Opportunistic protozoan parasites of man with special reference to 
*Pneumocystis carinii* and *Cryptosporidium parvum*

**PNEUMOCYSTIS CARINII**

*Pneumocystis carinii* is a pathogen of uncertain taxonomic status. The capability to produce spores and cysts, places *Pneumocystis* within either of the two protistan groups, fungi or protozoa. *Pneumocystis carinii* causes **interstitial plasma cell pneumonia** occurring almost exclusively in infants, children and immuno-compromised adults.

P. carinii was discovered in 1909 by Charles Chagas', who mistakenly described the organism as a trypanosome. Delanoe and Delanoe (1912) described the organism in rats and they were the first to identify the organism as a distinct aetiological agent. They named the organism as *Pneumocystis carinii* in the honour of Dr. Carinii, another early worker in the field. The parasite was later implicated as the aetiological agent of interstitial plasma cell pneumonia by Van der Meet and Brug (1942). Since then, there is an increased report of *P. carinii* infection, especially associated with the acquired immuno-deficiency syndrome (AIDS).

**MORPHOLOGY**

Three morphologically distinct stages are recognized: trophozoite or trophic form, pre-cyst and cyst.

**Trophozoite:** Trophozoites or trophic forms are always present in large numbers. These are small, (size: 1 to 5 μm), pleomorphic and usually occur in clusters. They are readily stained by Giemsa or acridine orange. In Giemsa stain, the nucleus is stained red and cytoplasm blue. The electron micrograph of a trophozoite shows a nucleus, mitochondria, a few other organelles and filopodia (tubular cytoplasmic extension). Trophozoites multiply asexually by binary fission.

**Pre-cyst:** It is an intermediate stage between the trophozoite and cyst. It is oval in shape and measures 4-6 μm in diameter. It lacks pseudopodia. Typically, it is surrounded by a thick limiting layer or cell wall. Periodic-acid schiff (PAS) and silver methenamine stain clearly the cell wall. It is difficult to demonstrate this stage in the tissue.
Cyst: It is spherical. 5-8 μm in diameter and is surrounded by a 70-140 nm thin cell wall. A mature cyst consists up to eight daughter forms or extra-cystic bodies called sporozoites. The sporozoites are spherical, crescent-shaped, measure 1-1.5 μm in diameter. Each sporozoite consists of a nucleus, mitochondria, ribosomes and endoplasmic reticulum.

LIFE CYCLE

P. carinii occurs as a saprophyte in the human and in a variety of mammals in nature. It is an extra-cellular parasite which inhabits the pulmonary alveoli of the lungs.

The life cycle of P. carinii is still incompletely understood. It is based on the morphologic studies of the lung sections obtained from the rat and on the parasites grown in culture.

Fig - Life cycle of Pneumocystis carinii

In man, the life cycle of Pneumocystis is extra-cellular and occurs in the lung alveoli, Intracellular stage has not been described. Trophozoites may develop into cysts either during sexual or asexual phase. The existence of a sexual phase is probable, and no evidence exists for an intracellular phase in the parasite life cycle.
The trophozoites multiply either by binary fission or endogeny. Trophozoite first develops into a single-nucleated structure called pre-cyst. The latter develops into a cyst by a process similar to sporogony. In this process, a single nucleus divides by meiosis into four haploid nuclei. These nuclei undergo post-meiotic mitosis to produce eight daughter nuclei. A membrane surrounds each daughter nucleus in the late phase during which pre-cyst develops into a cyst. Each cyst contains eight sporozoites or daughter cells. The mature cyst on rupture releases these daughter cells.

The specific factors that cause excystation of cysts or encystation of trophozoites are not known. The mature cyst with eight intra-cystic bodies is believed to be the infective form of the parasite responsible for transmission of infection from man to man. Congenital infection may also be caused by trophozoites.

