IMMUNODIAGNOSIS OF HUMAN AND CANINE ECHINOCOCCOSIS AND COMMUNITY STUDIES IN NORTHWESTERN CHINA

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Abstract

Echinococcosis is highly endemic in northwestern China. In order to improve sero-testing in support of community screening and for hospital use, a dot immunogold filtration assay (DIGFA) for rapid serodiagnosis of human CE and AE was developed. DIGFA incorporated four antigen preparations: crude *E. granulosus* cyst fluid, crude extract of *E. granulosus* protoscoleces, *E.granulosus* native antigen B, and a metacestode extract (Em2) from *E. multilocularis*. The overall sensitivity of DIGFA in a hospital diagnostic setting using archived sera was 80.7% for human CE (n=857 samples) and 92.9% for human AE (n=42 samples). In endemic communities (Qinghe, Hobukersaier, Wenquan, Xinyuan County and Bayanbulak Pasture in Xinjiang; Xiji County in Ningxia; Ganzi County in Sichuan; Dangxiong and Dingqing County in Tibet AR) in northwest China screened for echinococcosis, the sensitivity of DIGFA ranged from 71.8% to 90.7% in comparison to abdominal ultrasound as the gold standard; specificity for CE using AgB was 94.6% and for AE using Em2 was 97.1%. This simple eye-read rapid test was judged useful for both clinical diagnostic supports, as well as in conjunction with ultrasound for mass screening in endemic CE and AE areas. An immunochromatographic assay (ICA) test for rapid *E.granulosus* antigen detection showed AgB detection in human cyst fluid biopsy samples had a sensitivity of 93.6%. Application of ICA for rapid coproantigen detection in dog faeces, indicated a test sensitivity and specificity generally lower (66.7%) than for coproELISA (72.2%) after 20 days post infection (dpi). A faecal sample time-course from experimental *E.granulosus* in dogs (n=9) indicated ICA coproantigen detection by 16 dpi and coproDNA detection by 20dpi. Epidemiological results also showed the overall ultrasound prevalence of human echinococcosis was 3.28% (615/18766), with cystic echinococcosis (CE) 2.73% (513/18766) and alveolar echinococcosis (AE) 0.54% (102/18766) respectively. Meanwhile the DIGFA serological positive rate was 22.4% (2388/10684), females had a relative higher seropositive rate (p<0.05). Relative risk factors for human CE were dog and livestock ownership, occupation as herdsman, ethnic groups as Mongolian and Kazakh. Another risk factor for seropositive might be involved with the gender as a female. This research has implications for further development of rapid tests in support of human and canine echinococcosis diagnosis and for surveillance of transmission in China and elsewhere.

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Abbreviations

ABZ	albendazole
AgB or EgB	Antigen B
Arc 5	antigen-antibody precipitation line detected by double diffusion (DD)or IEP
AE	alveolar echinococcosis
AR	Autonomous Region in China (eg. Xinjiang, Tibet and Ningxia)
AP	Autonomous Prefecture in some provinces and ARs (i.e. Yili Kazakh AP in Xinjiang, Aba Tibetan AP in Sichuan)
CDC	Centers for Disease Control
CE	cystic echinococcosis
CT	Computed (computer assisted) tomography
DIGFA	Dot immuno gold filtration assay
DNA	Deoxyribonucelic acid
dpi	Days post infection
E. granulosus	*Echinococcus granulosus, or E. granulosus*
E. multilocularis	*Echinococcus multilocularis, or E. multilocularis*
EgCF	crude antigen of *E. granulosus* cyst fluid
EgP	crude extract of *E. granulosus* protoscoleces
EgWWE	*E. granulosus* whole worm extract antigen
EITB	Enzyme-linked immunoelectro transfer blot
ELISA	Enzyme-linked immunosorbent assay
IB	Immunoblot
ICA/IGCA	Immuno chromatographic assay
IEP	Immunoelctrophoresis
IHA	Indirect haemagglutination assay
kDa	Kilodalton
LAT	Latex agglutination test
MBZ	Mebendazole

