Introduction
1. INTRODUCTION

Echinococcosis in both humans and animals is a disease known since ancient times, caused by the small taeniid tapeworms of the genus *Echinococcus* (1). The infection is worldwide in distribution and is being increasingly recognized as zoonoses of public health importance in many countries of the world. The genus *Echinococcus* contains four well-recognized species namely *Echinococcus granulosus* causing cystic echinococcosis (CE), *Echinococcus multilocularis* causing alveolar echinococcosis (AE) and *Echinococcus oligarthrus* and *Echinococcus vogeli*, both causing polycystic echinococcosis (PE).

In India, *E. granulosus* is the most widespread species of medical importance that cause CE in humans (2) and also in farm animals (3). So far only few reports of *E. multilocularis* causing AE (4, 5, 6) have been reported from India. *E. oligarthrus* and *E. vogeli* is yet to be documented from India.

Cystic echinococcosis in humans is one of the three most important zoonotic diseases next only to rabies and brucellosis (7). The condition is present particularly in sheep rearing areas of the world. Although, India is not a sheep rearing country, the occurrence of the disease has been reported extensively throughout the country. CE in humans and in farm animals pose a major problem in India even at this millennium due to its difficulty at the level of diagnosis, treatment and overall containment as a result of which it has become public health importance.

Man is an accidental host and acquires the infection from dogs harbouring the adult worm. CE in humans is acquired by coming in contact with infected dogs harbouring adult *E. granulosus* in their intestine. The eggs of the parasite are the source of infection especially those present on perianal hair, muzzle and paws. The infection is transmitted orally by ingestion of these eggs from the hands, food or water (1). Infection acquired in childhood due to their close association with dogs, later manifests itself in adult after many years of infection. This has been well documented in the Indian studies with respect to abdominal hydatidosis and the age
group falls between 30-39 years, the incidence being noted to be rare in extreme age groups (8, 9).

The CE is widely distributed in farm animals throughout India in different states (10) and human infections occur in most of those areas. Life cycle of *E. granulosus* is maintained by two different cycles namely domestic or pastoral cycle which involves domestic dog as the definitive host and sheep, goat, cows and pig as domestic intermediate host and sylvatic cycle where the cycle is maintained between wolves and wild intermediate hosts like deer, elk and other animals. The sylvatic cycle produces accidental infections in humans whereas as domestic cycle leads to higher endemicity in the human population at risk and the infection causes higher morbidity and mortality.

### 1.1 The parasite

The adult *E. granulosus* resides in the intestine of dog. They have a small pyriform scolex, short neck and strobila always containing three segments and occasionally four segments. The adults show intraspecies variations in their morphology and developmental stage when it follows the goat-dog or buffalo-dog cycles (11). The adult worms also show differences in their morphology depending on the sources of the protoscolecites. Irshadullah et al. (2) have documented morphological variations in buffalo strains to other strains. These intraspecies variations are considered as subspecies. *E. granulosus granulosus* and *E. granulosus equines* are the examples of two morphological variations of sheep and horse origin respectively. In addition to this, genetic variations of *E. granulosus* collected from different intermediate hosts have been increasingly recognized by employing DNA analysis, southern blot hybridization. A number of strain variations in *E. granulosus* isolates from different parts of the world have been well documented (12). In India, the strain variation of *E. granulosus* of buffalo-dog origin has been reported by Singh et al. (13) and also by Chataopadhaya (14).
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Barring these reports of strain variations no reports however are documented from India on genetic variations of the parasite.

1.2 Pathogenesis and pathology

The presence of unilocular hydatid cyst in a tissue or organ is the main pathology of the CE in humans. Depending on the mode of development, the CE is of two types: primary cystic echinococcosis and secondary cystic echinococcosis. Hydatid cyst is primarily responsible for the pathogenesis of the disease.

Primary cystic echinococcosis occurs after per-oral infection with *E. granulosus* eggs, which subsequently gives rise to hydatid cysts in different parts of the body. In primary echinococcosis, the hydatid cysts can be found in any organ. Virtually, each and every organ in the human body can be affected by the cysts. Most commonly these cysts are found in the liver followed by lung and other organs. In 80% of cases cysts are found only in one organ. In rest cysts can be found in multiple organs or sites (9, 15, 16). The hydatid cysts cause symptoms of the infection mostly due to mechanical pressure at the site of their lodgement. The morphological characters of the cysts are determined by the resistance offered by the affected sites. The firm consistency of the liver exerts a restraining influence on the expansion of the cysts leading to the cysts which are relatively small. In contrary, the cysts expand rapidly and therefore become large in the soft parenchymal tissues of the lungs. In the bones, the cysts can not expand, hence assumes a bizarre shape (9, 15, 16).

The hydatid cysts in the liver, lung and other organs evoke a foreign body type reaction in the surrounding tissues. These neither infiltrate nor invade the tissues. The growing cyst is surrounded by three layers. The outer layer also known as pericyst which consists of only fibrous tissues. It does not form any organic part of the cyst. The middle layer contains numerous fibroblasts, eosinophils and newly formed blood vessels. The third inner layer consists of radially arranged giant cells and eosinophils. The growth rate of cysts is highly variable. On an average the cysts
grow at a rate of approximately 1-1.5 cm per year and may depend on strain differences (17). During the natural course of infection, the fate of the hydatid cysts is variable. Some cysts may grow to a certain size and then continue to remain without producing any pathological change for many years. Other cysts may rupture spontaneously or collapse and disappear completely (17).