**PATHOGENESIS AND CLINICAL MANIFESTATION**

*P. carinii* inhabits the lung alveoli. In man and other animal species, it causes disease by attaching itself to Type-1 alveolar epithelial cells. The specific factors involved in the process of attachment have not been recognised. The organism lives on the lining layer of the alveoli. It has been demonstrated that surface of the organism and alveoli epithelial cells are closely apposed to each other without any fusion of the cell membrane or changes in the intra-membranous particles.

The lungs, in pneumocystosis in humans are consolidated and appear reddish grey at necropsy. Histological studies of the lung shows the alveoli to be completely filled with pink frothy honey combed materials and a large number of *P. carinii*. Infected infants show extensive plasma cell infiltration of the alveolar space but immunosuppressed children and adults do not show these changes, instead they show only intestinal thickening.

*P. carinii* rarely causes symptomatic disease in healthy individuals. It causes diffuse pneumonia only in immunocompromised hosts. In these hosts, the organism as well as the disease always remain localised to the lungs. The severity of clinical manifestations, to some extent, depend on the age of the host as follows:

**Epidemic or infantile pneumoeystosis**:

This occurs in premature, malnourished and debilitated infants. Incubation period varies from 1 to 2 months. The symptoms of *P. carinii* pneumonia (PCP) include dyspnea, non-productive cough and fever. Chest radiography demonstrates bilateral
infiltrates. In 1 to 4 weeks, respiratory manifestations become well marked. The condition may last 4 to 6 weeks and shows a mortality of 25 to 50 percent.

**Sporadic pneumocystosis:**

P. carinii produces sporadic pneumocystosis in immunocompromised children and adults with acquired immunodeficiency syndrome (AIDS), or in persons receiving immunosuppressive therapy for the treatment of malignant conditions, organ transplantation, etc. The clinical manifestations are similar to that of epidemic pneumocystosis except that the onset of sporadic pneumocystosis is abrupt. Course of the disease is rapid and begins with fever, tachypnoea and respiratory distress. Extrapulmonary lesions occur in a minority (<3%) of patients, involving most frequently the lymph nodes, spleen, liver, and bone marrow. Typically, in untreated PCP increasing pulmonary involvement leads to death. The condition has a high mortality of 90-100 percent. The pathogenesis of pneumocystosis in AIDS and other immunodeficiency disease still remains unclear. It may be due to simple reactivation of latent infection or additional exposure to exogenous sources of the organism.

**DIAGNOSIS**

Clinical manifestations of P. carinii infection are nonspecific and can be observed in many different infectious and non-infectious conditions. Hence, the diagnosis of the condition depends mainly on the laboratory diagnosis.

**Pathogenic diagnosis**

The specific diagnosis is based on identification of P. carinii in bronchopulmonary secretions obtained as induced sputum or bronchoalveolar lavage material. In situations where these two techniques cannot be used, transbronchial biopsy or open lung biopsy may prove necessary. Microscopic identification of P. carinii trophozoites and cysts is performed with stains that demonstrate either the nuclei of trophozoites and intracystic stages (such as Giemsa) or the cyst walls (such as the silver stains).

**Specimen:** The methods of collection of specimens are essentially invasive procedures. These include:

1) Open lung biopsy.

2) Percutaneous needle biopsy or needle aspiration of the lung.
3) Bronchoalveolar biopsy and bronchoalveolar lavage. Fibre optic bronchoscopy with broncho-alveolar lavage and or transbronchial biopsy is the most commonly used procedure.

4) Inhalation of a saline mist. Frequently, in AIDS patients the organisms can be demonstrated it’s the sputum induced by inhalation of a saline mist.

**Serodiagnosis**

The indirect fluorescent antibody (IFA), complement fixation test (CFT) and enzyme-linked immunosorbent assay (ELISA) are being used for the demonstration of serum antibodies to P. carinii. These tests use whole parasites or soluble extracts of parasites as antigens.

