aquatic plants, frogs, tadpoles and crayfish. Human infection with the parasite was first found and described from the cecum of an Indian patient. Human infections have also been reported from Bengal, Bihar and Orissa in India, from Vietnam, the Philippines, Burma, Thailand, China, Kazakstan, and Indian immigrants in Guyana (Ahluwalia, 1960; Murty and Reddy, 1980; Harinasuta et al., 1987). In Assam, an infection rate of 41.2% was once reported.

9- Family Gymnophallidae:

9-1 *Gymnophalloides seoi* n. sp.

The first human infection with *Gymnophalloides seoi* was reported from a Korean woman who complained of epigastric discomfort. Further studies have shown that 49% of inhabitants of villagers of a southwestern island of the Republic of Korea were infected (Lee et al, 1994). Worm burden per individual ranged between 1 and 26,373 with an average of 3,119 (Lee et al., 1992). In one study, Kook et al (1997) were able to develop metacercariae obtained from naturally infected oysters into adult worm by in vitro cultivation of metacercariae (Kook et al, 1977). In 1996 a nationwide survey was performed to know the geographical distribution of *Gymnophalloides seoi* (Digenea: Gymnophallidae) metacercariae in Korea. Locally produced oysters, *Crassostrea gigas* were collected from 24 coastal areas and examined for metacercariae. In ten endemic areas, the infection rates for metacercariae were between 1.7% and 100% (Lee et al, 1996).

10- Family Lecithodendriidae:

In addition to the family Plagiorchidae, 3 species of family Lecithodendriidae, are transmitted by insect to human (Kaewkes et al, 1991 and 1992).

10-1 *Phaneropsolus bonnei* Lie Kian Joe, 1951

P.bonnei was first discovered in the small intestines of 15 out of 24 cases in Indonesia at autopsy. Human infection with *Phaneropsolus bonnei* was also found in northeast Thailand (Manning and Viyanant 1972). In one study in northern Thailand, 4356 flukes were detected from stool of a patient after treatment with praziquantel (Radomyos et al., 1984). The rate of infection in another study in Thailand was 15% (Radomyos et al, 1994). Morphological features of lecithodendriid eggs in human feces were described by Kaewkes et al (1991).

10-2 *Prosthodendrium molenkampi* Lie Kian Joe, 1951

Another species of the family is *P. molenkampi* which was also found during autopsy of an Indonesian. In 14 of 24 autopsies in northeast Thailand, eggs in feces and adult parasites were recovered from the intestines and also in human stool specimens from Laos. Human infection occurs by consumption of raw small fish contaminated with infected naiads (water nymph of dragonflies). The snail intermediate host is *Bithynia goniomphalus* and odonate insects, dragonflies and damselflies, are the second intermediate hosts. Monkeys, bats and rats are the natural reservoirs (WHP, 1994). Although heavy worm burden with lecithodendriids is found, it is difficult to separate the clinical symptoms caused by these worms from those caused by other helminth infections highly prevalent in endemic areas. In one study in Thailand the infection rate for *Prosthodendrium molenkampi* was 19.4% (Radomyos et al, 1994). In another survey in northern Thailand, cases of *Prosthodendrium molenkampi* were found among 431 residents from 16 provinces (Radomyos et al 1998).

10-3 *Phaneropsolus spinicirrus* n. sp.:

Human infection with *Phaneropsolus spinicirrus* was reported from northeast Thailand. Kaewkes et al, (1991) have described its morphology. The parasite was recovered from the feaces of a 44-yr-old woman from Kalasin Province in northeastern Thailand, after treatment (Kaewkes et al 1991). Treatment of 244 villagers were done with praziquantel and their stools were collected and the egg output and the worm burden were determined.
Five species of lecithodendriidae (including *P. spinicirrus* n. sp.), five species of heterophyid and one species of plagiorchiid Trematodes were identified from a total of 108,661 flukes collected. The prevalence of infection determined by finding eggs in the stool, and by worm recovery were 43.7% and 52.3%, respectively (Kaewkes et al., 1991; Kaewkes et al., 1992). Morphologically, It differs from *Phaneropsolus bonnei* Lie, 1951, in the presence of short spinose cirrus and the structure and distribution of tegumental spines. Human infection with two other species in this family i.e. *Paralecithodendrium obtusum* and *Paralecithodendrium glandulosum* has also been reported during the same investigation as P. spinicirrus in Thailand.

11- FAMILY MICROPHALLIDAE

Members of this family are intestinal parasites of a species of vertebrates.

11-1 *Spelotrema brevicaeca* (Africa and Garcia, 1935;

Tubangui and Africa,1939) Synonym: *Heterophyes brevicaeca* Africa and Garcia, 1935. Velasquez in 1975 considered this family as a genus Velasquez also studied life cycle of *Carneophallus brevicaeca* in 1975. He reported finding metacercariae of the parasite from the muscles of naturally infected shrimps, Macrobrachium sp., and recovering the worm after experimentally infecting albino rats. Because this species of shrimps are eaten raw in some regions of the Philippines, this trematode is medically important (Velasquez, 1975). The parasite has been reported on several occasions in humans in the Philippines. Eggs of the parasite were found in lesions of the heart, brain, and spinal cord of persons died of acute cardiac dilatation. Other species of microphallid Trematode were recovered from the small intestine of a cat from Central Thailand (Waikagul 1983). Human infection with species from the family microphallide was reported from parts of Southeast Asia (Waikagul, 1991).

12- FAMILY STRIGEIDAE

12-1 *Cotylurus japonicus* Ishii, 1932

Members of this family are normally intestinal parasites of aquatic birds. Human infection was reported from Hunan province in China in a 13-year-old girl. An adult worm was later found in her faeces(Chen & Cai, 1985). Studies on the eggs and adult worm confirmed that the worm was *C. japonicus.*

13- FAMILY BRACHYLAIMIDAE

13-1 *Brachylaima ruminae* n.sp.

(Trematoda: Brachylaimidae) is a parasite of rodents in some countries. The life cycle of the parasite is as follows: Eggs passed in the faeces of the definitive host are ingested by a specific first intermediate host, the land snail *Rumina decollata*. Cercariae shed by the snail are terrestrial and

penetrate the second intermediate hosts, which are land snails, such as Helicids species. The definitive host becomes infected when ingesting infective metacercariae together with the snail. In 1996, Butcher et al reported infection of two 21-month-old children from rural district of South Australia with species of Brachylaima. Clinical symptoms were mild abdominal pain and diarrhoea. The larval stage of the parasite infects helicid snails and definitive hosts are house mouse, and poultry. Recently another human infection with these species has been reported from a 78-year-old woman in a rural area of South Australia. The main symptom was intermittent diarrhoea. After treatment with praziquantel, adult Brachylaima species was recovered from her stool (Butcher et al, 1998). The source of infection was consumption of infested with helicid snails that are commonly infected with larval stages of brachylaimid.

مشروع دعم قطاع مياه الشرب والصرف الصحي
Water and Wastewater Sector Support, (WWSS)

الديدان الطفيلية في البيئة المائية

PARASITIC HELMINTHS IN THE AQUATIC ENVIRONMENT

INTRODUCTION TO PARASITOLOGY

- **PARASITOLOGY:**
  is that branch of biology which deals with the study of parasitism.

- **PARASITISM:**
  is a phenomenon of dependence of one living organism (parasite “usually small”) on another (host “usually larger”), inducing a certain degree of injury to that host.

- **COMMENSALISM:**
  ……without causing any harmful effect to the host

- **PARASITE:**
  is an organism (usually small) that lives at the expense of another one (usually larger) for food and shelter.

Definitions

- The organism that harbors the parasite and suffers a loss caused by the parasite is a **host**.
- The host in which the parasite lives its adult and sexual stage is the **definitive** host.
- The host in which a parasite lives as the larval and asexual stage is the **intermediate** host.
- Other hosts that harbor the parasite and thus ensure continuity of the parasite's life cycle and act as additional sources of human infection are known as **reservoir** hosts.
- An organism (usually an insect) that is responsible for transmitting the parasitic infection is known as the **vector**

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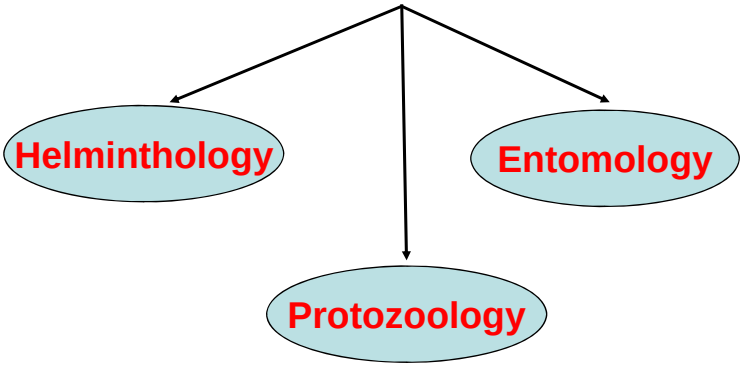
SYSTEMATIC CLASSIFICATION OF PARASITES



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Classification of helminthes

- Nemathelminths (round worms)
- Platyhelminths (flat worms)
- Trematodes (flukes)
- Cestodes (tape worms)
- Acanthocephala (thorned worms)
- Annelida

INFECTIVE STAGES OF HELMINTHS

1. NEMATODES:

- Egg (ovum) containing larva.
- Larva.

2. TREMATODES:

- Cercaria.
- Encysted metacercaria.

3. CESTODES:

- Egg containing hexacanth embryo.
- Intermediate host containing pleurocercoid.

ROUTE OF INFECTION

1. SKIN:

Some Nematodes → *Necator americanus*, *Strongyloides*

Some Trematodes → *Schistosoma spp.*

3. INGESTION

- Most of Nematodes.
- Most of Cestodes.
- Most of Trematodes.

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HELMINTHS TRANSMITTED VIA WATER

1. **WATER DEPENDANT**:

All Trematodes → *Schistosoma*
Some Nematodes → *Dracanculus medinensis*
Some Cestodes → *Diphyllobothrium latum*

2. WATER CONTAMINANT:

Most gastro-intestinal

→ Nematodes
→ Cestodes

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PHYLUM	NEMATHELMINTHES		ACANTHO-CEPHALA	PLATYHELMINTHES		ANNELIDA
	Nematoda	Nematomorpha	Thorny-headed worm	Trematoda	Cestoda	Segmented Worms
Free-living	Many	Adult stage	None	None	None	Mostly
Parasitic	Many	Larvae in insects	All	All	All	Few
Separate sexes	Yes	Yes	Yes	No, few exceptions	No	No
Shape	Spindle Tapered ends	Cylindrical, blunt ends	Tapering Posterior end	Oval, leaf-shaped	Ribbon, band-shaped	Cylindrical
Segmented	No	No	No	No	Yes	Mostly
Body cavity	Yes	Yes	Yes	No	No	Yes
Digestive tract	Complete	Atrophied	Absent	Yes, no anus	Absent	Present
Proboscis	No	No	Yes	No	No	Yes

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PARASITIC NEMATODES

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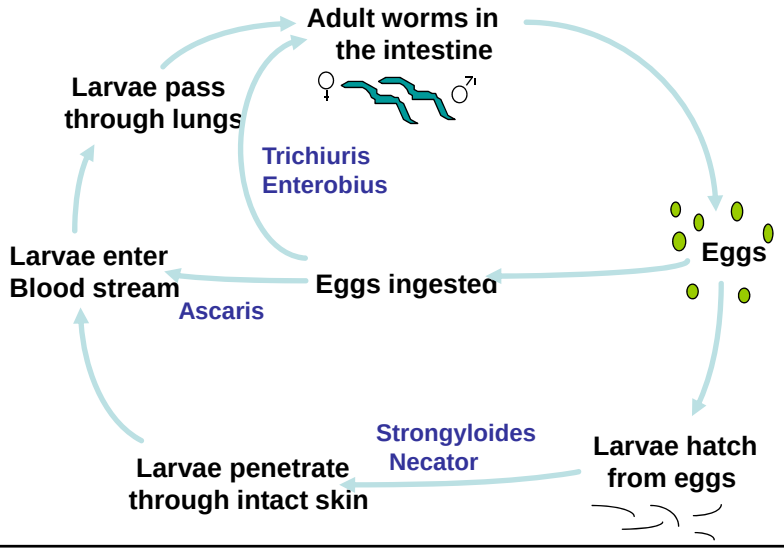
Life Cycles of Intestinal Nematodes



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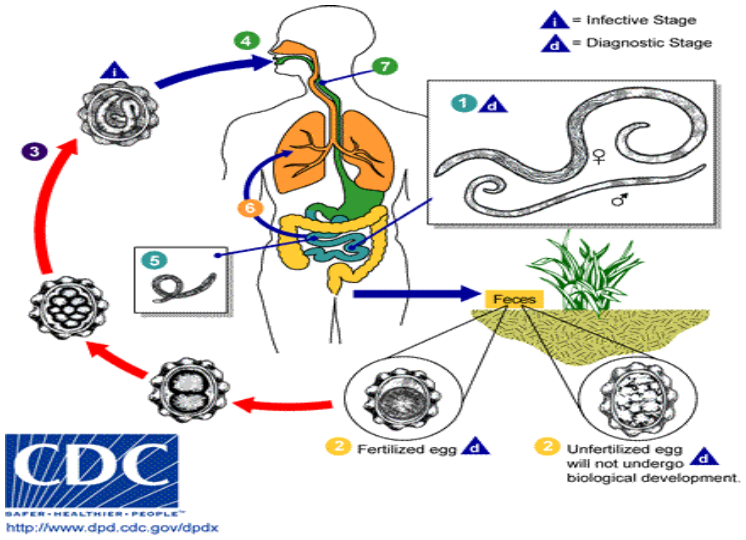
Ascaris lumbricoides



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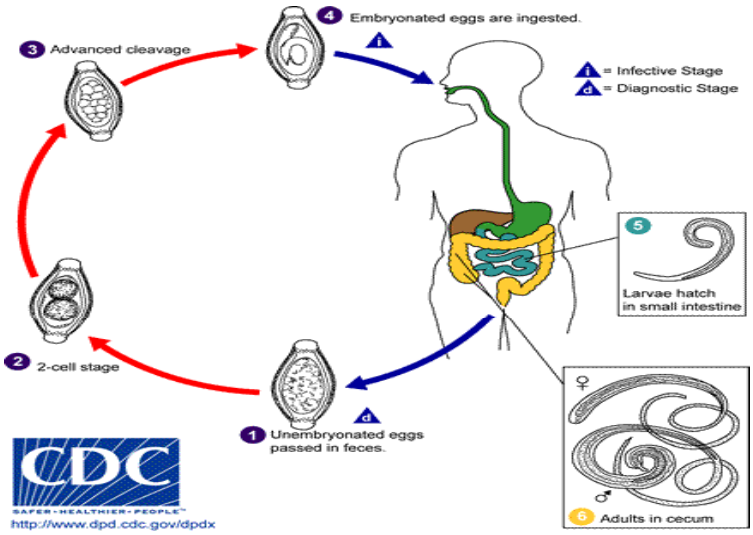
Trichuris trichiura



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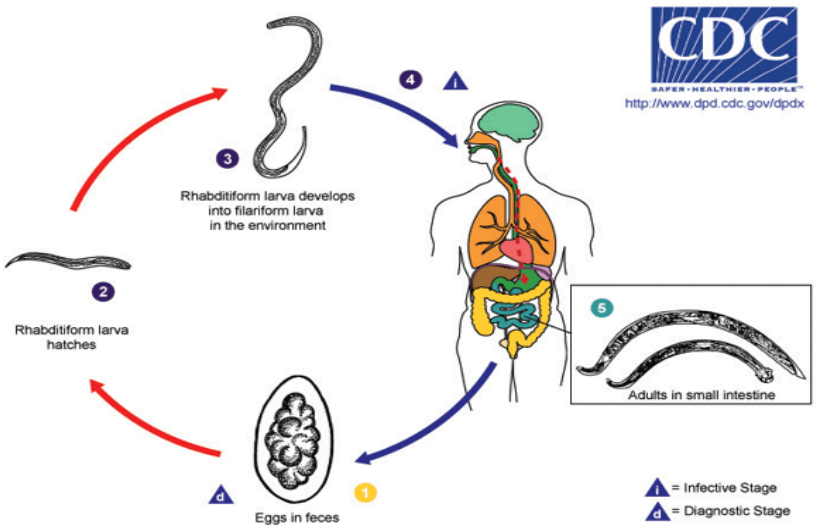
Trichostrongylus spp



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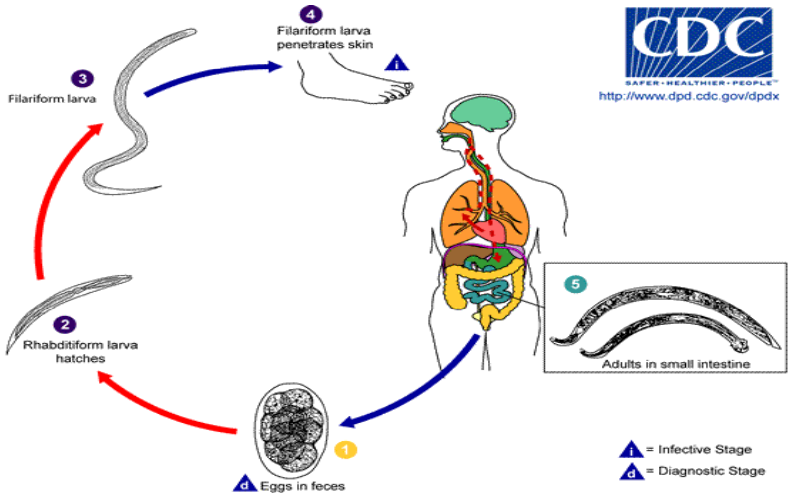
intestinal hookworm infection



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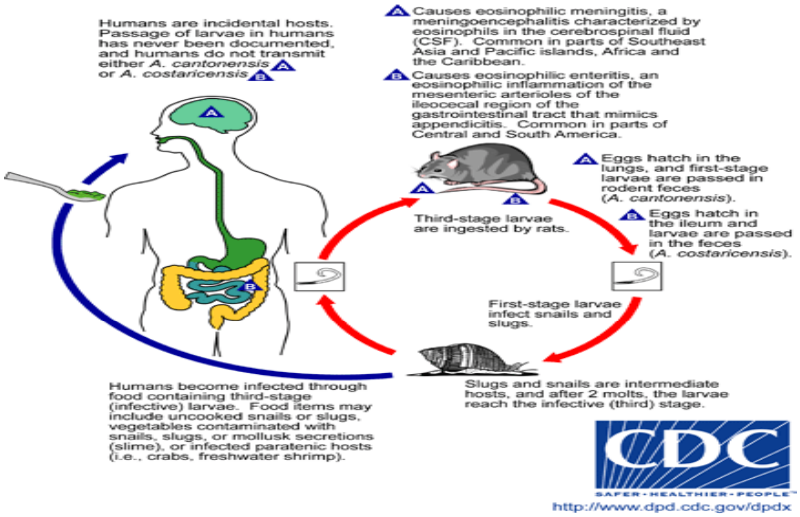
Angiostrongylus



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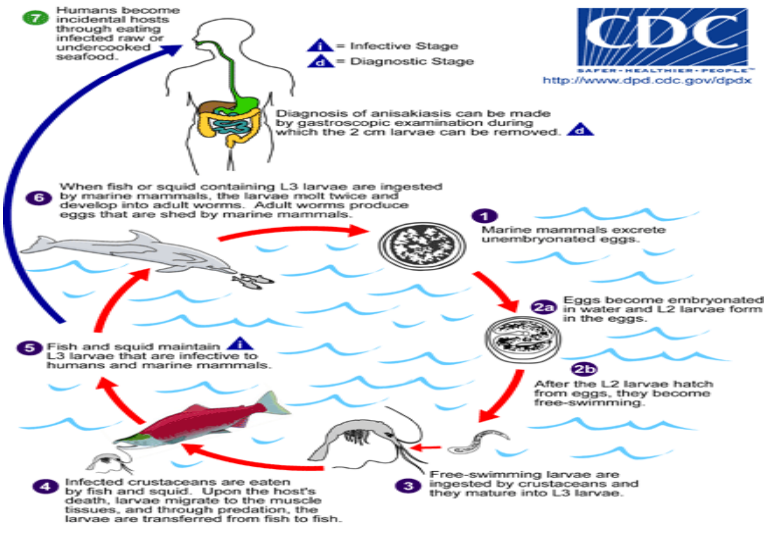
Anisakis simplex



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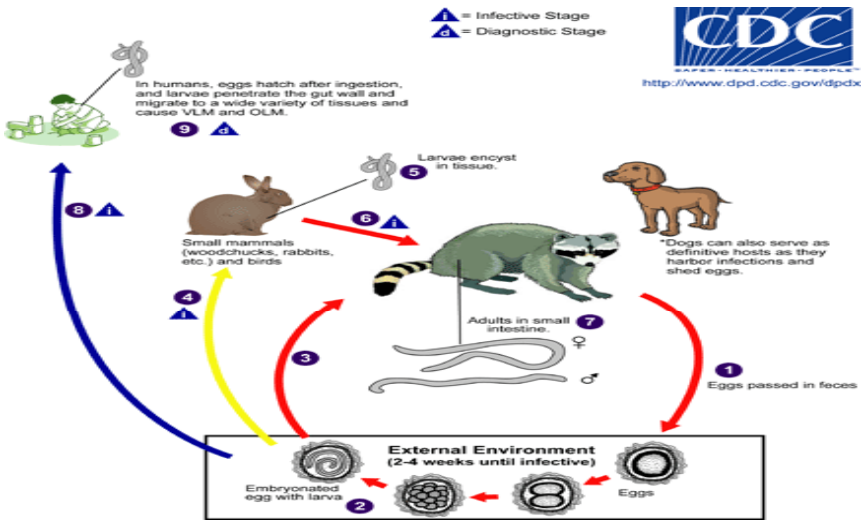
Baylisascaris procyonis



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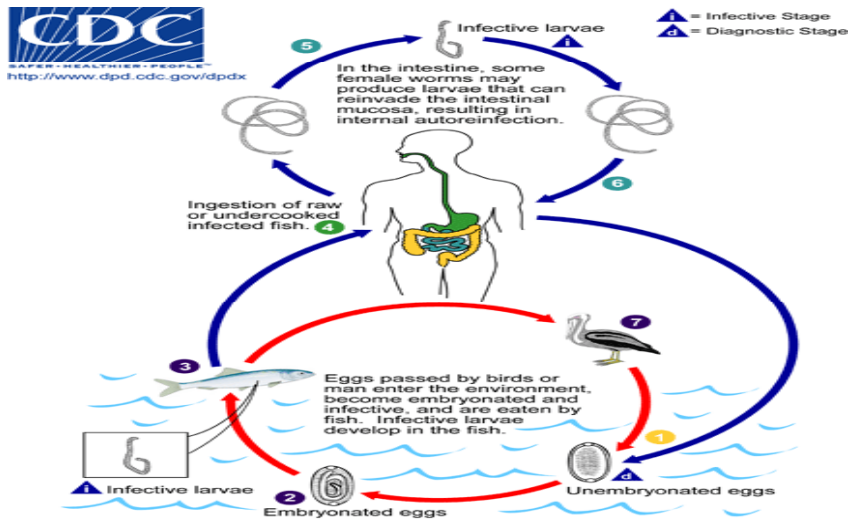
Capillaria hepatica



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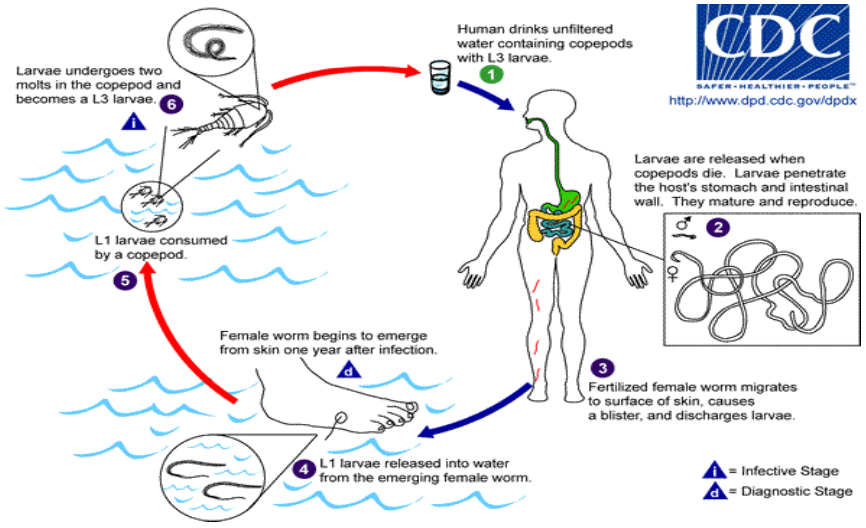

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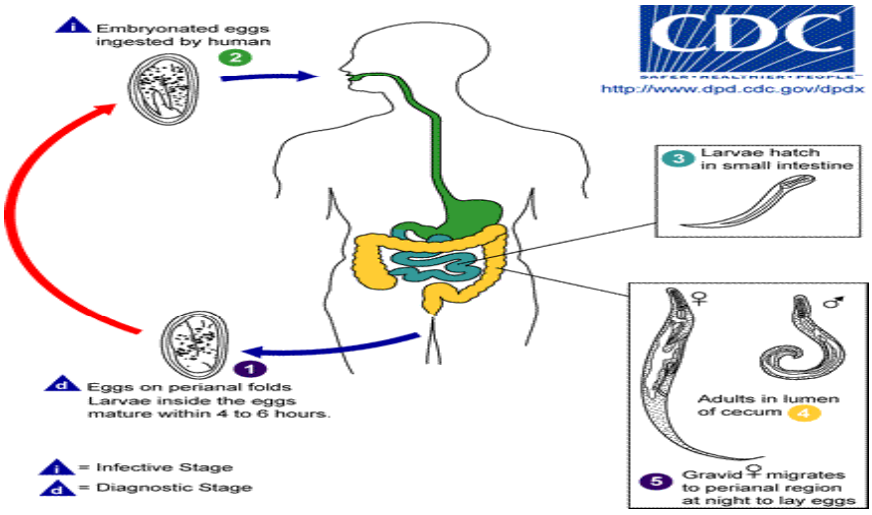
Enterobius vermicularis



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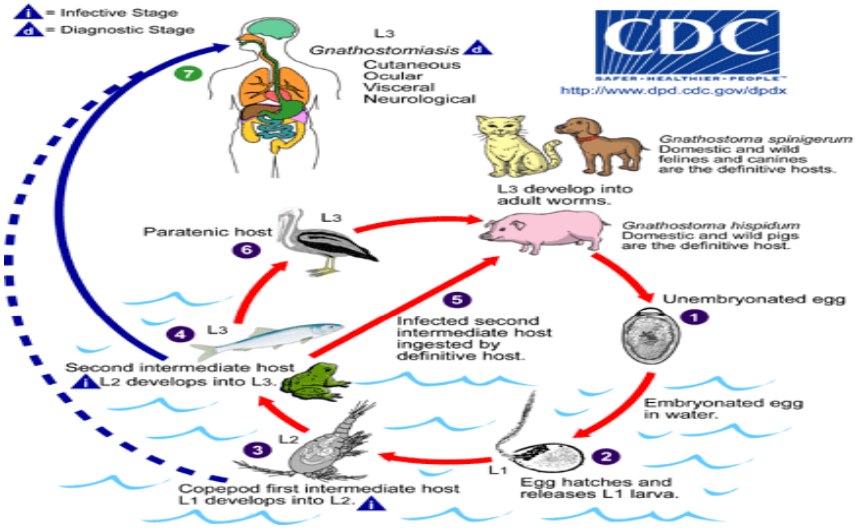
Gnathostoma spinigrum



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CESTODES

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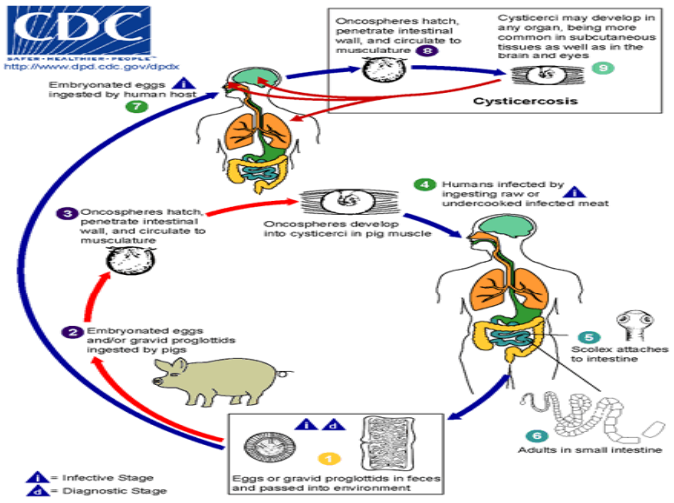
Taenia solium



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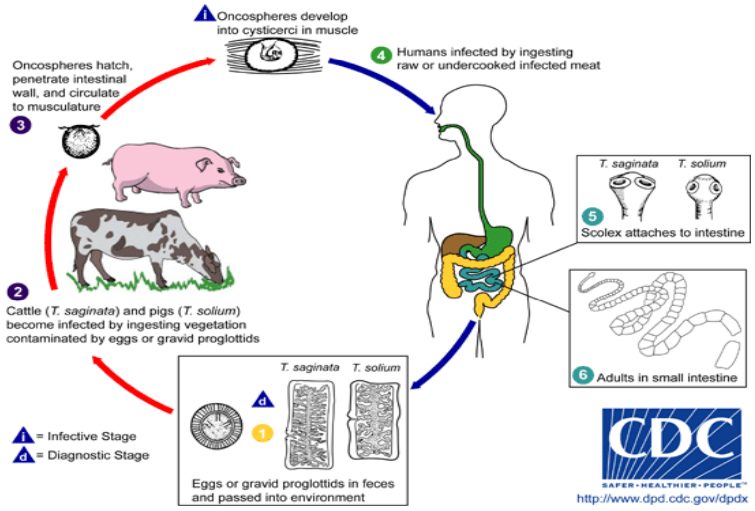
Taenia saginata



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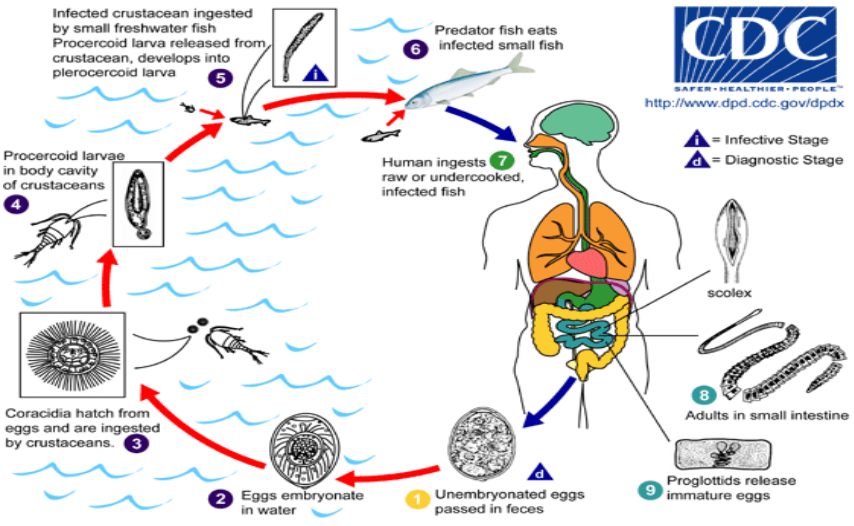
Diphyllobothrium latum



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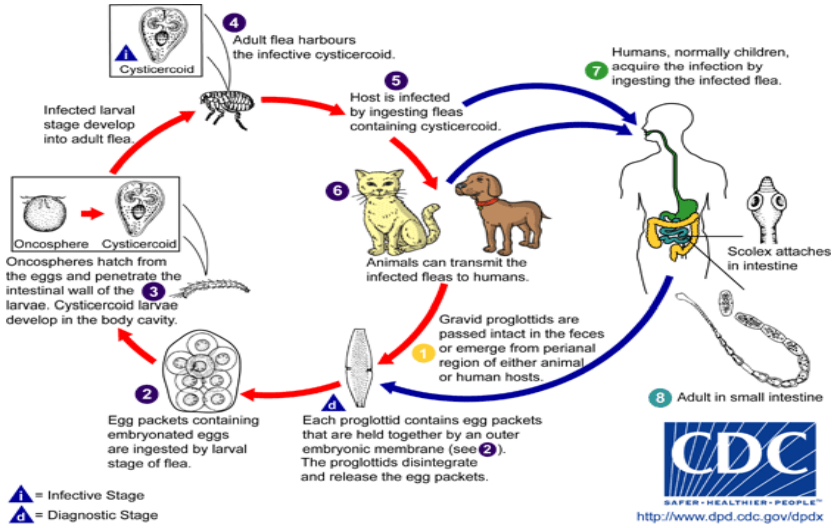
Dipyllidium caninum



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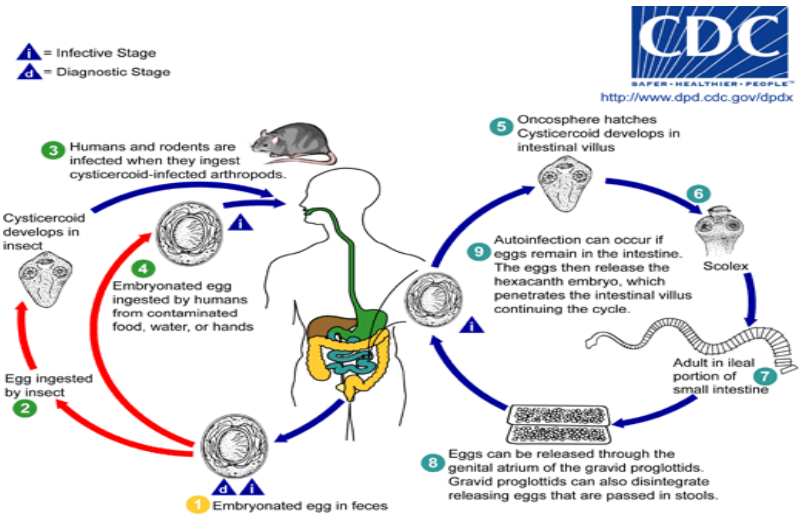
Hymenolepis nana



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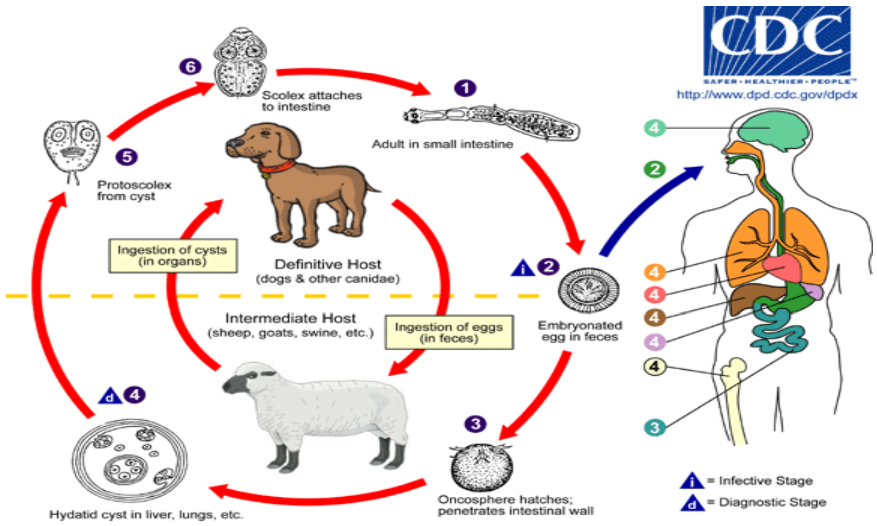
Echinococcus granulosus



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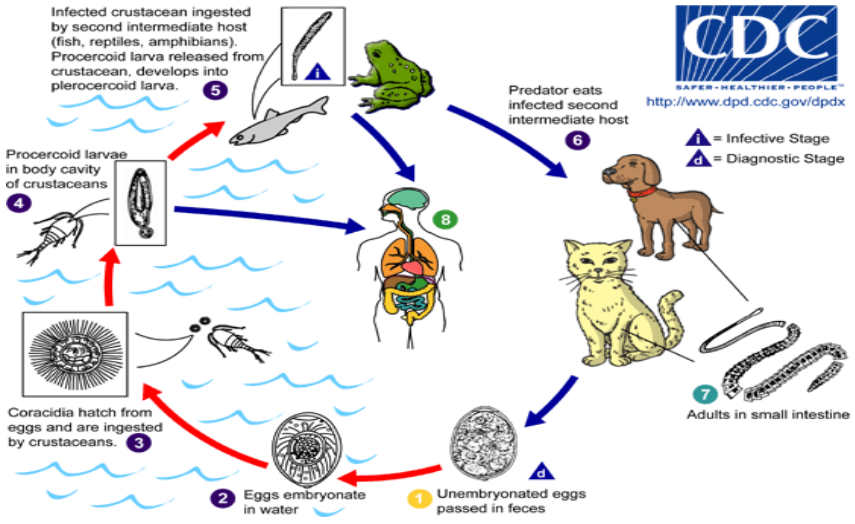
Spirometra



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TREMATODES

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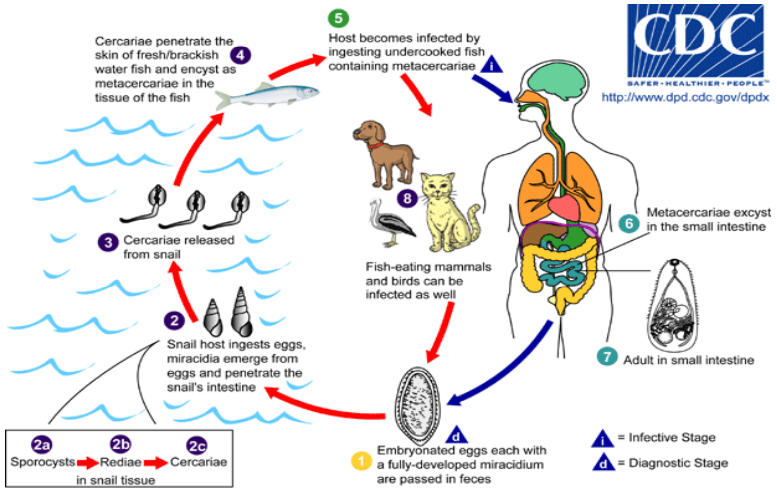
Heterophyes heterophyes



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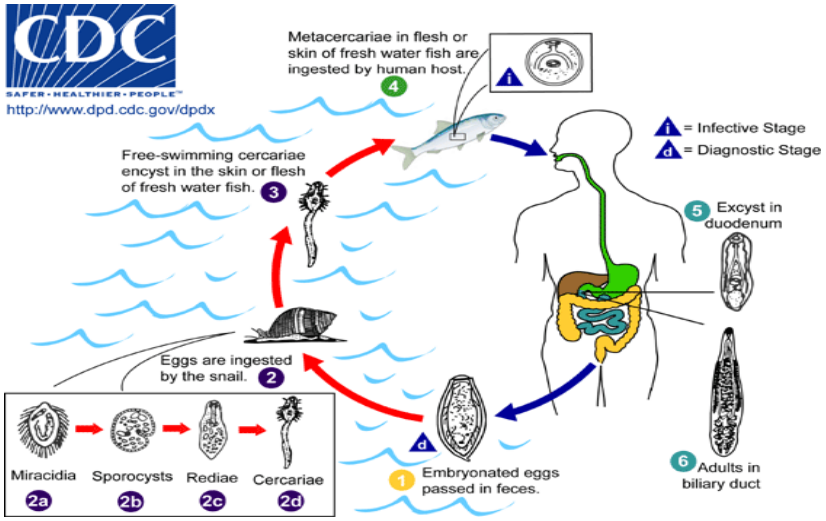
Clonorchis sinensis



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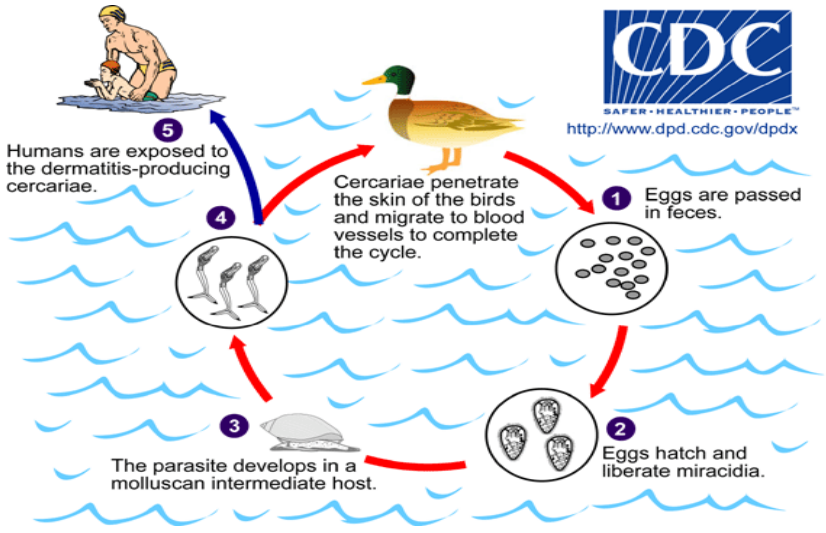
Swimming ichs



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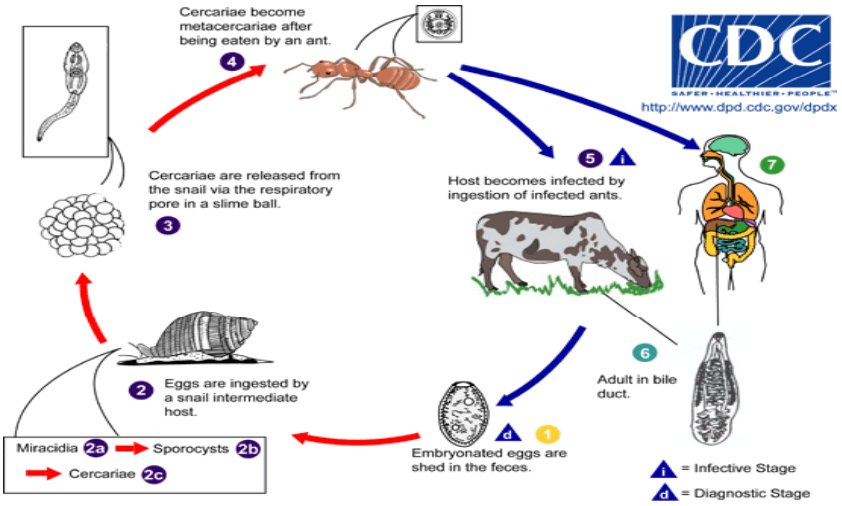
Dicrocoelium denderiticum