Secondary echinococcosis occurs by rupture of primary hydatid cysts by trauma or during surgery. In this condition, the protoscoleces are carried by blood circulation to different parts of the body at which these develop into secondary hydatid cysts. Sudden release of a large volume of fluid antigen causes anaphylactic reaction, shock or even death that are most serious complication of CE (18).

The presence of some trace elements during development of endogenous daughter (hydatid) cysts of *E. granulosus* of buffalo origin was determined by atomic absorption spectrophotometry (19). The study showed that the distribution of copper and cobalt was highest in the smaller cysts. These elements gradually decreased in the cysts as they enlarged. The distribution of zinc, iron and manganese was very high in the smaller cysts in comparison to copper and cobalt but these three elements decreased greatly in larger cysts. Iron and manganese are the only two elements found in very high concentration in thin walled cysts. Like all other trace elements both calcium and magnesium decreased as the cysts increased in size. In the thin walled cysts, magnesium increased by four times initially and this was followed by a two fold influx of calcium in the white spots in the above cysts (19).

1.3 Immunity

CE in humans is characterized by the manifestations of both humoral and cellular mediated immunities. Various studies have been carried out in India to study different components of the immunity both in humans and in experimental animal models.
Phagocytic function of monocytes was studied in *E. granulosus* infected Swiss albino mice by Wangoo and his associates (20). No significant difference in percentage of phagocytic activity was observed in mice in the early stage of infection when compared to non-infected controls. However, an increase in the phagocytic activity was noticed during the later stage of infection although the significance of these responses was not clear. This study showed an intact inflammatory response with certain degree of specificity (20). Other experiment used to study immune response in animal model includes cytotoxicity in experimental *E. granulosus* infected mice and lymphocyte subpopulation and blast transformation studies in experimental infection (21).

Wattal (22) has reported the specific and non-specific cellular immune status of the infected humans using *in-vitro* blast transformation assay. The author found that the patients with CE had suppressed cellular immune function to a non-specific T cell mitogen phytohaemagglutinin (PHA). Hydatid antigen directly inhibited *in-vitro* blast transformation at a concentration of 0.15 microgram protein / ml (22).

Baveja et al. (23) demonstrated a significant increase in the levels of IgG, IgA, IgM, IgE antibodies and complement C3 in patients with CE. Though there was adequate amount of humoral response, it did not provide any protection. The absolute lymphocyte counts was raised and mean percentage of T cells was reduced (23).

1.4 Clinical Manifestations

1.4.1 Primary cystic echinococcosis

CE in human is mostly asymptomatic throughout the life in a majority of cases. In endemic areas, any patient of any age who presents with a mass anywhere in the body is first suspected of having a hydatid cyst. The hydatid cysts in lung, liver or other organs may be detected accidentally during x-ray examination, body scanning, and surgery or for other clinical reasons. These cases are also detected at autopsy or when the cyst ruptures giving rise to anaphylactic reactions.
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In symptomatic cases, the clinical manifestations are highly variable and non-specific and depend on the organ infected with the cyst, number of the cysts, size of the cysts and their sites within the involved organ, interaction between the expanding cysts and adjacent organ and complications caused by rupture of cysts. Moreover, incubation period varies from few months to several years. Hence there is difficulty in the clinical diagnosis of this condition. *E. granulosus* producing morbidity differs with the strain of the parasite. Usually the strains affecting wild animals are less virulent than those occurring in domestic animals.

The clinical manifestations of the CE are variable and primarily depend on the organ or tissue affected as follows:

**Liver**

CE affects primarily the liver. The condition presents as hepatomegaly with or without palpable abdominal mass. The symptoms include dull abdominal pain caused by stretching of the liver capsule, lumpy abdomen and tender right hypochondrial mass with intercostal tenderness (9). In Indian studies, most commonly the right lobe (24) and occasionally left lobe (25) are found to be affected.

The uncomplicated primary liver hydatid cysts constitute the majority of cases of hepatic CE. In addition to this, primary hepatic CE associated with intrathoracic extension and primary hepato-renal CE have also been reported (26).

Secondary bacterial infections due to Gram-negative bacilli such as *Salmonella* (27) and *Yersinia enterocolitica* (28) are one of the most common complications of hepatic CE. Pain and fever are the common symptoms.

Rupture of liver hydatid cysts either due to pressure mounted by surrounding organ, or infection, or due to surgery is the second most common complication of liver hydatid cysts and constitutes 3% to 17% of cases (29). This condition, in some patients, may be associated with jaundice, pain and fever (30). Spontaneous rupture
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of liver hydatid cyst into inferior vena cava with resultant thrombosis of the inferior vena cava and left kidney, right common iliac and right external iliac veins (31) or into gall bladder causing emphysema with obstructive jaundice have also been reported (30).

Portal hypertension, bronchobiliary fistula, cysto-bilio bronchial communication and hepatic hydatid cyst communicating with a biliary system are the other noted complications of liver hydatid cysts (32). Spontaneous expulsion of cysts, a rare complication of sclerotherapy (33) and obstruction of inferior vena cava due to enlargement of hydatid cyst located in liver have also been documented (34). Hydatid cyst of liver presenting with ascites, misdiagnosed as peritoneal tuberculosis, is also reported by Singh et al. (35).