The counter-current immunoelectrophoresis (CIEP-) and latex agglutination test (LAT) also are frequently used for the detection of antigen in the serum to diagnose the refection.

**Chest x-ray** : In some cases, it shows bilateral diffuse infiltrates originating from the perihilar regions of the lungs.

**EPIDEMIOLOGY**

P. carinii is widespread in nature. It occurs in humans and many species of animals (rats, rabbits, mice, sheep, goats, dogs, guineapigs, horses, chimpanzees and monkeys). It has been reported from India, China, Japan, Iran, Israel, South America, Congo, Malaysia, Australia, New Zealand, USA, Canada, Brazil and erstwhile USSR.

**Reservoir of infection**

Infected man is the main source and reservoir of infection. Mature cyst containing eight intracystic bodies appear to be the infective stage.

**Transmission:**

P. carinii occurs in following ways:

1) Man-to-man transmission :
   It occurs by inhalation of mature cysts. Air-borne infection seems to be the major mode of transmission.

2) Congenital transmission occurs rare. Milk-borne transmission occurs less frequent. The populations at risk for P. carinii infection include:

   1) Premature malnourished infants;
   2) Children with primary immunodeficiency.
3) Patients receiving immnosuppressive drugs such as corticosteroids for treatment of malignancies, organ transplantations and other diseases; and

4) Protein malnutrition.

TREATMENT AND CONTROL

Treatment of P. carinii infection is broadly based on the supportive therapy and specific chemotherapy. It consists of aeration by high concentration of oxygen, blood transfusion and good nursing care. Pentamidine, trimethoprim and sulfamethoxazole are the drugs currently available for the treatment of P. carinii infection.

Treatment of P. carinii infection ill patients with AIDS relatively is complex. It requires treatment with higher dose and for a longer duration.

The control measures include respiratory isolation of high-risk cases susceptible to infection, and chemoprophylaxis by trimethoprim (5 mg/kg per day) and sulphamethoxazole (25 mg/kg per day).
CRYPTOSPORIDIUM

Cryptosporidium is a coccidian, which causes infection of the intestinal tract, particularly the small intestine. Once thought to be a non-pathogenic, this coccidian has been recognised recently as a cause of diarrhoea in man. Numerous species of Cryptosporidium are known to affect amphibians, fish, birds and mammals, but Cryptosporidium parvum is the only species known to cause infection in man.

The parasite was first described by Tyzzer in 1907, in the peptic glands of a laboratory mouse. He suggested its present name Cryptosporidium. When first described, the organism was thought to be non-pathogenic and only 15 reports of Cryptosporidium infections in animals were recorded prior to 1975. The first description of infection in man was reported in a three year old healthy girl in USA as late as 1976. Since then, the infection has been frequently diagnosed in patients with acquired immune deficiency syndrome (AIDS) and others receiving immuno-suppressive therapy.

MORPHOLOGY

The parasite shows six distinct morphological forms during its life cycle: oocyst, sporozoite, trophozoite, meront, microgamont, and macrogamont.

Oocyst: Cryptosporidium oocyst is the smallest coccidian known to cause infection in man. It is colourless, spherical to oval and measures 4.5 to 6 μm in diameter (Fig1). It does not stain with iodine and is acid-fast. The cyst is surrounded by a 50nm thin cyst wall. The latter consists of an electroluscent middle zone surrounded by two electron dense layers. Each oocyst contains up to four slender bow-shaped sporozoites and many small granules. The oocysts are excreted in small numbers in the faeces. The number of oocysts excreted bears no relationship with the severity of illness. The oocysts which sporulate inside the host are of two types: Thick-walled and Thin-walled. The thick-walled sporulating oocysts are infectious to susceptible human hosts, whereas thin-walled oocysts always cause autoinfection ill the same host only.