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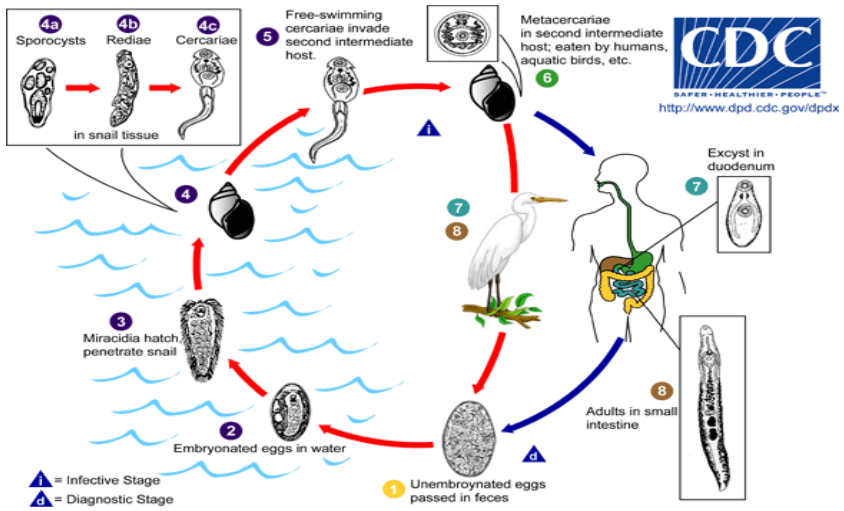
Echinostoma revolutum



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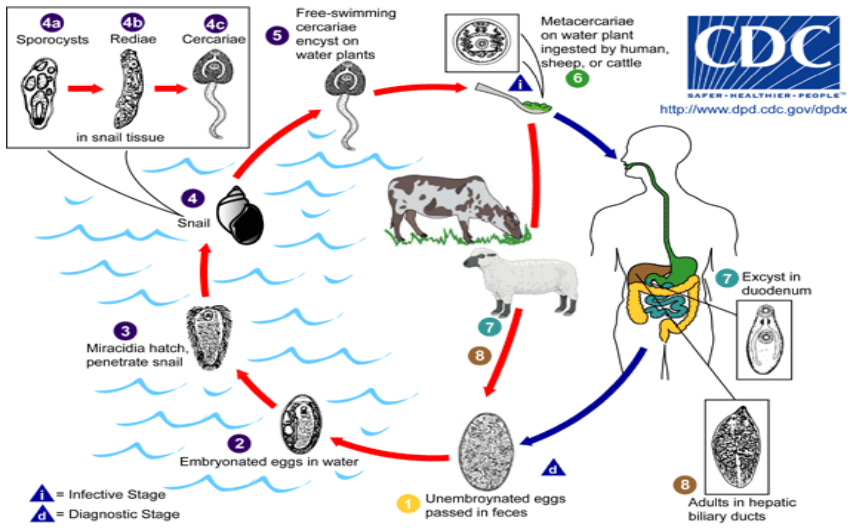
Fasciola hepatica



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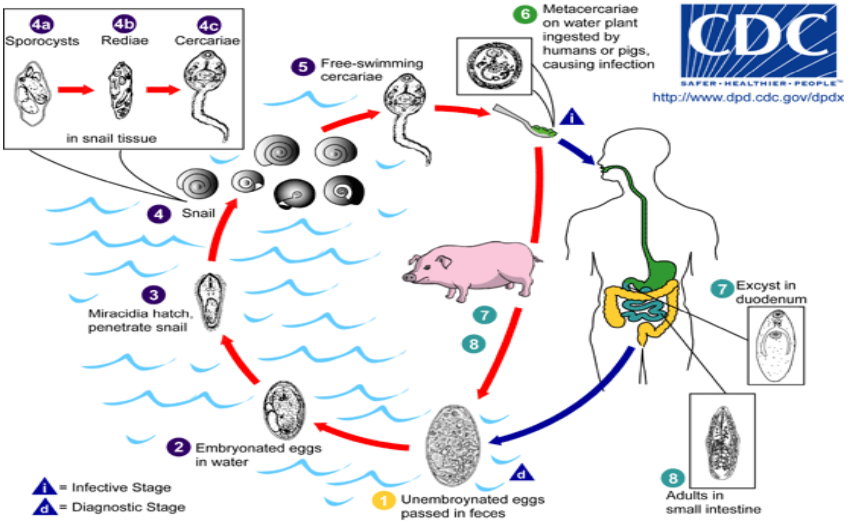
Fasciolppsis buski



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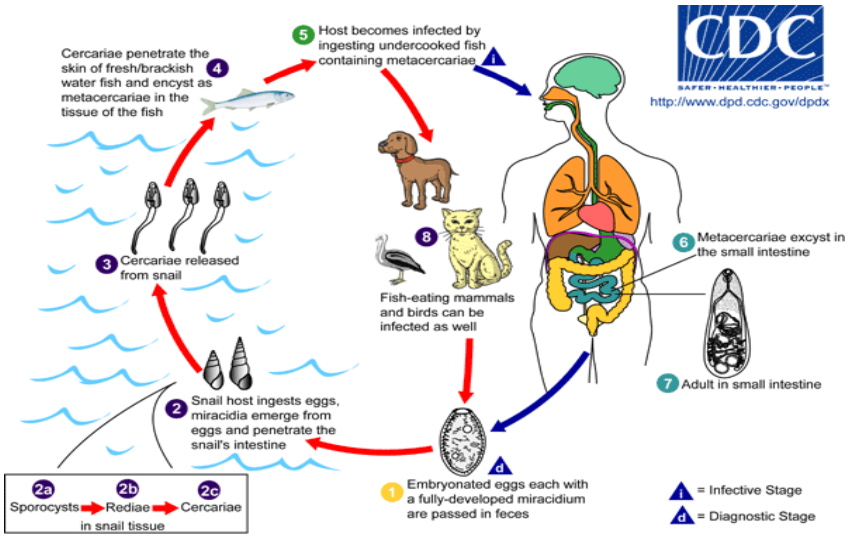
Metagonimus yokogawai



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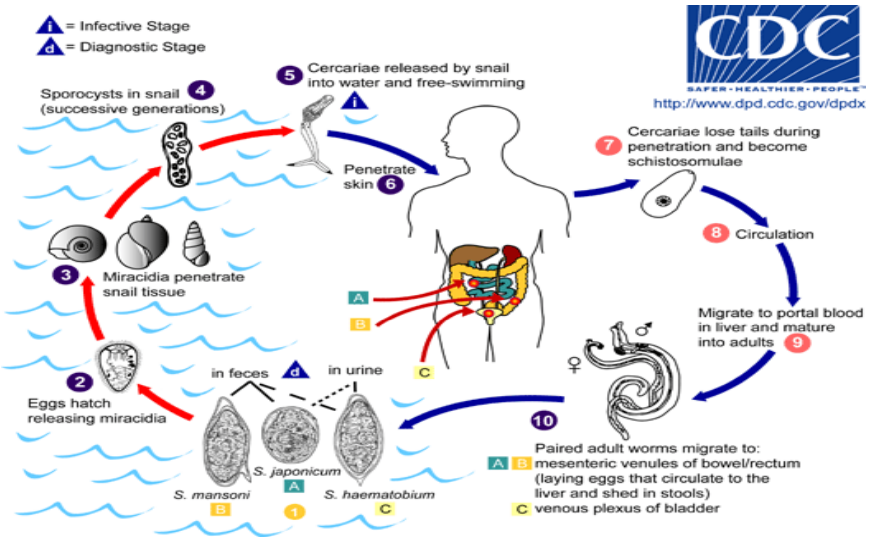
Schistosoma



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DETECTION OF HELMINTH OVA & LARVAE IN WATER

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Concentration

- Centrifugation
- Swinging centrifuge
- V-shape centrifuge tubes

- Filtration
- Membrane filters up to 3µm pore size
- Sucssion pump

FLOTATION

- Is based on the differences in the specific gravity of some chemical solutions and eggs, cysts ..... the solution is haevy and the parasitic objects are lighter, then these objects are going to the top of the tube.
- There is on the bottom of the tube some destritus (debris) and on the top surface of the chemical solution are parasitic objects.

Zinc Sulfate Centrifugal Flotation Technique (according to FAUST)

- Sulfate solution ($ZnSO_4$ 331g + 1000mL water)
- Specific gravity is 1, 180
- It is about 80% effective in detecting eggs and cysts of parasites.

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Flotation procedure

- Centrifuge for 10 min / at 2500 r.p.m.
- Discard the supernatant
- Add $ZnSO_4$ solution to sediment - 1 cm under margin of the tube
- Centrifuge 1 min/2500 r.p.m.- NOT REMOVE
- Add ZnSO4 solution to the tube for all of value,
- Cover with coverglass - wait for 20 minutes
- Put cover glass on the slide
- Add 1- 2 drops of Lugol's iodine solution to the sample on the slide.
- Observe under the microscope

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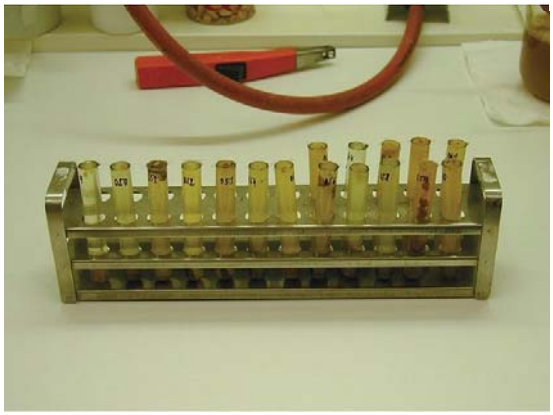
Flotation using zinc sulfate solution



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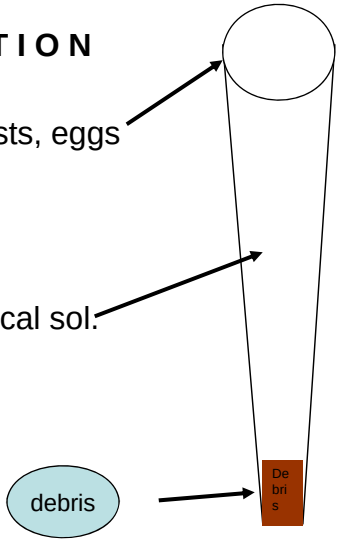
F L O T A T I O N

- coverglass with cysts, eggs
- the tube with chemical sol.
- detritus (debris)



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Lugol ´s solution on the slide

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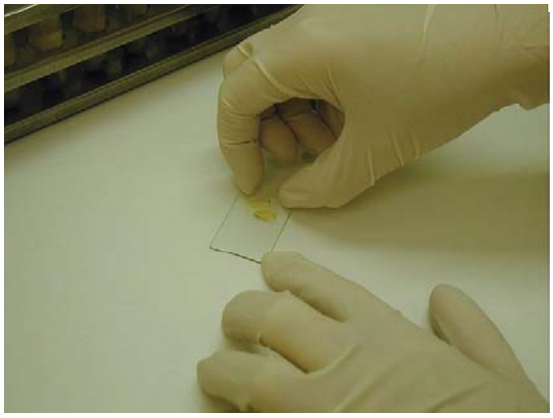
Put the coverglass after flotation



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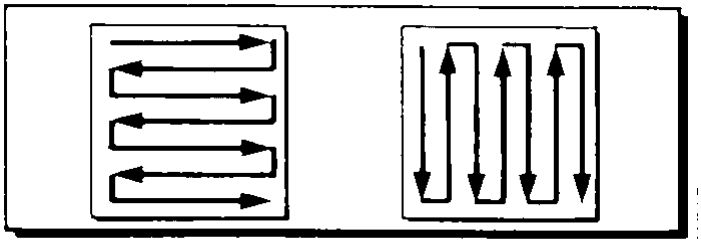
Microscpic examination



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Key to identification of eggs

The characteristics used to identify species of eggs are as follows:

1. *Size.* The length and width are measured and are generally within a specific range.
2. *Shape.* Each species has its own particular shape.
3. *Stage of development when passed.* In some species, the eggs consist of a single cell; in some, there may be several cells; and some species are usually embryonated (i.e., they contain a larva) when passed in the faeces.
   Occasionally, if the stool specimens are several hours or 1–2 days old, eggs may develop to more advanced stages. *Ascaris* eggs usually have only 1 cell when passed in the faeces; however, the single cell may divide and, in old specimens, eggs with 2 or 4 cells may be seen. Hookworm eggs in specimens that are several hours old may contain 16, 32, or more cells. In 12–24 hours, the egg may be embryonated and later still the larvae may hatch. Therefore, when observing the stage of development of helminth eggs, be sure that the stool specimen is freshly passed. If it is several hours or a day old, expect to see changes in the stage of development of some species. Ideally only fresh samples should be accepted for diagnosis.

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4. *Thickness of the egg shell.* Some species, like *Ascaris*, have thick egg shells; others, like hookworm, have thin shells.
5. *Colour.* Some eggs are colourless (e.g., hookworm, *Enterobius*), others are yellow or brown (*Ascaris, Trichuris*).
6. Presence of characteristics like opercula (lids), spines, plugs, hooklets, or mammillated outer coats.

If an egg, or an object that looks like an egg, is found, these features should be carefully observed in order to make a specific identification. Occasionally, atypical or distorted eggs will be seen. In such cases, it will be necessary to look for more typical forms in order to make a reliable diagnosis. Remember that more than one species of helminth may be present in an individual patient.

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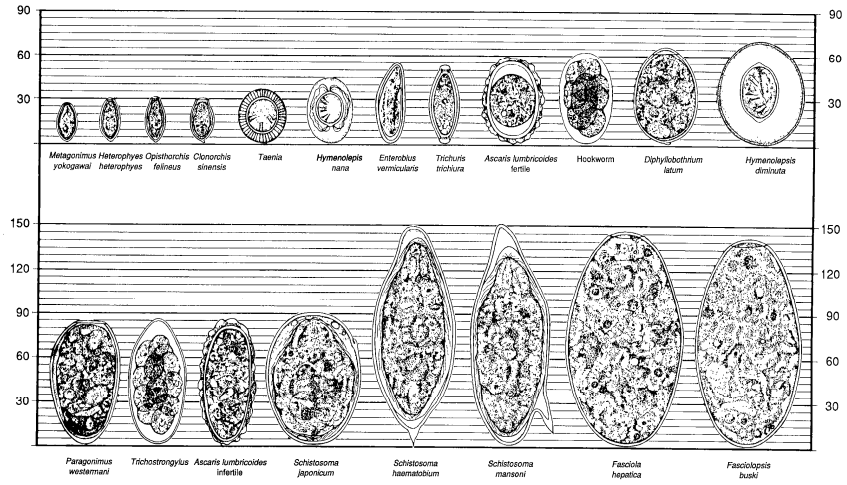
RELATIVE SIZES OF HELMINTH EGGS *



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Worm larvae

In fresh stool specimens, the larvae seen are usually rhabditiform (= first stage) larvae from *Strongyloides stercoralis*. However, if the stool has been passed for more than 12 hours, the larvae may hatch into filariform larvae (infective stage); these must be differentiated from hookworm larvae, which may hatch in stool within 12–24 hours. The appearance of filariform larvae of *Strongyloides stercoralis* may indicate a systemic hyperinfection.

The characteristics used to separate the species are shown in Fig. 5 and in Table 4.

In iodine preparations, the genital primordium will be more visible. Iodine will kill the larvae and you will be able to see the features better. You will need to use high-power, dry magnification to see these structures.

- If you see a larva with a short mouth opening and a prominent (clearly visible) genital primordium, it is *Strongyloides*.
- If you see a larva with a long mouth opening and do not see a genital primordium, it is *hookworm*.

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Fig. 5. Helminth larvae

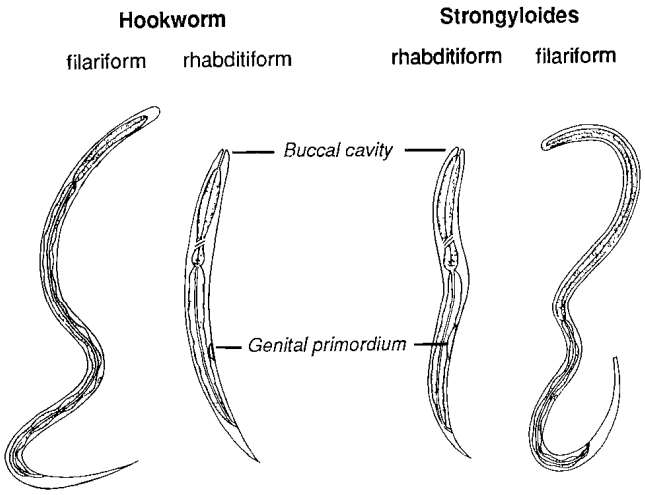

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Table 4. Characteristics of helminth larvae

Hookworm	*Strongyloides*
Filariform larvae	*Filariform larvae*
Size 500 × 14–20 µm	Size 500 × 14–20 µm
Sheathed	Unsheathed
Tail tapered	Tail forked or blunt
Oesophagus one-third of body length with no swelling	Oesophagus half of body length with no swelling
Rhabditiform larvae	*Rhabditiform larvae*
Size 100–150 × 15–17 µm	Size 200–300 × 15–18 µm
Buccal cavity long (15 µm)	Buccal cavity short (4 µm)
Oesophagus one-third of body length with two swellings	Oesophagus one-third of body length with two swellings
Genital primordium small (7 µm)	Genital primordium large (22 µm)
Anal pore 80 µm from posterior end	Anal pore 50 µm from posterior end

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Cleaning and storage of microscope slides

Cleaning

The availability of clean, good quality glass slides for the preparation of blood specimens for microscopic examination needs to be emphasized. All slides must be scrupulously clean and free from grease or moisture. This will prevent most of the artefacts which confuse malaria diagnosis and will avoid the detachment and washing away of thick blood films during the staining process. Reject defective materials such as:

- slides with an iridescent bloom or frosted appearance;
- imperfectly cleaned slides, whether new or old;
- old slides with surface scratches or notched edges.

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New slides

It is prudent to clean all new slides (including commercially pre-cleaned slides) by soaking in water with a reliable detergent[1] and then placing them in running tap-water, or several changes of clean water, for some hours. Each slide should be wiped dry and polished with a dry, clean, lint-free cloth. Always handle the cleaned slides by the edges to avoid finger marks.

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Used slides

Used slides must be soaked for at least 60 minutes in hypochlorite solution before washing. They should be washed in hot soapy water and both sides scrubbed with a brush. Wash only a few at a time to avoid scratching or chipping. The slides should then be cleaned one by one with gauze or cotton wool. Then the slides should be transferred to a fresh solution of detergent and later to running water, or several changes of clean water, before drying with a clean cotton cloth. Slightly scratched slides that are considered unsuitable for blood films may still be usefully passed on to the entomology section for routine laboratory use.

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Storage of slides

In the humid tropics, glass slides should not be kept in the ambient climate for more than a few weeks. Otherwise they will adhere to each other because of entrapped moisture and there will be a loss of transparency due to "frosting". After cleaning, slides are best stored in a dry place or a warm air cabinet.

It is recommended that cleaned slides be stored in packages of 10, which have been wrapped in thin paper and secured with adhesive tape or rubber bands, so that they are ready for use. Packages of slides can be put in the original cardboard boxes or other suitable boxes for mailing or transportation, but should be protected with corrugated cardboard, expanded polystyrene, or cotton wool.

الأميبات حرة المعيشة الممرضة في البيئة المائية

Pathogenic Free-Living Amoebae in the Aquatic Environment

المحتويات

أولا: نظرة عامة على البرنامج التدريبي (الأميبات حرة المعيشة الممرضة في البيئة المائية)

١- الهدف العام للدورة التدريبية

تجرى الاختبارات المعملية المختلفة (فيزيائية - كيميائية – بكتريولوجية - بيولوجية – طفيلية) لمياه الشرب ومصادرها قبل وبعد تنقيتها ومعالجتها بغرض الوقوف على مدى جودة المياه ومعرفة مكوناتها ومواصفاتها قبل وبعد تنقيتها وذلك للتحقق من صلاحية عملية المعالجة وتطابق نوعية المياه المنتجة مع المعايير والمواصفات القياسية المحلية والدولية لمياه الشرب.

لذا تهدف الدورة إلى رفع كفاءة العاملين بالمعامل المركزية والمعامل الفرعية التابعة لشركة مياه الشرب والصرف الصحي بشمال وجنوب سيناء وتزويدهم بالمعلومات النظرية والعملية الواجب إتباعها في إجراء التحاليل المعملية المتعلقة بالكشف عن الأميبات حرة المعيشة الممرضة في عينات المياه والتدريب على الاختبارات والطرق المختلفة والمعتمدة من الجهات المنوط بها وضع المواصفات القياسية لمياه الشرب.

٢- المجموعة المستهدفة

العاملون بالمعامل الرئيسية والمعامل الفرعية التابعة لشركة مياه الشرب والصرف الصحي بشمال وجنوب سيناء (إحدى الشركات التابعة للشركة القابضة لمياه الشرب والصرف الصحي) والمنوط بهم إجراء التحاليل الخاصة بمياه الشرب.

٣- عدد المتدربين

يقدر عدد المتدربين المرشحين لحضور دورة "الأميبات حرة المعيشة الممرضة في البيئة المائية" حوالي متدرب من معامل المحطات والمعامل الفرعية التابعة.

٤- منهجية التدريب

تعتمد منهجية التدريب بالدورة على عدة أسس بحيث يكون الهدف الرئيسي منها توصيل المعلومة بسهولة ويسر للمتدرب , وكذلك ضمان المشاركة الفعالة من المتدربين أثناء جلسات التدريب والتأكد من الفهم الكامل لمحتويات وموضوعات الدورة مع التدريب العملي والشخصي على الموضوعات المتعلقة بمحتوى هذه الدورة.

هذا ويمكن تلخيص منهجية التدريب المتبعة في هذه الدورة فيما يلي:

- **التقييم الأولي:** حيث يقوم المدرب بعمل اختبار مبدئي للمتدربين لتحديد مستوى كل متدرب من حيث المعلومات المتعلقة بموضوع الدورة (الأميبات حرة المعيشة الممرضة) والخلفية العلمية لكل متدرب على حده.
- **المحاضرات:** والتي يقوم المدرب بتوصيل أحدث المعلومات النظرية والعملية المتعلقة بموضوع الدورة للمتدربين بما يؤدي إلى اكتساب معلومات صحيحة وموثقة عن الأميبات حرة المعيشة الممرضة وكيفية التعرف على الأطوار المعدية منها والتي قد تنتقل للإنسان عن طريق المياه.
- **الرسومات الإيضاحية:** التي يتم عرضها أثناء الشرح لتسهيل وصول المعلومة وإبراز النقاط الرئيسية لكل موضوع في تسلسل منطقي لتثبيت المعلومة لدى المتدرب.
- **التجارب المعملية:** التي تجرى في المعمل لتطبيق الاختبارات المتعلقة بموضوع الدورة بطريقة صحيحة وبناءا على أسس علمية موثقة بحيث يستطيع المتدرب من تلاشي الأخطاء التي تؤثر في صحة النتائج المتحصل عليها وكيفية التعامل بأدق الوسائل للوصول إلى أفضل النتائج المرجوة.
- **المناقشات المفتوحة:** التي يديرها المدرب لإتاحة الفرصة لتبادل الآراء وتوجيه الأسئلة والحصول على معلومات جديدة يتم من خلالها نقل المعارف والخبرات العلمية والنظرية من المدرب إلى المتدربين وذلك بغرض إصلاح المفاهيم غير الصحيحة أو غير الحديثة لد المتدربين بخصوص الأميبات حرة المعيشة الممرضة.
- **المشاكل الواقعية المتعلقة بالمتدربين**: وذلك عن طريق مناقشة العقبات العلمية التي تواجه المتدربين أو المشكلات التي سوف يواجهونها والمتعلقة بموضوع الدورة , وذلك بغرض الوصول إلى أنسب الطرق للتغلب عليها باستخدام الأسلوب العلمي الصحيح.
- **التدريب العملي:** حيث يتم إجراء الاختبارات والطرق القياسية الحديثة لعزل واستزراع الأميبات حرة المعيشة الممرضة في المعمل بما يتيح لكل متدرب المشاركة في إجراء التجارب لضمان الفهم التام والتطبيق السليم من المتدرب للمعلومات والطرق العلمية التي تلقاها واكتسبها من المحاضرات .
- **المراجع العلمية:** يتم تزويد المتدرب بالمراجع العلمية المتعلقة بموضوع الدورة (الأميبات حرة المعيشة الممرضة) والتي يمكن الرجوع إليها للتأكد من صواب المعلومة والتعمق في اكتساب معلومات أخرى متعلقة بنفس الموضوع.

- **التقييم النهائي:** حيث يتم عمل اختبار تحريري في نهاية الدورة يشتمل على أبرز ماتم دراسته خلال الدورة وذلك لتقييم المتدربين والوقوف على مدى الاستفادة الفردية لكل متدرب من حضور هذه الدورة.

٥- مساعدات التدريب

- جهاز عرض (Data show)
- شاشة عرض
- سبورة بيضاء وأقلام ملونة

٦- مكان التدريب وطريقة الجلوس بجلسات التدريب

يجلس المتدربون وفي مواجهتهم المحاضر في منتصف القاعة وعن يمينه جهاز الكمبيوتر لعرض ملفات الدورة على شاشة العرض , أما عن يساره توجد السبورة البيضاء والأقلام الملونة .

ويكون وضع كل من شاشة العرض والسبورة البيضاء واضح بحيث يسمح بسهولة الرؤية المتساوية لجميع المتدربين

تقدر مساحة قاعة التدريب المطلوبة بما لايقل عن ٥ x ٧ مترا لتستوعب المتدربين وتسمح للمتدرب بسهولة الحركة والوصول لأماكن جلوس المتدربين.

يجب أن تتوفر بالقاعة إضاءة مناسبة وأجهزة صوتية واضحة ونظام تهوية ملائم.

ثانيا: خطة التدريس بالدورة التدريبية (الأميبات حرة المعيشة الممرضة في البيئة المائية)

المحاضر: ا.د./ أحمد زكريا سالم الهراوي

١- أهداف الدورة:

- التعرف على الأجناس وأنواع الأميبات حرة المعيشة الممرضة
- دراسة دورة حياه كل نوع من الأميبات حرة المعيشة الممرضة
- دراسة التركيب المورفولوجي للأطوار المعدية للأميبات حرة المعيشة الممرضة
- المقارنة بين حويصلات الأميبات حرة المعيشة الممرضة وكيفية التفرقة بينها
- تحديد طرق التخلص من الأميبات حرة المعيشة الممرضة في مياه الشرب
- تأثير خطوات المعالجة الفيزيائية والكيميائية للمياه على إزالة حويصلات الأميبات حرة المعيشة الممرضة
- طريقة دخول الأميبات حرة المعيشة الممرضة إلى جسم العائل عن طريق المياه
- التدريب على طرق تركيز الأميبات حرة المعيشة الممرضة في المياه
- استخدام الميكروسكوب المقلوب والميكروسكوب الضوئي البحثي لفحص الأميبات المزروعة على أطباق الآجار غير المغذي
- تقييم النتائج المتحصل عليها من تحاليل للمياه الخاصة بالأميبات حرة المعيشة الممرضة
- كتابة تقارير تحاليل المياه الخاصة بالأميبات حرة المعيشة الممرضة والتعليق على النتائج المتحصل عليها

٢- موضوعات الدورة:

- نبذة عن الأميبات حرة المعيشة الممرضة وأنواعها وشرح الهدف من الدورة
- تقسيم الأميبات حرة المعيشة الممرضة
- مقدمة عن الأميبات حرة المعيشة الممرضة
- أنواع الأميبات حرة المعيشة الممرضة التي تسبب مرض الالتهاب السحائي الأميبي للإنسان
- كيفية اختراق الأميبات حرة المعيشة الممرضة لجسم الإنسان
- دور الأميبات حرة المعيشة في نقل مسببات الأمراض للإنسان
- الصفات المورفولوجية المميزة لحويصلات الأميبات حرة المعيشة الممرضة

- الأمراض الناجمة عن الأميبات حرة المعيشة الممرضة في الإنسان
- طرق تركيز الأميبات حرة المعيشة الممرضة من المياه
- استخدام الميكروسكوب المقلوب والميكروسكوب الضوئي البحثي للتمييز بين حويصلات الأميبات حرة المعيشة الممرضة المختلفة
- استخدام تقنية البيولوجيا الجزيئية للتعرف على الأنواع الممرضة من الأميبات حرة المعيشة
- طرق التخلص من الأميبات حرة المعيشة في المياه
- إعداد تقارير تحاليل الأميبات حرة المعيشة الممرضة والتعليق على النتائج

٣- مدة الدورة:

تستغرق الدورة مدة خمسة أيام متواصلة حيث يبدأ العمل يوميا من الساعة التاسعة صباحا حتى الساعة الرابعة عصرا بواقع سبع ساعات يوميا تتخللها نصف ساعة لتناول المشروبات والغداء

٤- البرنامج الزمني للدورة

الأميبات حرة المعيشة الممرضة

في البيئة المائية

PATHOGENIC FREE-LIVING AMOEBAE IN THE AQUATIC ENVIRONMENT

Introduction

The distinction between parasitic and free-living protozoa is generally sharply drawn with organisms falling readily into one or the other category. Some of the free-living amoebae are unusual in that they straddle the line separating the two groups of organisms and yet are as destructive as any of the classic parasitic protozoa. Unlike their parasitic counterparts, they are not well adapted for parasitism. They almost invariably kill their hosts instead of evolving, as have many parasites, to a state of de´tente with their hosts. Furthermore, being free-living and widely distributed in nature, they are not dependent upon a host for transmission and spread, nor does host-to-host transmission of these amoebic diseases occur. The amoebae include *Acanthamoeba spp., Balamuthia mandrillaris, Naegleria fowleri, Sappinia diploidea,* and several other representatives that, by virtue of their ability to survive within a mammalian host, are preadapted for a pseudoparasitic and potentially pathogenic life-style. These organisms have been called amphizoic amoebae in recognition of their ability to live endozoically, yet they are capable of free-living existence.

It was due to the prescient observations of Culbertson that the pathogenic potential of free-living amoebae was first realised. Cytopathology produced in monkey kidney tissue cultures used for growing poliovirus was shown to be caused by *Acanthamoeba* and not by a simian virus as originally thought. Later, it was confirmed that, when inoculated into mice or monkeys, the amoebae killed the animals. The first human case of amoebic meningoencephalitis was reported from Australia, but the etiologic agent, first thought to be *Acanthamoeba*, was later identified as *Naegleria*. Not long after, however, the first human infection by *Acanthamoeba* was described. *Acanthamoeba* infections were characterised as opportunistic infections by Martinez, who recognised their occurrence in debilitated or chronically ill patients, and who distinguished between the pathologies caused by *Acanthamoeba* and *Naegleria*. Keratitis cases caused by *Acanthamoeba* were diagnosed in the United Kingdom and in the United States, respectively. The first recognised case of a *Balamuthia* infection was identified in a baboon that died in a zoological park, but the amoeba was detected in humans soon after.

It should be noted that earlier reports of human infections by free-living amoebae have appeared in the literature, but they were ascribed to parasitic or commensal amoebae (e.g. *Iodamoeba bu¨tschlii*) and, only upon later review, were they found to be caused by free-living amoebae. Some reports, particularly early ones, of *Acanthamoeba* infections referred to the amoeba as *Hartmannella*, or used the two genus names interchangeably. *Hartmannella* is, however, a distinctly different amoeba, and no human pathologies have been reliably associated with hartmannellid amoebae.

At present, the taxonomy of the free-living amoebae is unsettled and subject to change, reflecting new data arising from genomic sequencing studies. The amoebae are a polyphyletic group, with stocks arising from different branches of the protozoal ancestral tree. *Acanthamoeba* and *Balamuthia,* based on 16S rRNA sequencing data, are closely related, but phylogenetically distant from *Naegleria* and *Sappinia*. The pathophysiology of the diseases they cause and the immunocompetence of the hosts also helps to distinguish between them. Opportunistic infections by *Acanthamoeba* and *Balamuthia* occur in immunocompromised or immunosuppressed individuals, and the HIV/AIDS epidemic gave rise to a number of cases of acanthamoebiasis and balamuthiasis. *Acanthamoeba* also causes amoebic keratitis (AK), a non-opportunistic ocular infection in otherwise healthy individuals. *Naegleria fowleri* causes a non-opportunistic, but devastating and rapidly fatal meningoencephalitis (primary amoebic meningoencephalitis) in immunocompetent children and young adults. *Sappinia*, based on a single case reported in the literature, was described from an immunocompetent individual. The encephalitides caused by *Acanthamoeba* and *Balamuthia* are of a granulomatous type that develop insidiously over an extended period of time. There is no host specificity for these amoebic diseases, and they have been described in a variety of animals as well as humans.

1. Acanthamoeba spp.

Acanthamoeba is the most common amoeba, if not the most common protozoon, to be found in soil and water samples. It has a cosmopolitan distribution and has been isolated from a wide variety of habitats including fresh, brackish, and seawaters, beach sands, sewage, and soils ranging from tropical to arctic regions. It can also be readily isolated in the home environment from flowerpot soils, home aquaria,

humidifiers, water taps and sink drains, and has been recovered from the hospital environment in shower heads, ventilators, hydrotherapy baths and heating, ventilation, and air conditioning units. Dental irrigation systems have also yielded isolates. Amoebae have appeared as contaminants in tissue cultures, probably as cysts carried through the air, where they have been mistaken for transformed cells or cytopathology caused by viral infections. In the laboratory, they have been isolated from emergency eye wash stations where they pose a danger to persons with a damaged cornea. Their wide distribution in nature brings humans into contact with these amoebae in soil or water, and evidence is found in the presence of antibodies to *Acanthamoeba* in human and animal populations. Despite the opportunities for infection, acanthamoebiasis in populations is rare and, except for AK, is mostly limited to immunocompromised hosts. Systemic acanthamoebiasis is slow to develop, with an insidious, subclinical course from the time of infection. *Acanthamoeba* keratitis is a more acute disease with rapid onset following infection.

1.1. Life-cycle, morphology, and growth in vitro

The organism exists in nature either as a trophic amoeba, feeding on bacteria in soil and water, or as a dormant cyst. The trophic amoeba is recognisable by its fine finger-like pseudopodia (acanthopodia) projecting outward from the cell surface (Fig. 1). The vesicular nucleus contains a large central nucleolus. The encysted amoeba is protected from desiccation, starvation and a variety of chemical (disinfectants and antimicrobials) and physical agents (heat, freezing, ultraviolet radiation), and cysts have been known to survive in vitro for 20 years. Cysts of species causing keratitis have survived for as long as 14 days in ophthalmic solutions, including disinfectants used in lens care. The cyst is a double-walled (endo- and ectocyst) structure in which cellulose is a component of the cyst wall.

Pores are found in the wall, enabling the dormant amoeba to emerge and resume trophic growth (Figs. 5 and 11). In its amoeboid or cystic stages, *Acanthamoeba* is remarkably tolerant of a wide range of environmental conditions. There are about 30 species of *Acanthamoeba,* and morphology, particularly that of the cyst stage, has long been the basis for their identification.

Morphologically *Acanthamoeba spp.* fall into three groups (I–III), distinguished from one another by differences in cyst morphology. Group I has large cysts (18 μm or more) with stellate endocysts and smooth or wrinkled ectocysts; Group II has smaller

cysts (less than 18 µm) with polyhedric, globular, ovoid, or stellate endocysts and wavy ectocysts; and Group III has cysts (up to 19 µm) with globular or ovoid endocysts and smooth or wavy ectocysts. Group II amoebae are the most abundant in nature, and includes many of the more commonly isolated and potentially pathogenic species such as *Acanthamoeba castellanii* and *Acanthamoeba polyphaga*.

Culture conditions, however, can affect cyst morphology making species identifications based on morphology alone unreliable. More recently, identifications have relied upon sequencing of the amoeba small subunit nuclear 18S rRNA genes. Based on sequence variations, 15 different evolutionary lines or clades are now recognised: T1–T12, T13, T14, and T15. As an example of some of the difficulties encountered in identification and terminology, two isolates of *Acanthamoeba* from two culture collections identified as *A. polyphaga* were found to have a number of different characteristics including growth at 37 °C, endocyst morphology, isozyme patterns, ability to produce cytopathology and endonuclease-derived fragments of rDNA. Confusion of this type may arise through misidentification of isolates, possible mislabeling of culture tubes, or cross-contamination of laboratory cultures. Both environmental and clinical isolates can be established in vitro using a non-nutrient agar and a suitable bacterial food source such as *Escherichia coli* or *Enterobacter aerogenes*.

Cultivation of clinical specimens where amoebal infection is suspected (biopsied tissues, corneal scrapings) is important for confirming that amoebae are the causal agents of infection, for the identification of the pathogen, and for testing antimicrobial sensitivity. Most isolates readily adapt to growth in an axenic (bacteria-free) medium following antibiotic treatment, though some of the clinical isolates may be fastidious in their growth needs, and require nutritional supplements such as serum and additional vitamins.

Defined media formulations are available for both pathogenic and non-pathogenic stocks. All isolates grow well at room temperature (25 °C); clinical isolates can grow at 37 °C. Some clinical isolates, particularly those from corneal infections, grow optimally at lower than mammalian body temperature (30 °C).

1.2. Ecology

The major factor in the distribution of *Acanthamoeba* in water and soil is the presence of an available bacterial food supply. Warm waters might enhance growth and spread of *Acanthamoeba,* particularly those strains that are thermotolerant, but the amoebae are found over a wide range of temperature, salinity and pH conditions. *Acanthamoeba* strains infective for humans (and other mammals) must be able to survive 37 °C and somewhat higher body temperatures. But there is no evidence to suggest that human infections result solely from exposure to amoebae in warm or heated waters. Relatively few species have been associated with human infections: *A. castellanii, A. culbertsoni, A. hatchetti, A. healyi,* and *A. polyphaga*. Other species, however, may be thermotolerant but nonpathogenic. Thus, thermotolerance is necessary, but not sufficient for mammalian infection.

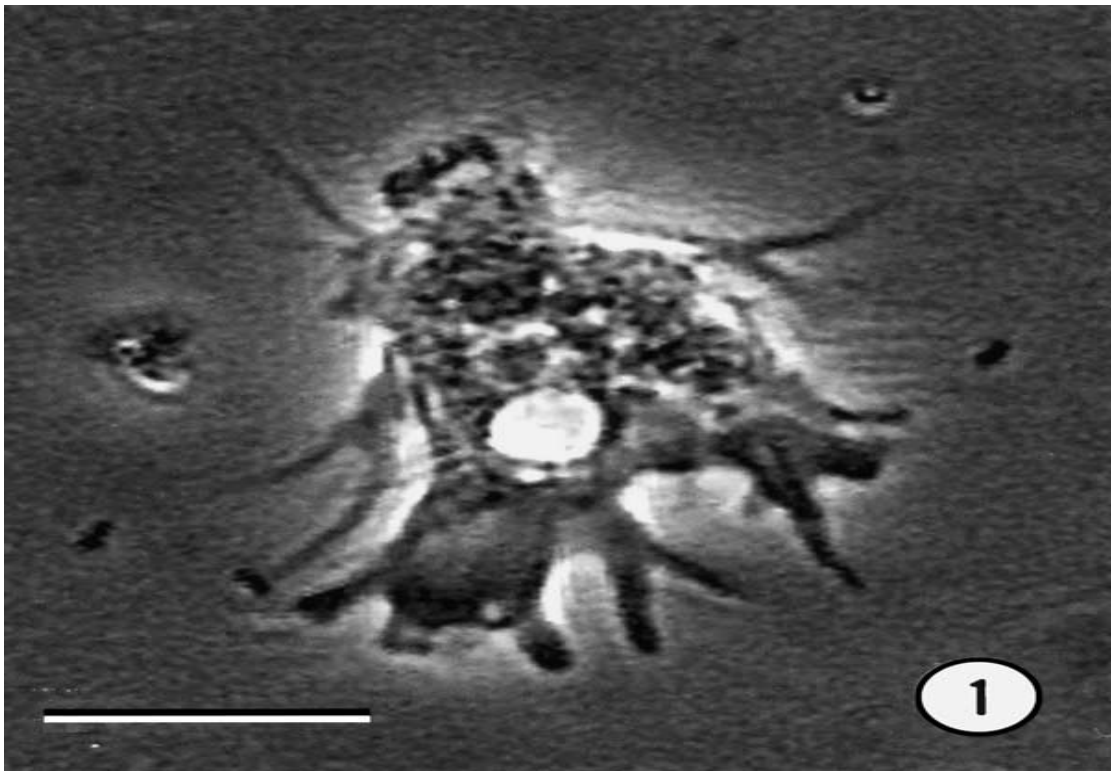

Fig. 1. *Acanthamoeba spp.* trophozoite. The distinctive feature is the presence of multiple finger-like acanthopodia projecting from the surface of the amoeba. The large clear vesicle in the cytoplasm is the contractile vacuole. Phase-contrast micrograph. Bar measures 10 µm.

