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Paper		**Clinical mycology and parasitology**
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Q1) >Write down the life cycle of Enterobius vermicularis.

Ans:- life cycle of Enterobius vermicularis :-

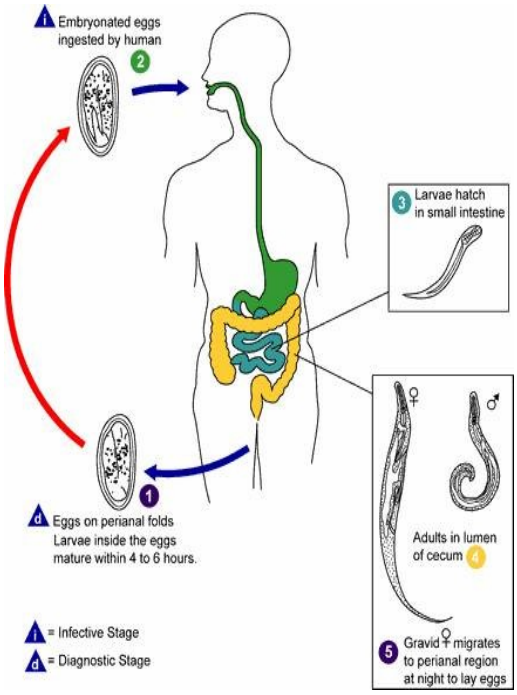
Life Cycle

*The life cycle involves the **reduviid bug** (*Triatoma,* cone-nose) as the vector and both humans and animals as reservoir hosts

*The animal reservoirs include domestic cats and dogs and wild species such as the armadillo, raccoon, and rat

*The cycle in the reduviid bug begins with ingestion of trypomastigotes in the blood of the reservoir host

*In the insect gut, they multiply and differentiate first into epimastigotes and then into trypomastigotes



*Eggs are deposited at night by the gravid females.
*Eggs are ingested via person-to-person transmission through the handling of contaminated surfaces (such as clothing, linen, curtains, and carpeting), or airbourne eggs may be inhaled and swallowed. Self-infection may also occur if eggs are transferred from to the mouth by fingers that have scratched the perianaln area.

*After ingestion, larvae hatch from the eggs in the small intestine. The adults then migrate to the colon. The life span of the adults is about two months. Adults mate in the colon, and the males die after mating.

*Gravid females migrate nocturnally to the anus and ovideposit eggs in the perianal area. The females die after laying their eggs. The time period from ingestion of infective eggs to the ovideposition of eggs by females is approximately one month.

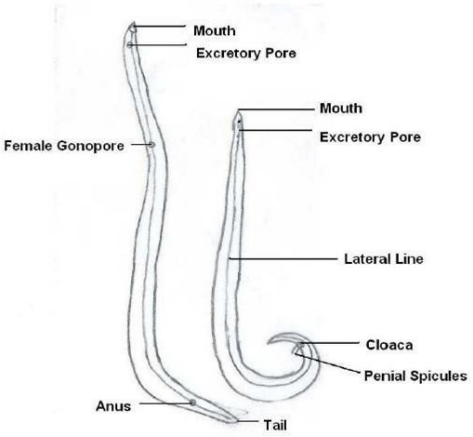
*The larvae develop and the eggs become infection within 4-6 hours. Newly hatched larvae may also migrate back into the anus, and this is known as retroinfection.

Q2) :Describe pathogenesis of Ascaris.
Ans) :

Pathogenesis:

1. Mode of transmission:

*faeco-oral route, by contaminated vegetables or water.



2. Pathogenesis:

Infection of *A. lumbricoide*s in man is known as Ascariasis. There are two phase in ascariasis.

Phase I: migrating larvae

*The migrating larvae causes pathological lesions. The severity of lesions depends upon the sensitivity of host, nutritional status of host and number of migrating larvae.
*During migration and molding through lungs, larvae may causes pneumonia with low grade fever, cough and other allergic symptoms.

Phase II: Adult worm

*Few worm in intestine produce no major symptoms and but some time give abdominal pain especially in children.
*The adult worm produce trauma in host tissue and the wandering adults may block the appendical lumen or common bile duct and even small intestine.
*Large number of adult worms affects the nutritional status of host by robbing the nutrition leading to malnutrition and growth retardation in children.
*The metabolites of living or dead worm are toxic and immunogenic.
*lumbricoides also produces various allergic toxin, which manifests fever, conjunctivitis and irritation.