Lung

The lung next only to the liver is the second major organ to be affected in CE. Hydatid cysts in lungs are always intracapsular and produce the signs and symptoms of an intra-thoracic growth. The condition may manifest as dry cough, chest pain, haemoptysis, pneumothorax and pneumonitis (36). Other symptoms include pain radiating to the medial fingers, miosis, ptosis, anhidrosis of face and wasting of small muscles of either one side associated with Homer’s syndrome (37).

Generally, lung hydatid cysts may be either unilateral or bilateral. Unilateral lung hydatid cysts present either as solitary or segmental consolidation with or without complications (36). Hydatid cysts in bilateral pulmonary CE may either be single or multiple and most of them resolve on treatment with albendazole (38).

Pulmonary hydatid cysts are more important than the liver hydatid cysts because of their potency to produce complications. Complications are either due to extension of the cyst and involvement of neighbouring areas, rupture, infection or surgery. Post-operative empyema and bronchopleural fistula are well known complications after surgical removal of the lung cysts (38). Hydatid cysts of the lung causing rib erosion, cyst extending into the chest wall with destruction and thinning of the first and
second rib on the right side (39), pulmonary disease with myopathy which resolved after administering high dose of mebendazole (40), hydatid cyst rupturing to produce a hydropneumothorax (41) and complicated pulmonary sequestration or non-resolving consolidation (42) are some of the complications of lung hydatid cysts noted in the Indian literature.

Lung hydatid cysts associated with pulmonary tuberculosis (43), phaeohypomycosis (44), saprophytic mycosis resembling aspergillosis (45), pyogenic chest infection or parasitic infection of the gut (46) are some reports of lung hydatid cysts associated with concurrent infections documented from India. Invasive mycosis of the hydatid cyst situated in an ectactic bronchus in immunocompetent normal hosts is another such report (47). Disseminated pulmonary CE presenting with multiple cannon ball shadows on chest x-ray has also been reported in patients with HIV infection (48).

Hydatid cysts of thorax, intra thoracic hydatid cyst (49), pleural hydatid cyst (50) and pulmonary and mediastinal hydatid cysts (51) are the other rare forms of hydatid cyst in the thorax being reported in several Indian studies.

Spleen
Hydatid cyst of the spleen although rare, is the third most common type of CE next only to the liver and lung. Splenic hydatid cysts constitute 3% to 5% of total cases of abdominal hydatidosis (52). Abdominal discomfort and palpable swellings in the left hypochondrium are the common clinical manifestations. Juxta splenic hydatid cyst, hypersplenism and giant hydatid cyst of gastro splenic ligament simulating massive ascites are some of the rare forms of CE of the spleen being reported in the literature (53). Splenectomy remains the treatment of choice because it is associated with low morbidity and mortality rates (54). In order to avoid the damage caused to spleen due to surgery, conservative surgery is being suggested (54).

Kidney
Hydatid cysts of the kidney and urinary bladder have been reported frequently (55). Many of these conditions are diagnosed by ultrasound. Percutaneous aspiration
injection reaspiration (PAIR) procedure remains the treatment of choice and this percutaneous drainage has been undertaken along with medical prophylaxis to decrease morbidity, shorten the duration of hospital stay and to assist in renal preservation. Conservative surgery can also be applied in the treatment of renal CE rather than nephrectomy (55). Disadvantage of surgery is that it is associated frequently with secondary infection and haematuria which could lead to loss or damage of the kidneys.

Complications of hydatid cysts of the kidneys include ureteric obstruction in renal CE following surgery, hydatiduria, anuria, nephropathy, hydatidcysturia and hypertension (56,57).

**Bone**

Hydatid cysts of the bone are well documented entities in India. Hydatid cyst of tibia, which manifested as a case of pathological fracture has been reported by Madiwale and his associates (58). Pain and swelling of the left lower limb with inability to bear the weight were the main features. Tender swelling was also noted at the upper and middle third of tibia. Albendazole treatment followed by curettage and bone grafting helped in improving the conditions. Hydatid cysts of sacrum are rare entities, characterized by chronicity without any clinical manifestation and are usually misdiagnosed in the early stage resulting in significant loss of bone and destruction of surrounding tissues. The symptoms are early sphincteric involvement with minimal sensorimotor deficits in the lower limbs and bone destruction on radiography (59).

**Mediastinum**

Primary mediastinal hydatid cyst is not common. Primary posterior mediastinal hydatid cysts are quite characteristic and can be readily distinguished from other cystic lesions of the mediastinum (60). A case has been reported in a patient with signs of Homer's syndrome and mild superior vena cava compression (61). The extension of primary retroperitoneal cyst into the posterior mediastinum, diagnosed
using magnetic resonance imaging (MRI), later confirmed to be of echinococcal origin, after surgery has also been documented (60).

**Eye**
Hydatid cysts of the eye can be unilocular or multilocular(62). Other manifestations of orbital hydatid cysts include subperiosteal hydatid cyst of orbit and hydatid cyst of the orbit causing proptosis (63).

**Breast**
Primary hydatid cyst of breast was diagnosed by fine needle aspiration cytology (FNAC).

Infected hydatid cyst leading to breast abscess in CE has also been reported (64).