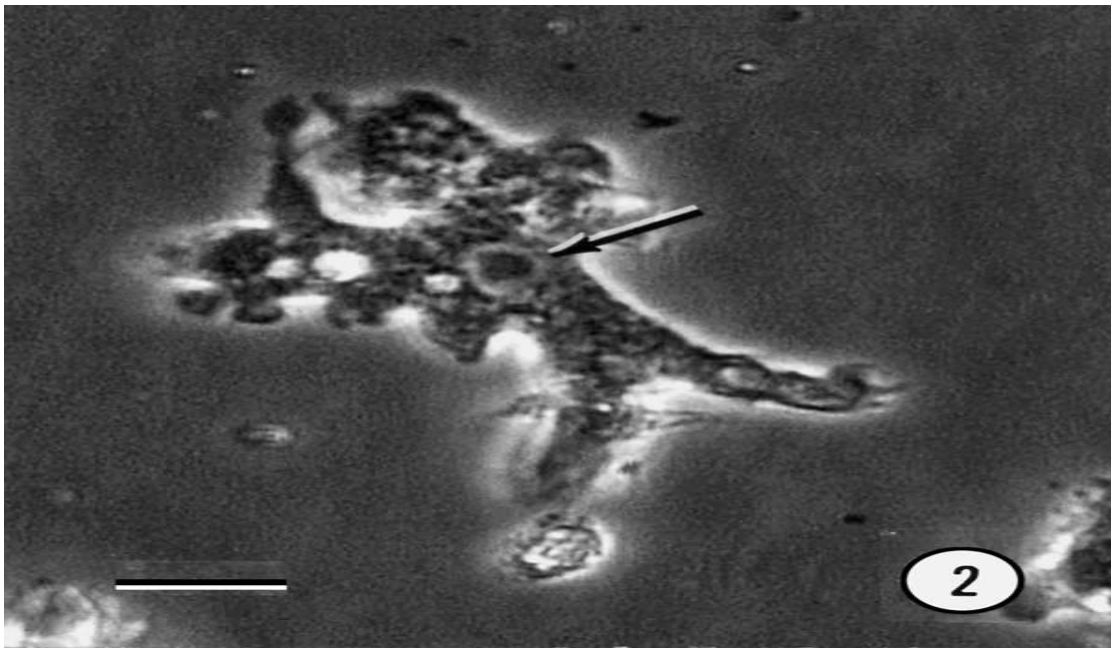

Fig. 2. *Balamuthia mandrillaris* trophozoite. The amoeba is seen with extended psuedopods, typical of the shape of the organism seen in vitro. Arrow indicates vesicular nucleus. Phase-contrast micrograph. Bar measures 10 µm.

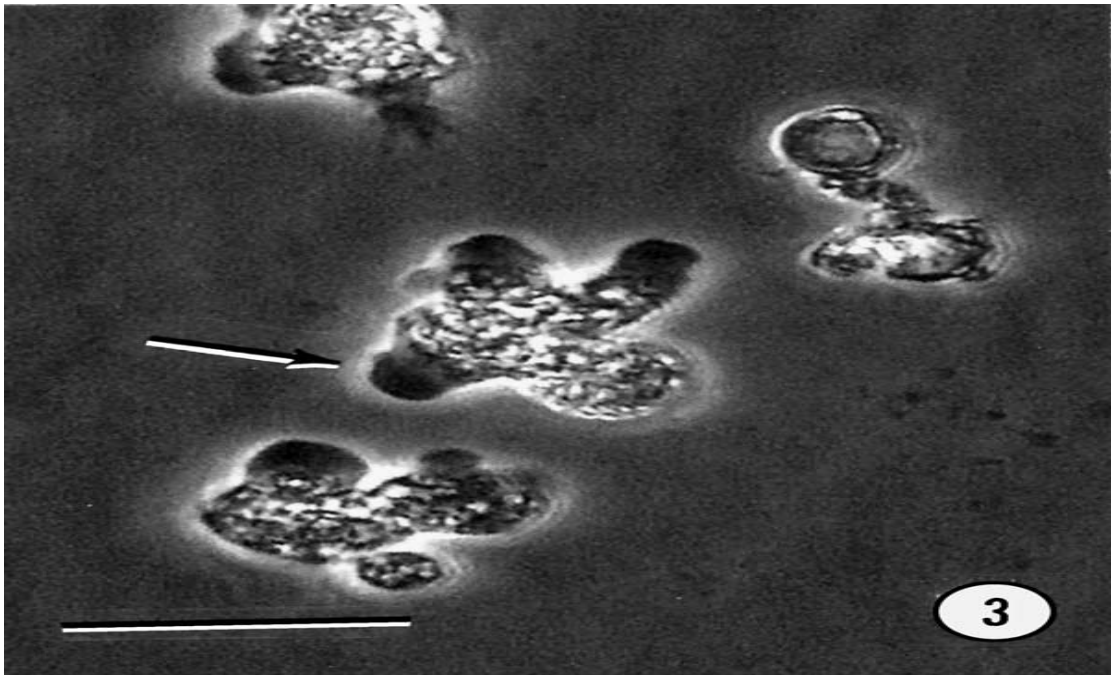

Fig. 3. *Naegleria fowleri* trophic amoebae. Bulging ectoplasmic pseudopods are a distinctive feature of the organism (arrow). Phase-contrast micrograph. Bar measures 20 µm.

It should be noted that there are non-pathogenic and thermosensitive strains. One such isolate is the Neff strain (*A. castellanii,* ATCC #30010) that has been a laboratory workhorse for morphological, biochemical, nutritional and molecular studies.
About 90% of amoebae (including *Acanthamoeba* and *Naegleria*) isolated from ponds (Oklahoma) during summer months were thermotolerant, of which 2% were pathogenic for mice. A study of amoebae in sources of drinking water (Germany) found 80 and 55% of *Acanthamoeba* isolates from river and reservoir waters, respectively, were thermophilic. *Acanthamoeba spp.* have also been isolated from poikilothermic animals (fish, amphibia, reptiles) where thermotolerance is not a critical factor for infection, and where amoebae may or may not be associated with existing pathology. Thermotolerance is less of a determinant for species that infect the human cornea. Corneal temperature is about 32–35 °C and, therefore, a wider variety of *Acanthamoeba spp.* are able to colonise the corneal surface.

1.3. Epidemiology

Considering the many opportunities for contact with amoebae, it is surprising that relatively few *Acanthamoeba* infections are encountered in humans and animals. The portal of entry can be through breaks in the skin contaminated by soil, or through the upper respiratory tract by cysts carried by air currents or wind. Once in the body, amoebae can spread haematogenous to the central nervous system (CNS) and various organs. The types of infections produced include granulomatous amoebic encephalitis, and nasopharyngeal, cutaneous and disseminated infections. *Acanthamoeba* (and *Naegleria*) have been isolated from the nasal epithelial surfaces of healthy humans, particularly during periods of high winds, such as the African harmattan. Some of these *Acanthamoeba* isolates have proven to be pathogenic in mice. *Acanthamoeba* has also been isolated from air samples. Swimming and other water activities have not been directly implicated as the cause of *Acanthamoeba* infections. Although cases of acanthamoebiasis have been reported from immunocompetent children, immunocompromised, immunosuppressed (HIV/ AIDS and organ transplant patients) and debilitated individuals (alcoholics, diabetics, patients with systemic lupus erythematosus) are primarily at risk of infection. Acanthamoebiasis has been reported from a variety of animal species, including dogs, monkeys, a bull, a kangaroo and an Indian buffalo.

Acanthamoeba isolates vary in their virulence (and antimicrobial sensitivity), which can be assessed using mice or tissue cultures. Studies using the mouse as a model for encephalitis have provided information about the course of the disease, variation in virulence found between *Acanthamoeba* isolates, and loss of virulence as a result of prolonged in vitro cultivation. The model is also useful for *Naegleria, Balamuthia,* and other pathogenic amoebae as well. Mice that are inoculated intranasally or intracranially with suspensions of *Acanthamoeba* develop symptoms similar to those in humans with amoebic encephalitis, usually within days to weeks depending on the inoculum size and the virulence of the amoeba strain. Virulence can be measured by the length of time before onset of symptoms and death, number of animals that died, and the dose of amoebae required to produce encephalitis. The ability of amoebae to cause cytopathology in tissue cultures has also been used as an indicator of virulence.
In addition to systemic infections, *Acanthamoeba* causes AK when amoebae directly attack the corneal surface. The disease is the result of corneal trauma or, more commonly, non-compliance in the care and wearing of soft contact lenses and maintenance of the lens case. Corneal trauma from physical damage to the cornea, either directly or indirectly introduces amoebae to the corneal surface and underlying stroma. For the contact lens user, a major source of infection has been presence of amoebae in non-sterile tap water used to prepare saline solutions for lens care. An infrequently cleaned lens case can develop bacterial biofilms, providing a source of food for the amoebae. Ophthalmic solutions, if they contain amoebae, would also be likely to contain bacteria that, in synergy with the amoebae, may promote corneal injury through secretions and metabolic wastes. Infecting amoebae have been ‘fingerprinted’ by endonuclease digestion of whole-cell DNA or sequencing of18S rRNA, and traced back to the tap water supply in the home of the contact lens wearer and/or the lens case (Section 2.8). Wearing contact lenses while swimming in pools or bathing in hot tubs, which may harbour a bacterial and amoebal fauna, is a high-risk activity. A case of keratitis has been traced to an outdoor hot tub in a garden setting. Elevated water temperatures, such as that be found in hot tubs, provide a selective environment for strains of amoebae that are able to tolerate mammalian body temperature. Domestic water storage tanks on the roofs of homes of 27 AK patients in the United Kingdom were found to be the source of free-living amoebae in 89%, and *Acanthamoeba* in 30% of in water taps in the homes tested. The use of the tanks as the domestic water supply was conjectured to be the cause for the high incidence of AK

in the United Kingdom (17–21 cases per million), w15-fold greater than the incidence of AK in the United States (one to two cases per million).

Data from the 1980s indicated that males were more likely to develop keratitis as the result of trauma, while infections in females arose mainly from contact lens use. A rough approximation of the number of AK cases worldwide in 2004 is 3000. A review of AK cases from 1985 to 1987 estimated annual incidence of infections to be one to two per million lens wearers.

Although several animal models (Chinese hamster, micropig, rabbit, rat) have been developed for study of AK, the models are more difficult to use than the mouse model for amoebic encephalitis. The technique requires abrading the corneal surface of the eye prior to application of an amoeba-carrying contact lens, or direct injection of amoebae into the cornea along with *Corynebacterium*. There appears to be a degree of selectivity in amoebal binding to and invasion

of the cornea. An in vitro study using corneal buttons from various animals, found specific binding to human, hamster and pig corneas, but not to horse, guinea pig, cow, chicken, dog, rabbit and rodent corneas.

Mannose blocked binding of amoebae to corneal epithelium in vitro, indicating a role for mannose residues in attachment to host tissue. Binding also stimulated release of cytolytic substances by the amoebae.

1.4. Systemic infections

As used here, systemic infections include *Acanthamoeba* granulomatous encephalitis (AGE), and nasopharyngeal and cutaneous infections. A cutaneous or nasopharyngeal infection can evolve into AGE as amoebae are carried to the CNS via the circulatory system. Typically, encephalitis is of the granulomatous type, but in immunocompromised individuals with an impaired cell-mediated immune response, granuloma formation may be suppressed and the typical pattern not seen. The amoebae attack brain tissue, producing hemorrhagic necrotizing lesions, most commonly found in the cerebrum, cerebellum and brain stem. In hematoxylin – eosin (H and E)-stained sections of brain tissue, the amoebae can be seen in large numbers in the perivascular spaces, and can be distinguished from host cells by their distinctive nuclear morphology. The thick-walled cysts may be seen in tissue sections (Fig. 5). Because, the disease has a chronic course with an extended incubation period, it is difficult to accurately identify a focal source of infection. More common symptoms

include behavioural abnormalities, fever, headache and hemiparesis. Premortem diagnosis, if made at all, usually follows biopsy of brain tissue and indirect immunofluorescence (IIF) staining for *Acanthamoeba* in the tissue sections. Magnetic resonance imaging and computerised tomography scans are helpful in recognizing and defining locations of space-occupying lesions. Because of a lack of familiarity with amoebae in H and E-stained sections, they can be easily overlooked upon routine histopathologic examinations of tissue sections. Although, recovery of *Acanthamoeba* from cerebrospinal fluid (CSF) has been reported following spinal taps of patients, amoebae are generally not found in the CSF. Amoebae can also be visualised in biopsied tissue from cutaneous (Fig. 11) and nasopharyngeal lesions by conventional H and E or immunofluorescence staining.

Under some conditions, *Acanthamoeba* is able to invade via an oral route. The amoeba has been identified in biopsy tissue of an ulcerated stomach wall from a female who died as the result of a perforated ulcer. Amoebae were assumed to have secondarily invaded the already damaged stomach lining, probably because of altered gastric acidity. Mice have been infected with *Acanthamoeba* inoculated orally, particularly after cimetidine dosing to reduce stomach acidity, or tetracycline to modify intestinal bacterial flora.

The infections spread to the CNS. Various substances have been reported as possible virulence factors in *Acanthamoeba spp.* Cysteine and serine proteases that are active against matrix proteins such as collagen have been identified in *Acanthamoeba* secretions. Serine protease activity may also protect amoebae from the host immune response by attacking and destroying immunoglobulins. In vitro, a cytopathic effect in corneal epithelial cell cultures was produced by corneal isolates, which was associated with pathogenic but not nonpathogenic *Acanthamoeba* species. Trophic amoebae have been reported to induce apoptosis in target cells as evidenced by membrane blebbing, and fragmentation and ‘laddering’ of DNA. Pathogenic strains also exhibited greater numbers of acanthopodia, better adhesion to corneal epithelial cells in culture, and formation of food cups for enhanced phagocytic activity. Based on comparative immunoblotting of pathogenic and non-pathogenic strains of

Acanthamoeba, excreted amoebal proteins (presumably cytolytic substances) correlated with strain pathogenicity. Contact with a human cell line in culture (HEp-2) stimulated protein release.

1.5. *Acanthamoeba* keratitis

Most, if not all cases of *Acanthamoeba* keratitis develop in immunocompetent persons when amoebae infect the corneal surface, either by trauma or by an improperly cared for contact lens or lens case. Typically, only one eye is involved. Although, AK infection usually does not develop into encephalitis, a case of uveitis and pharyngitis in a child was fatal. Early symptoms of AK include pain, lacrimation and photophobia that, unlike the early subclinical course of systemic infections, have an immediate effect upon the comfort and well being of the patient. Clinical diagnosis of the early stage of AK is based upon the presence of a dendritiform epithelial pattern. With time, a ring-shaped stromal infiltrate develops, caused by polymorphonuclear leukocytes streaming into the affected area. Staining of corneal scrapings with the fluorescent dye calcofluor white, has been used widely for visualizing amoebae microscopically.

Amoebae may be cultured from corneal scrapings. Once entrenched in the corneal stroma, the amoebae are difficult to eradicate. Trophic amoebae are more sensitive to antimicrobials used in treating AK than are cysts. While antimicrobial treatment can destroy trophic amoebae, dormant cysts can later give rise to amoebae once the drug level is reduced. Drug treatment has also been reported to induce encystation of amoebae in the stroma. Recurrence of infection is not unusual. Corneal grafting (penetrating keratoplasty) has been employed as a means of improving vision and/or reducing the amoeba load in the cornea. Often, however, repeated keratoplasties are performed because of flare-up of infection from surviving cysts in the area surrounding the graft. Treatment, at least in the initial stages, is intense with hourly topical drug applications, carried out for 3–4 months.

Topical application of steroids is common in the treatment of AK, both to relieve pain and to lessen inflammation, particularly following keratoplasty. Based on in vitro studies and use of the Chinese hamster model for AK, steroid use (dexamethasone) was found to stimulate proliferation of trophic amoebae and to enhance the cytopathic effect of amoebae on corneal epithelial cells in culture. Steroids are also responsible for suppressing the host immune response, allowing for further spread of amoebae into the corneal stroma. Dexamethasone was found to stimulate excystation of *Acanthamoeba* in vitro, but not

encystation. This can lead to an increase in the amoeba load in the cornea with a resultant increase in inflammation and corneal damage, but it also aids in keeping the organism in its trophic stage, which is more sensitive to antimicrobials. Thus, steroids can be helpful when used in conjunction with effective amoebacidal and cysticidal agents.

1.6. Molecular diagnostics

Development of molecular diagnostic techniques for acanthamoebiasis arose from studies of phylogeny and taxonomy of *Acanthamoeba* through analysis of nuclear and/or mitochondrial DNA. Much of the research was done with AK isolates, either to characterise the etiologic agent, to trace the source of the infection in the patient's environment, or to survey environmental samples for potentially pathogenic strains. AK is more common than systemic infections, and amoebae are more readily available for isolation without having to resort to invasive procedures such as biopsies. Isolation, cultivation, and subsequent identification of amoebae from corneal scrapings remain the gold standard of diagnosis. Cultivation, however, can be a slow process, and may present difficulties because of too few amoebae in the clinical sample to initiate a culture, inhibition of amoebal growth due to prior use of antimicrobial agents, embedment of amoebae in the corneal stroma so that they are not accessible to surface scraping, or an infecting strain that does not readily adapt to in vitro growth. Molecular techniques for identification of *Acanthamoeba*, as well as other pathogenic amoebae, are faster than cultivation, provide greater sensitivity, and can be done with a small amount of specimen.

Identification of infecting amoeba by molecular methodology is generally in good, though not perfect, agreement with results from cultivation of corneal scrapings; *Acanthamoeba* can be cultured from corneal epithelium, but may not be detected by molecular screening for DNA, or vice versa. The techniques that have been developed include: (i) PCR to amplify DNA of the nuclear small subunit 18S and the mitochondrial 16S rDNA genes, (ii) PCR-restriction fragment length polymorphism (PCR-RFLP) analyses of nuclear or mitochondrial rRNA and (iii) fluorescent probes to hybridise with *Acanthamoeba* DNA. PCR has been widely used as a means of improving the understanding of the phylogeny of members of the genus *Acanthamoeba* (Stothard et al., 1998, 1999) and identifying amoebae in culture. More importantly, the methodology has clinical relevance in detecting *Acanthamoeba* in corneal scrapings, and distinguishing AK from fungal and viral keratitis, allowing for early initiation of effective antimicrobial treatment. Using amplification of 18S rRNA, *Acanthamoeba* DNA has been detected in corneal epithelia and tear samples of contact lens wearers who developed AK. The use of 18S rRNA genes was also found effective as a diagnostic aid in cases of AK resulting from trauma, rather than lens-

wear. PCR-based analyses were sensitive enough to detect %5 trophic amoebae in samples.

Genus (*Acanthamoeba*) and subgenus-specific (T4 sequence type) fluorescent oligonucleotide probes complementary to 18S rRNA genes have been designed for in situ hybridisation. The probes were used successfully for whole cells from axenic cultures, corneal scrapings, or deparaffinised sectioned material and, to a limited extent, were able to distinguish between trophic amoebae and cysts. The probes were useful in ruling out amoebae other than *Acanthamoeba* in samples, since they did not hybridise with DNA from *Hartmannella* or *Balamuthia* amoebae. Another study identified *Acanthamoeba spp.* from corneal scrapings and sewage sludge from Scotland and South Africa using genus- and genotype-specific amplification products.

By means of DNA characterisation, it was possible to track the origin of resistance to the antimicrobial propamidine in an AK patient. Analysis of 18S rRNA was used to determine if the development of propamidine-resistance of a clinical isolate in the course of treatment for AK was due to mutation in a uniform population or appeared because of selection for drug resistance in a mixed population of amoebae. Analysis of three isolates from a patient over time showed sequence uniformity of 18S rRNA, confirming an earlier finding based on RFLP analysis of the population that drug resistance developed in a uniform amoeba population and not as a result of a mixed infection. In contrast to uniformity of sequence type in the previous isolates, amoebae of three recognisably different genotypes were isolated from the contact lenses of an AK patient.

Molecular techniques make it possible to track corneal infections back to the patient's environment. An uncommon AK isolate (*Acanthamoeba griffini*) was identified and traced back by 18S rRNA sequencing from cornea to the lens case, and ultimately to the domestic water tap in the home of the patient. Similar tracking of AK strains was done in studies in Hong Kong and Belgium. Mitochondrial PCR-RFLPs and sequencing of the cytochrome oxidase gene were used to survey domestic water sources (cold and hot water taps in bathroom and kitchen sinks) for *Acanthamoeba* in the homes of AK patients. Sequence similarities of water tap and corneal isolates pinpointed the domestic water supply as the source of the infecting amoebae in six of eight cases of AK in which *Acanthamoeba* was identified from the taps.

The T4 clade of *Acanthamoeba spp.* contains most of the keratitis pathogens and is also the most abundant in the environment of the 15 recognised clades. T4 amoebae

have a universal distribution and are agents of AK throughout the world, and they comprised a majority of *Acanthamoeba* isolates from south Florida beach sand.

The ability of amoebae to survive in a beach environment with seawater exposure may enable them to invade and colonise the corneal surface, where tear composition is roughly similar to that of dilute seawater. T4 amoebae were also isolated from asymptomatic freshwater fish and from a necrotic lesion in an iguanid lizard. Are the T4 amoebae inherently or potentially pathogenic, or are they isolated from infections because they are the most abundant sequence type of all *Acanthamoeba*? The answer to this question has yet to be determined. Other corneal pathogens are in groups T1 *(A. castellanii*), T3 (*A. griffini*), T6 (*A. palestinensis*), and Tll (*A. hatchetti*), but have been isolated infrequently.

A study of amoebae isolated from lens cases in southeastern Korea found that 88% of these were potential keratitis pathogens, with distribution of isolates reflecting geographic regions of the country. Likewise, a study using mitochondrial PCR-RFLP analysis concluded that isolates from lens cases (91%) and house dust (47%) were likely to be pathogenic, but that these strains were not generally found in soil. However, a study employing a similar approach found that many clinical isolates had environmental counterparts.

A complication of working with 18S rRNA is the presence of multiple alleles of the gene or the presence of Group I introns in the DNA of some species of *Acanthamoeba*. The use of shorter rRNA sequences found in mitochondrial 16S rRNA (1540 base pairs), rather than the longer 18S rRNA sequences (2300 base pairs) reduces the amount of variation seen. Analysis of sequences from the 16S rRNA gene validated the earlier sorting of *Acanthamoeba spp.* into genotype clusters based on the study of 18S rRNA, with most of the keratitis pathogens again falling into the T4 group. Riboprinting uses restriction fragments of endonucleasetreated DNA amplified by PCR, to produce profiles of isolates on agarose gels. Less effort is required for identifying isolates of *Acanthamoeba* than would be by the sequencing of the entire 18S rRNA gene. The technique has given rise to a phylogenetic tree compatible with the schemes based on sequencing of 18S and 16S rRNA.

1.7. *Acanthamoeba spp*. and endosymbiotic bacteria

An unusual property of environmental and clinical isolates of *Acanthamoeba spp*. is their ability to support growth of intracellular bacteria. This association remained something of an interesting curiosity until a connection was proposed between amoebae and pathogenic bacteria, in particular *Legionella pneumophila*, an agent of legionellosis (pneumonia) and Pontiac and humidifier fevers. *Legionella spp.* are widespread in the environment, and yet have growth requirements that make their survival in locations such as heating, ventilating and air conditioning units (HVAC) systems and plumbing lines unlikely.

In support of an amoeba-bacterium association, *Legionella* and *Legionella*-like bacteria have been isolated from a soil *Acanthamoeba*. The bacteria are phagocytised and proliferate in the amoeba cytoplasm much as they do in mammalian macrophages and are protected from digestion. Ultimately, they exit the amoebae, either by lysis or by an exocytic pathway, to infect other amoebae. Upon release from amoebae, bacteria may be enclosed in protective vesicles that can be carried in aerosols. About one-quarter of environmental, keratitis, and contact lens isolates have been found to harbour intracellular bacteria. In addition to serving as a source of nutrients, the intracellular location of the bacteria provides them with protection from hostile environmental conditions such as drying, and potentially bactericidal chlorine levels. Bacteria also survive within cysts of the amoebae, proliferating upon excystation and resumption of trophic growth. The bacteria are often obligate endosymbionts,

and resist attempts at in vitro cultivation. Among the endosymbionts detected in *Acanthamoeba* isolates are *Parachlamydia acanthamoebae* and other chlamydia-related prokaryotes (see below), some of which may be causal agents of pneumonia. Other bacteria isolated from amoebae are *Burkholderia picketti* and *Cryptococcus neoformans.*

In addition to HVAC units, the endosymbiont-containing amoebae have been isolated from sites in the hospital environment, including hydrotherapy baths, shower heads, faucet taps, ventilators and humidifiers. Amoeba-associated bacteria (mainly *Legionella anisa* and *Bosea massiliensis*) were isolated from more than one-third of water samples taken in a hospital intensive care unit. Hospital patients, many of whom are elderly, debilitated, or immunocompromised, are exposed to the bacteria released from amoebae in aerosols and are at risk for developing nosocomial pneumonias. Concern about amoebae in hospital hot water systems has engendered

quantitative and qualitative studies to evaluate the different genera and their degree of thermotolerance. Chlamydia are well-known as intracellular pathogens of humans, but an increasing number of chlamydia-related organisms are being isolated from environmental water samples in symbiotic association with amoebae. The isolation of one such bacterium from *Acanthamoeba* has offered insight into the origins of the symbiotic association. The symbiont is presumably an offshoot from the ancestral group that gave rise to extant pathogenic and environmental chlamydiae, and shares many coding sequences and virulence factors with the pathogens. The relationship points to an ancient association, with *Acanthamoeba* (or an evolutionary predecessor) serving as an available eukaryote host for perfecting virulence mechanisms found in present-day pathogenic chlamydiae. Endosymbionts without the adaptations to ensure survival within host amoebae would be destroyed.

Other studies on *Acanthamoeba* as host to endosymbiotic bacteria have attempted to establish pathogenic or potentially pathogenic bacteria in amoebae, including: *Afipia felis*, *Burkholderia cepacia*, *Burkholderia pseudomallei*, *E. coli O157*, *Chlamydia pneumoniae*, *Listeria monocytogenes*, *Mycobacterium avium*, *Mycobacterium bovis*, *Simkania negevensis* and *Vibrio cholerae*.

Given their ability to harbour bacteria, the ubiquity of *Acanthamoeba* in the environment makes it a potential incubator for infectious agents. Establishment of the pathogen *Francisella tularensis* in trophic amoebae has raised concerns that *Acanthamoeba* could be used as a bioterrorist weapon. The relationship between F. tularensis and amoebae is very similar to that described for *Legionella*, with bacteria being taken up into vacuoles and being released in vesicles upon lysis of the amoebae. Recently, a large DNA virus measuring 400 nm in diameter was identified from *A. polyphaga* isolated from a water-cooling tower. It was called *Mimivirus*, because it was first thought to be a Gram-positive coccus. Its relationship to the amoeba has yet to be characterised.

2. Naegleria fowleri

Naegleria fowleri is the causal agent of primary meningoencephalitis (PAM). *Naegleria spp.* are amoeboflagellates found in soil and water, but they are not as ubiquitous as *Acanthamoeba*. In general, they are more sensitive to environmental conditions such as drying and pH extremes, and cannot survive in seawater. Although,

some 30 species of *Naegleria* have been recognised based upon sequencing data, *N. fowleri* is the only one that has been isolated from cases of amoebic meningoencephalitis. Other species (*Naegleria australiensis, Naegleria italica, Naegleria philippinensis*) may be pathogenic in the mouse model of PAM, but have not been identified from any human cases. Because, it grows best at somewhat elevated temperatures, the amoeba has been isolated from warm-water bodies including man-made lakes and ponds, hot springs, and thermally polluted streams and rivers. In the United States, the summer months are the time of the year when cases are most likely to occur. *Naegleria fowleri* is thermotolerant, being able to survive temperatures up to 45 °C, preadapting the species to mammalian body temperature. Indeed, incubation at 45 °C is routinely used to isolate the amoeba from water samples, while suppressing growth of other amoebae in the samples. But thermotolerance is not the sole factor determining pathogencity of *Naegleria spp. Naegleria lovaniensis* is a thermotolerant species, but is non-pathogenic in the mouse model for PAM.

PAM is a fulminating disease, developing within several days following exposure to the water source, and causing death within 1–2 weeks after hospitalisation. Partly because of the rapid onset of the infection, and partly because of the delay in diagnosis, few individuals survive. The ability of *N. fowleri* to produce such a rapidly fatal infection has encouraged search for virulence properties of the amoeba that might account for its destructiveness. Among candidates that might serve as virulence factors is the release of the enzymes phospholipase, or neuraminidase, the creation of pores in target cell membranes that may have a lytic effect and aggressive phagocytotic activity. The amoeboid stage forms food cups (amoebostomes) that are capable of pinching off bits of target cell cytoplasm.

2.1. Life-cycle, morphology, and growth in vitro

The *Naegleria* life cycle includes amoeboid and cystic stages, as well as a flagellate stage which develops from the amoeba. The trophic amoeba has a distinctive limacine (slug-like) pattern of locomotion, with one or more ectoplasmic pseudopods (Fig. 3). The flagellate arises when the amoeba undergoes a morphogenetic transformation (over 30–60 min), triggered by suspension in water, into a transitory non-feeding and non-dividing flagellate stage (Fig. 4). The flagellate is believed to play a role in dispersion of the amoebae in their normal soil or water habitats. There are, however,

several species of *Naegleria*, identified as such by their genomic sequences, which lack the flagellate stage or in which the flagellate can divide. The cyst stage has a double wall with pores (Fig. 6). All *Naegleria spp*. are morphologically similar, if not identical to one another, although some differences among species may be found, as in cyst pore structure.

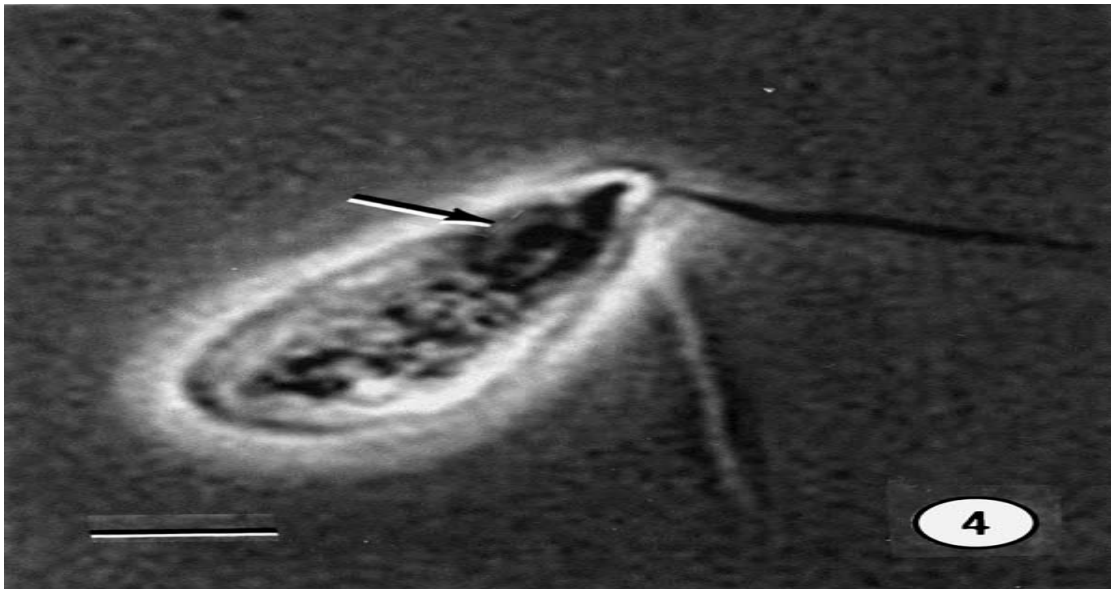

Fig. 4. *Naegleria fowleri* flagellate. Two flagella extend from the anterior end of the organism, where the vesicular nucleus (arrow) is located. A rhizoplast or rooting fiber (not seen in micrograph) extends posteriorly from the flagellar insertion. Phase contrast micrograph. Bar measures 8 µm.

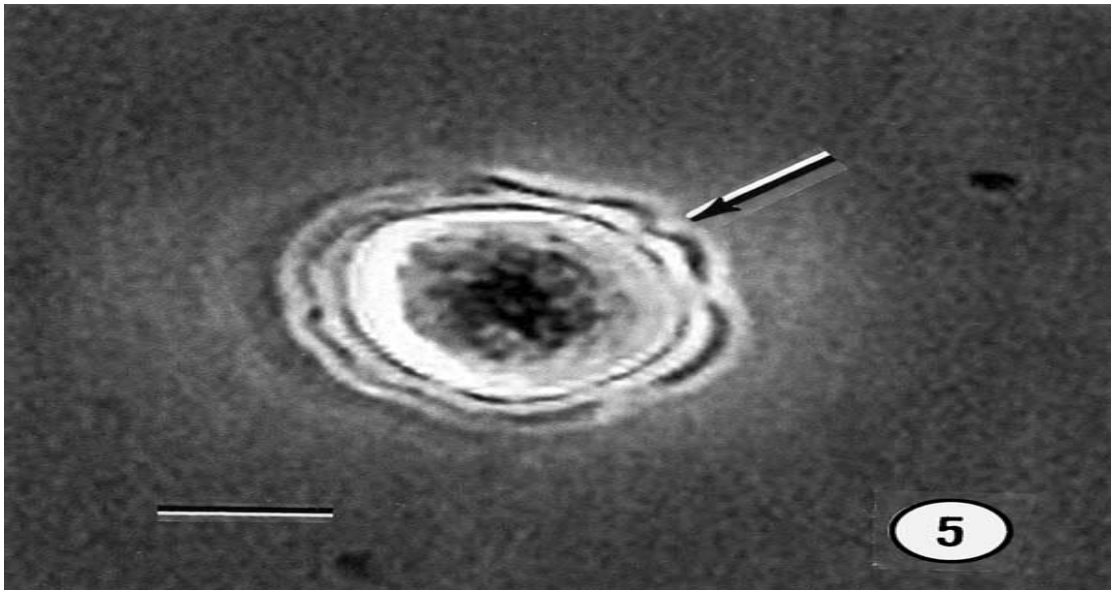

Fig. 5. Cyst of *Acanthamoeba spp.* The wavy outer wall or ectocyst is evident, as are the indentations in the wall (arrow) that indicate location of pores. The endocyst wall by comparison is smooth and closely covers the cytoplasm. Phase-contrast micrograph. Bar measures 5 µm.

In nature and in the laboratory, the amoeba feeds actively on bacteria. Isolations of *N. fowleri* from environmental soil or water samples are accomplished by growth on non-nutrient agar plates coated with *E. coli* at 45 °C.

Other bacteria (preferably nonmucoid strains), such as *Enterobacter aerogenes* or *Klebsiella pneumoniae*, may also be used. Once established in vitro, the amoebae can also be grown in an axenic medium (including a completely defined medium) following elimination of bacteria by antibiotic treatment of the culture. Surprisingly, *N. fowleri*, the pathogenic species, is less nutritionally demanding than *Naegleria gruberi*, its non-pathogenic counterpart. This difference in in vitro growth of pathogenic and nonpathogenic species has been exploited as a means of distinguishing between them.

Pathogenic strains can also be grown in tissue cultures at 37 °C, producing a cytopathic effect that varies with the virulence of the isolate.

2.2. Ecology

There has been great interest in the ecology of *N. fowleri*, since the presence of the amoeba in recreational waters is a threat to humans. Warm fresh waters coupled with a bacterial food supply are ideal habitats for these amoebae. Many infections have

occurred as the result of people swimming in man-made bodies of water, disturbed natural habitats, or areas in which soil and unchlorinated/unfiltered water are in contact. In France, for example, where cooling waters from nuclear power plants are released into rivers, awareness of the problem has led to downstream monitoring to guard against overgrowth and spread of *N. fowleri.*

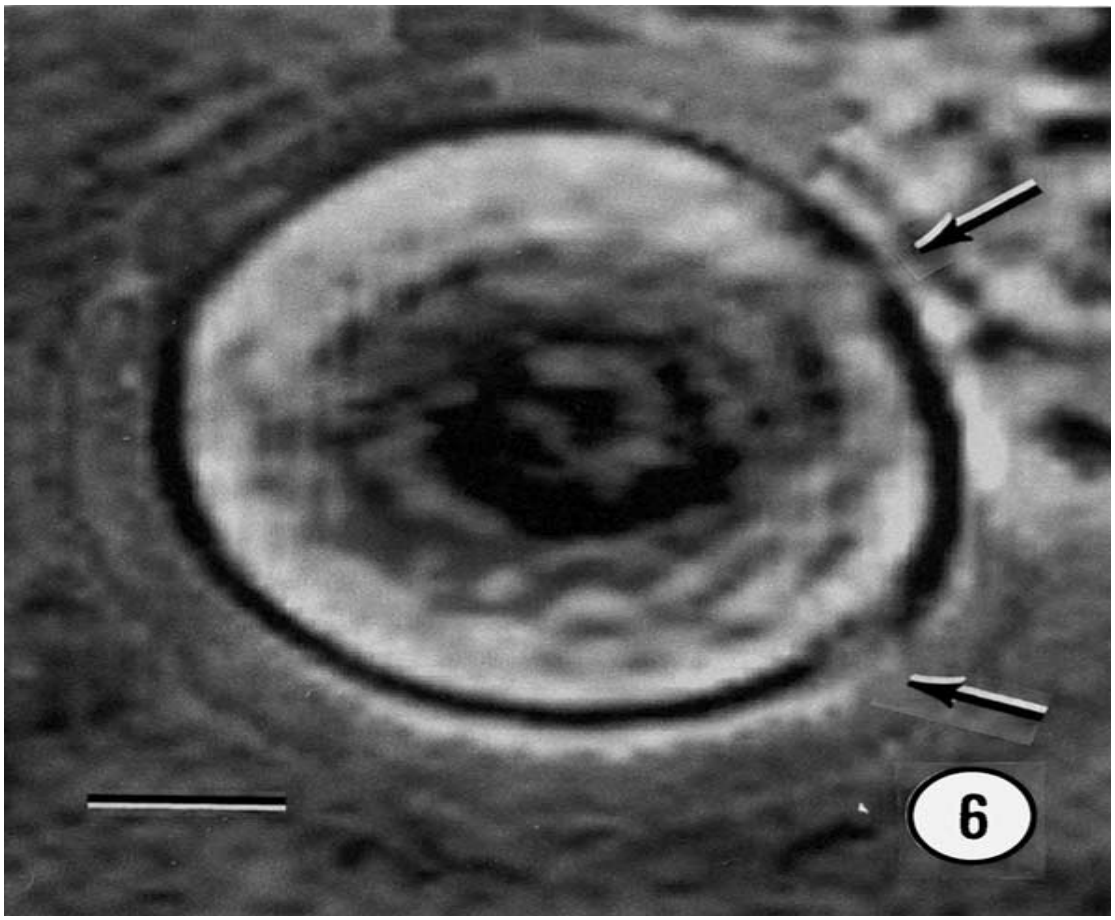

Fig. 6. Cyst of *Naegleria fowleri*. The two-layers of the cyst wall cannot be clearly seen in this micrograph. The pores in the wall (arrows) are flush with the surface of the cyst. The nucleus is visible in the center of the cyst. Phase-contrast micrograph. Bar measures 4 µm.

French authorities have deemed waters containing 100 *N. fowleri* amoebae/l unfit for swimming, because of the increased probability of infection, while a more stringent standard of five thermophilic *Naegleria*/l was adopted for a Western Australian water ski park. Presumably, local conditions and attendant risk factors would dictate local variations in standards. A survey of amoebae from a swimming pool (23 °C) implicated in a PAM outbreak (see below) revealed 10^2 to 10^3 amoebae/l of water,

none of which was pathogenic *Naegleria*. It took several more years before *N. fowleri* itself was isolated and an understanding developed of where the pathogen was 'hiding' in the pool.

The flagellate-empty hypothesis sought to explain the success of *N. fowleri* in these types of habitats. It suggests that disturbance of the environment by human actions (e.g. thermal pollution) results in suppression of the normal, thermosensitive protozoal fauna, thereby eliminating competition and opening the now-'empty' habitat to colonisation by *N. fowleri*. The thermotolerant amoeboflagellate has the added advantage of a motile flagellate stage that aids in its dispersal. Even the presence of a low concentration of chlorine in the water can open up the habitat to *N. fowleri* by destroying the resident protozoal community.

The relationship between the ecology of *N. fowleri* and disease is well illustrated by an outbreak of PAM associated with a swimming pool in Ustı´ nad Labem, Czechoslovakia. Here, a confluence of warm water, low free chlorine concentration, suspended organic matter and recreational swimmers, resulted in 16 deaths.

The PAM cases, which occurred over a 3-year period (1962–1965), were diagnosed retrospectively. In a subsequent investigation of the source of the infections, it was shown that a cracked false wall created a water-filled compartment in the swimming pool, where amoebae were sequestered at 27–30 °C, and protected from disinfectant concentrations used in the pool proper.

When the water level in the pool was raised for competitive swimming events, amoebae and organic matter were washed from the area behind the wall into the pool.

Adjoining the pool was a garden area through which swimmers could walk, possibly bringing soil and amoebae into the pool on their feet. The free chlorine level in the pool (0.3 mg/l) was inadequate to kill the amoebae. In order to be effective in preventing growth of *Naegleria,* the free chlorine concentration should be at 1 mg/ml.

2.3. Epidemiology

Naegleria amoebae (even *N. fowleri*) have been isolated from the nasal mucosa of healthy, asymptomatic children, and from nasal passages of patients with PAM. Most cases of PAM caused by *N. fowleri* are generally acquired by recreational activity in fresh-waters containing the amoebae. Some exceptions to this have been individuals bathing in hot springs, or washing with water containing amoebae. Individuals contracting the infection are children and young adults in ostensibly good health, with

a history of swimming or bathing in fresh water. Infection is through the nasal mucosa, by either the trophic amoeba or the cyst, the latter giving rise to the trophic amoeba. The flagellate stage (Fig. 4) has also been shown to be infective in the mouse model).

Apparent explanations for the incidence of PAM in young people, rather than other age groups, have been that they are more likely to remain in the water for longer periods of time, are more active in the water, and are more likely to engage in diving and underwater swimming, perhaps stirring up bottom sediment that may contain amoebae. Any activity that forces water into the nostrils would increase chances for amoebal invasion. In a recent selection of PAM cases from the United States and Mexico for DNA analyses, males (12) outnumbered females (one), suggesting that males may be more likely than females to engage in boisterous behavior while in water, with a greater chance for acquiring infection. Cases that have occurred in the United States are more likely to have developed in the warmer, southern tier of the country. In researching the history of a recent fatal case of PAM (Georgia, US) in one of a group of 15 children who had been swimming in a river (water temperature at 33°C), the child had spent O2 h in the water and had been actively engaged in diving, swimming, and horseplay. Also in the United States, unchlorinated water from a deep aquifer (Arizona) was the source of amoebae that caused PAM in two children; probably as the result of playing in a wading pool (Anonymous, 2002. *Naegleria* deaths in Arizona. Health Stream Article. http://www.waterquality.crc.org.au/hsarch/ HS28c.htm; Okuda et al., 2004). PAM cases have occurred in Florida following swimming in warm lake waters. The same lakes, however, have been frequented by millions of bathers, only a small fraction of which developed PAM. The risk of infection has been estimated at one case per 2.6 million exposures. Pathogenic *Naegleria* were isolated from 41% of 26 Florida lakes sampled, with isolations dependent upon time of the year and whether samples were taken from the water column or bottom sediment. Maximal risk of PAM for bathers in France was calculated by mathematical modeling to be 8.5!10K8, in waters containing 10 *N. fowleri* amoebae/l, with the risk rising (10K6 for 1000 amoebae/l) as the numbers of amoebae increase.