Q3):Explain the transmission and life cycle of Entamoeba histolytica in detail.

Ans*Transmission

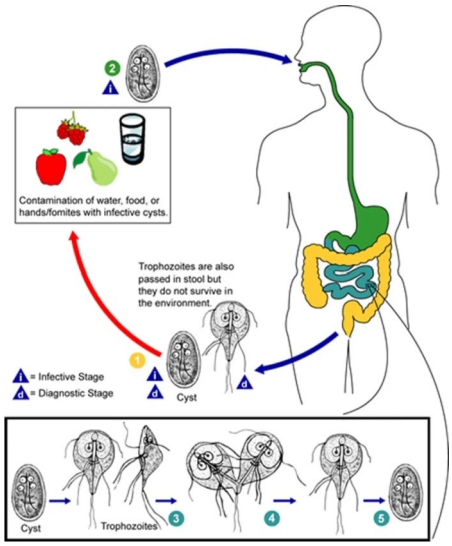
*Transmission occurs via the faecal–oral route, either directly by person-to-person contact or indirectly by eating or drinking faecally contaminated food or water.

*Sexual transmission is possible, especially in the setting of oral-anal practices (anilingus). Trophozoites if ingested **would not survive** exposure to the gastric environment.

*Life cycle of *Entamoeba histolytica*

*Infection by*Entamoeba histolytica* occurs by the ingestion of **mature quadrinucleate cysts** in fecally contaminated food, water, or hands. The quadrinucleate cyst is **resistant to the gastric environment** and passes unaltered through the stomach

*When the cyst of *E.histolytica* reaches caecum or lower part of ileum excystation occurs and an **amoeba with four nuclei emerges** and that divides by binary fission to form **eight trophozoites**.



*Trophozoites migrate to the large intestine and lodge into the submucosal tissue.

*Trophozoites grow and multiply by binary fission in the large intestine *(Trophozoite phase of the life cycle is responsible for producing characteristics lesion of amoebiasis).*

*Certain numbers of trophozoites are discharged into the lumen of the bowel and are transformed into cystic forms.

*The cysts thus formed are unable to develop in the same host and therefore necessitate a transference to another susceptible host. The **cysts are passed in the feces.**

Q4) :How will you diagnose Trypanosoma Cruzi inside a laboratory?
Ans) :*LABORATORY DIAGNOSIS
*The laboratory diagnosis of Chagas' disease is accomplished by methods which demonstrate the presence of the parasite in the blood, directly or indirectly (parasitologic methods) and through the detection of serum anti-*T.cruzi* antibodies.

*PARASITOLOGIC METHODS

*These methods can be employed to diagnose either the acute or chronic phase of disease.

*In the acute phase of Chagas' disease (first 6 weeks), methods which demonstrate the presence of trypanosomes in the bloodstream are employed. This can be accomplished through the examination of a thin peripheral blood smear under a microscope. Other variations of this method, such as preparing thick blood smears or concentrating the parasite, increase the likelihood of detection. A promising technique called Quantitative Buffy Coat (QBC method), which is widely used to diagnose infections with plasmodia, has been successfully applied to detect trypanosomes, especially in patients with very low levels of parasitemia.

*Blood culture, though an insensitive technique, is useful in isolating *T. cruzi* strains for studies of biochemical and immunochemistry typing. When used for diagnostic purposes, it detects approximately 50% of cases in the chronic disease.

*Other diagnostic methods such as animal innoculation (mice, guinea pigs) and in vitro cell culture are seldom employed.

*SEROLOGIC DIAGNOSIS

*Serologic tests are widely used to: screen suitable blood donors, as markers to monitor therapy, to confirm or exclude clinical suspicion of Chagas' disease, in epidemiologic studies and to screen infected industry workers. Serologic tests give only an estimate of probability of disease and their final interpretation is influenced by numerous factors, like the sensitivity and specificity of the test and the prevalence of Chagas' disease in the population being tested.