**Brain**
Intra cranial CE constitutes 1% to 2% of CE cases (65). Primary hydatid cyst of the brain in adults is rare and can pose various diagnostic problems. Hydatid cyst of the brain is included in the differential diagnosis when a cystic brain lesion is found in patients coming from an area endemic for CE. The cyst may be unilocular or multilocular often polygonal with perifocal oedema, calcification and membrane detachment (65). Multiplicity of the cyst is even rarer. Clinically most common findings include papilloedema, headache, vomiting with focal neurological deficit and raised intracranial pressure. The most important diagnostic tool is computed tomography (CT) scanning of the brain (66) and *in-vivo* proton magnetic resonance spectroscopy (67).

Cases of multiple solitary intracranial cysts in the lateral ventricle, commonly in parietal lobe, and intra cerebral hydatid cyst in a child with atrial septal defect have also been reported. Hydatid cyst of the brain located in other sites such as posterior cranial fossa and extraparenchymatous multiple cysts with cervico-medullary compression and jugular foramen syndrome have been documented (68).
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Spine
Primary spinal hydatid cysts are uncommon and if present are usually associated with complications. Primary spinal intradural hydatid cyst can present as incomplete dorsal cord compression (69). Another case of lower cauda equina compression caused by a primary solitary hydatid cyst of sacral spinal canal presented with lower motor neuron type of bladder bowel involvement has been reported (70). The complications include minimal urinary stress incontinence, paraplegia and spinal cord compression (71).

In an unusual case of CE of the spine, a young man presented with thoracic spinal cord compression, revealed an extradural cystic lesion involving spine and paraspinal musculature. Surgery confirmed echinococcosis and histopathological examination revealed Trichinella infection of the paraspinal musculature. This was an unusual report of primary CE of the spine with concomitant infestation with Trichinella (72).

Heart
Hydatid cyst of heart is a rare manifestation and is normally associated with complications which are highly fatal. Cardiac hydatid cysts have been reported in adults and also in children (73). The cases presented as intracardiac mass lesion in one patient (74), multiple cardiac hydatid cysts in one patient with left chest pain and left ventricular hydatid cyst in another patient who did not respond to oral mebendazole therapy and had a sudden death due to rupture of the cyst (75).

Some of the complications of cardiac hydatid cysts include ventricular tachycardia (76), interventricular septal hydatid cyst presenting as complete heart block and hydatid cyst of the right ventricle in association with rheumatic mitral stenosis (77).

Pancreas
Hydatid cyst of pancreas although is rare has been documented in the Indian literature (78). Hydatid cysts in the pancreas are commonly seen in body and tail (78). A rare case presented with a primary hydatid cyst in the head of pancreas along
with obstructive jaundice caused by extrinsic compression of the intra pancreatic portion of the bile duct (79). Another case of hydatid cyst in the head of the pancreas presented with obstructive jaundice, where the cyst was compressing the common bile duct and pancreatic duct. In this case, excision of the cyst with choleduodenostomy was performed (79).

Pelvis
A primary hydatid cyst in the pelvis is rare. The condition presents with pressure symptoms affecting adjacent abdominal organs usually urinary bladder and rectum, results in gluteal swelling, obstructive uropathy with chronic renal failure (80). In these cases, surgical excision and medical prophylaxis were effective in alleviating the symptoms and improving the renal function. Retroperitoneal primary hydatid cyst of pelvis and right sided female pelvic echinococcosis are the rare presentation of CE of the pelvis (81). Spontaneous rupture of pelvic hydatid cysts or pressure effect of the pelvic cysts leading to labour obstruction (82) are the noted complications. Pelvic hydatid cyst rupturing spontaneously into the bladder was managed conservatively and the resulting communication between the bladder and cyst was used advantageously for intravesical instillation of 20% saline to destroy the germinal layer of the hydatid cyst and daughter cysts (82).

Peritoneum
The cases of primary CE of the peritoneum, disseminated peritoneal hydatidosis and floating intra peritoneal hydatidosis have been documented in the Indian literature (83, 84). These cases usually present as fever and abdominal pain. Apart from this, hydatidemesis (hydatid cysts and membranes in the vomitus) and hydatidenteria (passage of hydatid membranes in the stool) are being observed in the cases of disseminated peritoneal hydatidosis (83).

Other unusual sites
Hydatid cysts of other unusual sites include hydatid cysts of thyroid glands (85), mandible (86), parotid gland (87), adductor longus muscle (88), sterno mastoid muscle (89) and thigh muscles.
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Hydatid cysts of testis (90), prostate (91), neck (92), mesenteric cysts (93), ovary (94), female genital viscera (95) and aorta (96) are reported from India. Hydatid cyst of common bile duct producing obstructive jaundice due to extrinsic compression of the bile duct (97), biliary sclerosis following formalin instillation into hydatid cyst (98) and obstructive azoospermia due to retrovesical hydatid cyst (99) have also been documented. Echinococcosis as a cause of infertility has also been reported (100).

An unusual case of a ruptured primary hydatid cyst of the gall bladder which on sonography revealed intra luminal mass with undulating membrane in the neck and body, an extremely rare condition has been reported (101).

Hydatid cysts of soft tissue diagnosed by FNAC have been reported in a patient presenting with gradually increasing swelling in the anterior inferior aspect of shoulder. The aspiration of fluid from the swelling showed protoscoleces, fully developed scolexes with rostellum and scattered hooklets, diagnostic of hydatid cyst. Histopathology of surgically removed cyst showed chronically inflamed echinococcal cyst (102).