In New Zealand, cases have been associated with bathing in geothermal pools and bathers are cautioned to avoid diving and immersing their faces in the water. Nevertheless, a recent case of PAM in a child was likely due to just such immersion).

In parts of South Australia, household water supply, carried above ground in pipes over long distances and warmed by the sun to 35–45 °C, was an important source of infections, mostly during the summer months.

With effective chlorination and monitoring of the domestic water supply, *Naegleria* disease cases as a result of washing have virtually disappeared. No cases of PAM have ever been known to develop from drinking water containing amoebae.

PAM has been reported in animal species including bovines and a tapir. The mouse model is used both to study the course of the disease and to evaluate the virulence of isolates in culture. Recently isolated clinical specimens are likely to exhibit a high degree of virulence, but this tends to diminish with continued in vitro cultivation.

Virulence can be up regulated by mouse intranasal inoculation and reisolation of the amoebae, or by serial passage of amoebae in tissue cultures. Different *N. fowleri* isolates exhibit varying degrees of virulence, as can be demonstrated by their ability to produce a cytopathic effect in tissue cultures or by killing mice. Even the nonpathogenic *N. gruberi* can produce cytopathogenicity in tissue cultures if incubated at 30^{o}C.

Endosymbionts have not been found in environmental and/or clinical isolates of *Naegleria spp. Naegleria fowleri* can support growth of *L. pneumophila* in vitro, but only under specific culture conditions; amoebae suspended in saline solution do not support bacterial proliferation, as do amoebae in culture medium (Newsome et al., 1985).

2.4. Primary amoebic meningoencephalitis

Once entering into the nostrils of swimmers and others engaging in water sports, *N. fowleri* penetrates the mucosal epithelial layer and migrates along the olfactory nerve tracts, crossing the cribriform plate, to the brain. The cribriform plate in children is more porous than in adults, another possible reason for the higher incidence of PAM in young persons. Because of their proximity to the point of entrance of amoebae into the CNS, the frontal and olfactory lobes of the brain are the initial targets of amoebic destruction. Other areas affected are the base of the brain, the brainstem, and the cerebellum. Amoebae are found in large numbers in the perivascular regions in brain tissue (Fig. 9).

A purulent exudate containing trophic amoebae can be found in the subarachnoid space of the meninges. Involvement of the meninges is the basis for the distinction made between PAM and *Acanthamoeba* and *Balamuthia* encephalitides, which are typically granulomatous amoebic encephalitides.

Onset of symptoms follows within days after exposure, and includes severe headache, nausea and vomiting, fever (38.5–41 °C) and behavioural abnormalities. Rapid diagnosis is by microscopic examination, preferably phase-contrast, of freshly drawn CSF, to visualize motile amoebae. Suspending amoebae in 1 ml of distilled water can further confirm the identity of the amoebae as *Naegleria*, by watching for development of actively swimming flagellates. Further corroboration can be obtained by isolation of the amoeba (from CSF or macerated brain tissue) on a non-nutrient agar plate that has been spread with a lawn of E. coli, and incubated overnight at 37 °C. The amoebae will grow out in large numbers, feeding on the bacteria. Because this is an acute disease, early diagnosis is essential in order that appropriate antimicrobial therapy may be initiated before the amoebae do extensive damage. Diagnosis may be delayed when amoebae in CSF are mistaken for leukocytes. The pattern of locomotion is distinctly different, the leukocyte being sluggish in motion, while *Naegleria* amoebae move relatively swiftly with a distinctive ectoplasmic pseudopod.

Reports of three PAM cases gave amoeba counts in the CSF ranging from 26 to 118 amoebae/mm3, as compared to 330 to O9000 leukocytes/mm3. There are a few known cases of infection developing in individuals who were immunocompromised or in poor health. From the World War II era, a malnourished and disease-weakened Japanese prisoner-of-war was diagnosed, but which was later confirmed by immunofluorescence staining many years later to be due to *N. fowleri*. Perhaps because of his weakened condition, the infection had disseminated to include the brain (hemorrhagic necrotic lesions), stomach (ulcer), intestinal tract, mesenteric lymph nodes and both lungs. A patient with systemic lupus erythematosus with no history of swimming was diagnosed premortem with PAM.

2.5. Molecular diagnostics

The number of species of *Naegleria* has escalated from one (*N. gruberi*) in the pre-PAM literature, to five (Page, 1988), to 11 (De Jonckheere, 1998), to 27 (De Jonckheere, 2002), to the present number of about 30 (De Jonckheere, 2004), with more likely to come.

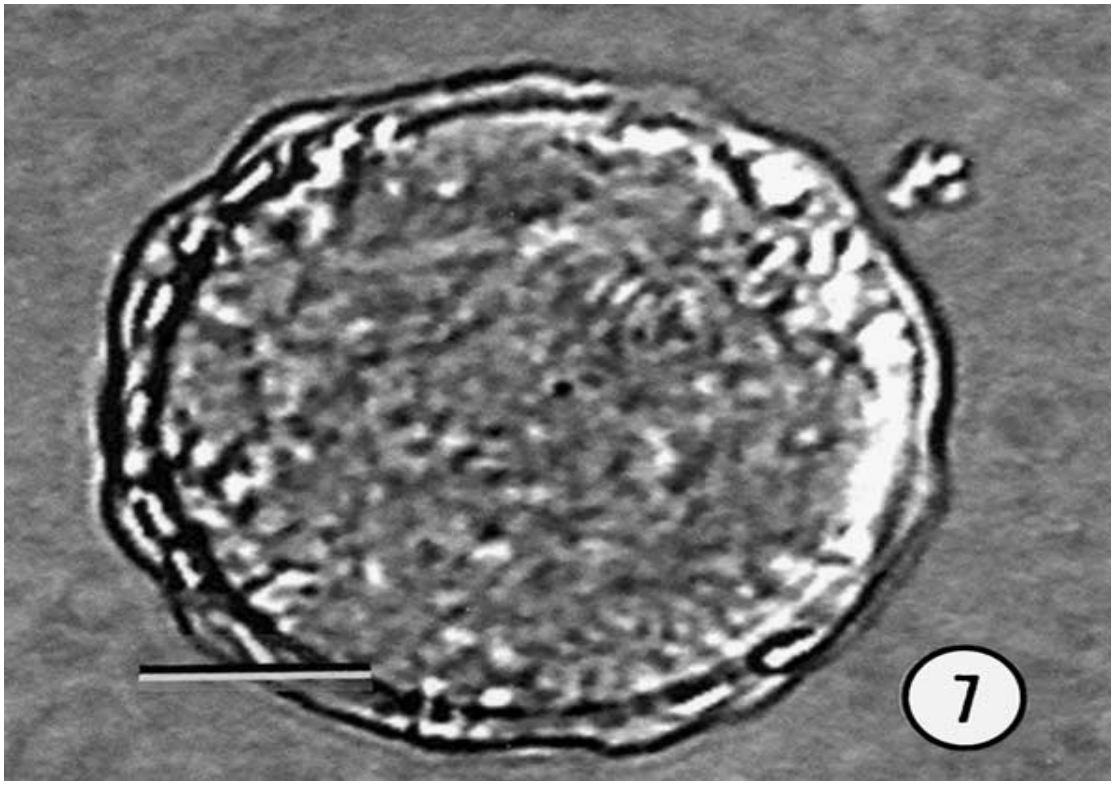

Fig. 7. Cyst of *Balamuthia mandrillaris*. The cyst wall is made up of three layers, the outer (ectocyst) of which is wrinkled. An inner wall (endocyst), which invests the cytoplasm, is separated from the ectocysts by a middle wall (mesocyst) of reticulate composition. Phase-contrast micrograph. Bar measures 7 µm.

While early distinctions between species were based on physiological properties (temperature tolerance, pathogenicity, ability to flagellate, etc.) of *Naegleria* isolates, current differences between species and strains are based upon molecular analyses of small subunit rRNA genes, leading to the current increase of species descriptions. The focus in this section will be on work done on the pathogenic species *N. fowleri*, and only obliquely on the non-pathogenic species.

Diagnosis of *Naegleria* infections presents fewer problems than *Acanthamoeba* infections. A single species is involved in all human infections, swimming in fresh

water is often the common denominator in all infections and the disease has a distinctive course, unfortunately often recognised only in retrospect. Thus, less effort has gone into molecular methodology for diagnostic purposes, but rather into: (i) distinctions between *Naegleria spp*. and *N. fowleri*, (ii) strain differences of *N. fowleri* isolates, (iii) methods for detecting amoebae in recreational waters and (iv) epidemiologic analyses for tracking infections from environmental source to patient.

Early application of PCR methodology was for the specific amplification of *N. fowleri* DNA in order to identify the amoeba in environmental samples. The method was sufficiently sensitive to detect single amoebae, and was also able to detect cysts. A species-specific probe was designed from cDNA for use with a nested PCR assay offering greater sensitivity in direct detection of *N. fowleri* in water samples. The technique does not require cultivation of, or DNA extraction from, the amoebae, and was sensitive enough to detect 5 pg (five trophic amoebae) of *N. fowleri* genomic DNA in spiked samples.

A novel application of PCR methodology has been in the corroboration of laboratory identification of *Naegleria spp*. based on the flagellation response of trophic amoebae. The flagellation test is often employed as a confirmatory test for recognition of environmental *Naegleria spp.* or to verify the identity of clinical amoebal isolates as *N. fowleri*. Some isolates, however, flagellate poorly under laboratory conditions and, in the absence of flagellates, might be misidentified. PCR and ELISA were used to examine *Naegleria spp.* that did not flagellate when subjected to the flagellation test. Of 28 amoeba isolates that did not flagellate, 11 were confirmed as *Naegleria spp* by non-specific PCR, and six as *N. fowleri* by a combination of species-specific PCR and ELISA.

Genetic heterogeneity of *N. folweri* strains was documented by restriction fragment length polymorphism (RFLP) analysis of whole cell DNA. Examination of a combination of isoenzyme profiles and RFLP analysis of geographically diverse *N. fowleri* isolates led to the recognition of groups of strains from Australia and from Europe, and the presence of both genotypic groups in the United States. *Naegleria fowleri* populations in the United States were postulated as the source of the other two groups that spread globally eastward and westward to Europe and Oceania (Australia/New Zealand), respectively. In another study, RFLP analysis confirmed genotypic heterogeneity among strains of *N. fowleri* leading to the recognition of three groups: European, Oceanic and American (US). The analyses also showed that

continental varieties were not sharply delineated; variations existed within populations from discrete geographic areas (e.g. among the European isolates). Further confirmation of strain differences among *N. fowleri* isolates was obtained from randomly amplified polymorphic DNA (RAPD), which also confirmed that different genotypic variants were present in the same general geographic region, and that strains from widely separated regions were genotypically identical. As examples of this diversity, a French isolate (Cattenom) was identical to a strain from Japan, while French isolate (Chooz) resembled strains from the South Pacific, a third (Golfech) resembled variants from Mexico, and a fourth (St Laurent) was similar to strains from the United States and Mexico.

Sequencing of the 5.8S rRNA gene and the internal transcribed spacers 1 and 2 (ITS1 and ITS2) of *N. fowleri* has shown that the genomic region can be used to differentiate strains. The ITS1 of different *N. fowleri* isolates shows sequence and length variations (42–142 bp long), while ITS2 (106 bp long) and the 5.8S rRNA gene (175 bp long) show only sequence variations.

Variability in the length of ITS 1 is a function of the number of repeats of motifs 1 and 2 in ITS 1. Two species-specific primers complementary to variable end-regions of ITS1 and ITS2 were designed, making it possible to distinguish *N. fowleri* from other *Naegleria spp.*. When applied to a phylogenetic analysis of the species, two major groups of *N. fowleri* amoebae were recognised. One group (genotype IV, according to Zhou et al., 2003) consisted of isolates from France and the South Pacific; the second group (genotype III) comprised isolates from Europe and America (including 'Widespread' isolates from France and Hong Kong). A study of *N. fowleri* isolates from the United States and Mexico confirmed the presence of two major groups of *N. fowleri* strains: the European–American group (genotype III of Zhou et al., 2003), and representatives of the Widespread group (genotype I). A third group (genotype II, restricted so far to America only) was also described, supporting De Jonckheere's (2002) hypothesis that some *N. fowleri* strains are geographically segregated. Phylogenetic trees based on either small subunit mitochondrial rRNA or the ITS 1 and 2 of *N. fowleri* strains gave consistent results with respect to assigning strains to different genotypes (I–III). It is now known that there are six genotypes of *N. fowleri,* based on the number and types of repeats in the ITS.

Molecular characterisation of strains is also useful in tracking infections to a source and recognising potential risks for swimmers or bathers in particular locales. A

species-specific DNA probe was designed to identify *N. fowleri* in environmental samples, followed by RFLP analyses of whole-cell DNA for confirmation. Epidemiological typing of *N. fowleri* was used in an analysis of the 5.8S rRNA gene and the ITS of clinical isolates. Two individuals who had visited the same hot spring in California at different times developed PAM. Amoebae isolated from patients' CSF were of the same genotype (II), and these in turn differed from genotypes of other *N. fowleri* strains examined.

3. Balamuthia mandrillaris

Following the recognition of *Acanthamoeba* and *Naegleria* encephalitides, infections by amoebae were turning up that were caused by neither of these two pathogens, or of any other known free-living or parasitic amoebae. In the late 1980s, an amoeba was isolated from the brain of a pregnant mandrill baboon that died in the San Diego (California) Wildlife Park. Once the amoeba was established in culture and antibodies against it raised in rabbits for immunofluorescence testing, it became evident that the amoeba that killed the baboon was also responsible for the encephalitis cases caused by previously unidentified amoebae. The amoeba was named *B. mandrillaris*. Soon, additional cases of *Balamuthia* granulomatous encephalitis (BGE) began to appear. The earliest human cases reported were in immunocompromised or debilitated individuals, and HIV/AIDS patients constituted a number of these. In more recent years, victims of BGE have been ostensibly immunocompetent children. When first established in culture, they were called leptomyxid amoebae because of their resemblance, particularly that of the cyst stage, to members of this group of soil organisms. But as noted previously, *Balamuthia* is closely related to *Acanthamoeba*, based on 16S rRNA sequencing (Amaral Zettler et al., 2000; Booton et al., 2003a), and causes infections similar to those of *Acanthamoeba spp*: granulomatous amoebic encephalitis and nasopharyngeal and cutaneous infections.

3.1. Life-cycle, morphology, and growth in vitro

The life-cycle comprises a trophic amoeboid stage (Fig. 2) measuring about 50–60 mm in diameter, larger than that of *Acanthamoeba* and *Naegleria*, and a cyst stage with a triple-layered wall lacking pores (Fig. 7). The shape of the amoebae is variable; with the trophic form appearing sometimes rounded, but often extended in a thread-

like configuration. The organism is uninucleate with a vesicular nucleus, often with multiple nucleoli.

Binucleate forms are occasionally encountered. Although, the amoebae move by means of broad pseudopodia, in tissue cultures they also move over the substrate by fingerlike pseudopodia in a kind of spider-like pattern of locomotion.

In vitro, *Balamuthia* amoebae can feed upon smaller soil amoebae (*Acanthamoeba*, *Naegleria*) and on tissue culture cells, but do not feed upon bacteria. Monkey kidney and rat glioma cells have been used as feeder layers in isolating amoebae from clinical samples, particularly biopsied brain tissue. They can be isolated from clinical samples by adding macerated necrotic brain tissue to a tissue culture and, if viable amoebae are present, they will emerge over several weeks to feed on the tissue culture cells. An enriched cell-free axenic medium containing fetal calf serum, liver concentrate, hemin, and amino acid and vitamin supplements has been developed. In axenic cultures, the amoebae grow with a doubling time of 25–50 h and produce populations of 10^5 amoebae/ml.

3.2. Ecology

Little is known about the ecology of *Balamuthia*. They are reclusive amoebae and are isolated from the soil habitat with difficulty. *Balamuthia* are slow-growing and appear in culture only after most other types of protozoa have grown out in the samples. They apparently feed on other protozoa (most likely amoebae) in soil samples applied to plates, using the conventional nonnutrient agar plus bacteria used for amoeba isolations. The amoebae are slow to appear in cultures, taking weeks before they become evident in any numbers. An environmental strain came from a sample of flowerpot soil in the home of a child who died of amoebic encephalitis (Schuster et al., 2003c) and a second environmental strain has also been isolated (Dunnebacke et al., 2003). They have been observed in other soil samples following plating, but are difficult to separate from contaminating fungi and other soil amoebae (Dunnebacke et al., 2003).

3.3. Epidemiology

Balamuthia amoebae are present in soil and, possibly, water. There has been no obvious history of swimming or other water activities for individuals who have developed BGE. Portals of entry of the amoeba into the human are similar to those for

Acanthamoeba: breaks in the skin contaminated by soil with subsequent haematogenous spread, or cysts transported by air currents to the respiratory tract.
One well-documented case involved an individual who was stuck by a rose thorn while digging a drain in his yard (Deetz et al., 2003). It is likely that the initial break in the skin was contaminated by soil. Amoebae were seen in a biopsy of the ulcerated lesion that developed at the site of injury, but recognised only retrospectively. The initial cutaneous infection progressed to amoebic encephalitis several weeks later. Contact with compost soil was also thought to be the source of infecting amoebae in a female who had no cutaneous lesions prior to developing encephalitis (Jung et al., 2004).

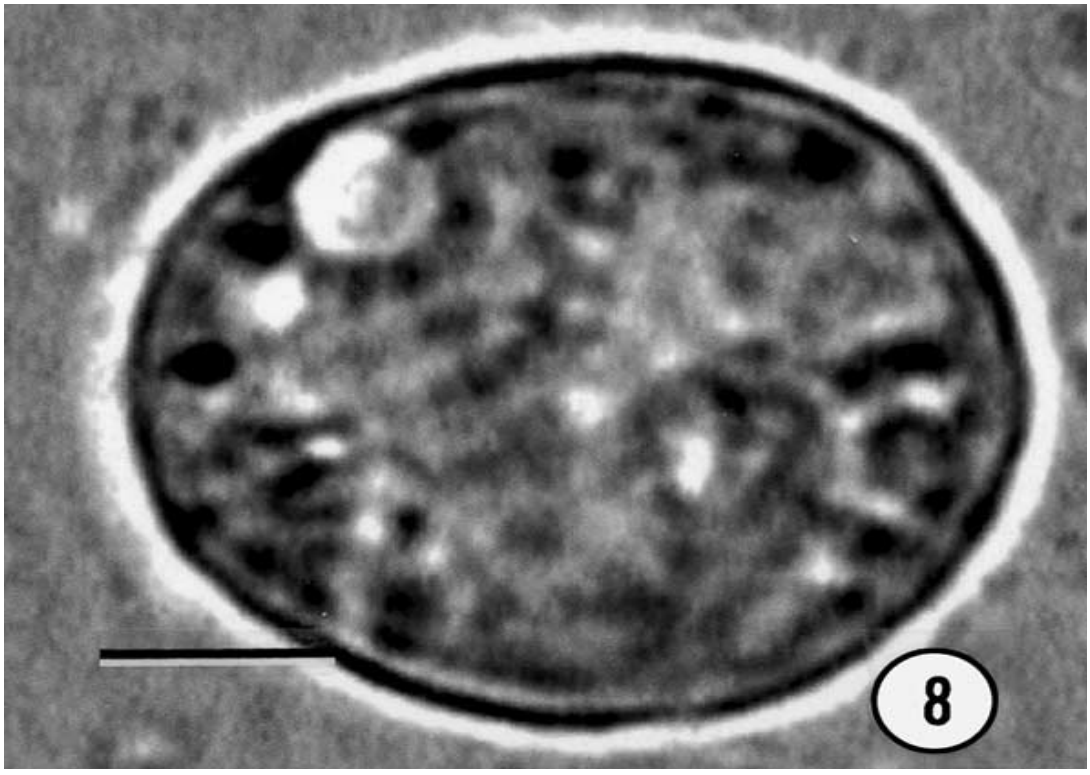

Fig. 8. Cyst of *Sappinia diploidea*. The cyst wall is smooth, and is without pores. Phase-contrast micrograph. Bar measures 7 µm.

A case of BGE in a male rancher developed first as a nodule on the nose, progressing to an ulcer with granulomatous inflammation and, over 5 months, reaching the CNS (Pritzker et al., 2004). The initiation of the lesion on the nose is suggestive of infection via the upper respiratory tract, perhaps through cysts carried by air currents, with progressive spread giving rise to intracranial lesions.

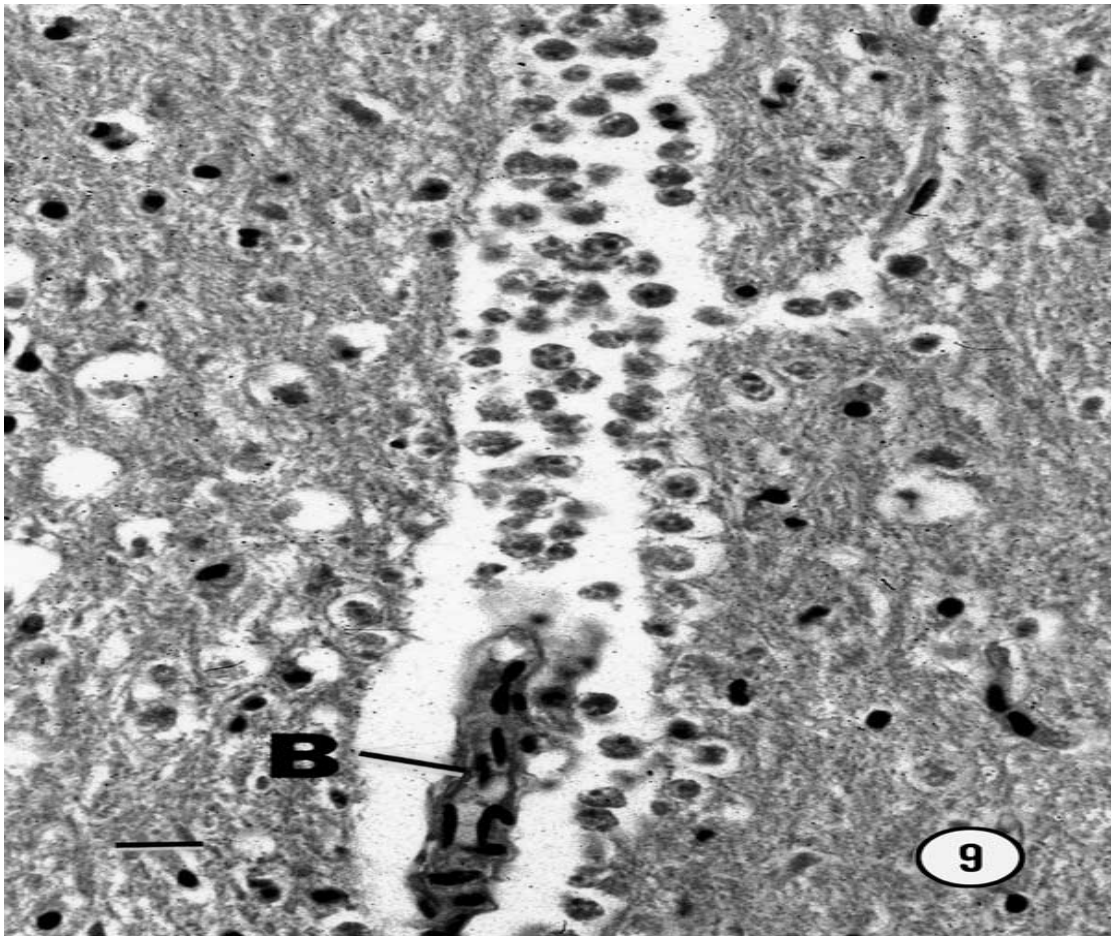

Fig. 9. Section of brain from a case of primary meningoencephalitis caused by *Naegleria fowleri*. A portion of a blood vessel (B) and the surrounding perivascular region are seen. Numerous trophic amoebae are present in the perivascular space. Hematoxylin-eosin stain. Bar measures 12 µm.

Cases have been reported from the United States (50), South America and Mexico (45), Australia (eight), Japan (two), and the Czech Republic (one). In the United States, about 50% of BAE cases have occurred in persons of Hispanic ancestry, though Hispanic Americans make up only 12%of the population. It is not clear if the over-representation is due to environmental exposure or to a genetic factor that renders Hispanics at greater risk.

Because of difficulty in diagnosing the infection, it is likely that the incidence of BGE is higher worldwide than the numbers of reported cases would suggest. *Balamuthia* encephalitis has been diagnosed in animals, including a horse, a sheep and dogs, but

most often in nonhuman primates. Many of the animals that have developed BGE were in zoological parks including the original mandrill baboon. The association, however, is due to the greater likelihood of autopsies being performed upon animals that have died in zoos. There is no evidence that animals are reservoirs for human infections.

3.4. *Balamuthia* amoebic encephalitis

Early signs of BGE include fever, personality change, stiff neck, cerebellar ataxia, hemiparesis, aphasia and seizures. The disease is difficult to distinguish from viral or tuberculous meningitis, tuberculoma, neurocysticercosis or, in some cases, neoplasia. Hemorrhagic necrotising lesions are seen in brain tissue (Fig. 10), which can also be detected by neuroimaging (computerised tomography and magnetic resonance imaging). CSF protein may be slightly to highly elevated (O1000 mg/dl), as is the number of leukocytes, although glucose level is normal or decreased. Amoebae may disseminate to other organs such as kidneys, adrenal glands, pancreas, thyroid and lungs, but are generally not found in CSF.

The disease is chronic and can develop over a period lasting from several weeks to as long as 2 years, allowing ample time for antibody production. Premortem diagnosis most often follows biopsy and histopathologic examination of brain tissue for trophic amoebae and/or cysts. In brain tissue, amoebae are typically seen clustered in the perivascular spaces. Antibodies can be detected in patients' sera by immunofluorescent staining.

Specific identification of *Balamuthia* in tissue sections can be done with IIF staining using rabbit anti-*Balamuthia* serum. Premortem diagnosis is no guarantee of survival, since the patients may have suffered extensive brain damage prior to diagnosis. Most diagnoses of BGE are made post-mortem by H and E or immunofluorescence staining of brain tissues.

There is little information about virulence factors produced by the amoeba. Amoebal lysates have been shown to produce membrane lesions in tissue culture cells causing them to swell and eventually disintegrate.

3.7. Molecular diagnostics

Balamuthia when first isolated into culture was described as a leptomyxid amoeba based on morphological similarity of the trophic amoeba and cyst to members of the Leptomyxidae, a little-studied group of organisms found in soil (Pussard and Pons, 1976). Early clinical reports on *Balamuthia* encephalitis cases referred to the amoeba as a 'leptomyxid' (Anzil et al., 1991). An indication that *Balamuthia* was not a leptomyxid was a by-product of a study of *Acanthamoeba* phylogeny using the 18S rRNA gene (Stothard et al., 1998).

The study pointed to a close affinity with *Acanthamoeba*. The relationship was affirmed in a subsequent study that sequenced and compared the 16S-like rRNA genes of *Balamuthia* to those of other protozoa. Based on genotype sequencing, *Balamuthia* was found to be closely related to *Acanthamoeba* and phylogenetically distant from the leptomyxids.

A more extensive treatment of seven different clinical isolates of *Balamuthia* involved both nuclear and mitochondrial rDNA, and found no variation in the nuclear rDNA and only minor variation in the mitochondrial rDNA.

The strains examined included isolates from both human and animal infections from the United States and Australia. In contrast, sequencing data have shown multiple genotypes for *Acanthamoeba* and *N. fowleri*. The degree of variation in the mitochondrial rDNA of the different isolates ranged from 0 to 1.8%, while sequence dissimilarity between *Balamuthia* and *Acanthamoeba* mitochondrial rDNAs was 19%. Thus, Balamuthia appears to represent a monophyletic genus distinct from *Acanthamoeba.*

A primer pair specific for *Balamuthia* (50Balspec 16S and 30Balspec 16S) was developed from sequence data of mitochondrial 16S rRNA genes (Booton et al., 2003b). Using this *Balamuthia*-specific pair, an amplimer of 1075 bp was produced. The primer did not amplify *Acanthamoeba* or human DNAs, the latter being an important factor in distinguishing *Balamuthia* DNA from human DNA in tissue samples. In an application of PCR amplification, mitochondrial 16S rDNAs from a clinical isolate and from an amoeba isolated from flowerpot soil in the home of the patient yielded the same 1075 bp amplimers (Booton et al., 2003c).

Furthermore, sequencing of the product demonstrated that the two *Balamuthia* isolates were identical.

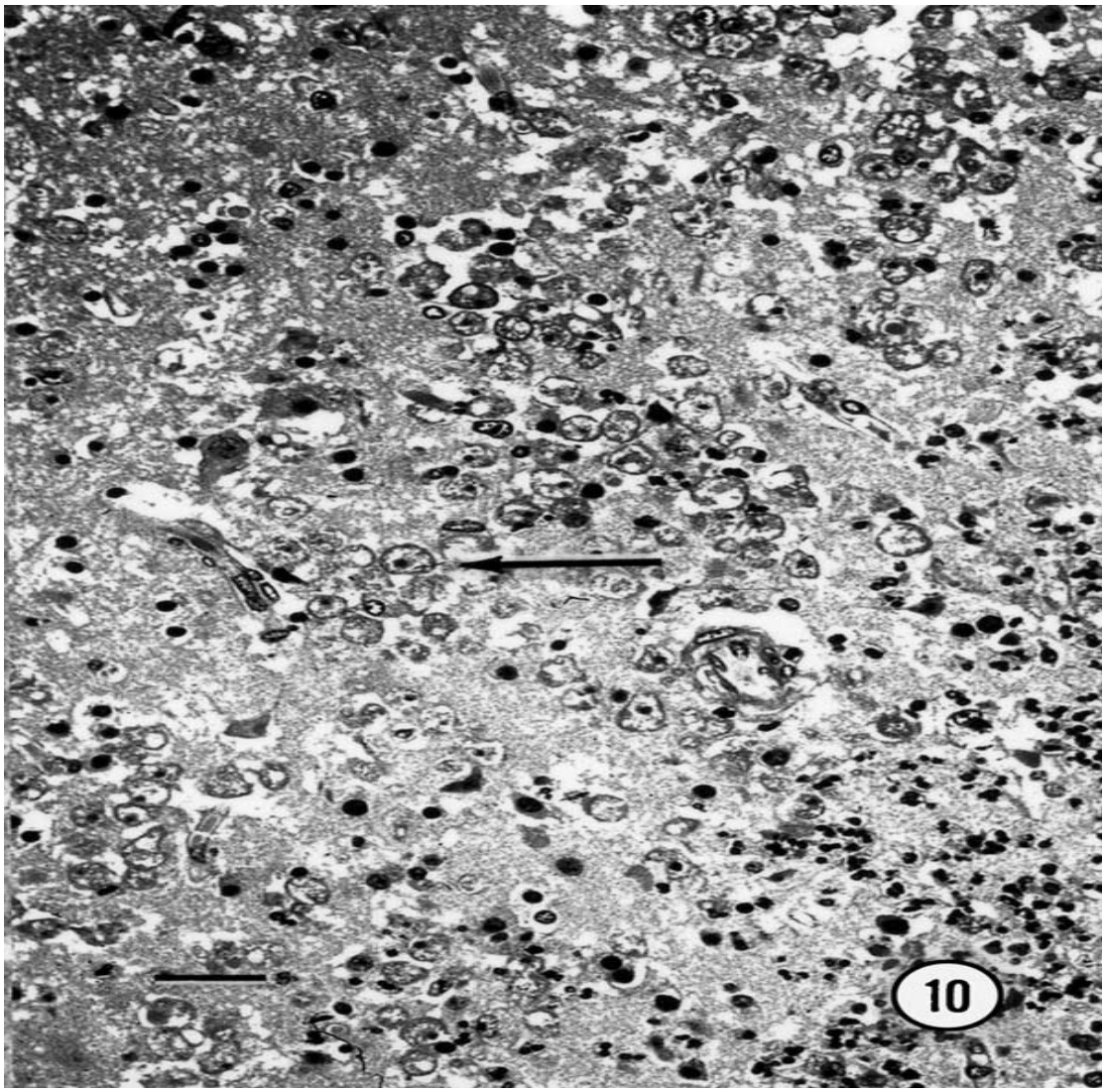

Fig. 10. Numerous *Balamuthia* trophic amoebae are present in brain section from a patient with amoebic encephalitis. Arrow points to a well-defined trophic amoeba, but many others are seen in the surrounding area. Hematoxylin-eosin stain. Bar measures 100 µm.

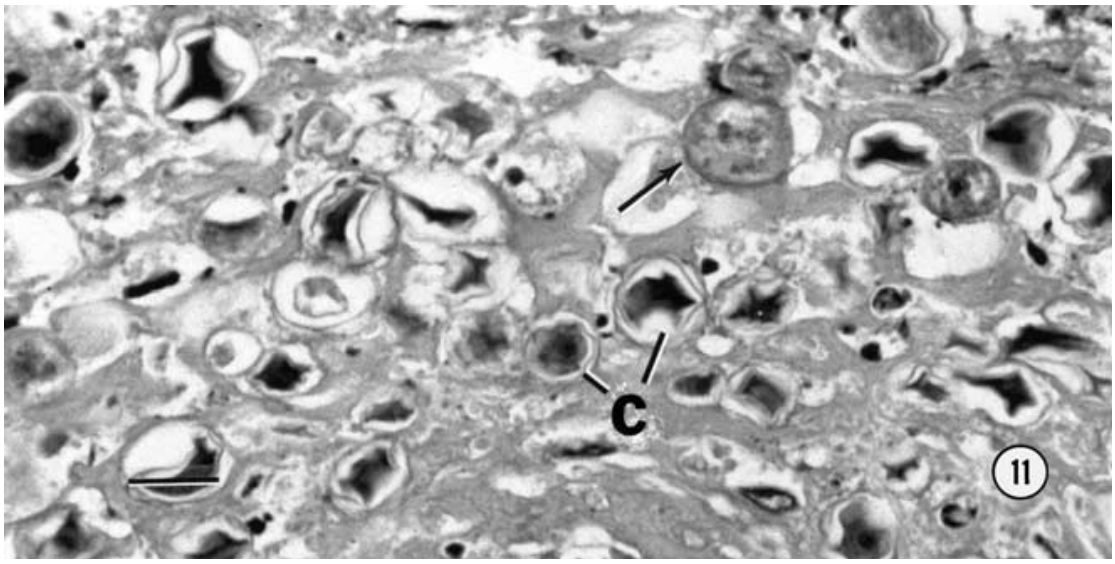

Fig. 11. Cutaneous infection with *Acanthamoeba spp.* Numerous cysts (some collapsed) can be seen in the section (C), recognisable by their irregular walls and the densely stained cytoplasmic content. Trophic amoebae can be seen in the adjacent area (arrow). Hematoxylin-eosin stain. Bar measures 20 µm.

4. Sappinia diploidea

A single case of encephalitis caused by the amoeba *S. diploidea* was reported in an immunocompetent male (Gelman et al., 2001, 2003). In addition to environmental isolations of the amoeba, it had been previously identified from feces of humans, elk, bison and perhaps cattle, but was never before implicated in pathology. The amoeba is relatively large with indistinct pseudopodia and a pellicle that may wrinkle as the amoeba moves (Fig. 13). A distinctive feature of the organism is the presence of two nuclei tightly apposed to one another (Fig. 12). A binucleate cyst stage also occurs in the life-cycle (Fig. 8). Because of its appearance in feces, the organism and other amoebal isolates from stools have been characterised as coprozoic.

The cysts can survive passage through the stomach with its gastric fluid, as well as withstanding the emulsifying properties of bile from the liver. Their presence is not indicative of infection since they have passed through the intestinal tract as cysts that, once deposited in the stool with an abundance of bacterial food, undergo excystation. Symptoms in this case were headache, seizure, blurred vision, photophobia and vomiting. Magnetic resonance imaging revealed a single space-occupying lesion that

was surgically excised. Histopathologic examination of brain tissue revealed trophic amoebae, but not cysts and no evidence of a granulomatous reaction. Following surgery, treatment with azithromycin, pentamidine isethionate, itraconazole and flucytosine was begun and led to a complete recovery with no neurological sequleae. The patient had contact with farm animals, and may have been exposed to *Sappinia* cysts carried on air currents into the nasopharynx. Significantly, a sinus infection preceded the patient's encephalitis, in support of the nasopharynx as the portal of entry.

5. Other amphizoic amoebae

Other free-living amoebae, able to adapt to mammalian body temperature, are also candidates for being facultative pathogens. Some of these have been isolated from homeotherms, some from poikilotherms, and still others from the environment.

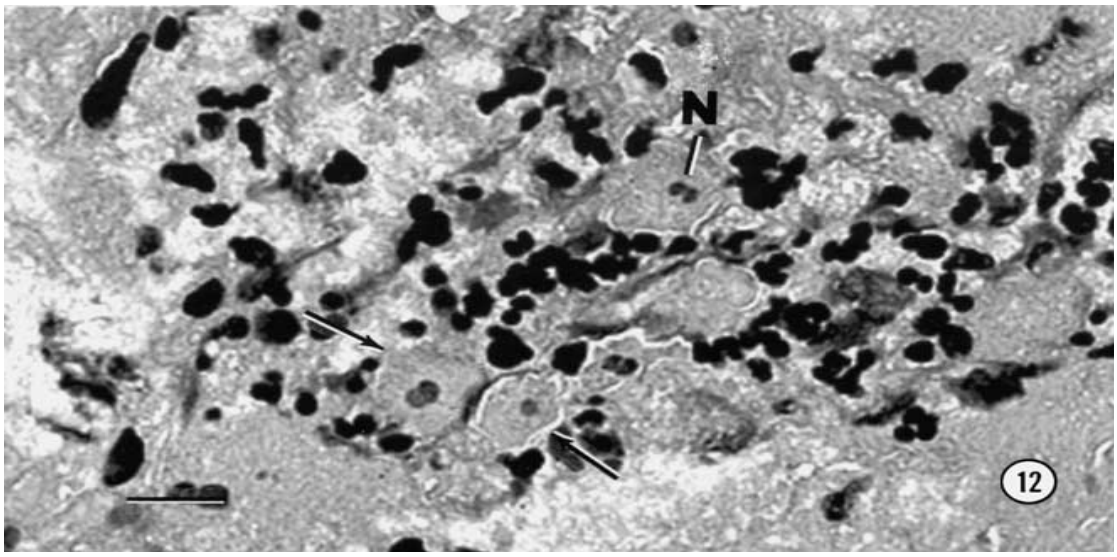

Fig. 12. Section of brain from patient with *Sapppinia* encephalitis. Arrows indicate trophic amoebae seen in the section. The double nucleus typical of *Sappinia diploidea* can be seen in one of the amoebae (N). Numerous heavily stained inflammatory cells are dispersed through the region. Hematoxylin-eosin stain. Bar measures 50 µm.

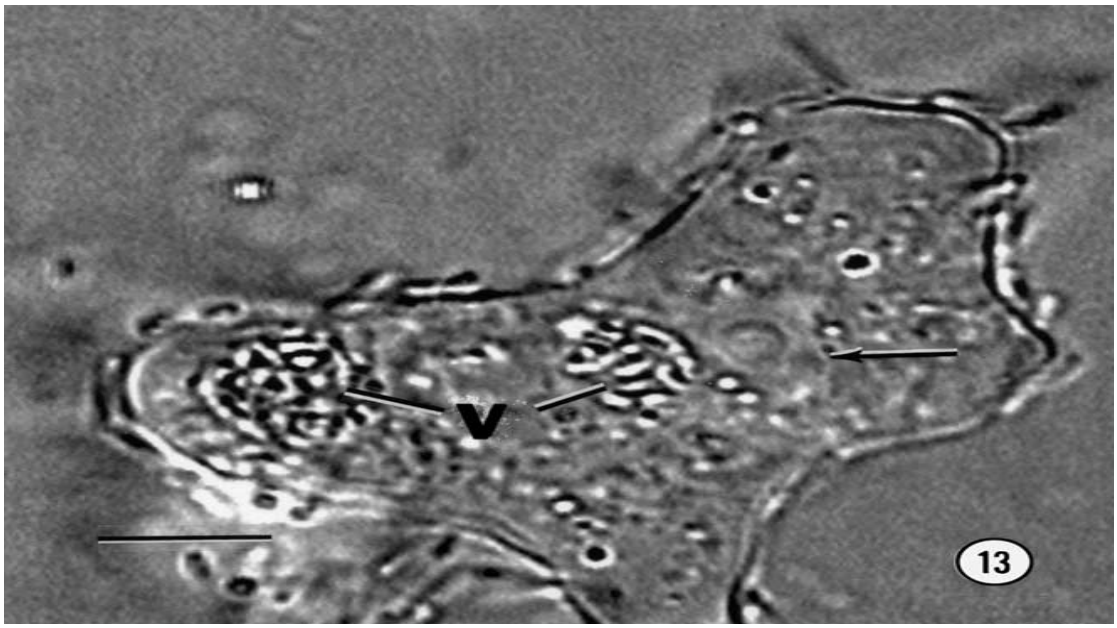

Fig. 13. *Sappinia diploidea* trophic amoeba. The nucleus can be seen in this image (arrow). Two food vacuoles (V) containing bacteria are also evident. The pseudopodia are indistinct. The surface pellicle of the amoeba may form wrinkles, which are not seen in this optical section, as it moves over the substrate. Phase-contrast micrograph. Bar measures 30 µm.

Acanthamoeba, Vahlkampfia, and *Hartmannella* have been isolated from the nasal cavity, intestine, vagina and other sites in dead cattle, swine, dogs, rabbits and turkeys, but not from the CNS or lungs (Kadlec, 1978). The isolates were not associated with any pathology, nor were they pathogenic in mice or guinea pigs.

Willaertia magna is a thermophilic amoeba, morphologically similar to *Naegleria*, but larger. The flagellate stage of the organism has four flagella, and undergoes repeated divisions, with cell volume diminishing in successive divisions. The amoeba has been isolated from bovine feces and thermally polluted waters. The organism has not been associated with human pathology nor is it pathogenic in the mouse model, but was identified by immunofluorescence staining in the ulcerated stomach of a dog that had been euthanised following corticosteroid treatment for other impairments. The administration of steroids as anti-inflammatory agents probably was a factor in promoting what might otherwise have been a benign amoebal infection, by suppression of the animal's immune system.