Sporozoite: The sporozoite is slender, crescent-shaped and measures 1.5 to 1.75 μm in diameter. The anterior end is pointed but the posterior end that contains a prominent nucleus is rounded. The sporozoites numbering four remain always parallel to each other within an oocyst and are released only after partial digestion of the oocyst.
**Trophozoite:** It is the intracellular transitional form of the parasite. It is round or oval and measures 2 to 2.5 μm in diameter. Each trophozoite consists of a large nucleus (1 to 1.3μm in diameter) with or without a conspicuous nucleolus. Unlike in the sporozoites and merozoites, the apical complex is not present in the trophozoite.

**Meront:** It is of two types: type I and type II meronts. These two forms morphologically are indistinguishable from each other. They are crescent-shaped and measure 1 to 5μm in diameter showing rounded anterior and posterior ends (Fig2.).

**Microgamont:** Microgamonts are the male sexual forms. These are wedge-shaped, 0.2 to 0.7 μm in length and are covered by a double layered membrane. Each microgamont contains a large compact nucleus and a polar ring. A single microgamont gives 1 to 4 microgametocytes.

**Macrogamont:** Macrogamonts are the female sexual forms. These are spherical, measure 3 to 5 μm and are covered by a double layer membrane. Each macrogamont consists of a single large nucleus and endoplasmic reticulum. The old macrogamonts characteristically contain dense polysaccharide granules.

**LIFE CYCLE**

Cryptosporidium completes its life cycle through the stages of asexual generation (schizogony) and sexual generation (gametogony) in a single host (Fig. II-X-3). All these stages of the parasite are truly intracellular and are being surrounded by a host cell membrane, which is extra-cytoplasmic. Man acquires infection on ingestion of food or drink contaminated with the faeces, containing sporulated thick-walled oocysts of Cryptosporidium. On ingestion, the infective sporozoites after being released from the oocysts in the small intestine, invade the epithelial cells in which they parasitise. Inside the epithelial cells, the sporozoites subsequently differentiate into intracellular trophozoites. These trophozoites multiply asexually by nuclear division to produce two types of meronts, type I and type II (sexual generation or schizogony). Each type I meront produces six to eight type I merozoites, which develop into type II meronts. These in turn produce four merozoites each, which are known as type II merozoites. Some of the type II merozoites invade new host cells and initiate sexual replication (gametogony). Inside the host cells, they differentiate either into female (macrogamont) or male (microgametocyte) forms. Each microgametocyte produces 16
sperm-like microgametes, which fertilize the macrogamonts resulting in the formation of oocysts (zygote). Four sporozoites are formed inside each oocyst in situ. The sporulating oocysts are of two types, thin or thick-walled. The thin-walled oocysts release the sporozoites inside the lumen of the intestine and cause auto-infection in the same host by repeating the cycle of schizogony and gametogony. The thick-walled oocysts excreted in the faeces are infective to other human hosts. The cysts under favourable conditions remain viable and infectious relatively for a long time. These cysts, when taken up by other susceptible human hosts, cause infection and the cycle are repeated.

**PATHOGENESIS AND SYMPTOMS**

The parasite inhabits the intestinal tract. It is found attached to the surface epithelial cells of villi or crypts of the small intestine. The organisms have also been found but less frequently in the stomach, appendix, colon, rectum and even pulmonary tree of the small intestine. Infection begins with the firm attachment of Cryptosporidium to the mucosal surface of the intestine followed by invasion of epithelial cells. The specific mechanism by which the parasite causes illness in man is not known. The cholera-like voluminous watery diarrhea, seen in Cryptosporidium infection in the immuno-suppressed hosts including, those with the AIDS is possibly caused by a toxin. Reduction in the mucosal surface and decrease in the mucosal enzymes frequently seen in this condition also may contribute to pathophysiology of osmotic diarrhea by lowering the absorbing capacity of the small intestine.