1.4.2 Secondary cystic echinococcosis

Secondary echinococcosis or disseminated CE, a notable feature that occurs due to the rupture of the primary cyst or by spillage during surgery has also been reported. These include the secondary echinococcosis of the orbit and spleen (103), heart (18), interarterial and interventricular septum of the heart (18) and diaphragm (104).

1.5 Epidemiology

CE is emerging as a public health problem of global importance.
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1.5.1 World scenario

Globally, few data are available on the overall prevalence of human CE. Most of the data available are based on retrospective case finding. This does not represent the most accurate description of human infection rates. Because hospital cases are always symptomatic and therefore asymptomatic cases are usually excluded and there is a bias of treatment in certain age groups, for e.g. most surgery for CE in developing countries occurs in the 20 to 40 years age group with relatively few operations for young and old patients.

The highest prevalence and annual incidence rates for human CE are around 2%-6% and > 50 per 100000 respectively, for example amongst pastoralists in northwest Kenya and Uruguay (105). Hospital reports revealed a higher incidence at around 220/100000. A combination of ultrasound and serology have indicated a prevalence of between 6% and 9% among people in northwest of the Kenya district. In rural Uruguay town, retrospective surgical data analysis has shown that annual CE incidence rates as high as 105 /100000 for some departments, national rate being around 18 per 100000 (106). An ultrasound survey recorded an abdominal CE rate of 1.4% which is also similar to the seroprevalence rate. Prevalence rate for confirmed asymptomatic abdominal CE was 3.6%. In China, annual surgical case rate for CE was 43.8 per 100000 and transmission is due to home slaughter which was practiced by 84% of villagers (107). In British Isles, endemic areas include England, Wales, Scotland and Ireland. The highest annual surgical incidence was 7 per 100000 in Breakneck district of Powys in midwales (108).

Other highly endemic areas include large parts of Chile, Argentina, Peru, Brazil, Morocco, Algeria, Tunisia, Libya, southern European countries especially Spain, Greece, parts of Italy, former Yugoslavia, Turkey, Iraq, Iran, Jordan, Syria, Bulgaria, Romania, Russia and the former soviet republics Uzbekistan, Kazakhstan, Tajikstan, Kirgizstan and Turkmenistan (109).
Transmission of CE to humans occurs when there is a close association of dogs, domestic livestock and people. Analysis of ultrasound detection rates indicates that there is a general pattern of increasing human prevalence with age where people under 20 years having the lowest rates of abdominal infection and over 40s having the highest (109). In rural Uruguay, the age specific prevalence of ultrasound detected human CE increased from 1.3% in the 5 to 9 years of age group to 14% in >60 years age group with no significant difference between males and females. Higher female infection rates have been recorded for CE in Turkana and Kenya (105). The reason suggested was frequent contact with dogs in the home area by women and children is more than men. In China, younger adult males spend considerable time with the dogs in the summer pastures and frequent handling may lead to significant exposure to *Echinococcus* eggs.

The European biotype of *E. granulosus* occurs in other regions of North and Central America, Europe, Africa, India, South East Asia and Australia, both in the dog-sheep cycle as well as other domestic animals e.g. pig, dog-horse, dog-camel and dog- cattle. Autochthonous human cases of CE occur in few countries like Peru (110), Spain, India and China (109).

1.5.2 Indian scenario

In India, the disease is widely distributed throughout the country both in the rural and urban areas. The large population of live stocks and large human populations in a agriculture-based country like India provides a congenial condition for establishment, propagation and dissemination of CE in both humans and animals (111).

The cases of CE in humans and domestic animals such as sheep, cattle, buffaloes, pigs and wild life are being increasingly reported from different parts of India (112 - 117). This is of much concern because of its potentiality to cause economic losses associated with loss of meat products. In many studies from the world the loss of food products due to discarding of viscera with hydatid cyst,
especially liver have been found to be enormous. It has been estimated that the viscera of 2 million cattle and 3.5 million sheep in Chile has to be discarded annually due to the animals suffering from CE. Unfortunately, the effect of CE on the production of wool, meat and milk is not well documented and is not widely studied. In one study Irsh abdullah et al. (118) has demonstrated that the condemnation of the liver of buffaloes at Aligarh, Uttar Pradesh, contributes to an economic loss of Rs. 131, 400 per year alone. The situation appears to be the same in other parts of India but no exact data is available. This is because studies carried out in this direction to know the effect of economic losses caused by hydatid cysts in domestic animals in India is lacking.

Another cause for concern is that the cost of medical and surgical care of human cases is also equally enormous. In one study in Argentina and in Chile it was estimated that the cost of hospitalization for a surgical case of CE without any complication was US 1500-2000 dollars. No such studies on cost of hospitalization and medical treatment of patients with CE in India have been carried out. Nevertheless, it is definite that cost due to such expenses would also be equally enormous.

1.5.3 Geographical distributions

In India, CE in humans is being increasingly recognized and reported from most parts of the country.