Acanthamoeba, Hartmannella, Naegleria, and *Entamoeba* were isolated from feces and, in some cases, the brains of reptiles, with the possibility that snakes and lizards

may be a potential source of human amoebic infection when kept as domestic pets. A variety of amoebae were isolated from the gut of reptiles, but *Acanthamoeba* and, to a lesser extent, *Naegleria* were the most common. A strain of *Paravahlkampfia ustiana*, from lizard intestine (skinks), grew at 42 °C and produced cytopathology in tissue culture monolayers, indicative of a potential for pathogenesis in mammals (Schuster et al., 2003b). Amoebae, including *Acanthamoeba, Naegleria*, and *Echinamoeba*, were isolated from a necrotic lesion on the tail of a basilisc lizard. Except for the *N. gruberi* isolate, the remaining two amoebae were thermotolerant, and the *Acanthamoeba* isolate produced a cytopathic effect in tissue cultures.

Acanthamoeba spp. isolated from fresh-water fish, were found to be members of the same evolutionary lineage (T4) as the amoebae that cause *Acanthamoeba* keratitis. It is not clear if the connection is due to the widespread distribution of the T4 group, or whether T4 amoebae are better adapted to develop commensal or even parasitic interactions with their hosts.

Are their other amoebal species involved in human pathogenesis? The question is a contentious one with reports of isolations of *Hartmannella* and *Vahlkampfia* from human keratitis cases. Although, these isolates are cytopathic in keratocyte tissue cultures (Kinnear, 2003), evidence is lacking that they are pathogenic in the keratitis animal model and, furthermore, that the claimants have not satisfied Koch's Postulates in demonstrating cause and effect of disease (De Jonckheere and Brown, 1998). Then, too, secondary invasion of lesions or contamination of clinical samples by free-living amoebae can occur. Opportunism by *Hartmannella* rather than etiology was the conclusion following isolation of the amoeba from CSF of a patient with meningoencephalitis (Centeno et al., 1996).

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مشروع دعم قطاع مياه الشرب والصرف الصحي
Water and Wastewater Sector Support (WWSS)

الأميبات حرة المعيشة الممرضة في البيئة المائية

PATHOGENIC FREE-LIVING AMOEBAE IN THE AQUATIC ENVIRONMENT

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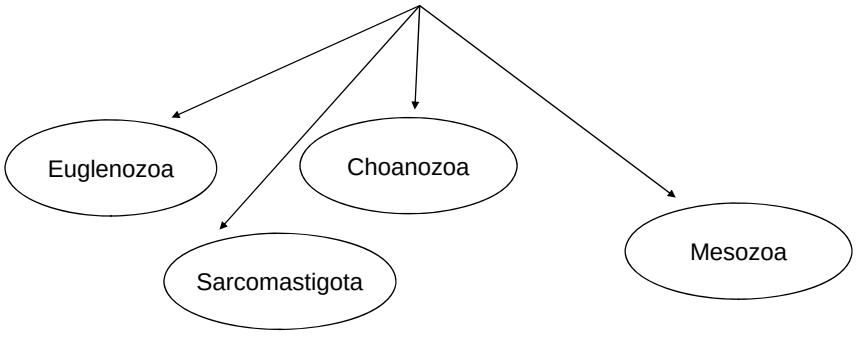
(1)

INTRODUCTION TO PATHOGENIC FREE-LIVING AMOEBAE

The 8 kingdoms of Life

- EUBACTERIA
- ARCHAEBACTERIA
- ARCHEZOA
- **PROTOZOA**
- PLANTAE
- ANIMALIA
- FUNGI
- CHROMISTA

PROTOZOA
(Cavalier-Smith, 1983)



- Unicellular
- Multicellular

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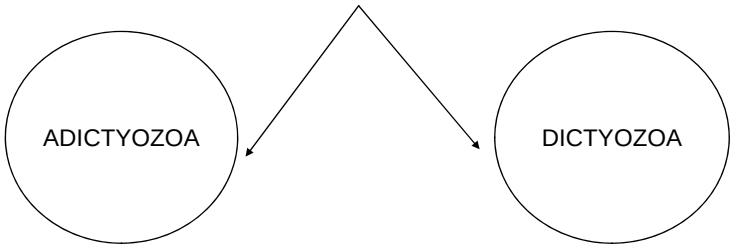
PROTOZOA
(Cavalier-Smith, 1993)



- Golgi dictyosomes

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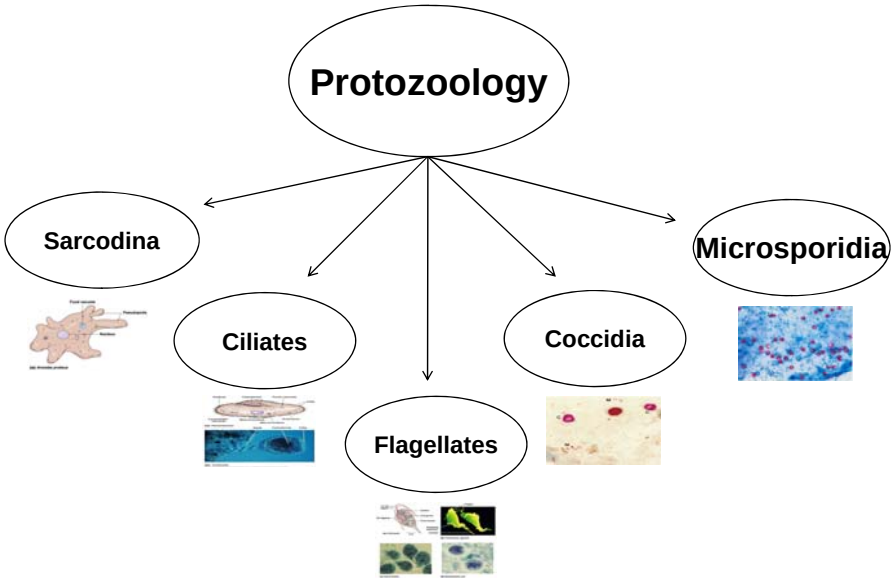

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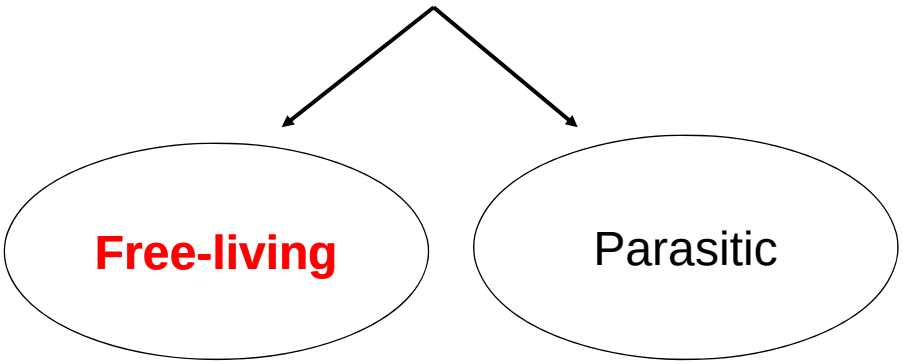
Protozoa



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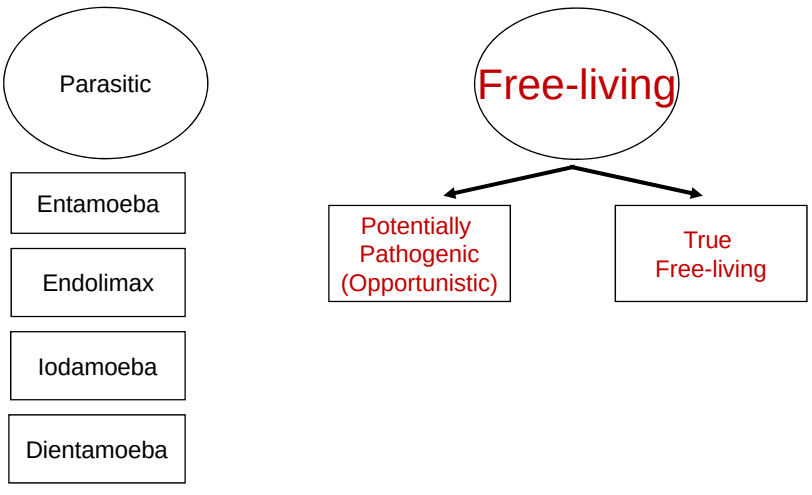
AMOEBAE
SARCODINA



AMOEBAE (SARCODINA)

ITEM	PARASSITIC	FREE-LIVING
NORMAL HABITAT	Inside host	Aquatic environment
FOOD	Tissue cells	* Environment …. Bacteria, viruses, algae * Host …. Tissue cells
MULTIPLICATION	Obligatory inside host	* Inside host * In environment
LIFE CYCLE	* Host …. Trophic * Environment …. Cystic	* Host …. Trophic and Cystic * Environment …. Trophic and Cystic
LOCOMOTION	Psuedopodia	Psuedopodia, acanthopodia, flagella, eruption
MORPHOLOGY	Generally normal, aclimatized with host cells	Comparatively larger nucleus and nucleolus, contractile vacoules

- **Free-living amoebae are a group of unicellular organisms belonging to Sarcodines, Protozoa.**
- Habitat ⟶ all types of water
- Movement ⟶ pseudopodia.
- Feeding ⟶ bacteria, fungi and algae.
- Developmental stages ⟶ trophozoite, cyst.
  - *Additional flagellate stage ⟶ *Naegleria*
  - *Non-cyst forming ⟶ *Mayorella*
- Multiplication ⟶ binary fission.
- Cyst wall ⟶ ecto , endo
  - *Additional mesocyst

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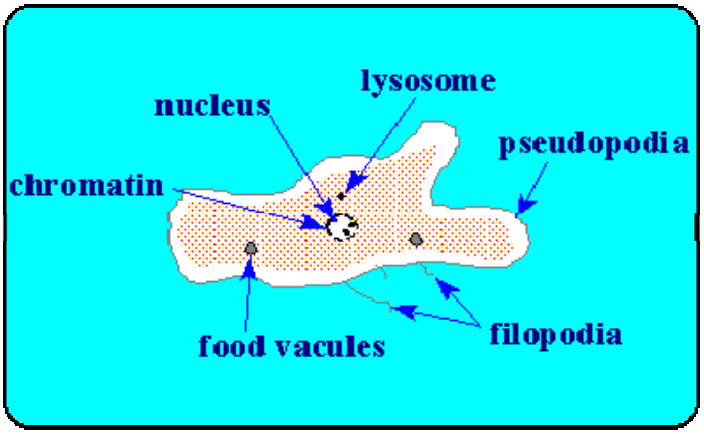
General morphology of amoeba



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- **Unlike** the true parasites, free-living amoebae survive, grow and replicate in the environment without the need for a host (**Schuster and Visvesvara 2004**)..
- These organisms can be isolated from soil, water, and are widely spread in the outdoor and home environment like flowerpots, aquaria, water taps, sink drains and humidifiers **(Jarolim et al., 2000).**
- Some species of free-living amoebae belonging to the genera ***Acanthamoeba***, ***Balamuthia***, ***Naegleria*** and ***Sappinia*** are responsible for opportunistic and non-opportunistic infections in humans and other animals **(Schuster and Visvesvara 2004).**


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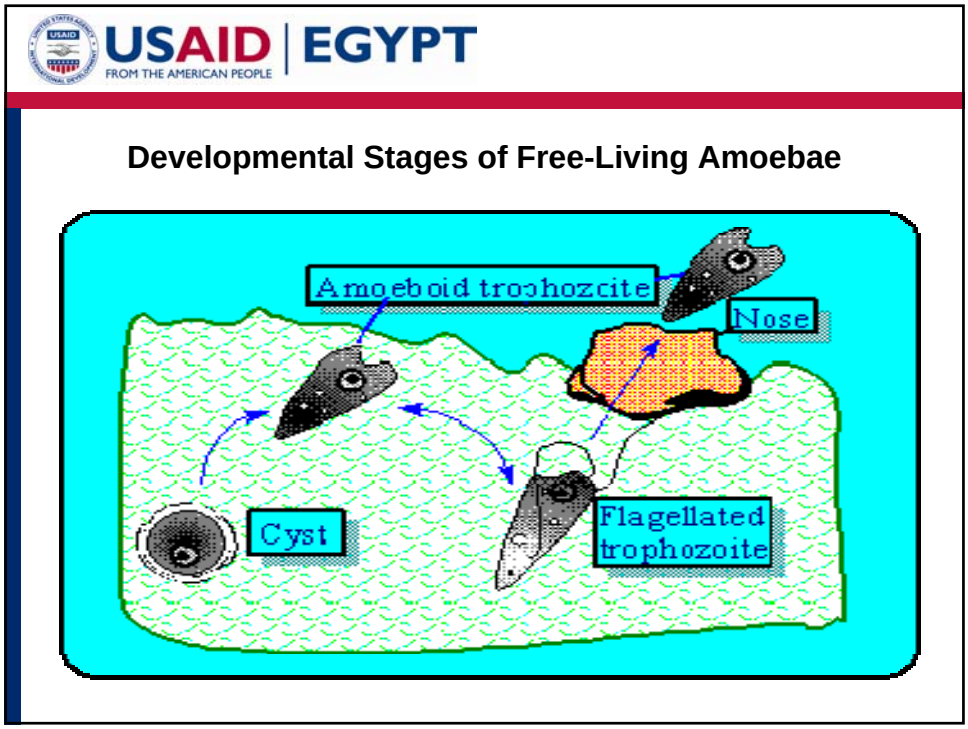
50 µm
uroide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Developmental Stages of Free-Living Amoebae
Amoeboid trophozcite
Nose
Cyst
Flagellated trophozoite

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(2)
ISOLATION AND IDENTIFICATION OF FREE-LIVING AMOEBAE

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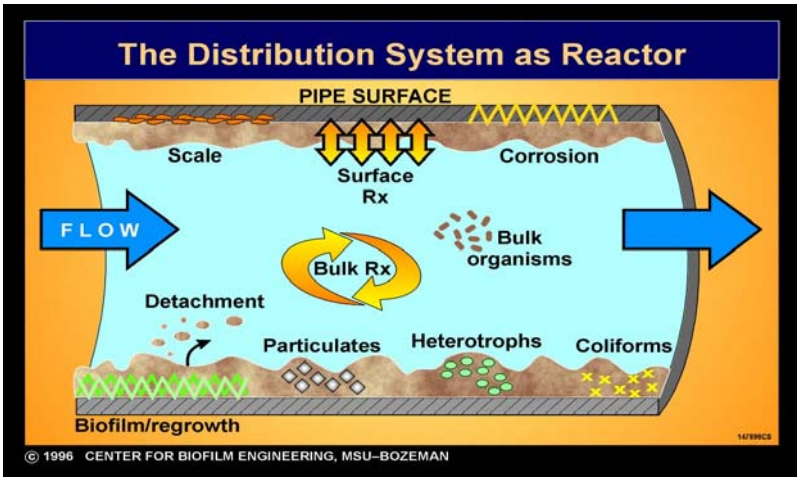
HABITAT OF FREE-LIVING AMOEBAE

- Water
- Air
- Soil



(http://www.erc.montana.edu/Res-Lib99-SW/Image_Library/Water/default.htm)

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SARCODINA (AMOEBAE)

- Parasitic
- Free-living
  - Opportunistic
  - Nonopportunistic

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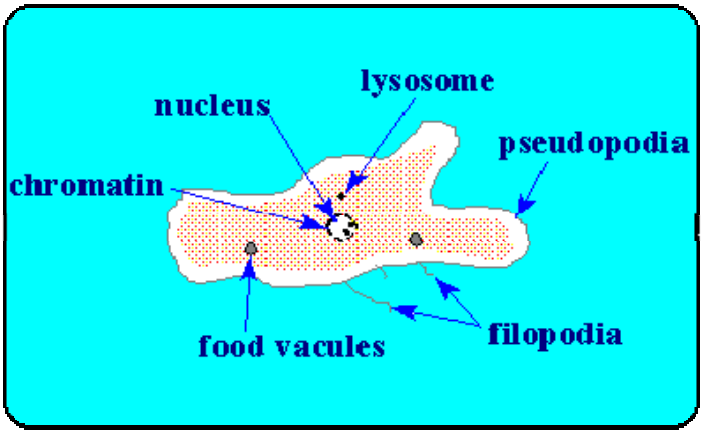
General morphology of free-living amoebae



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AMOEBAE (SARCODINA)

ITEM	PARASSITIC	FREE-LIVING
HABITAT	Inside host	Aquatic environment
FOOD	Tissue cells	* Environment …. Bacteria, viruses, algae * Host …. Tissue cells
MULTIPLICATION	Obligatory inside host	* Inside host * In environment
LIFE CYCLE	* Host …. Trophic * Environment …. Cystic	* Host …. Trophic and Cystic * Environment …. Trophic and Cystic
LOCOMOTION	Psuedopodia	Psuedopodia, acanthopodia, flagella, eruption
MORPHOLOGY	Generally normal, aclimatized with host cells	Comparatively larger nucleus and nucleolus, contractile vacoule

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Detection of Free-Living Amoebae

- A) Preparation of culture media for amoebae
- B) Preparation of food for amoebae
- C) Sample collection
- D) Sample concentration
- E) Culture
- F) Identification

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A) Preparation of culture media for amoebae

- **NN agar:**
  - Sodium chloride …… …… 0.120g
  - Magnesium sulphate …… 0.004g
  - Calcium chloride ……..…… 0.004g
  - Disodium hydrogen phosphate ..…….. 0.124g
  - Potassium dihydrogen phosphate ..…. 0.136g
  - Agar …… 15g
  - Distilled water …… up to 1000ml

B) Preparation of food for amoebae

- Gram –ve bacteria
- Nutrient broth or peptone water
- Incubation at 37ºC for 24h
- Slant nutrient agar

C) Sample collection

- Volume: 100 – 1000ml
- Container: Sterile glass or polypropylene
- Follow all microbiological precautions
- Convey in ice box
- Use the sample as soon as possible within 24 hours

D) Sample concentration

- Sterile stainless steel filter holder
- Membrane filtration using membrane filters (0.45 , 0.8 , 1 , 1.2 µm pore size)
- Suction pump

E) Culture

- Use sterile materials
- Apply culture under aseptic conditions
- NN agar + Gram –ve bacteria
- Or NN agar + 1% NaCl + Gram –ve bacteria
- Invert membrane filter on agar surface
- Seal plates with parafilm
- Incubate at a desirable temperature
- Inspect by inverted microscope

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F) Identification

- Morphology: using ordinary microscopy
- Encystation
- Heat tolerance
- Flagellation
- Osmolarity
- Fluorescent microscopy
- Electron microscopy
- PCR technology

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Detection of free-living amoebae in the aquatic environment

- **Sampling:** 100 ml to one liter
- **Concentration:** Membrane filter, 0.8 – 3 µm pore size
- **Elution:** Page´s amoeba saline
- **Centrifugation:** low speed at 500 rpm for 5 min.
- **Examination:**

-Xenic cultivation: monoxenic, polyxenic
-Direct examination: trophozoite, cyst
-Permanent stained preparation: Field stain, Geimsa stain
-Flagellation: distilled water
-Heat tolerance: subculture
-Enzyme pattern: proteolytic enzymes
-Animal infectivity: mice
-Axenic cultivation: SCGYEM
-Tissue culture: African green monkey kidney cells (vero cells)
-Fluorescence microscopy: direct & indirect
-Molecular biology techniques: PCR

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Microscope, 17 th century

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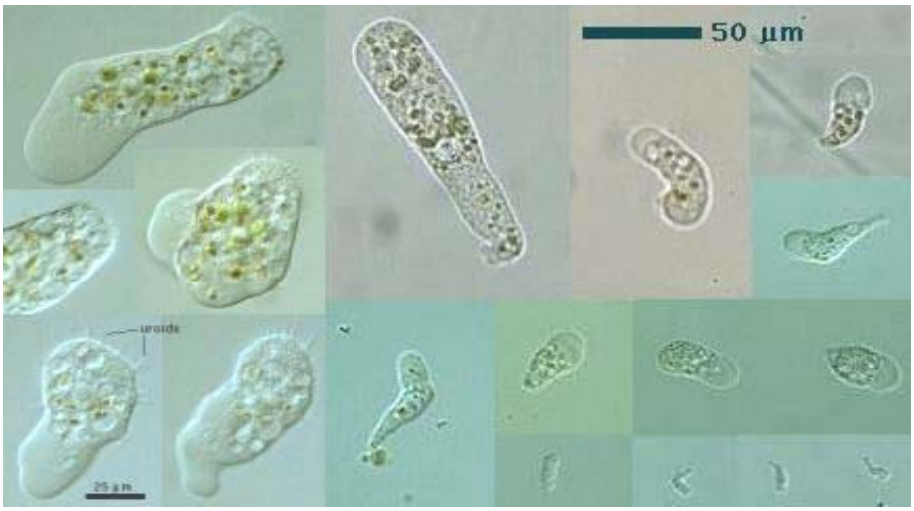
Differences in size of amoebae



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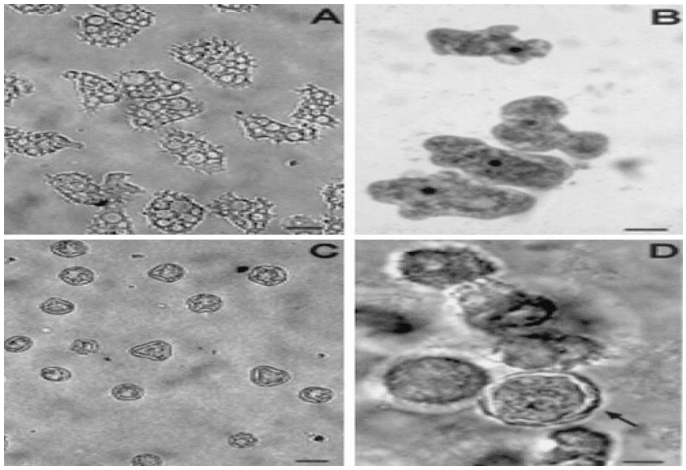
Ordinary microscopy



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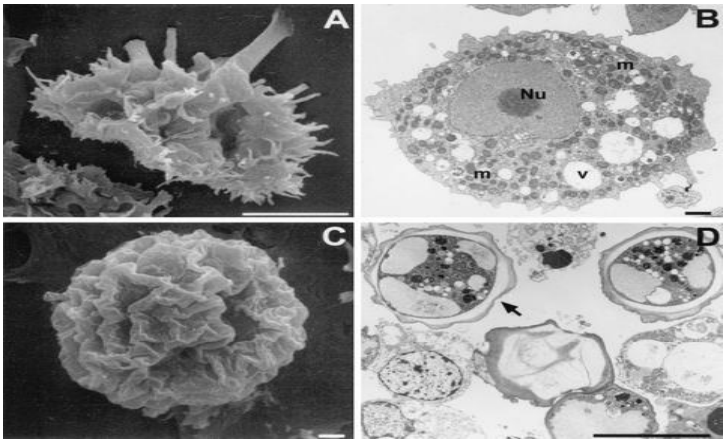
Electron microscopy,



Scanning (A and C) and transmission (B and D) electron micrographs depicting the life cycle stages of Acanthamoeba spp. (A) A. polyphaga trophozoite; (B) trophozoite of A. castellanii showing the prominent central nucleolus (Nu), mitochondria (m).

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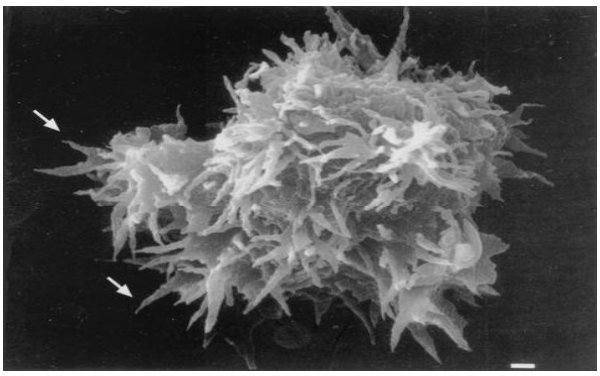
Scanning EM showing acanthopodia



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Acanthamoeba spp. isolated from the environment and from humans

- *A. astronyxis* 30137T7 I Water from termite colony 340, 366
- *A. castellanii* 30011T4II Yeast culture 70, 119, 463
- *A. commandoni* 30135T9I Garden humus 90, 362
- *A. culbertsoni* 30171T10III Monkey kidney cell culture 99, 413
- *A. divionensis* / 50238—*I* II Soil 363
- *A. echinulata* 50239—I Compost 363
- *A. griffini* 30731T3II Seawater bottom sample 389
- *A. hatchetti* 30730T11III Harbor sediment 393
- *A. healyi* CDC:1283:V013 *g* T12III Brain tissue 320
- *A. jacobsi* 30732—III Marine sediment 392
- *A. lenticulata* 30841T5III Swimming pool 313
- *A. lugdunensis* 50240T4II Pool 350, 363
- *A. mauritaniensis* 50253T4II Sewer sludge 363
- *A. palestinensis* 30870T2III Soil 340, 367
- *A. pearcei* 50435T3I Sewage sediments 327
- *A. polyphaga* CCAP1501/3A *h* T4II Pond 340, 361
- *A. pustulosa* / 50252 (GE3a)T2III Pool 112, 350, 363
- *A. quina* 50241—II Swimming pool 350, 363
- *A. rhysodes* 30973T4II Soil 418
- *A. royreba* 30884T4III Human choriocarcinoma cells 478
- *A. stevensoni* 50438T11II Shellfish beds 391
- *A. triangularis* 50254T4II Human feces 363
- *A. tubiashi* 30867T8I River water 259

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Naegleria spp. isolated from the environment and from humans

- *N. australiensis* (De Jonckheere, 1981) related to *N. tihangensis.*
- *N. andersoni* (De Jonckheere, 1988) related to *N. jamiesoni.*
- *N. carteri* (Dobson *et al*, 1997) No strain available at ATCC or CCAP.
- *N. clarki* (De Jonckheere, 1984) ATCC 30544 sewage effluent, Ohio, 1969 *N. chilensis* (De Jonckheere *et al*, 2001). related to *N.pussardi.* No known flagellar stage *N. fowleri* (Carter, 1970)
- *N. fultoni* (De Jonckheere *et al*, 2001) related to *N. pringsheimi.*
- *N. galeacystis* (De Jonckheere, 1994a)
- *N. gruberi* (Schardinger, 1899)
- *N. indonesiensis* (De Jonckheere *et al*, 2001) No known flagellar stage *N. italica* (De Jonckheere *et al*, 1984)
- *N. jadini* (Willaert & Le Ray, 1973)
- *N. jamiesoni* (De Jonckheere, 1988)
- *N. lovaniensis* (Stevens *et al*, 1980) ATCC 30467 domestic water supply, Kadina, Australia, 1972. ATCC30569 Bowling Green, OH, 1970.
- *N. minor* (De Jonckheere & Brown 1995) Flagellar stage has four flagella and can divide but only once. *N. morganensis* (Dobson *et al*, 1997)
- *N. niuginesis* (Dobson *et al*, 1997) No strain available at ATCC or CCAP.
- *N. pagei* (De Jonckheere, 2002)
- *N. philippinensis* (De Jonckheere, 2002)
- *N. pringsheimi* (De Jonckheere, 2002)
- *N. pussardi* (Pernin & De Jonckheere, 1996)
- *N. robinsoni* (De Jonckheere & Brown, 1999). *N.robinsoni* is related to *N.indonesiensis.N. sturti* (Dobson *et al*, 1997)
- *N. tihangensis* (De Jonckheere, 2002)

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Hartmannella

- **Described species:-**
- *H. abertawensis* (Page, 1980). Marine. 7.4 - 19mm (x 11.5mm). Glycocalyx arranged as "truncated pyramids". This amoeba is sluggish moving at 10-13mm/minute, this may be related to the fact that he often fail to adhere at the anterior. Floating forms are irregular rounded and extent pseudopods (3.8 - 5.4mm). No cyst mentioned.*H. agricola* not valid according to Page (Page, 1988).
- *H. cantabrigiensis* (Page, 1974).
- *H. crumpae* (Singh & Hanumaiah, 1979) not valid according to Page (Page, 1988).
- *H. hibernica* (Page, 1980). Marine. Now reclassified as *Nollandella hibernica* (Page, 1983).
- *H. hyalina* not valid according to Page (Page, 1988).
- *H. lobifera* (Smirnov, 1996). Marine. 28 - 42mm (x 35.2mm). Hyaline cap often obscured by granuloplasm . Nucleus 3.5 - 6mm with central nucleolus (1-2mm). Cyst present 10-12mm in diameter.
- *H. vacuolata* (Anderson *et al*,1997). Marine. *H. vermiformis* (Page, 1967). This appears to be the most commonly isolated species. Many strains are available (see below). The species is diagnosed through the ultrastructure of the trophozoite, its locomotion and the morphology of the cyst although this later indication has been questioned as a strain (C3/8) exhibits markedly different cyst morphology and yet its SSUrDNA gene indicates that it is a *H. vermiformis* strain (Walochnick *et al*, 2002).

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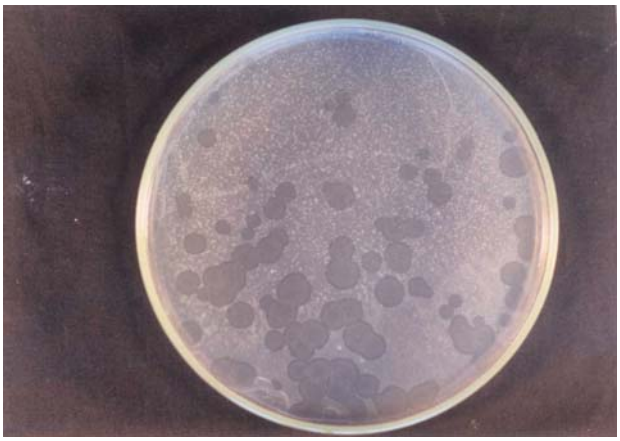
Amoebic Plaques (Macroscopically) on monoxenic cultured plates



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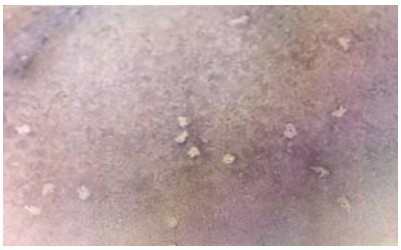
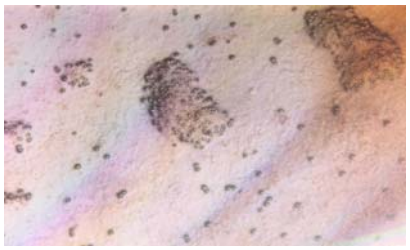
Freshwater Amoebae
with Inverted Microscope



Trophozoites



Cysts

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Water based (opportunistic) pathogenic free-living amoebae

Organism	Habitat	Disease	Route
Naegleria	Soil, Freshwater	PAM, Keratitis	Inhalation (olfactory bulb)
Acanthamoeba	Soil, Freshwater, Salt water	GAE, Bronchitis, Keratitis, Otitis	Inhalation (haematoginous), Contact
Balamuthia	Freshwater	GAE	Inhalation (haematoginous)
Sapinnia	Freshwater	GAE	
Hartmanella	Freshwater	Keratitis	Contact
Vahlkampfia	Freshwater	Keratitis	Contact

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Occurrence of pathogenic free-living amoebae in water supplies in Egypt

Amoebae	Water type	Reference
Acanthamoeba spp	Nile Sewage Tap water Ground water	Al-Herrawy, 1992
Naegleria australiensis	Nile	Al-Herrawy, 1992
Vahlkampfia sp	Nile Ground water	Al-Herrawy, 2001 Personal communicaion
Unclassified freshwater amoebae	Nile Finished	Ali & Al-Herrawy, 2001
Hartmannella sp	Nile	El-Hawaary et al., 1999

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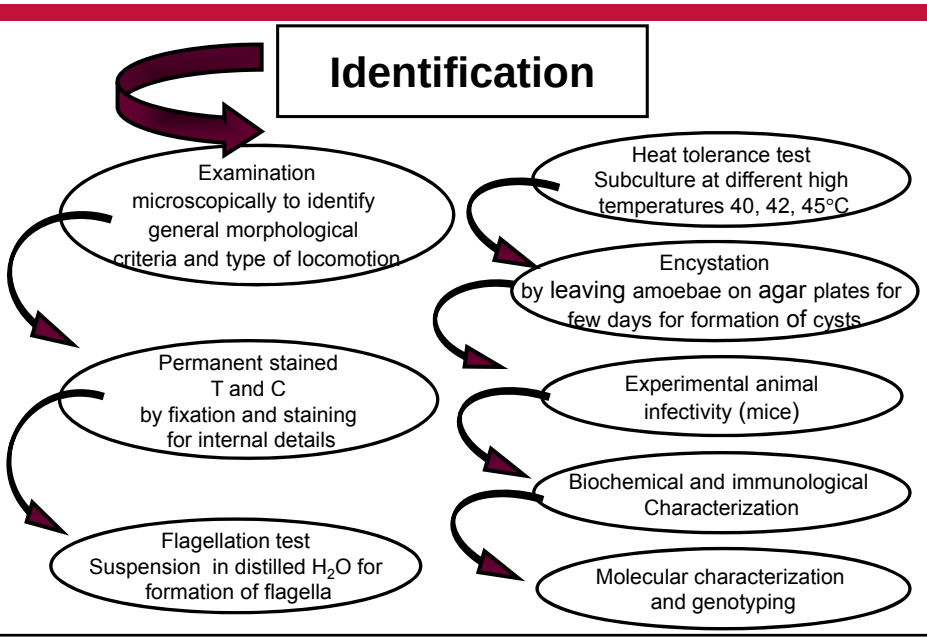

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Laboratory for PCR

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Safety cabinet

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Thermal cycler

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Electrophoresis

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UV illuminator

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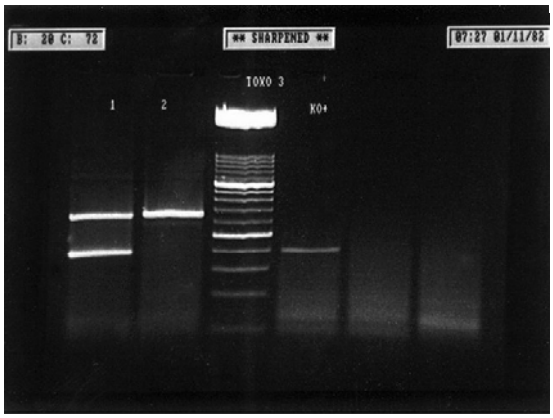
Agarose gel



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Acanthamoeba spp.

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Acanthamoeba species exists in nature in two forms: the trophic form (15 to 45µm long) feeding on bacteria in soil and water and the resistant cyst form (15 to 20µm in diameter) **(Spanakos *et al.*, 2006)**.

Acanthamoeba is the most common amoeba, to be found in soil, water, air and dust, in fresh water sources such as lakes, rivers, and in hot springs and hot tubs, brackish water and in sea water, beach, sewage and soil ranging from tropical to arctic regions.

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- ✓ There are about **28 species** of *Acanthamoeba,* and morphology, particularly that of the cyst stage, has long been the basis for their identification (Page, 2004).
- ✓ Relatively few species have been associated with human infections: ***A. castellanii***, ***A. culbertsoni***, ***A. hatchetti***, ***A. healyi*** and ***A. polyphaga***. Other species may be thermo-tolerant but non-pathogenic **(Khan, 2003)**.
- ✓ Majority of patients are chronically ill, immuno-compromised, or debilitated with other diseases.

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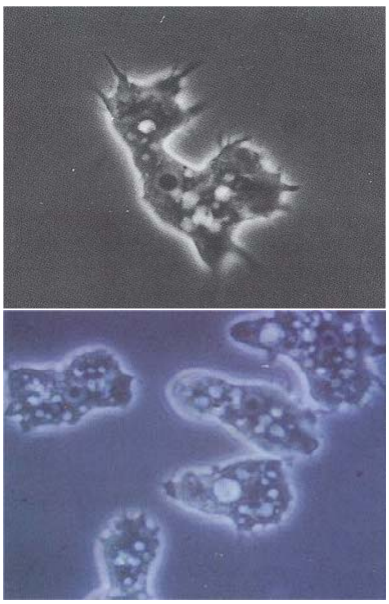
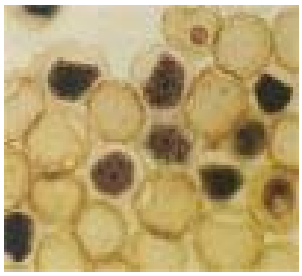
Trophozite:

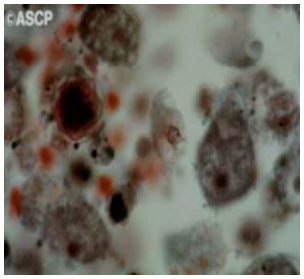
- Has irregular shape
- 15 to 45 µm long
- Has micropseudopodia called acanthopodia
- Trichrome Stain
  - Greenish pink cytoplasm
  - Pink or red centrally located karyosome



Cysts:

- Spherical.
- 15 to 20 µm in diameter
- Thick double wall
  - Outer wall: spherical or wrinkled
  - Inner wall: stellate or polyhedral
- Trichrome stain:
  - Cysts stain red





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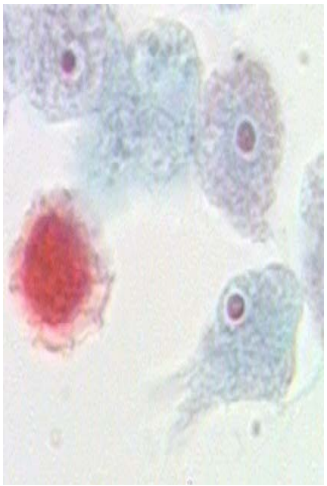
- Species identification is based on:
- Morphology of cysts:
  - Stellate
  - Polyhedral
- Both *Naeg & Acan* have:
  - Single Nucleus
  - Large centrally located nucleolus.



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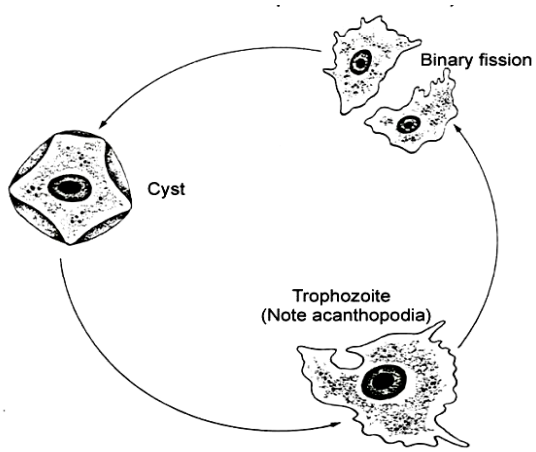
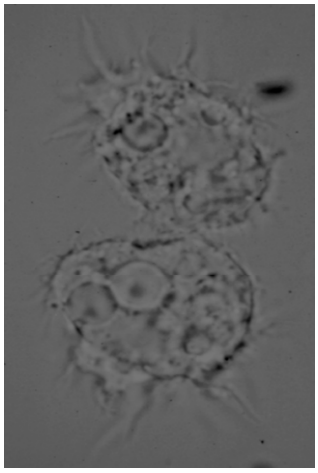
Life cycle of *Acanthamoeba*





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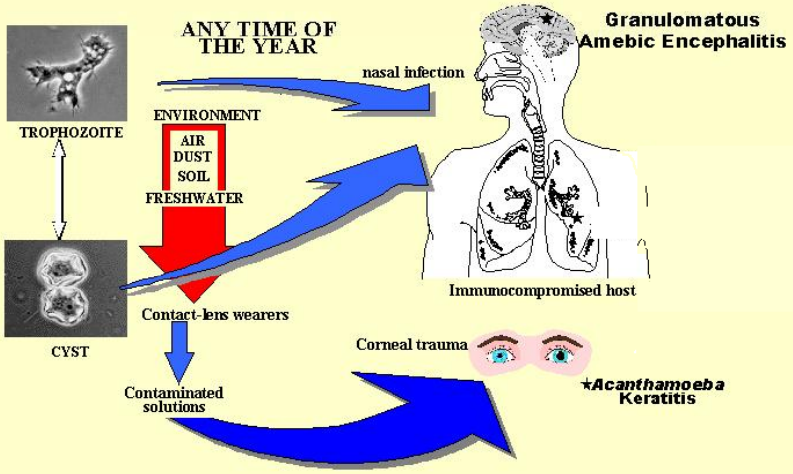
Infections Caused by *Acanthamoeba* spp.



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Swimming Pools:

- Isolation of *Acanthomeoba* is not unusual.
- No correlation between *Acanthomeoba* & bacterial quality of pool water.
- *Acanthomeoba* cysts are very resistant to chlorine.
- Greater % are pathogenic in pools than in fresh water isolates.

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Pathology of Acanthamoebiasis

☺ **Most common cause of corneal ulcers and keratitis in contact lens wearers**.

☺ **Keratitis is an inflammation of the cornea.**

Can lead to blindness.

Most common in people using home-made contact lense saline solutions.

May be acquired through abrasions by the contact lens and/or swimming with contact lenses.

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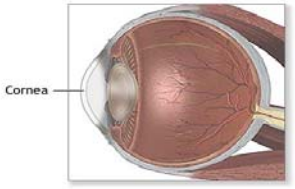
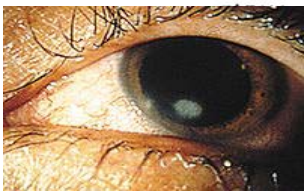
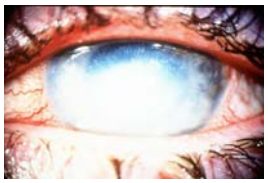
Keratitis

- Symptoms:
  - * Severe pain.
  - * Photophobia.
  - * Tearing.
  - * Blepharospasm.
  - * Conjunctivitis.
  - * Blurred vision

.