Bacterial fermentation of sugars and fatty acids of the unabsorbed nutrients present in the lumen of the intestine, cause offensive and foul smelling stool, characteristically seen in Cryposporidium diarrhea. Cryptosporidium is found attached to tile brush border of the small intestine particularly the jejunum. In the immunocompromised hosts, the parasites are also found in the uncommon sites such as pharynx, oesophagus, stomach, gall bladder, ileum, colon or rectum. They appear as small, basophilic round structures, staining readily with Giemsa and haematoxylin eosin stain. They are arranged in a row or clusters, along the border of the epithelial cells alone or in association with other intestinal parasites such as Giardia intestinalis.

Blunting and loss of villi, lengthening of the crypts and infiltration of lamina propria by lymphocytes, polymorphonuclear cells and plasma cell are tile pathological changes of the intestinal tract, in cryptosporidiosis.
Incubation period ranges from 2 to 14 days. The prepatent period (time between infection and oocysts shedding) ranges from 5 to 21 days in man. The patent period (duration of oocysts shedding) may last for more than 30 days in an immunocompetent host. The clinical manifestations of Cryptosporidium infection vary depending upon the immune status of the host.

**Cryptosporidiosis in immunocompetent host:**

It is a mild infection in normally healthy patients. The duration of symptoms is relatively short and recovery is complete. The condition rarely is fatal. Flue-like illness with watery diarrhoea, malaise, nausea, fever and crampy abdominal pain are the characteristic features of the condition in immuno-competent hosts. Diarrhoea is foul smelling with 2 to 10 motions per day, beginning on the first or second day of the illness. In some other cases, it is accompanied by prostration and weight loss even up to 10%. The oocysts may continue to be excreted in the faeces of the cases, twice as long, an average, as they had diarrhoea.

**Cryptosporidiosis in immunocompromised host:**

Cryptosporidiosis is a serious condition in patients with depressed immunity due to AIDS, congenital hypogammaglobulinaemia or severe combined immunodeficiency syndrome, patients receiving immunosuppressive drugs such as corticosteroids and cyclophosphamide, and persons with severe malnutrition. Cryptosporidium produces a cholera-like watery or mucus diarrhea in these groups of patients. Diarrhea relatively is more severe, profuse and watery with as many as 70 stools per day and loss of body fluids even up to 17 litres/day. Diarrhoeic stool may contain mucus but rarely blood or leucocytes. The main duration of diarrhea is 20 weeks with variability between 1 to 48 weeks. The prolonged diarrhea may lead to significant weight loss. Low grade fever (39~C), nausea, vomiting and crampy abdominal pain are other but less frequent symptoms of the condition. Occasionally, non-specific symptoms such as malaise, myalgia and headache may be present. In some of the immuno-suppressed patients, Cryptosporidium affects the entire gastrointestinal tract including the gall bladder, bile duct and pancreas and even pharynx and bronchial tree. Cryptosporidium infection is of long duration and death is the frequent outcome of the infection in AIDS and AIDS related diseases. A few cases of spontaneous recovery have also been reported. The patients of reversible immune
deficiencies show recovery from the infection when the cause of immune suppression is removed.

**DIAGNOSIS**

Clinical diagnosis of cryptosporidiosis is difficult as the condition clinically mimics giardiasis, isosporiasis and a few other infections caused by enteropathogens. The absence of blood, pus cells, Charcot-Leydencrystals in the faeces may rule out amoebiasis, isosporiasis and bacillary dysentery and suggest the possibility of cryptosporidiosis. The laboratory diagnosis of cryptosporidiosis is aided by parasitic and serologic methods.

**Pathogenic diagnosis**

The specific diagnosis of the condition is made by identification of oocysts in the faeces and less frequently the non-faecal specimens by microscopy and direct fluorescent antibody test.

1) **Microscopy examination:** The microscopic examination of direct faecal smear (wet smear and stained smear preparations) is adequate to demonstration of oocysts in the acute cases shedding a large number of oocysts in their faeces.