CE in humans has been reported in Agra (8), Aligarh (94), Allahabad (41, 56, 77), Ahmedabad (8), Aurangabad (8), Assam (8), Bankura (8), Baroda (8), Bellary (8), Bhopal (8, 76), Bangalore (34, 47, 73), Burdwan (119), Calcutta (8), Cuttack (8), Chandigarh (37, 52, 55, 96), Chennai (8), Delhi (18, 62, 66), 71, 103), Guntur (8), Gwalior (8), Hyderabad (120), Himachal Pradesh (121), Jamnagar (8, 122), Jaipur (61), Kozhikode (8), Kullu (64), Lucknow (39, 67), Maharashtra (8), Madurai (69), Manipal (34), Mumbai (58, 65, 95, 104), Nagpur (8, 123), Vellore (124, 125), Pondicherry (9, 114, 115, 126 – 130), Mysore (8), Nalgonda (8).
Patiala (8), Patna (53, 84), Punjab (8), Pune (48), Secunderabad (8), Rohtak (34, 74, 75, 99), Srinagar (29, 33, 78, 79), Visakapatnam (8), Kurnool (8), Shimla (93) and Uttar Pradesh (118).

In India, like other places of the world persons of all age groups, ranging from 1-2 years to 80 years are susceptible to infection, although high incidence of infection may be seen in persons between 30 to 40 years of age. Both males and females are equally affected. CE in children is being recognized in India with the involvement of the liver (131), lung (132), pelvic cavity, spleen and transverse mesocolon and brain (8).

The increased incidence of CE in both humans and live stocks throughout India, is not only due to increased detection of the cases as a result of improved imaging techniques and immunodiagnostic methods, but also many factors which possibly contributes to a true increase in the number of cases. Unhygienic slaughtering of food animals, a free access of dogs to infected offals in slaughtered houses and allowing them to eat these infected offals contribute a lot to perpetuate transmission of the disease in the community. Irrigation of vegetables with contaminated water and soiling of vegetables by dogs are the other factors. The habit of certain tribal people in the North eastern India to consume dog intestine as a food delicacy also a habit of concern for high incidence of CE in those communities (133).

1.6 Diagnosis

Cystic echinococcosis is a zoonotic disease that is common in rural population of underdeveloped countries because of their close association with domestic and wild animals. CE in human is mostly asymptomatic throughout the life in a majority of cases. In symptomatic cases, the clinical manifestations are highly variable and non-specific and depend on the organ infected with the cyst, number of the cysts, size of the cysts and their sites within the involved organ, interaction between the expanding cysts and adjacent organ, and complications caused by
rupture of cysts (8, 9). Moreover, incubation period varies from few months to several years. Hence, there is difficulty in the clinical diagnosis of this condition.

In the absence of specific clinical features, early detection of the developing hydatid cyst in patient depends more upon the laboratory procedures that is essential to initiate appropriate chemotherapy. Diagnosis of CE involves imaging methods, supplemented by immunological methods. Imaging methods in combination with immunological methods are now extensively used to support clinical diagnosis of the condition (134 - 137). During the last two decades, tremendous advance has been made in both these fields which have a direct bearing on the diagnosis of CE in humans as well as in animals.

The use of imaging methods such as ultrasonography (US), computerized axial tomography (CAT), and magnetic resonance imaging (MRI) have helped in the diagnosis of space occupying lesions caused by many parasitic conditions including the CE. However, in most endemic areas, newer imaging techniques are not available or even if available are often too costly. Atypical appearance of the visualized lesions, failure to provide information about the viability of the parasite and difficult to differentiate from abscesses or neoplasms are also some of the limitations of the imaging methods (138). Serodiagnosis based on serum antibody or antigen detection is of immense value to confirm clinical findings or render diagnostic help in radiologically unclear cases and determination of patients immune status in CE infection (139). Hence serological tests are most useful for the diagnosis of CE because of their low cost and ease of performance in endemic areas.

1.6.1 Serodiagnosis

CE is one of the few parasitic infections in which serology was used primarily for the diagnosis. These tests primarily detect specific hydatid antibodies in the serum and also hydatid antigen in the serum, urine and other body fluids. There are numerous serological tests that have been developed for the detection of circulating anti echinococcal antibodies (117).
INSensitive and nonspecific tests including the Casoni’s intradermal test, the complement fixation test, indirect haemagglutination test and the latex agglutination test have been used for four to five decades are replaced by enzyme linked immunosorbent assay (ELISA), the indirect immuno-fluorescence antibody test, immunoelectrophoresis (IEP) and enzyme linked immuno-electrotransfer blot (EITB) in routine laboratory applications for serodiagnosis of CE.

As there is considerable difference in composition between fertile and non-fertile hydatid cyst (140), fertile hydatid cyst fluid was the most commonly used source of antigen for serodiagnosis of CE. The hydatid cyst is obtained from different sources such as surgically removed cyst of humans (127,141-144), cattle (145-147), sheep (148-150), swine (151), camel (152 - 154) after slaughtering.

Though majority of the studies have used cyst fluid antigens, still there is a continued need for the identification and characterization of ‘new’ *E. granulosus* antigens from metacestode parasites. The identification of newer antigens may help to develop serological assay with greater immunodiagnostic sensitivity and specificity than those currently in use and this may facilitate post-treatment immunosurveillance and studies on the immunopathology of CE (155).

Reports are scanty on the usefulness of the hydatid protoscolex and cyst wall as antigen for use in hydatid serology. *E. granulosus* protoscolex as a source of antigen have been evaluated recently for antibody detection in CE patients using EITB (156). Gul Ahmed et al. (157) demonstrated six fractions (F1-F6) in the purified soluble proteins of the protoscoleces obtained from the lung hydatid cysts of Indian water buffaloes. Of these, three fractions F1, F2 and F3 induce production of serum antibodies after four days of post-infections. Mahned and Gamra (158) used *E. granulosus* alkaline phosphatase (EgAP) extracted from hydatid cyst membrane as antigen in ELISA and EITB. Both the tests showed a higher sensitivity and specificity using EgAP antigen extracted from hydatid cyst membrane than using hydatid cyst fluid in the serodiagnosis of CE.
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The use of crude antigen in serodiagnosis leads to false positive reactions. Thus, purification of the parasitic antigen for their use as antigen may lead to higher specificity in the serodiagnosis. The use of purified antigens for the diagnosis of CE has also been emphasized by some authors with good sensitivity (159-162).