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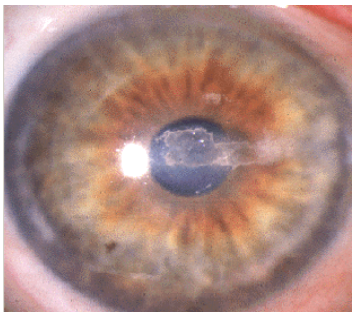
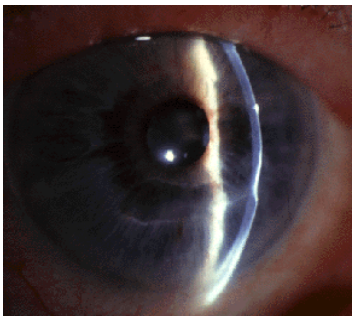
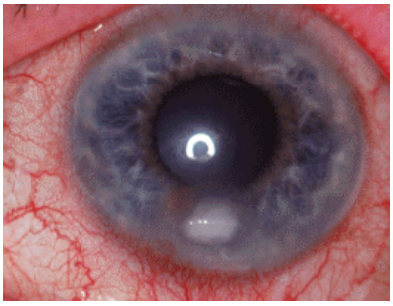


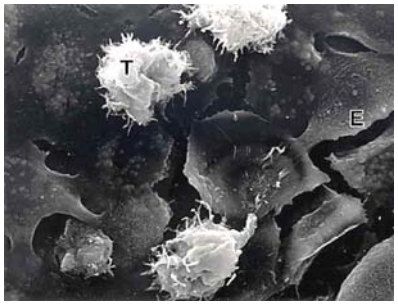
Corneal Exposure



Corneal Melt



Corneal ulcer



Acanthamoeba killing corneal epithelial cells

GAE

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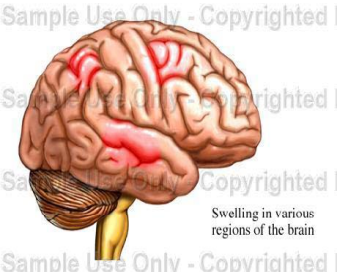
☆GAE symptoms:

- Fever
- Head ache
- Skin lesions
- ulcers, nodules, or subcutaneous abscesses
- Seizures (66%)
- Visual disturbances (26%)
- Ataxia (20%)
- Mental status changes (86%)
- Hemiparesis (53%)
- Maningismus (40%)



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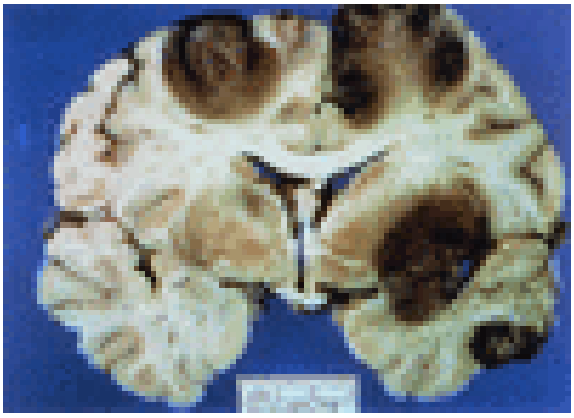

Coronal section of the cerebral hemispheres with cortical and subcortical necrosis from a fatal case of GAE

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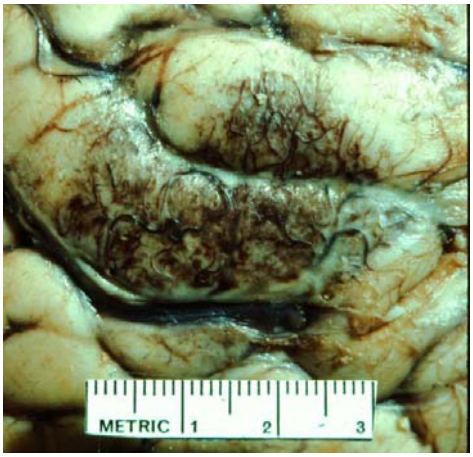

Brain damage due to *Acanthamoeba*

Pathogenic *Acanthamoeba* spp.

- ☆ Act as opportunistic pathogens in immuno-compromised or debilitated individuals.
- ☆ Enter blood stream through lungs, injured skin or sinuses .
- ☆ Result in systemic infection and eventually reach CNS.
- ☆ Usually occur in Immunocompromised patients (AIDS, liver disease, organ transplant, diabetes mellitus, etc).

Naegleria fowleri

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- Although some **40 species** of *Naegleria* have been recognized, based on sequencing data, ***N. fowleri*** is the only species of genus *Naegleria* that is pathogenic to humans.
- Ubiquitous in nature, fresh water, lakes and ponds (especially warm water) .
- Exists in 3 forms:
  1. Trophozoite
  2. Temporary flagellate
  3. Cyst

(Morales *et al.*, 2006)

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Forms of *Naegleria*

1. **Trophozoite form:** Invasive, reproductive form (7 - 20 µm in size), characterized by eruptive movement
2. **Flagellate form:** Transient (temporary 2 - 4 flagella) and does not feed or divide.
3. **Cyst form:** Resistant, rounded (7 – 15 µm in size).

(Morales *et al.*, 2006)

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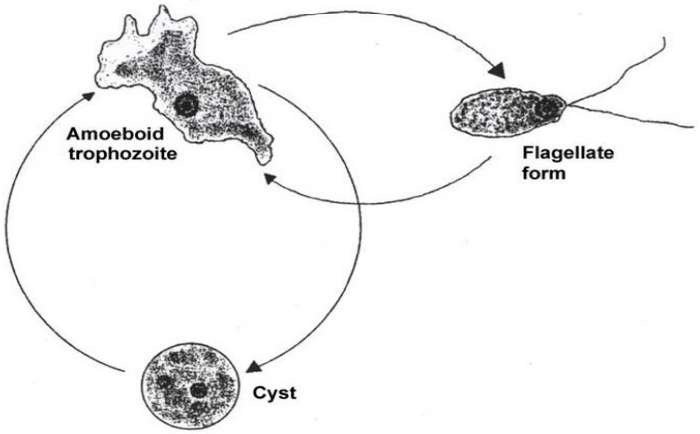
Life Cycle of *Naegleria*



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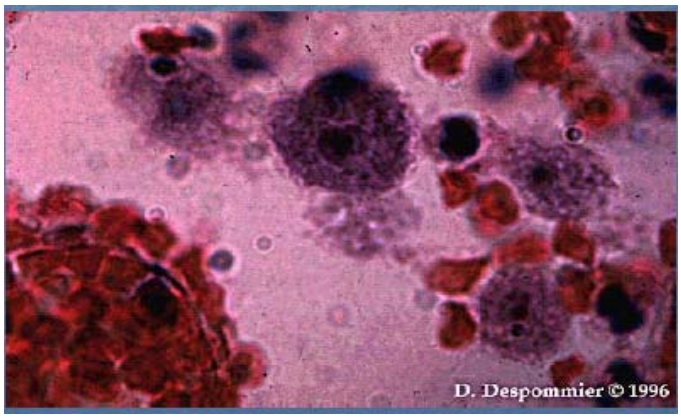
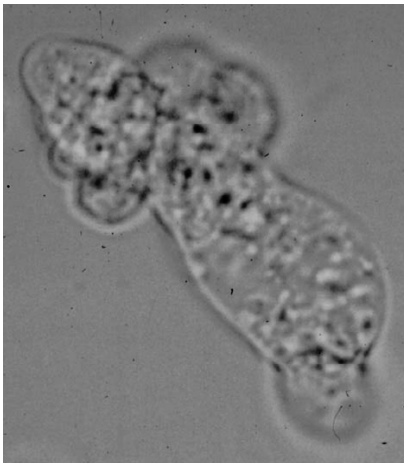
Primary Amoebic Meningo-Encephalitis (PAME)

- Acute, suppurative infection of the brain meninges caused by *N. fowleri.* First described in 1965 by R.F. Carter and M. Fowler in Australia.
- Usually affects immuno-competent children and young adults.
- As of 1997, approximately 200 cases have been reported worldwide with 81 cases in the US (primarily in central and southeast).
- Males/females =3/1.
- Mortality rate > 95%

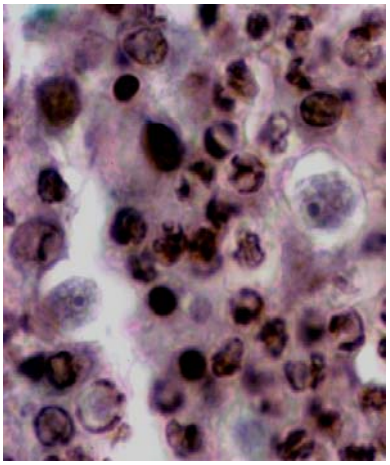
- Infection believed to be introduced through nasal cavity and olfactory bulbs.
- Incubation period 1-14 days .
- Symptoms usually within a few days after swimming in warm still waters.
- Symptoms include headache, lethargy, disorientation, coma.
- Rapid clinical course, death in 4-5 days after onset of symptoms.
- Trophozoites can be detected in spinal fluid, but diagnosis is usually at autopsy.



Naegleria fowleri **in brain tissue.**
Magnification X 1000



In vitro culture



Naegleria trophozoites in brain section

Balamuthia mandrillaris

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New facultative amoeba recently identified

Balamuthia mandrillaris has been incriminated in some 80 cases of amoebic meningo-encephalitis in humans since 2001.

- Only 2 survive.

- Clinically easy misidentified so some of the cases of granulomatous amoebic meningo-encephalitis caused by *Acanthamoeba* may well have been caused by ***Balamuthia mandrillaris*** .

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- Firstly reported in mandrill baboon (1990).
- Genus/species named 1993.
- Morphology similar to *Acanthamoeba.*
- Many *Acanthamoeba* GAE cases retrospectively assigned to ***Balamuthia***

  As of 1997, 63 cases of *Balamuthia* were identifiied (30 of them in U.S.).

- Thus far only identified post-mortem.

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- The life cycle comprises a **trophic stage** measuring about 50-60 μm in diameter and a **cyst stage** with a triple layered wall-lacking pores **(Pritzeker *et al.*, 2004)**.
- Portal of entry of the amoebae into the human are similar to those for *Acanthamoeba* **(Deetz et al., 2003).**
- Infect healthy persons and all died within days or weeks of neurological symptoms.
- Primary lesions: 8 nasal, 3 dermal.
- Symptoms of *Balamuthia* granulomatous encephalitis include fever, personality change, stiff neck, cerebellar ataxia, hemiparesis, aphasia and seizures **(Martinez and Visvesvara, 2001)**

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- *Balamuthia mandrillaris, unlike Acanthamoeba, cannot be cultivated* on bacterium-coated agar plates, and its food source in nature is not clearly known.
- It has, however, been isolated from the environment and is believed to feed on small amebae because it can feed on *Acanthamoeba* and *Naegleria* in vitro.
- It can be isolated from human or animal tissue using mammalian cell cultures, such as monkey kidney (E6), human lung fibroblasts (HLF), and human brain microvascular endothelial cells (HBMEC).

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- Clinical manifestations of *Balamuthia* granulomatous amebic encephalitis (GAE) are similar to those of GAE caused by *Acanthamoeba.*
- Cutaneous lesions or ulcers may appear initially, followed by neurological symptoms as amebae invade the central nervous system(CNS).
- The lesions are commonly seen in the center of the face, but they may occur on the trunk, hands, and feet accompanied by rhinitis before CNS involvement.
- *Balamuthia, like Acanthamoeba,* probably invades human tissue by producing enzymes and ingesting host tissue as a food source.

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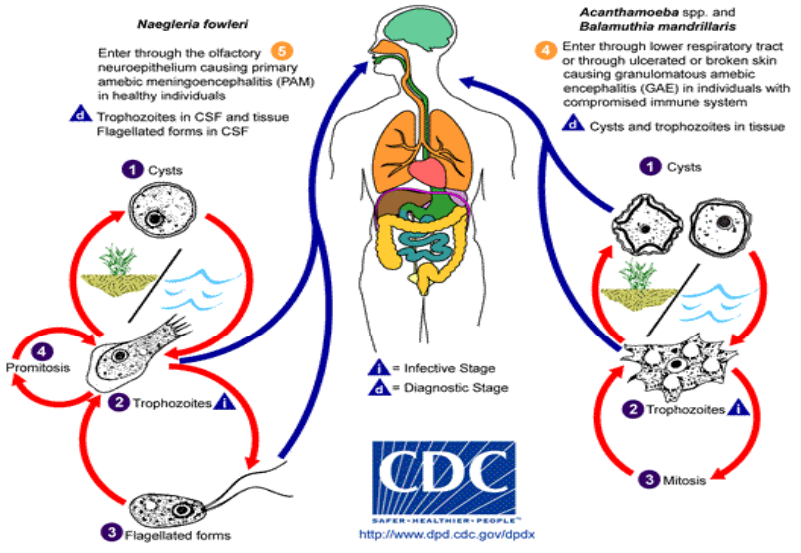

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Sappinia diploidea

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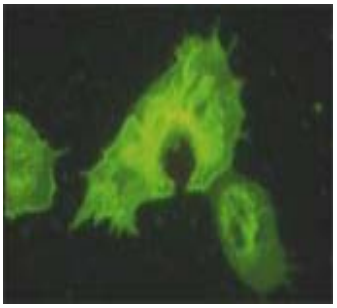
- A single case of encephalitis caused by ***Sappinia diploidea*** was reported in an immuno-competent male (Gelman *et al.*, 2001, 2003).
- The amoeba is relatively large with indistinct pseudopodia and a pellicle that may wrinkle as the amoeba moves.
- Presence of two nuclei tightly apposed to one another.
- **Binucleate cyst.**



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- The genus *Sappinia* currently comprises two distinct species, *Sappinia pedata* and *Sappinia diploidea*.
- The trophozoites are usually 50–60 µm long and 20–30 µm wide.
- They have a monopodial locomotion with a large hyaloplasma in the anterior part of the cell and a cell surface without any sub-pseudopodial projections.
- The cysts are generally smaller, with around 18–25 µm in diameter.

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- Sappiniae can be cultured at room temperature on non-nutrient agar plates coated with bacteria (e.g. *Enterobacter cloacae*).
- Sappiniae, particularly *S. diploidea,* are assumed to have sexual reproduction, although the fusion of nuclei has never convincingly been documented.
- Initially, *Sappinia* was assumed to be coprozoic, as first isolates derived from animal dung **(Hartmann and Nagler, 1908; Noble, 1958; Levine, 1961; Goodfellow et al., 1974).**
- Meanwhile, however, *Sappinia* is considered a typical free-living amoeba widely distributed in the environment. In 2001, a case of amoebic encephalitis caused by *Sappinia* has been reported **(Gelman et al., 2001).**

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Symptoms

- Headache.
- Seizure.
- Blurred vision.
- Photophobia.
- Vomiting.

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	Acanthamoeba spp. (systemic infections)	*Acanthamoeba* spp. (keratitis)	*Naegleria fowleri*	*Balamuthia mandrillaris*	*Sappinia diploidea*
Trophic amoeba	15–45µm	15–45µm	15–30 µm	12–60 µm	45–85 µm binucleated
Flagellate stage	Not found	Not found	Found (2 flagellae)	Not found	Not found
Cyst stage	10–15µm	10–15µm	7–15µm	10–30 µm	13–37 µm binucleated
Diseases	GAE Cutenus, lung	AK	PAME	GAE Skin lesion, lung	AE
Incubation period	Weeks to months	Days	Days	Weeks to months	No data
Symptoms	Headache, fever, abnormal behavior	Intense pain, lacrimation, photophobia	Headache, fever, abnormal behavior	Headache, fever, abnormal behavior	Headache, photophobia, vomiting, unconsciousness

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Other amphizoic amoebae

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- Other free-living amoebae, able to adapt to mammalian body temperature, are also candidates for being facultative pathogens.
- *Willaertia magna* is a thermophilic amoeba, morphologically similar to *Naegleria*, but larger. The amoeba has been isolated from bovine feces and thermally polluted waters.
- A strain of *Paravahlkampfia ustiana*, from lizard intestine (skinks), grew at 42 ° C and produced cytopathology in tissue culture monolayers, indicative of a potential for pathogenesis in mammals (Schuster et al., 2003b).

- *Acanthamoeba, Hartmannella, Naegleria*, and *Entamoeba* were isolated from feces and, in some cases, the brains of reptiles, with the possibility that snakes and lizards may be a potential source of human amoebic infection when kept as domestic pets

- *Acanthamoeba, Vahlkampfia*, and *Hartmannella* have been isolated from the nasal cavity, intestine, vagina and other sites in dead cattle, swine, dogs, rabbits and turkeys, but not from the CNS or lungs (Kadlec, 1978).

- Amoebae, including *Acanthamoeba, Naegleria*, and *Echinamoeba*, were isolated from a necrotic lesion on the tail of a basilisc lizard. Except for the *N. gruberi* isolate, the remaining two amoebae were thermotolerant, and the *Acanthamoeba* isolate produced a cytopathic effect in tissue cultures.

- Then, too, secondary invasion of lesions or contamination of clinical samples by free-living amoebae can occur.

- Opportunism by *Hartmannella* rather than etiology was the conclusion following isolation of the amoeba from CSF of a patient with meningoencephalitis (Centeno et al., 1996).

- isolations of *Hartmannella* and *Vahlkampfia* from human keratitis cases.

- *Acanthamoeba spp*. isolated from fresh-water fish, were found to be members of the same evolutionary lineage (T4) as the amoebae that cause *Acanthamoeba* keratitis.

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Microorganisms associated with free-living amoebae

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- Viral-like particles
- *Chlamidia pneumoniae*
- *Burkholderia cepacia*
- *Afipia felis*
- *E. coli 0157*

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- Viral-like particles
- *Chlamidia pneumoniae*
- *Burkholderia cepacia*
- *Afipia felis*
- *E. coli 0157*
- *Helicobacter pylori*
- *Legionella spp.*
- *Listeria monocytogenes*
- *Mycobacterium avium*
- *Pseudomonas aeruginosa*
- *Vibrio cholerae*
- *Richettsia-like*
- *Erlichia-like*
- *Francisella tularensisCytophaga spp.*
- *Sarcobium lyticumMolibuncus curtisii*
- Coliforms including *Salmonella, Shigella, Yersinia, Campylobacter*

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DRINKING WATER TREATMENT TECHNOLOGY

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DRINKING WATER TREATMENT TECHNOLOGY

- Conventional treatment using chlorine
- Use of UV
- Use of ozone
- Ultrafiltration & Nanofiltration
- Pulsed electric field

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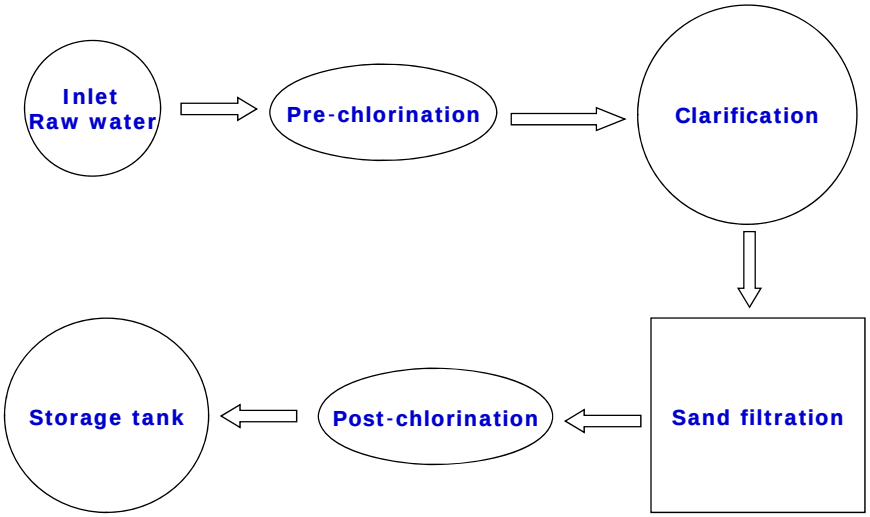
CONVENTIONAL DRINKING WATER TREATMENT



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Standard Criteria for Drinking Water

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قرار وزير الصحة والسكان رقم (458) لسنة 2007م

مسلسل	نوع الفحص	طريقة القياس المتبعة	الحد الأقصى المسموح به
ج-	**الفحص البيولوجي:** عند فحص عينات المياه للطحالب		
	عند فحص عينات المياه ميكروسكوبيا		يجب أن تكون خالية تماما من البروتوزوا الحية وجميع أطوار الديدان المسببة للأمراض

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ملحق رقم (1) دورية الفحوص

1-تجرى الفحوص الخاصة بالخواص الطبيعية – المواد الغير عضوية ذات التأثير على الاستساغة والاستخدامات المنزلية والمعايير الميكروبيولوجية والبيولوجية والأمونيا – النيتريت – النترات روتينيا لجميع العينات.

4-تجرى جميع الفحوص والتحاليل طبقا لطرق القياس الواردة في كتاب Standard Methods على أن تتولى الإدارة المركزية للمعامل بوزارة الصحة والسكان اختيار أنسب الطرق الواردة في الكتاب المذكور ويتم طبعها وتوزيعها على جميع معامل المحافظات وتدريب العاملين بها وتوفير إمكانيات تطبيقها من أجهزة ومعدات وكيماويات مع تطبيق الرقابة على القياسات على مستوى جميع المعامل المشتركة بالمحافظات.

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المواصفات القياسية الخاصة بالمياه المعدنية الطبيعية المعبأة والمعدة للشرب

- **3- الاشتراطات العامة:**

3-4 تكون خالية من المواد والكائنات الضارة بالصحة

3-5 يكون مصدر المياه بعيدا عن التلوث

- **4- المواصفات:**

4-26 تكون خالية من الطفيليات والكائنات الدقيقة المسببة للأمراض

4-31 تكون خالية تماما من الطحالب والفطريات وأي كائنات حية أولية غير بكتيرية

البروتوزوا الطفيلية في البيئة المائية

Parasitic Protozoa
in the Aquatic Environment

المحتويات

أولا: نظرة عامة على البرنامج التدريبي (البروتوزوا الطفيلية في البيئة المائية)

١- الهدف العام للدورة التدريبية

تجرى الاختبارات المعملية المختلفة (فيزيائية - كيميائية – بكتريولوجية - بيولوجية – طفيلية) لمياه الشرب ومصادرها قبل وبعد تنقيتها ومعالجتها بغرض الوقوف على مدى جودة المياه ومعرفة مكوناتها ومواصفاتها قبل وبعد تنقيتها وذلك للتحقق من صلاحية عملية المعالجة وتطابق نوعية المياه المنتجة مع المعايير والمواصفات القياسية المحلية والدولية لمياه الشرب.

لذا تهدف الدورة إلى رفع كفاءة العاملين بالمعامل المركزية والمعامل الفرعية التابعة لشركة مياه الشرب والصرف الصحي بشمال وجنوب سيناء وتزويدهم بالمعلومات النظرية والعملية الواجب إتباعها في إجراء التحاليل المعملية المتعلقة بالكشف عن البروتوزوا الطفيلية في عينات المياه والتدريب على الاختبارات والطرق المختلفة والمعتمدة من الجهات المنوط بها وضع المواصفات القياسية لمياه الشرب.

٢- المجموعة المستهدفة

العاملون بالمعامل الرئيسية والمعامل الفرعية التابعة لشركة مياه الشرب والصرف الصحي بشمال وجنوب سيناء (إحدى الشركات التابعة للشركة القابضة لمياه الشرب والصرف الصحي) والمنوط بهم إجراء التحاليل الخاصة بمياه الشرب.

٣- عدد المتدربين

يقدر عدد المتدربين المرشحين لحضور دورة "البروتوزوا الطفيلية في البيئة المائية" حوالي متدرب من معامل المحطات والمعامل الفرعية التابعة.

٤- منهجية التدريب

تعتمد منهجية التدريب بالدورة على عدة أسس بحيث يكون الهدف الرئيسي منها توصيل المعلومة بسهولة ويسر للمتدرب , وكذلك ضمان المشاركة الفعالة من المتدربين أثناء جلسات التدريب والتأكد من الفهم الكامل لمحتويات وموضوعات الدورة مع التدريب العملي والشخصي على الموضوعات المتعلقة بمحتوى هذه الدورة.

هذا ويمكن تلخيص منهجية التدريب المتبعة في هذه الدورة فيما يلي:

- **التقييم الأولي:** حيث يقوم المدرب بعمل اختبار مبدئي للمتدربين لتحديد مستوى كل متدرب من حيث المعلومات المتعلقة بموضوع الدورة (البروتوزوا الطفيلية) والخلفية العلمية لكل متدرب على حده.
- **المحاضرات:** والتي يقوم المدرب بتوصيل أحدث المعلومات النظرية والعملية المتعلقة بموضوع الدورة للمتدربين بما يؤدي إلى اكتساب معلومات صحيحة وموثقة عن البروتوزوا الطفيلية وكيفية التعرف على الأطوار المعدية منها والتي قد تنتقل للإنسان عن طريق المياه الملوثة بها .
- **الرسومات الإيضاحية:** التي يتم عرضها أثناء الشرح والمتعلقة البروتوزوا الطفيلية وذلك بغرض تسهيل وصول المعلومة وإبراز النقاط الرئيسية لكل موضوع في تسلسل منطقي لتثبيت المعلومة لدى المتدرب.
- **التجارب المعملية:** التي تجرى في المعمل لتطبيق الاختبارات المتعلقة بموضوع الدورة (البروتوزوا الطفيلية) وذلك بطريقة صحيحة وبناءا على أسس علمية موثقة بحيث يستطيع المتدرب من تلاشي الأخطاء التي تؤثر في صحة النتائج المتحصل عليها وكيفية التعامل بأدق الوسائل للوصول إلى أفضل النتائج المرجوة.
- **المناقشات المفتوحة:** التي يديرها المدرب لإتاحة الفرصة لتبادل الآراء وتوجيه الأسئلة والحصول على معلومات جديدة يتم من خلالها نقل المعارف والخبرات العلمية والنظرية من المدرب إلى المتدربين وذلك بغرض إصلاح المفاهيم غير الصحيحة أو غير الحديثة لد المتدربين بخصوص البروتوزوا الطفيلية.
- **المشاكل الواقعية المتعلقة بالمتدربين:** وذلك عن طريق مناقشة العقبات العلمية التي تواجه المتدربين أو المشكلات التي سوف يواجهونها والمتعلقة بموضوع الدورة (البروتوزوا الطفيلية) وذلك بغرض الوصول إلى أنسب الطرق للتغلب عليها باستخدام الأسلوب العلمي الصحيح.
- **التدريب العملي:** حيث يتم إجراء الاختبارات والطرق القياسية الحديثة الخاصة بالبروتوزوا الطفيلية وذلك في معامل الشركة مما يتيح لكل متدرب المشاركة في إجراء التجارب لضمان وصول المتدرب إلى مرحلة الفهم التام والتطبيق السليم للمعلومات والطرق العلمية التي تلقاها واكتسبها من المحاضرات .

- **المراجع العلمية:** يتم تزويد المتدرب بالمراجع العلمية المتعلقة بالبروتوزوا الطفيلية والتي يمكن الرجوع إليها للتأكد من صواب المعلومة والتعمق في اكتساب معلومات أخرى متعلقة بنفس الموضوع.
- **التقييم النهائي:** حيث يتم عمل اختبار تحريري في نهاية الدورة يشتمل على أبرز ماتم دراسته خلال الدورة وذلك لتقييم المتدربين والوقوف على مدى الاستفادة الفردية لكل متدرب من حضور هذه الدورة.

٥- مساعدات التدريب

- جهاز عرض (Data show)
- شاشة عرض
- سبورة بيضاء وأقلام ملونة

٦- مكان التدريب وطريقة الجلوس بجلسات التدريب

يجلس المتدربون وفي مواجهتهم المحاضر في منتصف القاعة وعن يمينه جهاز الكمبيوتر لعرض ملفات الدورة على شاشة العرض , أما عن يساره توجد السبورة البيضاء والأقلام الملونة .

ويكون وضع كل من شاشة العرض والسبورة البيضاء واضح بحيث يسمح بسهولة الرؤية المتساوية لجميع المتدربين

تقدر مساحة قاعة التدريب المطلوبة بما لايقل عن ٥ x ٧ مترا لتستوعب المتدربين وتسمح للمتدرب بسهولة الحركة والوصول لأماكن جلوس المتدربين.

يجب أن تتوفر بالقاعة إضاءة مناسبة وأجهزة صوتية واضحة ونظام تهوية ملائم.

ثانيا: خطة التدريس بالدورة التدريبية (البروتوزوا الطفيلية المعوية في البيئة المائية)

المحاضر: ا.د./ أحمد زكريا سالم الهراوي

١- أهداف الدورة:

- التعرف على الأجناس والأنواع المختلفة للبروتوزوا الطفيلية
- دراسة دورة الحياه كل والطور المعدي لكل نوع من البروتوزوا الطفيلية على حده
- دراسة التركيب المورفولوجي للأطوار المعدية للبروتوزوا الطفيلية
- المقارنة بين الأطوار المعدية للبروتوزوا الطفيلية وكيفية التفرقة بينها
- تحديد طرق تلوث المياه بالطور المعدي للبروتوزوا الطفيلية
- تأثير خطوات المعالجة الفيزيائية للمياه على إزالة الأطوار المعدية للبروتوزوا الطفيلية
- تأثير خطوات المعالجة الكيميائية للمياه على إزالة الأطوار المعدية للبروتوزوا الطفيلية
- تحديد طريقة دخول الأطوار المعدية للبروتوزوا الطفيلية إلى جسم العائل عن طريق المياه الملوثة بها
- التدريب على طرق تركيز الطور المعدي للبروتوزوا الطفيلية في المياه
- استخدام الميكروسكوب الضوئي لفحص الأطوار المعدية للبروتوزوا الطفيلية في المياه المركزة
- تقييم النتائج المتحصل عليها من تحاليل المياه الخاصة بالبروتوزوا الطفيلية
- كتابة تقارير التحاليل الطفيلية للمياه والتعليق على النتائج المتحصل عليها

٢- موضوعات الدورة:

- مقدمة عن البروتوزوا الطفيلية مع شرح الهدف من الدورة
- اللحميات (Sarcodina) التي تصيب الإنسان
- السوطيات (Flagellates) التي تصيب الإنسان
- الهدبيات (Ciliates) التي تصيب الإنسان
- البوغيات (Apicomplexa) التي تصيب الإنسان

- الميكروسبورا (Microspora) التي تصيب الإنسان
- البروتوزوا الطفيلية المشتركة التي تنتقل من الحيوانات والطيور إلى الإنسان
- تحديد العائل الأساسي والعائل الوسيط للبروتوزوا الطفيلية ذات العوائل المتعددة
- الصفات المورفولوجية المميزة للأطوار المعدية للبروتوزوا الطفيلية
- تلوث المياه بالطور المعدي للبروتوزوا الطفيلية
- طرق تركيز الأطوار المعدية للبروتوزوا الطفيلية في المياه
- استخدام الميكروسكوب الضوئي للتمييز بين الأطوار المعدية للبروتوزوا المختلفة
- إعداد تقارير تحاليل البروتوزوا الطفيلية والتعليق على النتائج

٣- مدة الدورة:

تستغرق الدورة مدة خمسة أيام متواصلة حيث يبدأ العمل يوميا من الساعة التاسعة صباحا حتى الساعة الرابعة عصرا بواقع سبع ساعات يوميا تتخللها نصف ساعة لتناول المشروبات والغداء

٤- البرنامج الزمني للدورة

البروتوزوا الطفيلية في البيئة المائية

PARASITIC PROTOZOA
IN THE AQUATIC ENVIRONMENT

PARASITIC PROTOZOA

The majority of protozoa are free-living aquatic organisms of no significance to public health. Protozoa can be differentiated into 3 general types: ciliates, flagellates and amoebae. They generally feed on other micro-organisms such as bacteria, algae, cyanobacteria or other protozoa.

Protozoa likely to be found in drinking-waters and of public health significance can be grouped into those of enteric or environmental origin:

• enteric protozoa occur widely as parasites in the intestine of humans and other mammals and involve at least two stages (trophozoite and (oo)cyst) in their life cycle

• some free-living protozoa (FLP) are opportunistic pathogens in humans and are responsible for some serious diseases of the nervous system and the eye

ENTERIC PARASITIC PROTOZOA

The most prevalent enteric protozoan parasites associated with waterborne disease include *Giardia intestinalis, Cryptosporidium hominis* and *C. parvum. Toxoplasma gondii, Entamoeba histolytica,* and *Balantidium coli* have also been associated with waterborne outbreaks. Other emerging protozoan parasites of concern include *Cyclospora cayetanensis* and *Isospora belli.* Microsporidia is also an emerging pathogen of public health importance and, although recently classified as fungi, its fate and behaviour in water can be similar to that of the parasitic protozoa.

The transmissive/infective stages of these parasites are cysts (*Giardia, Balantidium, Entamoeba*), oocysts (*Cryptosporidium, Cyclospora, Isospora, Toxoplasma*) or spores (Microsporidia). These forms are excreted in faeces of infected hosts as fully infectious agents (*Giardia, Cryptosporidium, Micropsporidia, Balantidium*) or as immature stages (*Cyclospora, Isospora, Toxoplasma*) requiring a short period of development in the environment to reach the mature stage. They can get into get into drinking-water supplies by contamination with human or animal faeces. All are widely dispersed and have been associated with outbreaks of infection resulting from drinking contaminated water

Giardia and *Cryptosporidium* are the most widely reported causes of waterborne parasitic disease in developed countries. In New Zealand giardiasis and cryptosporidiosis are the third and fourth most commonly notified disease respectively. These organisms cause varying degrees of enteric condition that can be manifested from violent diarrhoea symptoms to being asymptomatic.

The (oo)cysts of *Giardia* and *Cryptosporidium* are widespread in environmental waters of New Zealand especially in water from areas of intensive stock farming and they can occur in high concentrations. Coliforms, faecal coliforms, and E coli have been shown to be poor indicators of the presence of pathogenic protozoa in drinking-water, so *Giardia* and *Cryptosporidium* are considered as Priority 1 determinands in the DWSNZ.

The organisms can survive for a long time in cold water. Medema et al. (1997) conducted bench scale studies of the influence of temperature on the die-off rate of Cryptosporidium oocysts. Die-off rates were determined at 5°C and 15°C. Both excystation and vital dye staining were used to determine oocyst viability. At 5°C, the die-off rate was 0.010 $\log_{10}$/day, assuming first order kinetics. This translates to 0.5 log reduction at 50 days. At 15°C, the die-off rate in natural river water approximately doubled to 0.024 $\log_{10}$/day (excystation) and 0.018 $\log_{10}$/day (dye staining).

Sattar et al. (1999) evaluated factors impacting *Cryptosporidium* and *Giardia* survival. Microtubes containing untreated river water were inoculated with purified oocysts and cysts. Samples were incubated at temperatures ranging from 4 to 30°C; viability of oocysts and cysts was measured by excystation. At 20°C and 30°C, reductions in viable Cryptosporidium oocysts ranged from approximately 0.6 to 2.0 log after 30 days. Relatively little inactivation took place when oocysts were incubated at 4°C.

The significance of waterborne transmission in New Zealand is still not clear. The prevalence of *Giardia* and *Cryptosporidium* infection in livestock, domestic, and feral animals suggests a significant reservoir for zoonotic transmission. However, information is needed on the presence of human and animal specific genotypes in water in order to clarify the relative importance of human or animal derived waterborne infections.

OPPORTUNISTICALLY PATHOGENIC FREE-LIVING PROTOZOA

Free-living protozoa (FLP) are numerous in open surface waters including water supply sources but greatest numbers can be found in nutrient enriched environments where their bacterivorus feeding activities are of great benefit, e.g. in biological wastewater treatment systems. FLP are ubiquitous in aquatic environments with a wide tolerance to environmental conditions ranging from geothermal waters, thermally polluted waters, to water distribution pipes.

DISEASE FROM WATERBORNE PATHOGENS

Drinking-water is an important source of infectious agents, particularly ones that cause enteric infections. Many of the great epidemics of history have been caused by faecal contamination of drinking-water. While person-to-person contact is equally important it is common for the population to indicate water as a source of disease. The significance of any particular organism varies with the disease caused under local water supply conditions. Not all individual members of any population will be susceptible to a pathogenic organism in the water. Waterborne infections will depend on the following:

• the concentration of any pathogenic organism in drinking-water

• the virulence of the strain

• the amount of water taken in by individuals which has not been adequately disinfected

• the minimum infectious dose (MID) of the pathogen in question

• the immune capability or susceptibility of individuals

• the incidence of the infection in a community, thus determining the number of enteric pathogens that would be shed into a potential receiving water source.

Paradoxically, if a particular infection has been received repeatedly from a contaminated water source the community may have become immune to some of the pathogens. This situation develops in countries where the number of pathogens in water is high and the standard of drinking-water is low. Conversely, visitors who drink from such water frequently become ill while the locals have no ill effects. This is a population immunity but it is acquired at the cost of illness and death among children and is not considered acceptable in developed countries.

Where indicators of faecal pollution are found in water the population using that water may not be showing enteric disease. However, the presence of indicators of faecal pollution means that the likelihood of faecal pathogens occurring in that water is high. Continual vigilance is required to determine the need for treatment. If an infection occurs in a community, follow-up epidemiological studies should be carried out such that the source and route of infection can be determined and treated

Entamoeba histolytica

General description

Entamoeba histolytica is the most prevalent intestinal protozoan pathogen worldwide and belongs to the superclass Rhizopoda in the subphylum Sarcodina. *Entamoeba* has a feeding, replicative trophozoite (diameter 10–60mm), which, under unfavourable conditions, will develop into a dormant cyst (diameter 10–20mm). Infection is contracted by the ingestion of cysts. Recent studies with RNA and DNA probes demonGUIDELINES strated genetic differences between pathogenic and non-pathogenic *E. histolytica*; the latter has been separated and reclassified as *E. dispar*.

Human health effects

About 85–95% of human infections with *E. histolytica* are asymptomatic. Acute intestinal amoebiasis has an incubation period of 1–14 weeks. Clinical disease results from the penetration of the epithelial cells in the gastrointestinal tract by the amoebic trophozoites. Approximately 10% of infected individuals present with dysentery or colitis. Symptoms of amoebic dysentery include diarrhoea with cramping, lower abdominal pain, low-grade fever and the presence of blood and mucus in the stool.

The ulcers produced by the invasion of the trophozoites may deepen into the classic flask-shaped ulcers of amoebic colitis. *Entamoeba histolytica* may invade other parts of the body, such as the liver, lungs and brain, sometimes with fatal outcome.

Source and occurrence

Humans are the reservoir of infection, and there would not appear to be other meaningful animal reservoirs of *E. histolytica*. In the acute phase of infection, patients excrete only trophozoites that are not infectious. Chronic cases and asymptomatic carriers who excrete cysts are more important sources of infection and can discharge up to 1.5 ¥ 107 cysts daily. *Entamoeba histolytica* can be present in sewage and contaminated water. Cysts may remain viable in suitable aquatic environments for several months at low temperature. The potential for waterborne transmission is greater in the tropics, where the carrier rate sometimes exceeds 50%, compared with more temperate regions, where the prevalence in the general population may be less than 10%.

Routes of exposure

Person-to-person contact and contamination of food by infected food handlers appear to be the most significant means of transmission, although contaminated water also plays a substantial role. Ingestion of faecally contaminated water and consumption of food crops irrigated with contaminated water can both lead to transmission of amoebiasis. Sexual transmission, particularly among male homosexuals, has also been documented.

Significance in drinking-water

The transmission of *E. histolytica* by contaminated drinking-water has been confirmed. The cysts are relatively resistant to disinfection and may not be inactivated

by chlorination practices generally applied in the production of drinking-water. Within a WSP, control measures that can be applied to manage potential risk from *E. histolytica* include prevention of source water contamination by human waste, followed by adequate treatment and protection of water during distribution. Owing to the resistance of the oocysts to disinfectants, *E. coli* (or, alternatively, thermotolerant coliforms) cannot be relied upon as an index of the presence/absence of *E. histolytica* in drinking-water supplies.

Selected bibliography

Marshall MM et al. (1997) Waterborne protozoan pathogens. *Clinical Microbiology Reviews*, 10:67–85.

Balantidium coli

General description

Balantidium coli is a unicellular protozoan parasite with a length up to 200µm, making it the largest of the human intestinal protozoa. The trophozoites are oval in shape and covered with cilia for motility. The cysts are 60–70 m in length and resistant to unfavourable environmental conditions, such as pH and temperature extremes.

Balantidium coli belongs to the largest protozoan group, the ciliates, with about 7200 species, of which only *B. coli* is known to infect humans.

Human health effects

Infections in humans are relatively rare, and most are asymptomatic. The trophozoites invade the mucosa and submucosa of the large intestine and destroy the host cells when multiplying. The multiplying parasites form nests and small abscesses that break down into oval, irregular ulcers. Clinical symptoms may include dysentery similar to amoebiasis, colitis, diarrhoea, nausea, vomiting, headache and anorexia. The infections are generally self-limiting, with complete recovery.

Source and occurrence

Humans seem to be the most important host of *B. coli*, and the organism can be detected in domestic sewage. Animal reservoirs, particularly swine, also contribute to the prevalence of the cysts in the environment. The cysts have been detected in water sources, but the prevalence in tap water is unknown.

Routes of exposure

Transmission of *B. coli* is by the faecal–oral route, from person to person, from contact with infected swine or by consumption of contaminated water or food. One waterborne outbreak of balantidiasis has been reported. This outbreak occurred in 1971 when a drinking-water supply was contaminated with storm water runoff containing swine faeces after a typhoon.

Significance in drinking-water

Although water does not appear to play an important role in the spread of this organism, one waterborne outbreak is on record. *Balantidium coli* is large and amenable to removal by filtration, but cysts are highly resistant to disinfection. Within a WSP, control measures to reduce potential risk from *B. coli* should focus on prevention of source water contamination by human and swine waste, followed by adequate treatment. Due to resistance to disinfection, *E. coli* (or, alternatively, thermotolerant coliforms) is not a reliable index for the presence/absence of *B. coli* in drinking-water supplies.

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Cryptosporidium

General description

Cryptosporidium is an obligate, intracellular, coccidian parasite with a complex life cycle including sexual and asexual replication. Thick-walled oocysts with a diameter of 4–6µm are shed in faeces. The genus *Cryptosporidium* has about eight species, of which *C. parvum* is responsible for most human infections, although other species can cause illness. *Cryptosporidium* is one of the best examples of an "emerging disease"-causing organism. It was discovered to infect humans only in 1976, and waterborne transmission was confirmed for the first time in 1984.

Human health effects

Cryptosporidium generally causes a self-limiting diarrhoea, sometimes including nausea, vomiting and fever, which usually resolves within a week in normally healthy people, but can last for a month or more. Severity of cryptosporidiosis varies according to age and immune status, and infections in severely immunocompromised people can be life-threatening. The impact of cryptosporidiosis outbreaks is relatively high due to the large numbers of people that may be involved and the associated socioe11. conomic implications. The total cost of illness associated with the 1993 outbreak in Milwaukee, USA, has been estimated at US$96.2 million.