2) **Acid-fast staining methods:** Acid-fast staining methods, with or without stool concentration, are most frequently used in clinical laboratories. A large number of staining procedures have been employed to demonstrate acid-fast oocysts in the faecal smears. The hot Ziehl-Neelsen carbol fuschin staining method, a modification of acid-fast staining, is most frequently used to detect red-stained oocysts in the faeces. Red-stained oocysts also can be demonstrated in the sputum, bronchial washings and duodenal or jejunal aspirations by acid-fast staining method.

3) **Direct fluorescent antibody examination:** It is a specific method for accurate identification of Ctyptosporidium in the faeces. It is particularly useful to diagnose those doubtful cases, which are negative by faecal smear examinations.

**Histopathological diagnosis**

This is based on the demonstration of the developmental stages of the parasite (cysts 2 to 5 um in diameter, arranged in single or clusters in the intestinal mucosa) in the biopsy specimen from the jejunum and occasionally from the rectum. The invasiveness of the procedure and need for immediate processing of the specimen to avoid autolysis are the inherent disadvantages of the method.
Serodiagnosis

The indirect fluorescent antibody (IFA) and enzymelinked immunosorbent assay (ELISA), using purified oocysts as antigens have been used to detect circulating antibodies specific to Cryptosporidium in the serum. These antibodies appear in about six to eight weeks after onset of the infection. At the moment, these tests are carried out only in few laboratories to diagnose cryptosporidiosis.

EPIDEMIOLOGY

Cryptosporidium infection in the immunocompetent hosts has been described in more than 26 countries. The condition is worldwide with a prevalence of 0.6-20 percent in western countries and 4-20 percent in developing countries. In China, the condition has been described from 19 provinces with a vary prevalence.

Reservoir, source of infection

Man is the key reservoir of infection. Livestock’s such as cattle and pet animals (cat, dog) are other reservoirs. Human and animal faeces containing thick-walled oocysts are the important sources of infection.

Transmission: Cryptosporidium infection can be transmitted to man in the following ways:

1) **Person-to-person transmission**: This infection takes place by faecal-oral route through ingestion of sporulated thick-walled oocysts excreted in the human faeces, by drinking contaminated water. Rarely, it may be acquired by ingestion of milk or food contaminated with oocysts.

2) **Zoonotic transmission**: The infection is transmitted from the live stock, cattle or pet animals (cat, dog) either directly by ingestion of the oocysts derived from the faeces of these animals or indirectly by close contact with these animals.

3) **Auto-infection**: This is caused by sporozoites released from the thin walled oocysts inside lumen of the intestine. It is primarily responsible for persistence of infection in the infected host.

4) **Other mode of infection**

Rarely, the injection can be transmitted by aerosols, sexual contact and possibly by accidental laboratory infection. Children between 1 to 5 years of age are at greater risk to the infection. Cryptosporidiosis has been de tested more frequently in the urban children. In the rural areas where breast feeding is more common, the infection has been detected less frequently in the children below 1 year of age. The disease, which
appears on increase in the last decade, tends to be more common during the warm rainy and humid months of the year. *Cryptosporidium* oocysts show extreme resistance to the destruction by level of tree chlorine present in potable water.

**PREVENTION AND CONTROL**

Cryptosporidium infection in the immunocompetent hosts is self-limiting and requires supportive treatment to prevent dehydration. Infections in the immunosuppressed hosts with severe diarrhoea and symptoms of malabsorption require supportive therapy with replacement of fluid, electrolytes and nutrients. Antidiarrhoeal agents are of no value. For persons with AIDS, anti-retroviral therapy, which improves immune status, will also decrease or eliminate infection. Paromomycin is approved for treatment. The reduction or elimination of oocysts from the environment forms the mainstay of control of cryptosporidiosis but is difficult.

Freezing and heating at 65°C for 30 minutes kill all the oocysts. The care to avoid contamination of food and water with faecal oocysts prevent transmission of infection to man. Hand washing, use of gloves and improved personal hygiene will minimise risk of acquiring the infection in a hospital.