Various studies have demonstrated the specific antigen components of cyst fluid for the diagnostic use. Kanwar et al. (161) demonstrated a low molecular mass protein (8kDa) obtained from crude hydatid cyst fluid to be highly genus specific. Purified antigen was used in ELISA for the detection of specific antibody in sera from the cases of sheep hydatidosis. In another study, antigen was purified by chromatography on DEAE cellulose and sephadex-G 200 from hydatid cyst fluid and was found to be thermostable and apparently a lipoprotein. The antigen was electrophoretically pure and found to be sensitive and specific for *E. granulosus* (162).

The most recent approach is the isolation of parasite antigen excreted in the urine and their use as antigen following purification in the serodiagnosis. The characterization of purified protein from the parasite or urine of infected individual is important for production of recombinant antigen and to design synthetic peptides that paves way for adequate alternate source of antigen. This helps not only to enhance the serological test sensitivity but also to know the origin and significance of infection. In studies with other parasites like schistosomes (163) and trypanosomes (164), urinary antigen was purified and characterized for their use in serodiagnosis. In CE, characterization of parasite antigen has been reported with diagnostically specific polypeptides obtained from cyst fluids (165, 166). Reports are scanty on the characterization of purified protein isolated from urine of CE infected cases.

In the present study, therefore, an attempt will be made to evaluate the hydatid cyst wall as well as protoscolex as source of antigen in addition to the cyst fluid by serological assays such as ELISA, Dot-ELISA and EITB for the diagnosis of CE. The attempt will be also made to identify diagnostically relevant antigens
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from protoscolex, cyst wall and cyst fluid antigens and also from the urine of confirmed CE cases.

The mere demonstration of hydatid antibody doesn’t confirm clinical diagnosis because of non-specific reactions and persistence of anti-hydatid antibodies for several years after surgical removal of hydatid cyst. Hence, diagnostic methods based on the detection of parasite antigens are more useful as it helps in more direct measure of parasite burden, giving an indication of the activity and intensity of infection and also in assessing medical and chemotherapeutic treatment of CE (130, 167).

Detection of hydatid antigen in the serum or other body fluid is suggested to be more efficacious in the diagnosis of CE (168) than antibody detection. Circulating hydatid antigen has been reported from experimental E. granulosus infection of animals and in human CE (169). The circulating serum antigen was detected by double antibody sandwich ELISA (168). Subsequently, simple techniques such as countercurrent immunoelectrophoresis (CIEP) (137), bacterial Co-agglutination (Co-A) test (129) and latex agglutination test (LAT) (170) were used in detection of hydatid antigen in serum. Polyclonal antibodies raised against crude and semi purified hydatid cyst fluid antigen was used in many of these study for detection of hydatid antigen in the serum (129,137, 170).

To increase the specificity of antigen detection -based serological tests of CE, monoclonal antibodies (MAb) have been employed for detection of hydatid antigen in serum. Monoclonal antibodies produced against cyst fluid components, particularly the immunodominant antigens, antigen B (171) and antigen 5 (171, 172) have been evaluated for detection of circulating serum antigens in CE patients. Reports are scanty on the use and evaluation of the monoclonal antibodies raised against protoscolex and hydatid cyst wall antigens. Therefore in the present study, an attempt will be made to study usefulness of monoclonal antibodies in the detection of hydatid antigen in serum and urine.
The Dot-ELISA and EITB have been reported earlier in few studies for detection of antibodies for diagnosis of CE (152-154, 173-178). However, there are no reports on either the use of Dot-ELISA or EITB for detection of hydatid antigen in serum for diagnosis of CE. Therefore, in the present study, an attempt will be made to evaluate both the Dot-ELISA and EITB for detection of circulating hydatid antigen in the serum.

Detection of antigen in urine is a recent approach in diagnosis of many of the parasitic infections including Chaga's disease (164), urinary schistosomiasis (163), filariasis (179) and malaria (180).

Hydatid antigen detection in the urine for diagnosis of CE was first reported by Parija et al. in 1997 (181) from India by CIEP using hyperimmune antiserum raised against crude human hydatid cyst fluid. Subsequently, Co-A was also used to detect the urinary hydatid antigen (7). However, no reports are available in the literature on the evaluation of the more sensitive serological assays, the sandwich ELISA, Dot-ELISA and EITB for the detection of hydatid antigen in the urine for diagnosis of CE. In the present study, an attempt will be made to evaluate sandwich ELISA, Dot-ELISA and EITB for detection of circulating hydatid antigen in the urine for the diagnosis of CE.