*Stolf, in 1992, described the ideal antigen as the one which would be present in all strains from different endemic areas, highly immunogenic, not present in other pathogenic microorganisms, stable and easy to be obtained for use in serologic tests (19). Next, we will make a discussion of the different tests employed in the serologic diagnosis of Chagas disease. It is worth remembering that, for the different serologic tests, there is an overlap in the reactivity curves of infected and non-infected individuals. By changing the cut-off point of the test, one can achieve maximum sensitivity

or specificity. The point where the curves intersect defines the values of sensitivity and specificity (18).

1) Complement fixation

*Introduced by Guerreiro and Machado in 1913, the complement fixation test has only historic value, though it is still used by some to screen blood donors and for diagnostic purposes. The technical complexity of this test, which requires the daily standardization of its components - antigen, hemolytic system and complement - interferes in its reproducibility. Its low level of sensitivity and specificity have also contributed to the low popularity of this test.

2) Precipitation test

*The different variations of this test, though highly specific, have poor sensitivity.

*It is mainly used in the study of the different antigenic components of *T. cruzi*. Counterimmunolectrophoresis has been the preferred method for diagnosis and seroepidemiologic studies. Breniere, using serum with the component 5 of *T. cruzi* obtained from rabbits, obtained a sensitivity of 85% and specificity of 100%, even when testing sera from patients with leishmaniasis *(1). Requejo et al. recently standardized and tested the DIG-ELISA assay, which is an association between the immunoassay and the agar diffusion test. The authors found the test to have high sensitivity and specificity and recommended it for screening and for seroepidemiologic surveys. Multicenter studies are necessary to validate this test (18).

3) Agglutination tests

*This test, with its variants, has been widely used in the diagnosis of Chagas disease.

3a) Direct Agglutination

*Several authors have described the use of this test, comparing it to immunofluorescence. Vattuone and Yanowsky, employing a suspension of epimastigote forms of *T. cruzi*, treated with enzymes and fixed with formalin, obtained good sensitivity in the detection of antibodies in the acute phase of Chagas' disease (20). Harith et al. standardized the microagglutination test, which employed epimastigote forms of *T. cruzi*, treated with tripsin and stained with Coomasie-Blue. Treatment of the 2 sera with mercaptoethanol was critical for the detection of specific antibodies. The sensitivity and specificity of the test were very high. A drawback of the agglutination test is the large amount of parasites necessary to prepare the antigenic suspension (12).

3b) Hemagglutination

*This test is widely used for diagnosis, screening and seroepidemiologic studies. In the test, erythrocytes from mammals or fowl are treated with formalin and sensitized with antigenic components, partially or completely soluble. The product, lyophilized or in suspension, has excellent stability in adverse temperature conditions (5). The test, either quantitative or qualitative, is performed in microtiter plates (13), with the use of different antigenic extracts. The best results are obtained with alkaline and sonicated extracts. The treatment of the 2 sera with mercaptoethanol increases the specificity of the test. The hemagglutination test was studied by Neal and Miles, who used the Y strain of *T. cruzi*, grown in LIT media. They tested diluted blood, collected in paper filter, from different populations from Latin American countries (16).

*No regional differences in antibody response were observed. Because of its simplicity and low cost, the hemagglutination test is recommended for screening blood donors. In Brazil, well standardized kits from different manufacturers are commercially available.

3c) Latex agglutination

*Though a very promising test, the latex agglutination test was released in Brazil without proper standardization. False-positive and false-negative results, coupled with poor reproducibility, led to the discontinuation of this test by the manufacturer. With the current possibility of creating covalent bonds between *T. cruzi* antigens and free radicals present in the latex particles, new perspectives for obtaining a new test with good stability, low cost, ease of use and reliable sensitivity and specificity have appeared.

4) Immunofluorescence

*The indirect immunofluorescence test is usually performed with epimastigote forms of the Y strain of T cruzi, obtained from cultures of the parasite in LIT medium. The formalin treated trypanosomes are then fixed in glass slides and incubated in diluted serum for 30 minutes at 37°C. After proper washings, the slides are incubated with fluorescent conjugate (sheep or goat serum anti-human IgG or IgM, labeled with fluorescein isothiocyanate). After another incubation and washings, the slide is read with the use of a fluorescence microscope (6). The immunofluorescence test for the detection of IgG anti-*T. cruzi* antibodies is considered to be the gold standard in the serologic diagnosis of Chagas' disease (3).