Source and occurrence

A large range of animals are reservoirs of *C. parvum*, but humans and livestock, particularly young animals, are the most significant source of human infectious organisms. Calves can excrete 1010 oocysts per day. Concentrations of oocysts as high as 14 000 per litre for raw sewage and 5800 per litre for surface water have been reported. Oocysts can survive for weeks to months in fresh water. *Cryptosporidium* oocysts have been detected in many drinking-water supplies. However, in most cases, there is little information about whether human infectious species were present. The currently available standard analytical techniques provide an indirect measure of viability and no indication of human infectivity. Oocysts also occur in recreational waters.

Routes of exposure

Cryptosporidium is transmitted by the faecal–oral route. The major route of infection is person-to-person contact. Other sources of infection include the consumption of contaminated food and water and direct contact with infected farm animals and possibly domestic pets. Contaminated drinking-water, recreational water and, to a lesser extent, food have been associated with outbreaks. In 1993, *Cryptosporidium* caused the largest waterborne outbreak of disease on record, when more than 400 000 people were infected by the drinking-water supply of Milwaukee, USA. The infectivity of *Cryptosporidium* oocysts is relatively high. Studies on healthy human volunteers revealed that ingestion of fewer than 10 oocysts can lead to infection.

Significance in drinking-water

The role of drinking-water in the transmission of *Cryptosporidium*, including in large outbreaks, is well established. Attention to these organisms is therefore important.

The oocysts are extremely resistant to oxidizing disinfectants such as chlorine, but investigations based on assays for infectivity have shown that UV light irradiation inactivates oocysts. Within a WSP, control measures to reduce potential risk from *Cryptosporidium* should focus on prevention of source water contamination by human and livestock waste, adequate treatment and protection of water during distribution.

Because of their relatively small size, the oocysts represent a challenge for removal by conventional granular media-based filtration processes. Acceptable removal requires well designed and operated systems. Membrane filtration processes that provide a direct physical barrier may represent a viable alternative for the effective removal of *Cryptosporidium* oocysts. Owing to the exceptional resistance of the oocysts to disinfectants, *E. coli* (or, alternatively, thermotolerant coliforms) cannot be relied upon as an index for the presence/absence of *Cryptosporidium* oocysts in drinking-water supplies.

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Cyclospora cayetanensis

General description

Cyclospora cayetanensis is a single-cell, obligate, intracellular, coccidian protozoan parasite, which belongs to the family Eimeriidae. It produces thick-walled oocysts of 8–10 µm in diameter that are excreted in the faeces of infected individuals. *Cyclospora cayetanensis* is considered an emerging waterborne pathogen.

Human health effects

Sporozoites are released from the oocysts when ingested and penetrate epithelial cells in the small intestine of susceptible individuals. Clinical symptoms of cyclosporiasis include watery diarrhoea, abdominal cramping, weight loss, anorexia, myalgia and occasionally vomiting and/or fever. Relapsing illness often occurs.

Source and occurrence

Humans are the only host identified for this parasite. The unsporulated oocysts pass into the external environment with faeces and undergo sporulation, which is complete in 7–12 days, depending on environmental conditions. Only the sporulated oocysts are infectious. Due to the lack of a quantification technique, there is limited information on the prevalence of *Cyclospora* in water environments. However, *Cyclospora* has been detected in sewage and water sources.

Routes of exposure

Cyclospora cayetanensis is transmitted by the faecal–oral route. Person-to-person transmission is virtually impossible, because the oocysts must sporulate outside the host to become infectious. The primary routes of exposure are contaminated water and food. The initial source of organisms in foodborne outbreaks has generally not been established, but contaminated water has been implicated in several cases. Drinking- water has also been implicated as a cause of outbreaks. The first report was among staff of a hospital in Chicago, USA, in 1990. The infections were associated with drinking tap water that had possibly been contaminated with stagnant water from a rooftop storage reservoir. Another outbreak was reported from Nepal, where drinking-water consisting of a mixture of river and municipal water was associated with infections in 12 of 14 soldiers.

Significance in drinking-water

Transmission of the pathogens by drinking-water has been confirmed. The oocysts are resistant to disinfection and are not inactivated by chlorination practices generally applied in the production of drinking-water. Within a WSP, control measures that can be applied to manage potential risk from *Cyclospora* include prevention of source water contamination by human waste, followed by adequate treatment and protection of water during distribution. Owing to the resistance of the oocysts to disinfectants, *E. coli* (or, alternatively, thermotolerant coliforms) cannot be relied upon as an index of the presence/absence of *Cyclospora* in drinking-water supplies.

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Giardia intestinalis

General description

Giardia spp. are flagellated protozoa that parasitize the gastrointestinal tract of humans and certain animals. The genus *Giardia* consists of a number of species, but human infection (giardiasis) is usually assigned to *G. intestinalis*, also known as *G. lamblia* or *G. duodenalis*. *Giardia* has a relatively simple life cycle consisting of a flagellate

trophozoite that multiplies in the gastrointestinal tract and an infective thickwalled cyst that is shed intermittently but in large numbers in faeces. The trophozoites are bilaterally symmetrical and ellipsoidal in shape. The cysts are ovoid in shape and 8–12µm in diameter.

Human health effects

Giardia has been known as a human parasite for 200 years. After ingestion and excystation of cysts, the trophozoites attach to surfaces of the gastrointestinal tract. Infections in both children and adults may be asymptomatic. In day care centres, as many as 20% of children may carry *Giardia* and excrete cysts without clinical symptoms.

The symptoms of giardiasis may result from damage caused by the trophozoites, although the mechanisms by which *Giardia* causes diarrhoea and intestinal malabsorption remain controversial. Symptoms generally include diarrhoea and abdominal cramps; in severe cases, however, malabsorption deficiencies in the small intestine may be present, mostly among young children. Giardiasis is self-limiting in most cases, but it may be chronic in some patients, lasting more than 1 year, even in otherwise healthy people. Studies on human volunteers revealed that fewer than 10 cysts constitute a meaningful risk of infection.

Source and occurrence

Giardia can multiply in a wide range of animal species, including humans, which excrete cysts into the environment. Numbers of cysts as high as 88 000 per litre in raw sewage and 240 per litre in surface water resources have been reported. These cysts are robust and can survive for weeks to months in fresh water. The presence of cysts in raw water sources and drinking-water supplies has been confirmed. However, there is no information on whether human infectious species were present. The currently available standard analytical techniques provide an indirect measure of viability and no indication of human infectivity. Cysts also occur in recreational waters and contaminated food.

Routes of exposure

By far the most common route of transmission of *Giardia* is person-to-person contact, particularly between children. Contaminated drinking-water, recreational water and, to a lesser extent, food have been associated with outbreaks. Animals have been implicated as a source of human infectious *G. intestinalis*, but further investigations are required to determine their role.

Significance in drinking-water

Waterborne outbreaks of giardiasis have been associated with drinking-water supplies for over 30 years; at one stage, *Giardia* was the most commonly identified cause of waterborne outbreaks in the USA. *Giardia* cysts are more resistant than enteric bacteria to oxidative disinfectants such as chlorine, but they are not as resistant as *Cryptosporidium* oocysts. The time required for 90% inactivation at a free chlorine residual of 1 mg/litre is about 25–30 min.Within a WSP, control measures that can be applied to manage potential risk from *Giardia* include prevention of source water contamination by human and animal waste, followed by adequate treatment and disinfection and protection of water during distribution. Owing to the resistance of the cysts to disinfectants, *E. coli* (or, alternatively, thermotolerant coliforms) cannot be relied upon as an index of the presence/absence of *Giardia* in drinking-water supplies.

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Isospora belli

General description

Isospora is a coccidian, single-celled, obligate parasite related to *Cryptosporidium* and *Cyclospora*. There are many species of *Isospora* that infect animals, but only *I. belli* is known to infect humans, the only known host for this species. *Isospora belli* is one of the few coccidia that undergo sexual reproduction in the human intestine. Sporulated oocysts are ingested, and, after complete asexual and sexual life cycles in the mucosal epithelium of the upper small intestine, unsporulated oocysts are released in faeces.

Human health effects

Illness caused by *I. belli* is similar to that caused by *Cryptosporidium* and *Giardia*. About 1 week after ingestion of viable cysts, a low-grade fever, lassitude and malaise may appear, followed soon by mild diarrhoea and vague abdominal pain. The infection is usually self-limited after 1–2 weeks, but occasionally diarrhoea, weight loss and fever may last for 6 weeks to 6 months. Symptomatic isosporiasis is more common in children than in adults. Infection is often associated with immunocompromised patients, in whom symptoms are more severe and likely to be recurrent or chronic, leading to malabsorption and weight loss. Infections are usually sporadic and most common in the tropics and subtropics, although they also occur elsewhere, including industrialized countries. They have been reported from Central and South America, Africa and south-east Asia.

Source and occurrence

Unsporulated oocysts are excreted in the faeces of infected individuals. The oocysts sporulate within 1–2 days in the environment to produce the potentially infectious form of the organism. Few data are available on numbers of oocysts in sewage and raw and treated water sources. This is largely because sensitive and reliable techniques for the quantitative enumeration of oocysts in water environments are not available.

Little is known about the survival of oocysts in water and related environments.

Routes of exposure

Poor sanitation and faecally contaminated food and water are the most likely sources of infection, but waterborne transmission has not been confirmed. The oocysts are less likely than *Cryptosporidium* oocysts or *Giardia* cysts to be transmitted directly from person to person, because freshly shed *I. belli* oocysts require 1–2 days in the environment to sporulate before they are capable of infecting humans.

Significance in drinking-water

The characteristics of *I. belli* suggest that illness could be transmitted by contaminated drinking-water supplies, but this has not been confirmed. No information is available on the effectiveness of water treatment processes for removal of *I. belli*, but it is likely that the organism is relatively resistant to disinfectants. It is considerably larger than *Cryptosporidium* and should be easier to remove by filtration. Within a WSP, control measures that can be applied to manage potential risk from *I. belli* include prevention of source water contamination by human waste, followed by adequate treatment and disinfection and protection of water during distribution. Owing to the likely resistance of the oocysts to disinfectants, *E. coli* (or, alternatively, thermotolerant coliforms) cannot be relied upon as an index of the presence/absence of *I. belli* in drinking-water supplies.

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Microsporidia

General description

The term “microsporidia” is a non-taxonomic designation commonly used to describe a group of obligate intracellular protozoa belonging to the phylum Microspora. More than 100 microsporidial genera and almost 1000 species have been identified. Infections occur in every major animal group, including vertebrates and invertebrates. A number of genera have been implicated in human infections, including *Enterocytozoon*, *Encephalitozoon* (including *Septata*), *Nosema*, *Pleistophora*, *Vittaforma* and *Trachipleistophora*, as well as a collective group of unclassified microsporidia referred to as microsporidium. Microsporidia are among the smallest eukaryotes. They produce unicellular spores with a diameter of 1.0–4.5µm and a characteristic coiled polar filament for injecting the sporoplasm into a host cell to initiate infection. Within an infected cell, a complex process of multiplication takes place, and new spores are produced and released in faeces, urine, respiratory secretions or other body fluids, depending on the type of species and the site of infection.

Human health effects

Microsporidia are emerging human pathogens identified predominantly in persons with AIDS, but their ability to cause disease in immunologically normal hosts has been recognized. Reported human infections are globally dispersed and have been documented in persons from all continents. The most common clinical manifestation in AIDS patients is a severe enteritis involving chronic diarrhoea, dehydration and weight loss. Prolonged illness for up to 48 months has been reported. Infections in the general population are less pronounced. *Enterocytozoon* infection generally appears to be limited to intestinal enterocytes and biliary epithelium. *Encephalitozoon* spp. infect a variety of cells, including epithelial and endothelial cells, fibroblasts, kidney tubule cells, macrophages and possibly other cell types. Unusual complications include keratoconjunctivitis, myositis and hepatitis.

Source and occurrence

The sources of microsporidia infecting humans are uncertain. Spores are likely to be excreted in faeces and are also excreted in urine and respiratory secretions. Due to the

lack of a quantification technique, there is limited information on the prevalence of microsporidia spores in water environments. However, microsporidia have been detected in sewage and water sources. Indications are that their numbers in raw sewage may be similar to those of *Cryptosporidium* and *Giardia*, and they may survive in certain water environments for many months. Certain animals, notably swine, may serve as a host for human infectious species.

Routes of exposure

Little is known about transmission of microsporidia. Person-to-person contact and ingestion of spores in water or food contaminated with human faeces or urine are probably important routes of exposure. A waterborne outbreak of microsporidiosis has been reported involving about 200 cases in Lyon, France, during the summer of 1995. However, the source of the organism and faecal contamination of the drinking water supply were not demonstrated. Transmission by the inhalation of airborne spores or aerosols containing spores seems possible. The role of animals in transmission to humans remains unclear. Epidemiological and experimental studies in mammals suggest that *Encephalitozoon* spp. can be transmitted transplacentally from mother to offspring. No information is available on the infectivity of the spores.

However, in view of the infectivity of spores of closely related species, the infectivity of microsporidia may be high.

Significance in drinking-water

Waterborne transmission has been reported, and infection arising from contaminated drinking-water is plausible but unconfirmed. Little is known about the response of microsporidia to water treatment processes. One study has suggested that the spores may be susceptible to chlorine. The small size of the organism is likely to make them difficult to remove by filtration processes. Within a WSP, control measures that can be applied to manage potential risk from microsporidia include prevention of source water contamination by human and animal waste, followed by adequate treatment and disinfection and protection of water during distribution. Owing to the lack of information on sensitivity of infectious species of microsporidia to disinfection, the reliability of *E. coli* (or, alternatively, thermotolerant coliforms) as an index for the presence/absence of these organisms from drinking-water supplies is unknown.

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Toxoplasma gondii

General description

Many species of *Toxoplasma* and *Toxoplasma*-like organisms have been described, but it would appear that *T. gondii* is the only human infectious species. *Toxoplasma gondii* is a coccidian parasite, and the cat is the definitive host. Only cats harbour the parasite in the intestinal tract, where sexual reproduction takes place. The actively multiplying asexual form in the human host is an obligate, intracellular parasite (diameter 3–6µm) called a tachyzoite. A chronic phase of the disease develops as the tachyzoites transform into slowly replicating bradyzoites, which eventually become cysts in the host tissue. In the natural cycle, mice and rats containing infective cysts are eaten by cats, which host the sexual stage of the parasite. The cyst wall is digested, and bradyzoites penetrate epithelial cells of the small intestine. Several generations of intracellular multiplication lead to the development of micro- and macrogametes.

Fertilization of the latter leads to the development of oocysts that are excreted in faeces as early as 5 days after a cat has ingested the cysts. Oocysts require 1–5 days to sporulate in the environment. Sporulated oocysts and tissue-borne cysts can both cause infections in susceptible hosts.

Human health effects

Toxoplasmosis is usually asymptomatic in humans. In a small percentage of cases, flu-like symptoms, lymphadenopathy and hepatosplenomegaly present 5–23 days after the ingestion of cysts or oocysts. Dormant cysts, formed in organ tissue after primary infection, can be reactivated when the immune system becomes suppressed, producing disseminated disease involving the central nervous system and lungs and leading to severe neurological disorders or pneumonia. When these infection sites are involved, the disease can be fatal in immunocompromised patients. Congenital toxoplasmosis is mostly asymptomatic, but can produce chorioretinitis, cerebral calcifications, hydrocephalus, severe thrombocytopenia and convulsions. Primary infection during early pregnancy can lead to spontaneous abortion, stillbirth or fetal abnormality.

Source and occurrence

Toxoplasmosis is found worldwide. Estimates indicate that in many parts of the world, 15–30% of lamb and pork meat is infected with cysts. The prevalence of oocystshedding cats may be 1%. By the third decade of life, about 50% of the European population is infected, and in France this proportion is close to 80%. *Toxoplasma gondii* oocysts may occur in water sources and supplies contaminated with the faeces of infected cats. Due to a lack of practical methods for the detection of *T. gondii* oocysts, there is little information on the prevalence of the oocysts in raw and treated water supplies. Details on the survival and behaviour of the oocysts in water environments are also not available. However, qualitative evidence of the presence of oocysts in faecally polluted water has been reported, and results suggest that *T. gondii* oocysts may be as resistant to unfavourable conditions in water environments as the oocysts of related parasites.

Routes of exposure

Both *T. gondii* oocysts that sporulate after excretion by cats and tissue-borne cysts are potentially infectious. Humans can become infected by ingestion of oocysts excreted by cats by direct contact or through contact with contaminated soil or water. Two outbreaks of toxoplasmosis have been associated with consumption of contaminated water. In Panama, creek water contaminated by oocysts from jungle cats was

identified as the most likely source of infection, while in 1995, an outbreak in Canada was associated with a drinking-water reservoir being contaminated by excreta from domestic or wild cats. A study in Brazil during 1997–1999 identified the consumption of unfiltered drinking-water as a risk factor for *T. gondii* seropositivity. More commonly, humans contract toxoplasmosis through the consumption of undercooked or raw meat and meat products containing *T. gondii* cysts. Transplacental infection also occurs.

Significance in drinking-water

Contaminated drinking-water has been identified as a source of toxoplasmosis outbreaks. Little is known about the response of *T. gondii* to water treatment processes. The oocysts are larger than *Cryptosporidium* oocysts and should be amenable to removal by filtration.Within a WSP, control measures to manage potential risk from *T. gondii* should be focused on prevention of source water contamination by wild and domesticated cats. If necessary, the organisms can be removed by filtration. Owing to the lack of information on sensitivity of *T. gondii* to disinfection, the reliability of *E. coli* (or, alternatively, thermotolerant coliforms) as an indicator for the presence/absence of these organisms in drinking-water supplies is unknown.

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مشروع دعم قطاع مياه الشرب والصرف الصحي
Water and Wastewater Sector Support (WWSS)

البروتوزوا الطفيلية في البيئة المائية

PARASITIC PROTOZOA IN THE AQUATIC ENVIRONMENT

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Introduction to Protozoology

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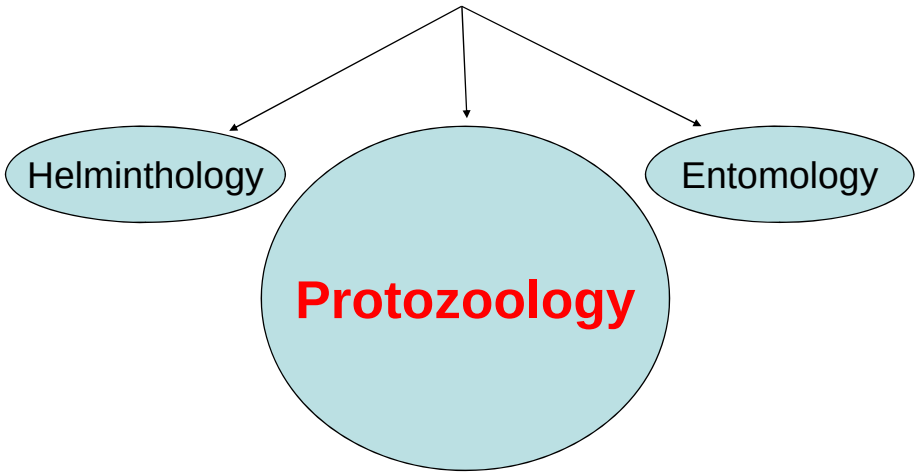
SYSTEMATIC CLASSIFICATION OF PARASITOLOGY



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- More than 50,000 different types of protozoa have been described.
- Their name comes from two Greek words, *protos,* or "first" and *zoön,* or "animal“
- The vast majority of protozoa are microscopic, many measuring less than 1/200 millimeter.
- The vast majority of protozoa are heterotrophic. That is, they cannot manufacture their own food, but must obtain it by eating other organisms.
- A few protozoa contain the green pigment chlorophyll, which allows them to make their own food.

- Some are free-living, while others are attached to other organisms. Some species are parasites of plants and animals, ranging from other protozoa to humans.
- All protozoa reproduce asexually, by dividing into two parts at regular intervals. Some species, however, have evolved the ability to reproduce sexually also.
- Protozoa have evolved mechanisms that allow them to live under a great range of environmental conditions.

- When these conditions are unfavorable, most species are able to enter an inactive, or dormant, phase. They secrete a thick protective outer wall that prevents them from losing water and protects the cell from extreme temperatures.
- This tough little package, called a cyst, may also serve as a means of dispersal (to spread widely). Cysts are carried away on the wind or on the feet of animals.
- Once a cyst reaches a more favorable environment, its outer wall breaks down and the cell resumes normal activity.

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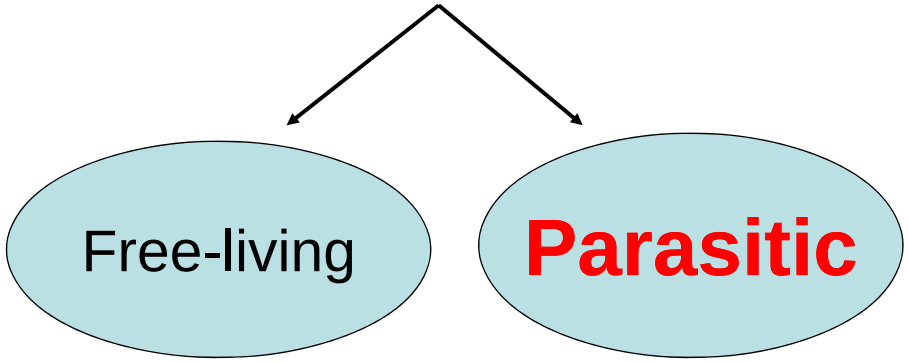
Protozoa



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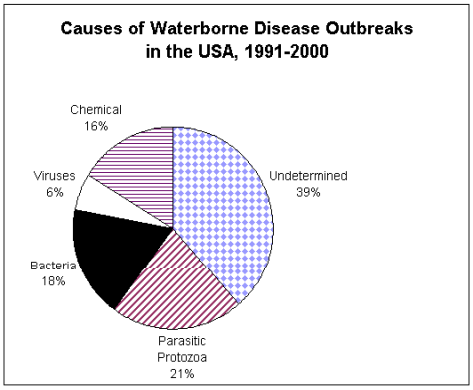
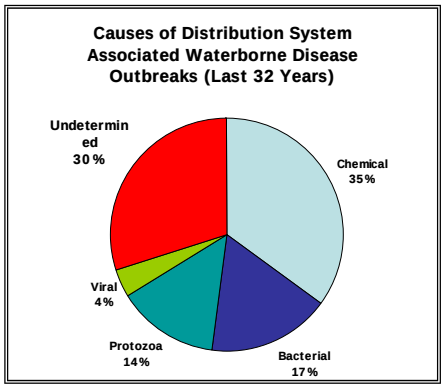
SYSTEMATIC CLASSIFICATION OF PROTOZOA

- Sarcodines
- Ciliates
- Flagellates
- Apicomplexa
- Microspora

Waterborne Disease Outbreaks



www.water and health.org/newsletter/new/spring 2003/waterborne.html



Craun, M.F., et al. 2006. Waterborne outbreaks reported in the United States.

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ACCORDING TO SITE OF INFECTION

- Intestinal protozoa.
- Urogenital protozoa.
- Respiratory protozoa.
- Tissue protozoa.

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Infective stages of protozoa

- Trophozoite.
- Cyst.
- Oocyst.
- Sporocyst.
- Sarcocyst.
- Spore.

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Routes of entrance

- Ingestion & drinking ⟹ all enteric & tisuue protozoa
- Venereal (sexual) ⟹ *Trichomonas vaginalis*
- Maternally ⟹ *Toxoplasma gondii*
- Inhalation ⟹ *Pneumocystis carinii*

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Group	Organism	Habitat	I.S.	Route
Amoebae	*Entamoeba histolytica*	Intestine, liver, brain	Cyst	Oral
Flagellates	*Giardia spp.* *Trichomonas vaginalis* *T. hominis* *T. tenax*	Intestine Genital organs Intestine Buccal cavity	Cyst Trophzoite Trophzoite Trophozoite	Oral Sexual Oral Oral
Ciliates	*Balantidium coli*	Intestine	Cyst	Oral
(Coccidia) Apicomplexa	*Isospora belli* *Cryptosporidium spp.* *Cyclospora caeytanensis* *Toxoplasma gondii* *Sarcocystis spp.*	Intestine Intestine Intestine Tissue	Oocyst Oocyst Oocyst Oocyst Sporocyst	Oral Oral Oral Oral Oral
Microsporidia	*Encephalitozoon spp.* *Enterocytozoon spp.* *Nosema ocularum* *Vittaforma corneae* *Pleistophora spp.* *Trachpleistophora spp.* *Microsporidium spp.* *Brachiola spp.*	Intestine, eye, kidney, liver Intestine, bile duct, nasal mucosa Eye Eye Skeletal muscle Eye, spleen, kidney, liver, brain, heart Eye Skeletal muscle, kidney, liver, lung	Spore Spore Spore Spore Spore Spore Spore Spore	Oral Oral Remain unclear
Unclassified	*Blastocystis hominis* *Pneumocystis carinii* *Prototheca spp.*	Intestine Lung Intestine	Cyst Cyst Cyst	Oral Inhalation Oral

PARASITIC PROTOZOA

1. Intestinal protozoa
2. Urogenital protozoa
3. Pulmonary protozoa
4. Tissue protozoa

1- INTESTINAL PROTOZOA

- Sarcodina
- Flagellates
- Ciliates
- Apicomplexa (Coccidia)
- Microspora

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SARCODINA (AMOEBAE)

- Protozoa are classified according to the ways in which they move about.
- One phylum, the Sarcodina, moves by pushing out portions of their cytoplasm forming pseudopods, or "false feet."
- They capture their food by extending their pseudopods around it, engulfing it, and digesting it. Probably the best known example of the Sarcodina is the amoeba.
- *Entamoeba*

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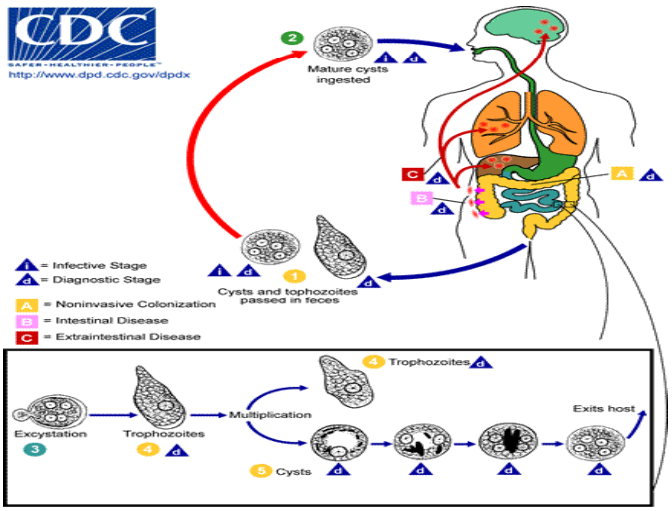
Entamoeba histolytica

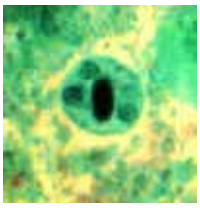
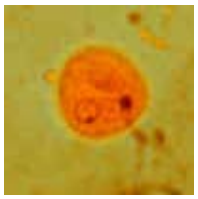
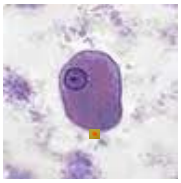
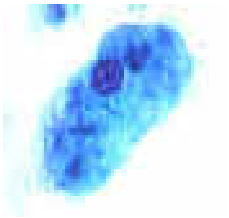
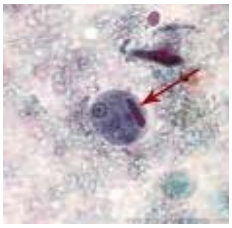


Epidemiology

- 0.5 to 50% of the population world wide harbors *E. histolytica* parasites with the higher rates of infection being in underdeveloped countries.
- 1 to 3% of the population of the USA are infected. Infection is associated with poor hygiene.
- Humans are the principal host, although dogs, cats and rodents may be infected

Stages of life

- **Trophozoite**: This form has an ameboid appearance and is usually 15-30 micrometers in diameter, although more invasive strains tend to be larger. The organism has a single nucleus with a distinctive small central karyosome (Figure 1A,B). The fine granular endoplasm may contain ingested erythrocytes (Figure 1C). The nuclear chromatin is evenly distributed along the periphery of the nucleus.
- **Cyst:** Entameba histolytica cysts are spherical, with a refractile wall; the cytoplasm contains dark staining chromatoidal bodies and 1 to 4 nuclei with a central karyosome and evenly distributed peripheral chromatin

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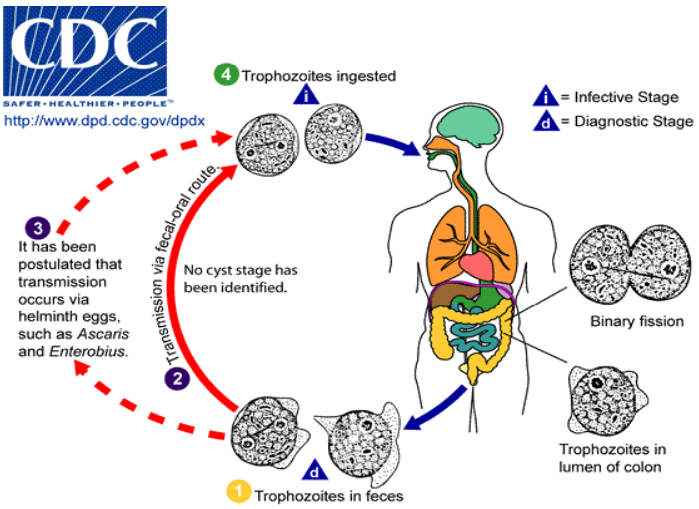
Dientamoeba fragilis



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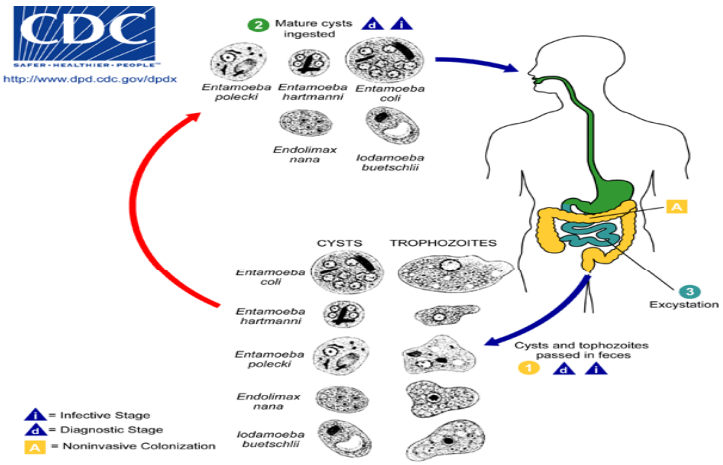
Endolimax nana



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FLAGELLATES

- The phylum Mastigophora consists of one-celled organisms that move about by means of flagella.
- Flagella are whiplike structures somewhat similar to cilia. The major difference between the two structures is that flagella are much larger than cilia.
- Also, flagellates have anywhere from one to several hundred flagella, while cilia never occur individually.
- The majority of flagellates live inside other organisms in either a symbiotic (mutually beneficial) or parasitic relationship.
- *Giardia & Enteromonas*

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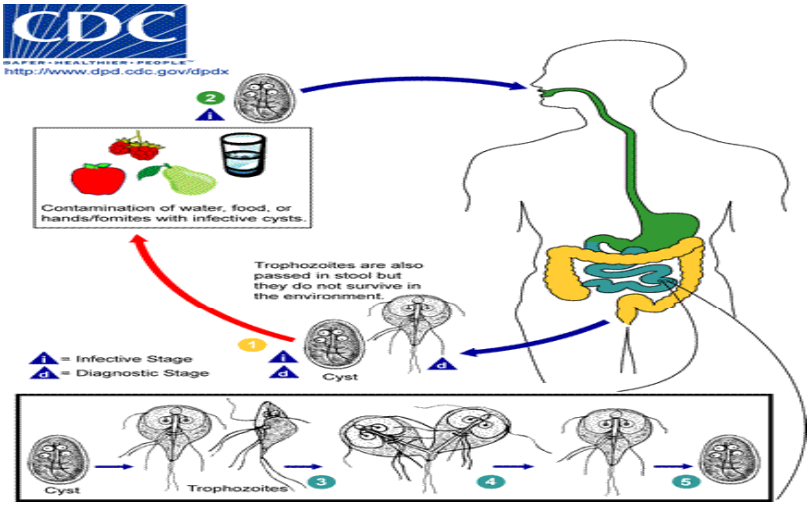
Giardia lamblia



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Epidemiology

- *Giardia* has worldwide distribution and is not uncommon in South Carolina.

- It is the most frequent protozoan intestinal disease in the US and the most common identified cause of water-borne disease associated with breakdown of water purification systems, drinking from contaminated streams, travel to endemic areas (Russia, India, Rocky Mountains, etc.) and day care centers .

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Stages

- **Trophozoite**: *Giardia* is a 12 to 15 micrometer, half pear-shaped organism with 8 flagella and 2 axostyles arranged in a bilateral symmetry. There are two anteriorly located large suction discs. The cytoplasm contains two nuclei and two parabasal bodies.

- **Cyst**: *Giardia* cysts are 9 to 12 micrometer ellipsoidal cells with a smooth well-defined wall. The cytoplasm contains four nuclei and many of the structures seen in the trophozoite.

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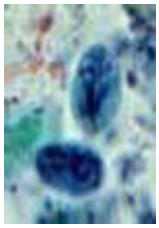
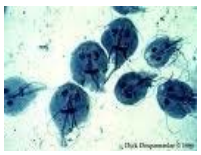

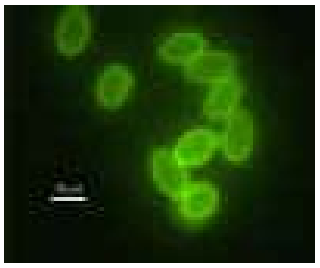
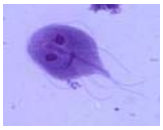

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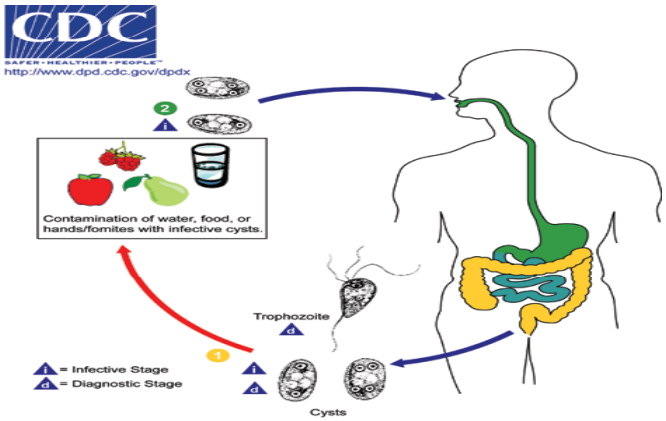
Enteromonas hominis (non pathogenic)



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CILIATES

- Members of the phylum Ciliophora get their name from tiny hair-like projections known as cilia on the surface of the cell.
- These protozoa swim around by waving their cilia back and forth, like the oars on a boat. Cilia are also used to obtain food.
- As they beat back and forth, the cilia create a whirlpoollike effect that brings food close enough for the organism to ingest.
- *Balantidium*

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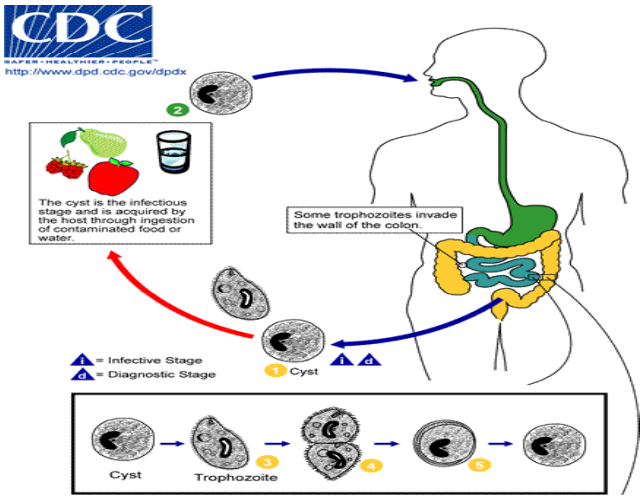
Balantidium coli



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Morphology

- This is a parasite primarily of cows, pigs and horses. The organism is a large (100 x 60 micrometer) ciliate with a macro- and a micro-nucleus.

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Epidemiology

- The infection occurs mostly in farm workers and other rural dwellers by ingestion of cysts in fecal material of farm animals. Man-to-man transmission is rare but possible.
- Symptoms and pathogenesis of balantidiasis are similar to those seen in entamebiasis, including intestinal epithelial erosion. However, liver, lung and brain abscesses are not seen.
- Metronidazole and iodoquinol are effective.

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APICOMPLEXA (COCCIDIA)

- Members of the Apicomplexa have no means of movement.
- They form environmentally resistant stage named oocyst.
- They are intracellular parasites and depend on their hosts for all their food and survival.
- *Cryptosporidium*
- *Cyclospora*
- *Cystiospora*

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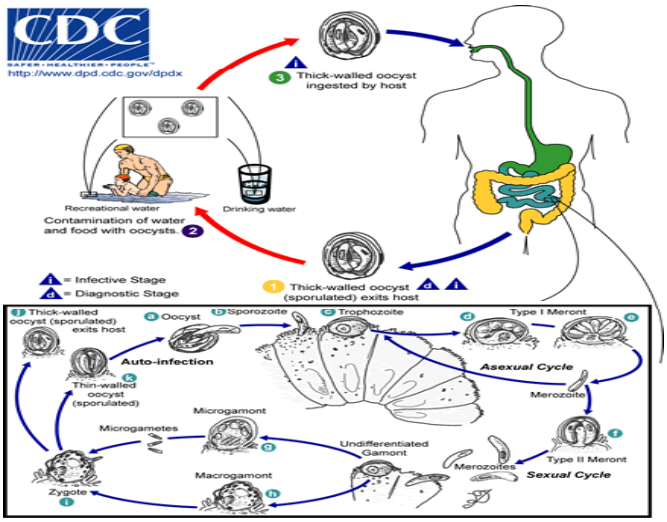
Cryptosporidium



Morphology

- A small round parasite measuring 3 to 5 micrometers which is found in the gastrointestinal tract of humans, many animals and birds.

Epidemiology

- It causes epidemics of diarrhea in humans via contaminated food and water.
- Humans are infected by ingestion of *C. parvum* oocysts containing sporozoites. The sporozoites are released in the upper GI tract and attach to the gut mucosal cells where they divide to produce merozoites. The merozoites invade other mucosal cells and further multiply asexually.
- Some of the merozoites differentiate into male and female gametocytes and form an oocyst in which they multiply and differentiate into sporozoites.
- The mature oocyst is excreted with fecal material and infects other individuals

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The potential for waterborne transmission of Cryptosporidium comes from:

1. Large number of oocysts excreted by infected host (10^7 oocysts per gram of faeces in calves).
2. Low infectious dose (30 oocysts).
3. The small size of oocyst “3-6µm”.
4. Oocysts are excreted fully sporulated at the time of excretion and can cause the infection.
5. Zoonosis.
6. Transmission (water, food and air).
7. Capability of oocysts to survive and remain infectious for several months under various environmental conditions.

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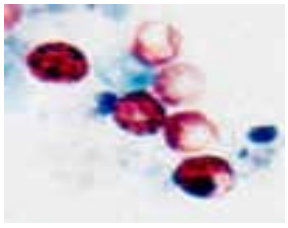

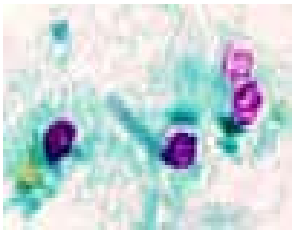

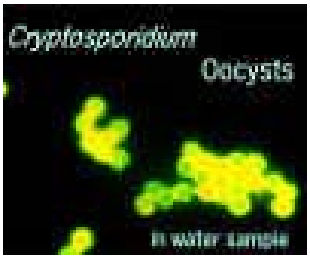

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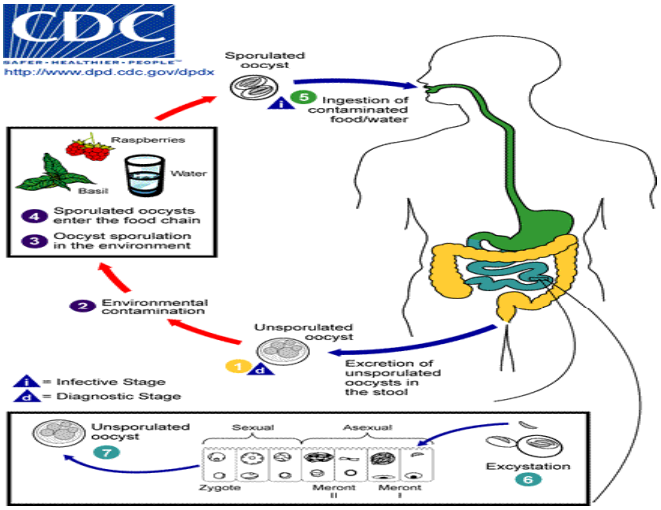
Cyclospora



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Epidemiology

- More commonly associated with consumption of contaminated produce in North America. Two waterborne outbreaks have been reported. It produces oocysts shed in the feces and require an external maturation period (5-14 d at 22 to 32° C) to become infective.
- Infection can result in diarrheal illness that may persist for several weeks and is normally self-limiting in immunocompetent individuals.