1.7 Treatment
1.7.1 Surgery

Surgery still remains the mainstay of the treatment of CE. Surgical removal of the cysts is indicated for the cysts located in the operable sites such as the liver (182, 183), lung (184), etc., and the cysts which may enlarge and likely to interfere with functioning of the vital organs. Conservative surgery plays an important role in preserving the vital organs. Low recurrence rate of hydatid cyst and complete evacuation of daughter cysts are the main advantages. Disadvantages of surgery are that it is associated with considerable morbidity and mortality (up to 2%). Puncture-Aspiration-Injection-Reaspiration (PAIR) (125) of the cyst is an effective therapeutic
alternate to surgery in terms of cost containment and hospitalization time. Management of the cases of CE by surgery, following different modifications and approaches have been extensively applied in India.

1.7.2 Percutaneous aspiration injection reaspiration (PAIR)

PAIR is a minimally invasive therapeutic option (185) used in the management of CE. It has been used successfully to treat the hepatic hydatid cyst due to its safety and efficacy. PAIR treatment enables one to observe a reduction in cyst size, change in echo pattern on ultrasonography during follow up. Some of the complication like cyst infection, cyst biliary rupture and urticaria were managed successfully without any mortality and with less morbidity (185, 186).

Ultrasound guided percutaneous drainage of the cyst using either hypertonic (20% or 15%) saline or ethanol (95%) as scolicidal agent and albendazole and praziquantel chemoprophylaxis before and after the drainage has been used as safe and effective method in management of hydatid cysts of the pancreas (79) and liver (187).

1.7.3 Laparoscopy

Laparoscopic intervention appears to be a minimally invasive management and is an alternative to open surgery because they result in less morbidity (188). Usually, this procedure of laparoscopic excision of a hydatid cyst is done after treatment with scolicidal agents such as praziquantel and albendazole to prevent spillage of scolices during evacuation of contents and to excise the entire germinal epithelium with no complication (189). Laparoscopy has been adopted in the treatment of hydatid cyst of liver (190). Short term complications are prolonged tube drainage, intracystic bile connection and intracystic pus.
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1.7.4 Chemotherapy

Treatment of the hydatid cyst by chemotherapy is the recent approach. It is especially useful for small sized cysts situated in un-operable sites.

Albendazole and mebendazole are the only anthelmintics effective against CE (189, 191). Albendazole is the drug of choice because its penetration into hydatid cysts is superior compared to mebendazole and is more effective in treatment of liver hydatid cysts. Pre-surgical use of albendazole in CE reduces the risk of recurrence. Albendazole given in combination with PAIR showed a significant reduction in size of the cysts (80, 192). Albendazole is given orally in a dose of 15 mg / kg body weight daily in two divided doses (not to exceed total daily dose of 800 mg) for a period of 4 weeks.

Mebendazole given orally in a dosage of 400-600 mg, thrice daily for 21 to 30 days has proved beneficial in regression of the cysts. Albendazole used both pre-operative and post-operative prophylaxis helps in reduction of the cyst size with no recurrence. Mebendazole was extensively used in treatment of hydatid cysts of the liver (182) and of other sites. Goel et al. (191) reported the management of non-communicating hydatid cyst of the kidney by PAIR. This was performed under medical prophylaxis with mebendazole and praziquantel. The treatment was found to be safe and effective, and prevented renal loss (191). Low dose mebendazole therapy has also been used in treating pulmonary hydatid cysts (193).

1.8 Prevention and Control

The preventive and control measures of CE include: 1) Regular treatment of infected dogs to reduce worm load and is the key component. 2) Elimination of stray and infected dogs. Since no suitable vaccines against adult infection in dogs are available, this will enable in preventing spread of infections to humans. 3) Prevention of dogs from eating infected offal's of domestic animals (sheep, cattle, etc.) in the slaughter houses in India, indiscriminate disposal of infected offals, a
practice mostly followed by butcherers after slaughtering of infected animals are primarily responsible for transmission of infection to dogs. There is a need for hygienic slaughtering of goat, sheep, cattle, etc. 4) Strict personal hygiene (e.g., such as washing hands after playing with or feeding the dogs). The eggs of *E. granulosus* are easily killed by boiled water or even by dry heat. These are killed at 60°C in 10 minutes or immediately at 100°C and 5) Avoidance of contamination of food and water with dog faeces are absolutely necessary. Washing of green salads thoroughly with boiled water before used as salads will reduce transmission of infection by eggs.

Studies are few in India on vaccination. In an experimental animal study, Singh and Dhar (194) infected pups with protoscolices attenuated by irradiation to study protection against the *E. granulosus* infection. A progressive decrease in worm load was observed in pups infected with 100-600 Gy irradiated protoscolices and no worm infection was established in the pups infected with 400-600 Gy irradiated protoscolices.
AIM AND OBJECTIVES

2. AIMS AND OBJECTIVES

Aims

1. To evaluate the ELISA, Dot-ELISA and EITB for demonstration of hydatid antigen in urine and sera for diagnosis of CE.

2. To evaluate different components of hydatid cyst (hydatid cyst fluid, protoscolex and cyst wall) for their use as antigens in the ELISA, Dot-ELISA and EITB for demonstration of serum antibodies in diagnosis of CE.

Objectives

1. To identify, isolate and characterize diagnostically relevant antigen from hydatid cyst wall antigen and urinary hydatid antigen.

2. To detect hydatid antibodies in the serum of cases of CE and controls by ELISA, Dot-ELISA and EITB using various hydatid antigen preparation.

3. To raise monoclonal antibodies against affinity purified specific hydatid cyst wall antigen.

4. To detect hydatid antigen in the urine and serum of cases of CE and controls by using sandwich ELISA, Dot-ELISA and EITB.