*Antigenic variation has been observed in the different parasitic stages of *T. cruzi*. Camargo found higher antibody titers with the tripomastigote than with epimastigote forms (2). Primavera et al. compared epimastigote with tripomastigote forms of *T. cruzi* and concluded that the amastigotes are more reactive for the detection of IgA antibodies, especially in patients with the digestive form of the disease (17). Levy standardized the in situ immunofluorescence test with tripomastigote forms, in order to detect membrane epitopes and to follow persistent infections. The test has effectively substituted the complement lysis reaction (15).

Different factors may affect the results of the immunofluorescence test: the quality of the optical equipment and of the antigens, the definition of what constitutes a positive test. These factors should be taken into account and the test should be rigorously standardized in order to obtain reliable results (10).

5) Enzyme immunoassay

*In 1975, Ferreira standardized the immunoperoxidase test, using formalin fixed epimastigote forms of *T. cruzi*, formalin fixed in glass slides as antigen and enzymatic conjugate (sheep or goat serum conjugated to peroxidase).

*The use of the enzyme linked immunosorbent assay (ELISA) for the diagnosis of Chagas disease was described by Voller et al. The test was standardized in microtiter plates, adsorbed with soluble *T. cruzi* antigens. After incubation with serum and enzymatic conjugate, color develops in the supernate after addition of substrate and hydrogen donors. The intensity of color development is measured by spectrophotometry (21).

*Due to its good sensitivity and specificity and because it is automated, the standardized enzyme immunoassay has opened new perspectives in the serologic diagnosis of Chagas' disease. The

possibility of employing very specific antigenic components, obtained through physicochemical or recombinant methods, is under investigation by several scientists around the world (4). Like the hemagglutination test, well standardized reagents are commercially available and are especially useful for screening blood donors (9).

*METHODS FOR DETECTING ANTIGEN IN BODY FLUIDS

*The detection of *T. cruzi* antigens in body fluids is of great importance in the confirmation of infection, especially when the serologic tests or parasitologic methods are negative.

*Tests based on precipitation, counterimmunoelectrophoresis or immunodifusion, though highly specific, have low sensitivity, becoming useful in the confirmation and prognosis of disease. The enzyme immunoassay, capture or double sandwich, has proved useful in the detection of *T. *cruzi* antigens in blood or urine, confirming a suspected diagnosis and monitoring the efficacy of anti-Chagas therapy. Some authors believe that the detection o *T. cruzi* antigens in urine is useful in the diagnosis of congenital Chagas' disease. Katzin et al.

Q5) : Enlist Leishmania species names. Summarize the clinical findings of all species of Leishmania

Ans) :Enlist leishmania :

*Important species :

1)Leismania donovani.
2) Leismania tropica.
3) Leismania Mexicana.
4) Leismania braziiensis.
5) Leismania major.
6) Leismania guyansis.
7) Leismania lainsoni etc.

*clinical findings Leishmania species

*Clinical finding of Leishmania donovani :

Symptoms begin with intermittent fever,weakness,and weight loss .

*Splenomegaly :

Massive enlargment of the spleen is characteristic.

*Hyperpigmentation:of the skin is seen in light-skinned patient (Kala-azar means black sickness)

*The course of the disease runs for months to years.

*Initially,patients feel reasonably well despite persistent fever.

*As anemia ,leukopenia,and thrombocytopenia become more profound ,weakness,infection and gastrointestinal beleeding occur.

*Untreated severe disease is nearly always fatal as a result of secondary infection.

*Clinical finding of Leishmania Tropica ,Mexicana and Brazillensis :

*Clinical finding of thes three species are same :

*The initial lesion of cutaneous leishmanasis is a red papule at the bite site, usually on an exposed extremity.

*This enlarges slowly to from multiple satellite nodules that coalesce and ulcerate.

*There is usually a single lesion that heals spontanously in patients with a competten immune system.

However, in certain individuals, if cell-mediated immunity does not develop, the lesions can spread to involve large areas of skin and contain enormous numbers of organisms

* Mucocutaneous leishmaniasis begins with a papule at the bite site, but then metastatic lesions form, usually at the mucocutaneous junction of the nose and mouth
* Ulcerating lesions destroy nasal cartilage but not adjacent bone
* These lesions heal slowly