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Morphology

- Round oocysts measuring 8-10 microns in diameter

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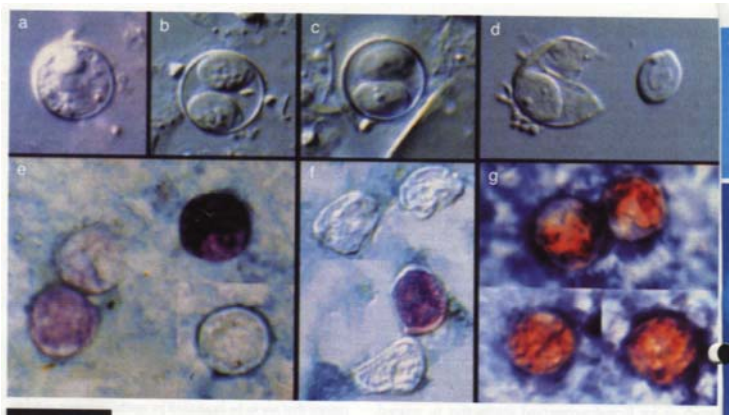

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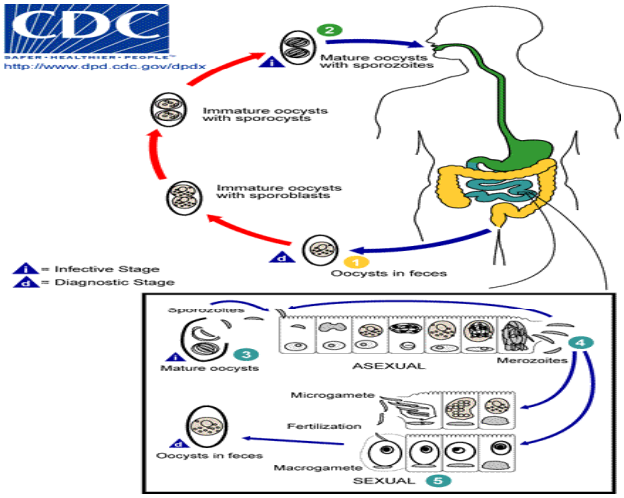
Cystoispora belli



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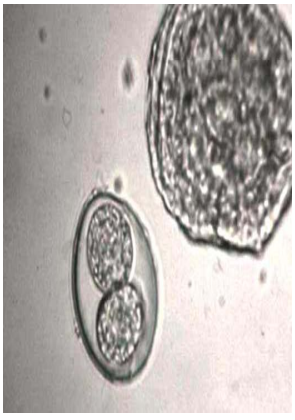
Morphology

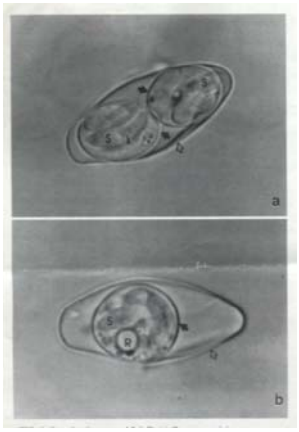
* **Oocyst is about 13 x 20µm, up to 30µm.**

* **Sporulated oocyst has 2 sporocysts.**

Epidemiology

- It is a rare infection of normal humans, although it is being seen in increasing numbers in AIDS patients.
- The infection occurs via the oro-fecal route.
- The infective stage of the organism is an oval oocyst which, upon ingestion, follows the same course as *C. parvum*.
- The disease produces symptoms similar to those of giardiasis.
- In normal individuals, mild infections resolve themselves with rest and mild diet and heavier infections can be treated with sulpha drugs.
- The treatment may have to be carried on for a prolonged period in AIDS patients.





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MICROSPORA

- Members of the Microspora are parasites of vertebrate and invertebrate hosts.
- They form environmentally resistant stage named spore.
- They are intracellular parasites and depend on their hosts for all their food and survival.
- *Encephalitozoon intestinalis*
- *Enterocytozoon bieneusi*

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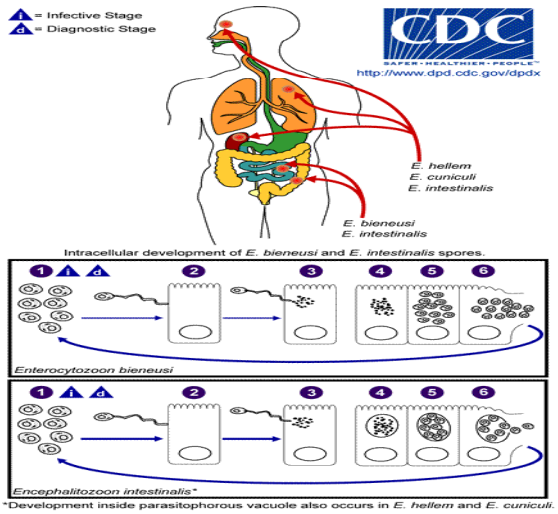
Intestinal microsporidia



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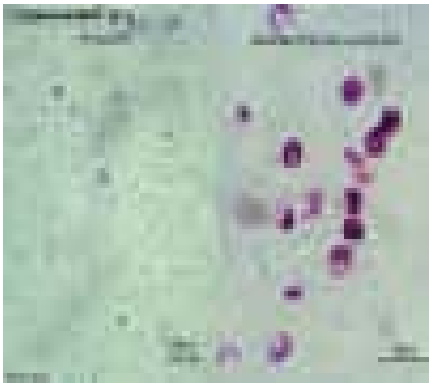

2- UROGENITAL PROTOZOA

- Flagellates
- Microspora

FLAGELLATES

- *Trichomonas vaginalis*

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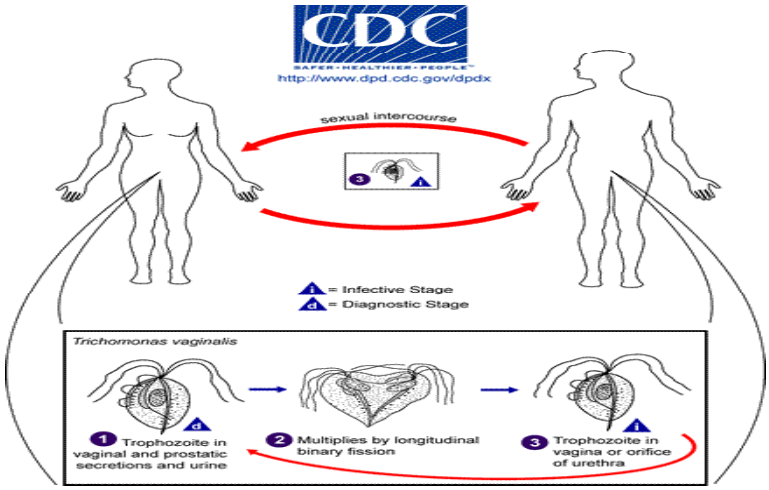
Trichomonas vaginalis



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Microspora

- *Encephalitozoon*
- *Enterocytozoon*

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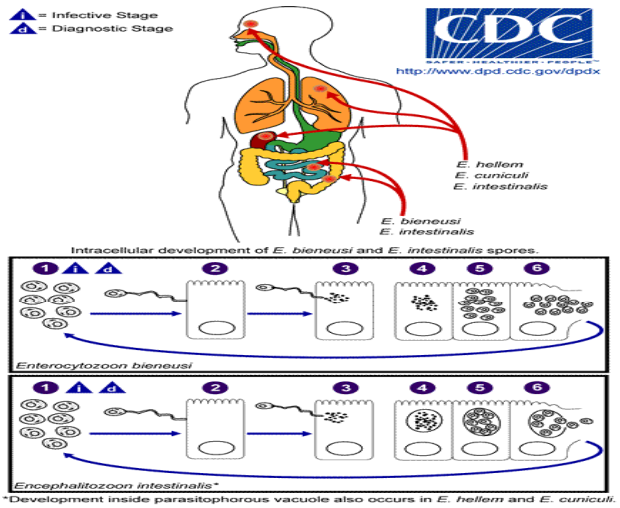
Encephlitozoon & Enterocytozoon



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Morphology

- *Entrocytozoon* spp. are small coccobacilli measuring 0.7 – 1.2µm
- *Encephlitozoon* spp. are small bacilli measuring 1.5 – 4 µm
- Polar tubules

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Epidemiology

- Although the potential for waterborne transmission exists, only one water-associated outbreak has been reported.
- Infection with microsporidia may result in diarrhea and self-limiting illness in immunocompetent individuals.
- Infection can be more problematic in the elderly and in the immunocompromised.

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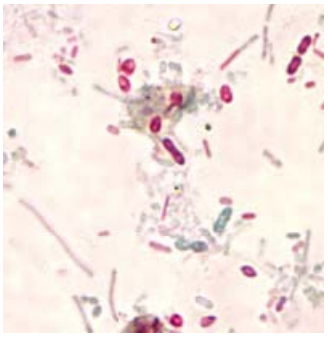

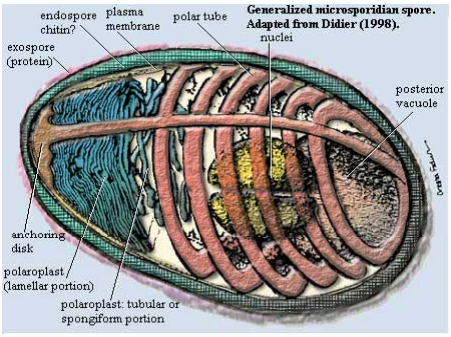

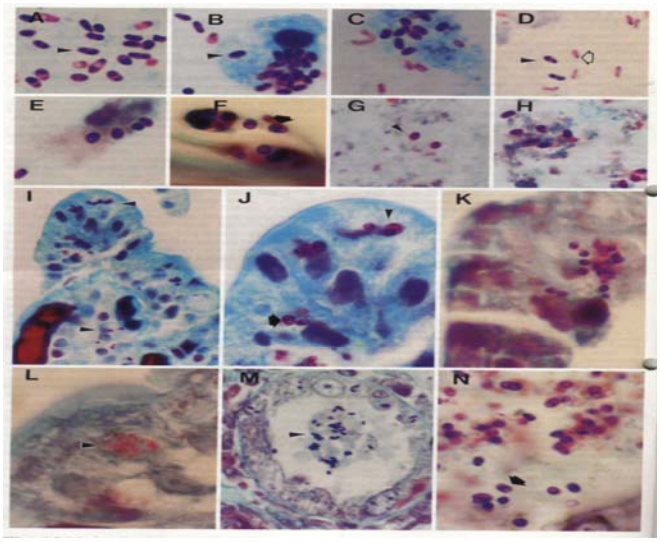

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3- PULMONARY PROTOZOA

- Pneumocystis carinii

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Pneumocystis carinii

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4- TISSUE PROTOZOA

- Apicomplexa

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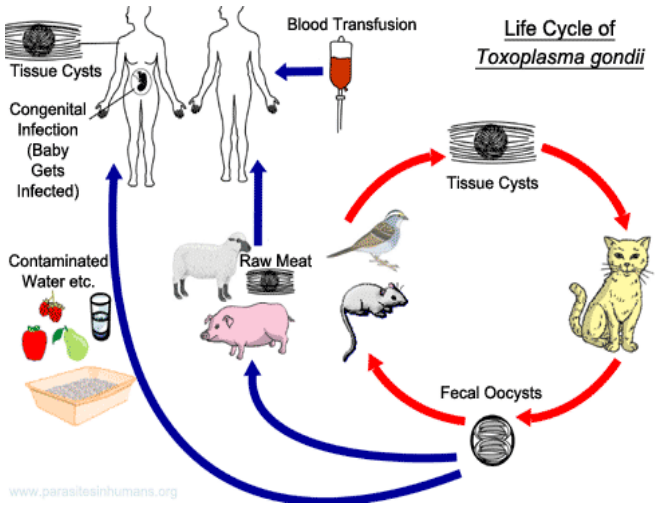
Toxoplasma gondii



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Stages of *Toxoplasma*

- Tachyzoites
- Bradyzoites
- Oocysts

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Epidemiology

- Two waterborne outbreaks have been reported in Panama (USA) and British Columbia (Canada).
- It requires intermediate and final hosts to complete its life cycle.
- Felines are the only known final hosts. Oocysts require an external maturation period (1 – 5 days) to sporulate and become infective.
- Pregnant women can pass infection to the fetus

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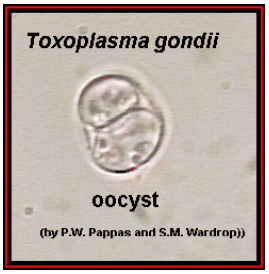

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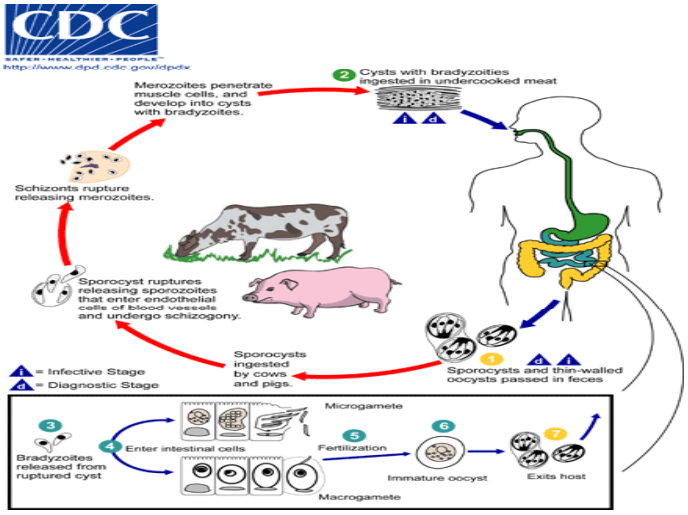
Sarcocystis



ETIOLOGY

- *Sarcocystis hominis* ⟹ cattle
- *Sarcocystis swihominis* ⟹ swine

Epidemiology

- Man contaminates the environment with feces containing sporocysts.
- Cattle and swine ingest contaminated pasture and water and develop muscular cysts known as sarcocysts.
- Human become infected by eating meat containing sarcocysts.

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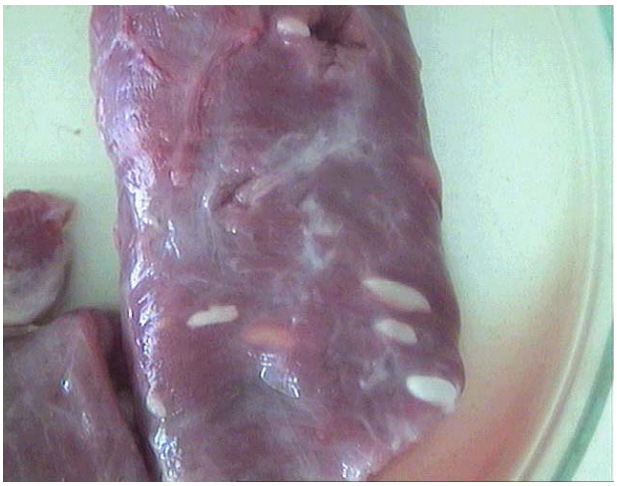
Infective stage: Sarcocyst



PROTOZOAN PARASITES OF ZOONOTIC IMPORTANCE

Zoonotic Diseases

- Diseases transmitted from animals to humans
- Zoonotic parasitic diseases are transmitted to humans either by ingesting environmentally robust transmissive stages (spores, cysts, oocysts, ova, larval and encysted stages) or by eating raw or undercooked meat containing infective tissue stages.
- Humans can be final, intermediate, paratenic or accidental hosts

- **Some parasite zoonoses complete their life cycles in the human host:**
  - * Cryptosporidiosis
  - * Giardiasis
  - * Microsporidiosis
- **Others do not:**
  - * Toxoplasmosis

Environmental Contamination with Zoonotic protozoa

- Number of infected non-human hosts
- Number of transmissive stages excreted
- Agricultural practices
- Host behavior and activity
- Socio-economic and ethnic differences in human behavior
- Geographic distribution
- Sanitation
- Safety of drinking water sources and supplies
- Climate and hydrogeology of the area

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Monitoring Methods

- USEPA method 1623
- Filtration
- Flocculation
- Flow cytometry
- Immunomagnetisable separation
- Immunofluorescence detection with monoclonal antibodies
- PCR
- Fluorescence in situ hybridisation
- Electrorotation

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Viability Determination

- In vitro excystation
- Animal infectivity
- Fluorogenic vital dyes
- PCR of inducible heat shock protein 70
- Reverse transcription PCR
- Fluorescence in situ hybridization

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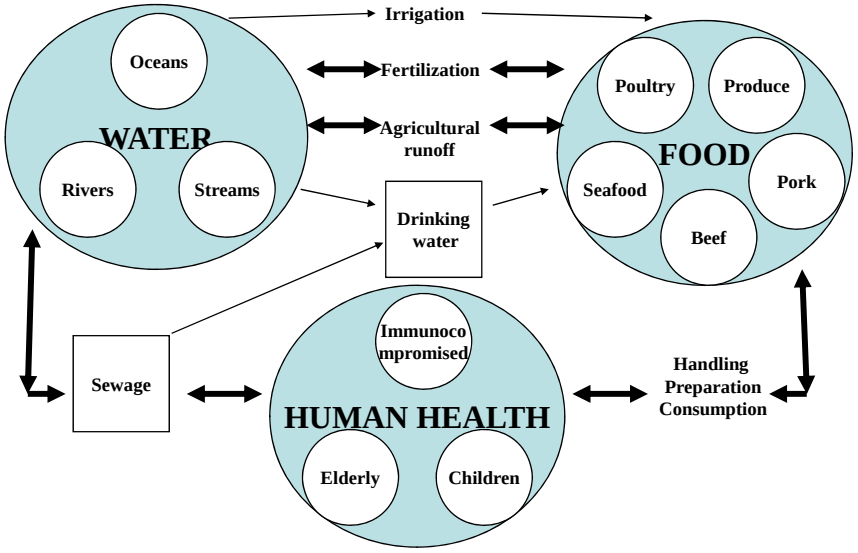
Food-water connection between human health and the environment



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Zoonotic Protozoa

Parasite	Transmission	Route	Infective stage	Final host
Cryptosporidium parvum **(genotype 2)**	Water, food	Ingestion	Oocysts in water and undercooked food	Humans, other mammals
Giardia duodenalis	Water, food	Ingestion	Cysts in water and undercooked food	Humans, other mammals and birds
Toxoplasma gondii	Food, water	Ingestion	Oocysts in water and undercooked food	Felines
Balantidium coli	Water, food	Ingestion	Cysts in water and undercooked food	Humans, pig, cat, nonhuman primates, rodents
Blastocystis hominis ***Blastocystis spp.***	Water, food	Ingestion	Cysts in water and undercooked food	Humans, other mammals

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Zoonotic Microspora

Parasite	Transmission	Route	Infective stage	Final host
Enterocytozoon bieneusi	Water?, food	Ingestion	Spores in water and undercooked food	Humans, rhesus monkey
Encephalitozoon cuniculi ***E. Intestinalis*** ***E. hellem***	Water?, food	Ingestion	Spores in water and undercooked food	Humans, pets, rabbits, mice, goat, cattle, canines. Parrot, Parakeet.
Pleistophora- **like**	Food	Ingestion	Spores in water and undercooked food	Humans, fish, crustacea

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WATERBORNE DISEASES CAUSED BY PROTOZOAN PARASITES

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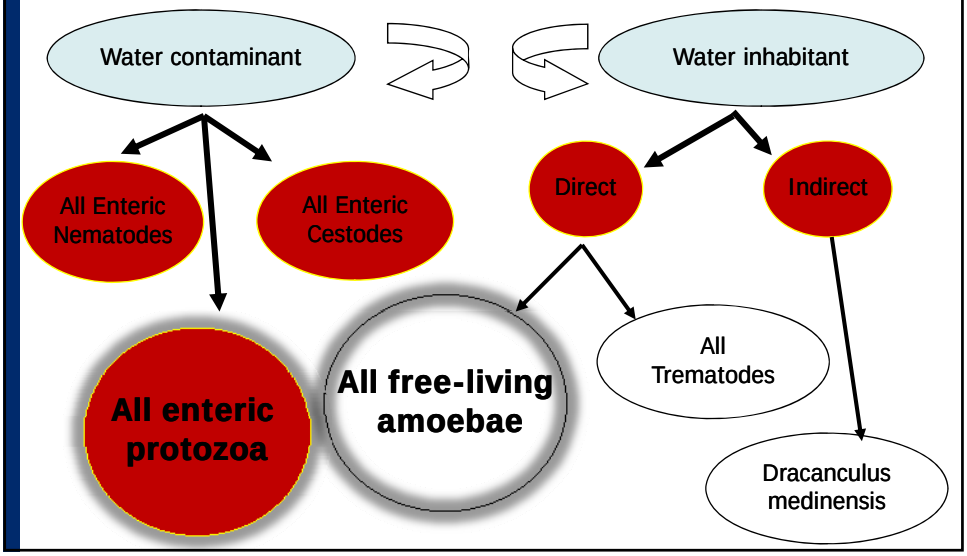
Waterborne parasitic diseases



Group	Organism	Habitat	Route
Amoebae	*Entamoeba histolytica*	Intestine, liver, brain	Oral
Flagellates	*Giardia spp.* *Trichomonas vaginalis* *T. hominis* *T. tenax*	Intestine Genital organs Intestine Buccal cavity	Oral Sexual Oral Oral
Ciliates	*Balantidium coli*	Intestine	Oral
Coccidia	*Isospora belli* *Cryptosporidium spp.* *Cyclospora caeytanensis* *Toxoplasma gondii*	Intestine Intestine Intestine Tissue	Oral Oral Oral Oral
Microsporidia	*Encephalitozoon spp.* *Enterocytozoon spp.* *Nosema ocularum* *Vittaforma corneae* *Pleistophora spp.* *Trachpleistophora spp.* *Microsporidium spp.* *Brachiola spp*	Intestine, eye, kidney, liver Intestine, bile duct, nasal mucosa Eye Eye Skeletal muscle Eye, spleen, kidney, liver, brain, heart Eye Skeletal muscle, kidney, liver, lung	Oral Oral Remain unclear
Unclassified	*Blastocystis hominis* *Pneumocystis carinii* *Prototheca spp.*	Intestine Lung Intestine	Oral Inhalation Oral

Occurrence of Parasitic Protozoa in Water Supplies in Egypt

Organism	Water type	Reference
Cyclospora caeytanensis	Treated water tanks	Abou-El-Naga, 1999
Cryptosporidium spp	Freshwater	Ali et al., 2004
Giardia lamblia	Fresh, finished, tap	Ali et al., 2004
Entamoeba histolytica	Tap	Khairy et al., 1987
Microsporidia	Nile, raw sewage	Ashmawy et al., 2005

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Parasitic Waterborne pathogens and their significance in water supplies

Parasite	Health significance	Persistence in water supplies	Resistance to chlorine	Relative infectivity	Important animal source
Cryptosporidium parvum	High	Long	High	High	Yes
Cyclospora cayetanensis	High	Long	High	High	No
Entamoeba histolytica	High	Moderate	High	High	No
Giardia intestinalis	High	Moderate	High	High	Yes
Toxoplasma gondii	High	Long	High	High	Yes

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Waterborne disease outbreaks surveillance

- Waterborne disease outbreak surveillance can help in identifying important waterborne agents, sources of contamination, and water system deficiencies.
- They have also provided information about failures in water treatment and distribution and sources of contamination for source and recreational waters.

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Prevention of waterborne disease

- Several nations, have the financial resources to prevent waterborne disease outbreaks. Source water protection, advances in water treatment, and real time monitoring of water quality parameters are some of the preventative measures
- Global organizations are also assisting developing nations to provide safe drinking water to all people.
- Despite advances in preventing waterborne disease, severe outbreaks still occur, even in developed nations like the United States (***Cryptosporidium,*** Milwaukee, 1993), Canada (***Escherichia coli*** O157:H7, Walkerton, Ontario, 2000), the United Kingdom and Europe (several outbreaks of ***Cryptosporidium***).

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CONCLUSIONS

1. Waterborne diseases have a major public health and socio-economic impact and are the most important concern of water quality.
2. Strategies for the control of waterborne disease must be based on the selection of appropriate water sources and treatment systems.
3. A wide variety of disinfection systems and use of more than one (chlorine, ozone, UV) can be reliable for production of microbiological safe drinking water.
4. Density of pathogenic bacteria, parasitic stages and living viruses are still less than the minimal infectious dose.
5. Development of microbiological water quality control by using new bioindicators (e.g. *Aeromonas hydrophila*, coliphage, *Cryptosporidium* and enteric viruses such as enteroviruses and rotaviruses) should be determined in addition to classical bioindicators.

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DETECTION OF PARASITIC PROTOZOA IN WATER

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DETECTION AND IDENTIFICATION METHODS

- Sampling and concentration
- Purification／Separation
- Assay

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Sampling and concentration

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Procedure	Sample volume (L)	Water turbidity	Advantages	Disadvantages
Grab samples	≤ 20	Low to high	Easy to collect, no filter cost	Larger volumes may be difficult
Flocculation	≤ 20	Low to high	Simple to perform	Not compatible with viability or sucrose flotation
Continuous-flow centrifugation	≤ 200	Low to high	High recovery rate	High initial equipment cost
Wound filters	≤ 100 raw ≥ 1000 finished	Low to high	High filtration rate	Poor retention & recovery. Time consuming
Pleated membrane filters	≤ 100 raw ≥ 1000 finished	Low to moderate	Good retention and recovery. Easy field concentration & elution	High cost. Slow filtration rate
Disc membrane filters	≤ 10	Low	Lower cost than pleated filters. Good recovery & retention	Low sample volume & filtration rate
Hollow fiber filters	≤ 100	Low to moderate	Good retention and recovery. Moderate cost. Reusable	Method not fully developed
Compressed foam filters	≤ 50 raw ≥ 1000 finished	Low to high	Excellent retention & recovery. Moderate cost	Special equipment for elution & concentration

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Purification / Separation

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Guidance for Selection of Purification / Separation Techniques

Technique	Advantages	Disadvantages
Flotation	Inexpensive Quick	Poor recovery. Background debris. High interlaboratory variability. Efficiency depend on matrix type. Target organism is not selective
Immunomagnetic separation	Improved recovery. Relatively inexpensive. Reduced interlaboratory variability. Minimal background debris. Selective target organism.	Interference from matrices containing iron or magnetic substances. High turbidity may interfere
Fluorescence activated cell sorting	Excellent separation High sample throughput Relatively rapid	High initial equipment cost High maintenance costs Requires highly trained analyst

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Assays

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Guidance for Selection of *Giardia* and *Cryptosporidium* Assay

Procedure	Advantages	Disadvantages
Immunofluorescence (IFA)	Established method Quantitative Relatively easy Viability assessment Capable of detecting dead and empty cysts and oocysts	Expensive equipment Labor-intensive Antibody cross-reactivity Limited species specificity and no genotype specificity Staining does not always correlate well with viability
Polymerase chain reaction (PCR)	Very specific High throughput Inexpensive equipment Viability determination (RT-PCR) Genotyping Standard PCR detects viability	Inhibition by materials in environmental samples Non-quantitative (semiquantitative) Some RNA targets are not good indicators of viability Can not detect empty cysts and oocysts

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Laboratory Procedures for the Detection of Protozoa in Water

- **Sampling and Concentration:**
  - -10 – 1000 liters
  - -Filtration unit 0.8µm pore size.
  - -Elution with a detergent solution (Triton x100, Tween 80)
- **Purification/Separation:**
  - -Sucrose gradient centrifuge – concentrated salt solution
- **Assay:**
  - -Ordinary Stains: Morphology & Viability
  - -Immunofluorescence :
  - -Molecular biology techniques: PCR

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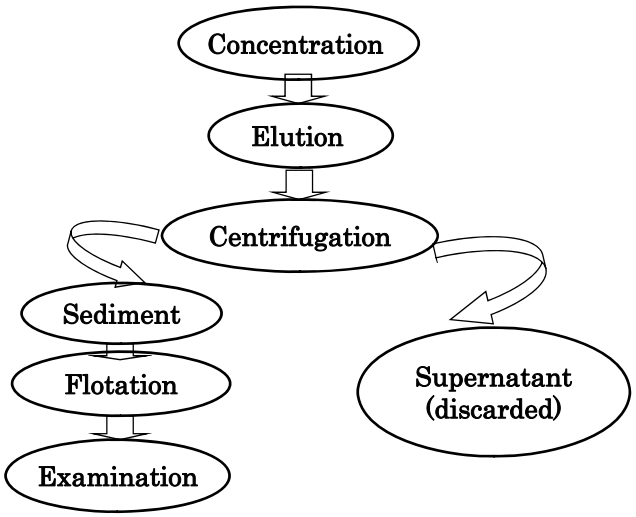
Water Protocol



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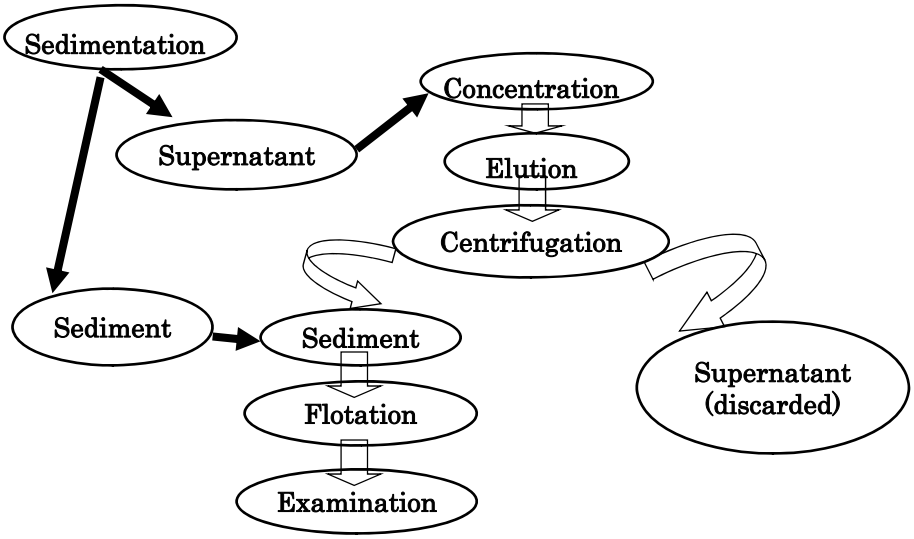
Wastewater Protocol



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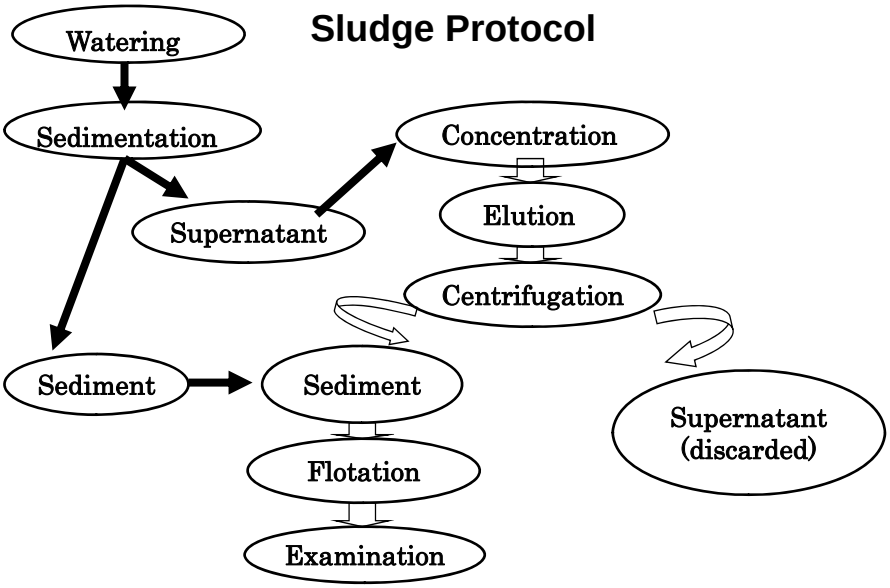
Sludge Protocol



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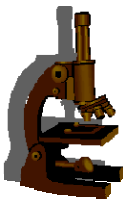
Microscopic Examination



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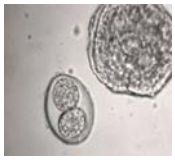
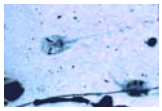
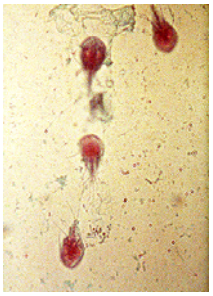
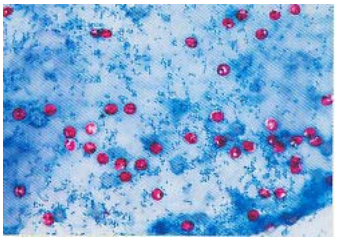
Some Parasitic Protozoa



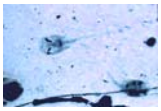
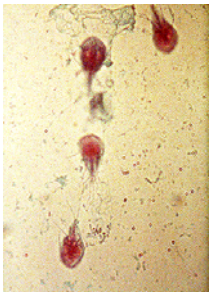
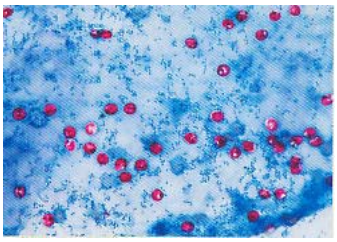


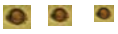




Some Parasitic Protozoa



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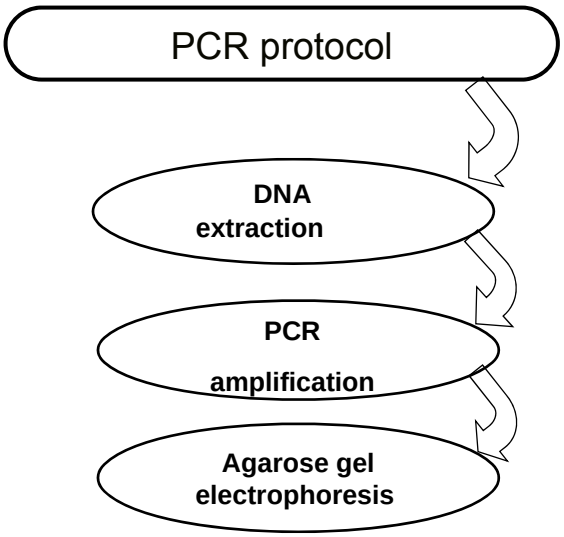

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PCR

Polymerase Chain Reaction

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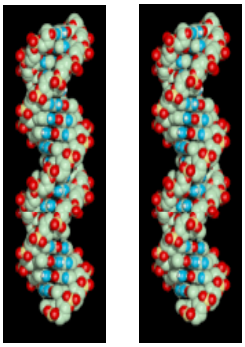
Basic PCR Protocol

A) DNA extraction

B) Amplification

C) Gel electrophoresis



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A) DNA Extraction

CRYPTOSPORIDIUM

- Tris + KCl + Tween 20
- 5 – 15 cycles of freezing and thawing
- Centrifugation at 16000 g for 2 min
- Keep supernatant at -20° C

GIARDIA

- Tris-HCl + EDTA + Proteinase K + Sarkosyl at 37° C for 1 hr
- CTAB + NaCl at 65° C for 30 min ⟶
- Isopropanol for precipitation ⟶
- 70% ethanol for washing
- Resuspend in sterile RNase-free water
- Keep at -70° C

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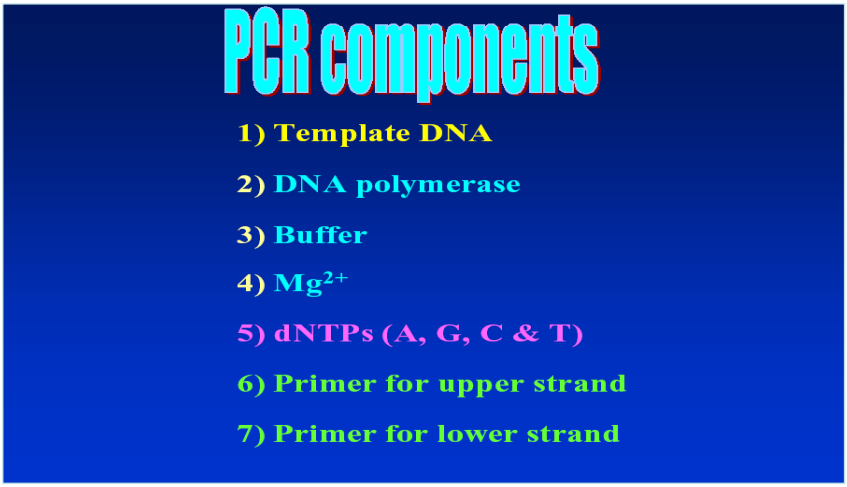
B) PCR Amplification



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1) Template DNA

- **The amount of DNA should be as small as possible.**

- 1 µg of human DNA = 3. 10^5 single copy target molecule
- 10 ng of yeast DNA = 3. 10^5 single copy target molecule
- 1 ng of *E. coli* DNA = 3. 10^5 single copy target molecule

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DNA nucleotide sequences

- **1 tccgcctgcg ctcctcagcc tgacggtccg cctttcgggg ctcctcagcc ttgtcacccg**
- **61 ctcttggttt tccttttctc ttcatctttg gctcctttga ccactcgaag ccgcgcagcg**
- **121 ggttccagcg gacctcacag cagccccaga agtggtgcgc caagcacagc ctctgctcct**
- **181 cctcgagccg gtcgggaact gctgcctgcc gccatcatgt ctactgcaaa tcctgaaact**
- **241 ccaaactcaa ccatctccag agaggccagc acccagtcct catcagctgc aaccagccaa**
- **301 ggctatattt taccagaagg caaaatcatg ccaaacactg tttttgttgg aggaattgat**
- **361 gttaggatgg atgaaactga gattagaagc ttctttgcta gatatggttc agtgaaagaa**
- **421 gtgaagataa tcactgatcg aactggtgtg tccaaaggct atggatttgt ttcatttttt**
- **481 aatgacgtgg atgtgcagaa gatagtagaa tcacagataa atttccatgg taaaaagctg**
- **541 aagctgggcc ctgcaatcag gaaacaaaat ttatgtgctt atcatgtgca gccacgtcct**
- **601 ttggttttta atcatcctcc tccaccacag tttcagaatg tctggactaa tccaaacact**
- **661 gaaacttata tgcagcccac aaccacgatg aatcctataa ctcagtatgt tcaggcatat**
- **721 cctacttacc caaattcacc agttcaggtc atcactggat atcagttgcc tgtatataat**
- **781 tatcagatgc caccacagtg gcctgttggg gagcaaagga gctatgttgt acctccggct**
- **841 tattcagctg ttaactacca ctgtaatgaa gttgatccag gagctgaagt tgtgccaaat**
- **901 gaatgttcag ttcatgaagc tactccaccc tctggaaatg gcccacaaaa gaaatctgtg**
- **961 gaccgaagca tacaaacggt ggtatcttgt ctgtttaatc cagagaacag actgagaaac**
- **1021 tctgttgtta ctcaagatga ctacttcaag gataaaagag tgcatcactt tagaagaagt**

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2) DNA polymerase

- *E. coli* DNA Polymerase
- *Thermus aquaticus* DNA Polymerase

  Taq 1-2.5U

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3) Buffer — Delivered as 10x, do not mix buffers with enzymes from different companies. Non ionic detergent

4) Mg^{2+} — Co-factor for the enzyme 0.5-2.5 mM

Mg concentration can affect:

primer annealing
denaturation temp for the template and the PCR products
product specificity
formation of primer dimer artifacts
enzyme activity
enzyme specificity
enzyme fidelity

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5) dNTPs (A, G, C & T)
20-200 mM

A = Adenine

G = Guanine

C = Cytosine

T = Thymine

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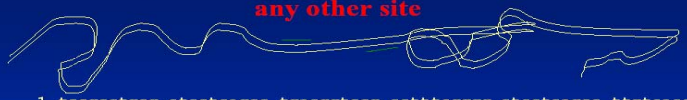
6) Primer for upper strand 7) Primer for lower strand

Ability of the primer to form a stable complex with a specific site and no duplex formation at any other site



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  61 ctcttggttt tccttttctc ttcatctttg gctcctttga ccactcgaag ccgcgcagcg
 121 ggttccagcg gacctcacag cagccccaga agtggtgcgc caagcacagc ctctgctcct
 181 cctcgagccg gtcgggaact gctgcctgcc gccatcatgt ctactgcaaa tcctgaaact
 241 ccaaactcaa ccatctccag agaggccagc acccagtcct catcagctgc aaccagccaa
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 481 aatgacgtgg atgtgcagaa gatagtagaa tcacagataa atttccatgg taaaaagctg
 541 aagctgggcc ctgcaatcag gaaacaaaat ttatgtgctt atcatgtgca gccacgtcct
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 781 tatcagatgc caccacagtg gcctgttggg gagcaaagga gctatgttgt acctccggct
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 901 gaatgttcag ttcatgaagc tactccaccc tctggaaatg gcccacaaaa gaaatctgtg
 961 gaccgaagca tacaaacggt ggtatcttgt ctgtttaatc cagagaacag actgagaaac
1021 tctgttgtta ctcaagatga ctacttcaag gataaaagag tgcatcactt tagaagaagt
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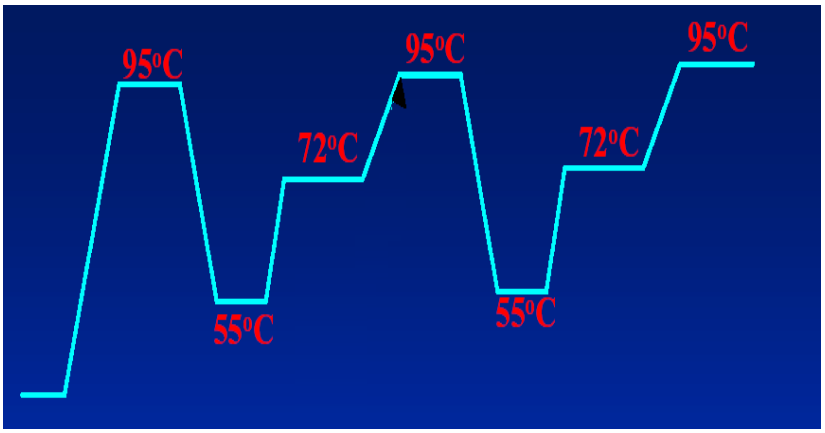
Selection of forward and reverse primer

- ATGGCACTCAGGGCAAAGGCAGAGGTGTGCATGGCAGTGCCCTG
- TACCGTGAGTCCCGTTTCCGTCTCCACACGTACCGTCACGGGAC
- GCTGTCCCTGCAAAGGGCACAGGCACTGGGCACGAGAGCCGCCC
- CGACAGGGACGTTTCCCGTGTCCGTGACCCGTGCTCTCGGCGGG
- GGGTCCCCAGGACAGTGCTGCCCTTTGAAGCCATGCCCCGGCGT
- CCCAGGGGTCCTGTCACGACGGGAAACTTCGGTACGGGGCCGCA
- CCAGGCAACAGGTGGCTGAGGCTGCTGCAGATCTGGAGGGAGCA
- GGTCCGTTGTCCACCGACTCCGACGACGTCTAGACCTCCCTCGT
- GGGTTATGAGGACCTGCACCTGGAAGTACACCAGACCTTCCAGG
- CCCAATACTCCTGGACGTGGACCTTCATGTGGTCTGGAAGGTCC
- AACTGGGGCCCATTTTCAGGTACGATTTGGGAGGAGCAGGCATG

GGCAAAGGCAGAGGTGTGCAT
TTCATGTGGTCTGGAAGGTCC

PCR Cycles



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C) Gel Electrophoresis

- Agarose – acrylamide – DGGE
- Ethidium bromide
- Buffer

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