MRI	Magnetic resonance imaging
N or n	Number
NC membrane	Nitrocellulose membrane
OD	Optical density
OIE	Office International des Epizooties (World Organisation for Animal Health)
PAIR	Puncture, aspiration, injection, reaspiration
PCR	Polymerase chain reaction
sp., spp.	Species (singular and plural)
US	Ultrasonography
WHO	World Health Organization
X-ray	Radiography
XUAR	Xinjiang Uygur Autonomous Region

Chapter 1. Introduction

1.1 General background of *Echinococcus* spp. and Echinococcosis

Echinococcosis, which also called hydatidosis, or hydatid disease, is an ancient chronic zoonosis, with a worldwide distribution caused by adult or larval (metacestode) stages of tapeworms (cestodes) belonging to the genus *Echinococcus* Rudolphi, 1801, family Taeniidae, order Cyclophyllidea, subclass Eucestoda, Class Cestoda, Phylum Platyhelminthes, and Kingdom Animalia. (Ding et al, 2000, WHO/OIE, 2001; Oxford Medical Dictionary, 2007)

The Scientific classification:
Kingdom: *Animalia*
Phylum: *Platyhelminthes*
Class: *Cestoda*
Order: *Cyclophyllidea*
Family: *Taeniidae*
Genus: *Echinococcus*

The classic 4 species (WHO/OIE, 2001) of *Echinococcus* are recognized as *Echinococcus granulosus* (Batsch, 1786), *E. multilocularis* (Leuckart, 1863; Rausch, 1995; 1997), *E. oligarthrus* (Diesing, 1863), and *E. vogeli* (Rausch and Bernstein, 1972). Other species however have recently been described or proposed (see below 1.2). The parasites have life-cycles which utilize carnivores as definitive hosts, harbouring the adult egg-producing stage in the intestine; and ungulates, rodents or other small mammals as intermediate hosts, developing the metacestode stage (also called larval stage) in inner organs (mostly liver and lung) after egg infection. The two major species of medical and pubic health importance in northwestern China are *Echinococcus granulosus* and *Echinococcus multilocularis*, which cause cystic echinococcosis (CE) and alveolar echinococcosis (AE) respectively (Craig et al., 1995, 2000, 2003, 2006, 2007; Wen et al., 1997, 2000; Zhou et al., 2000, Ito, 2003). Both are serious and severe life-threatening diseases, the latter especially with high fatality rates and poor prognosis if careful clinical treatment is not available in early stages. Human CE

often occurs as a fluid-filled cyst (bladder-like, single or multiple), with or without daughter cysts. It was occurred in most internal organs of humans but especially the liver (around 70%), lung (around 20%), peritoneal cavity, spleen, kidney, brain, bone, pelvic, heart, and also muscle or subcutis (Ding & Wen, 2000, WHO/OIE, 2001). Meanwhile human AE mainly occurs as a tumor-like lesion mostly in the liver (>99%) (Ammann et al, 1996, Sato, et al 1993, WHO/OIE, 2001, Craig 2000, 2001, 2003; McManus 2003), with possible lung and/or brain secondary lesions in late stages. Mixed human CE and AE cases are rare but have been described (Wen et al.1992, Yang, et al 2006). Mortality for human CE varied between 0.5% and 4.5%, and for human AE between 10-15% (WHO Guidelines, 1996; Ito, 2003; Vuitton, 2003; McManus, 2003).

Early diagnosis becomes difficult because human CE or AE cases usually have no signs or symptoms during the first few years. The clinical diagnosis of CE or AE mainly relies on imaging techniques such as ultrasound (US), X-ray, computerized tomography (CT) or magnetic resonance imaging (MRI). Surgery is currently the main initial choice for the treatment of most CE and AE cases. Medical treatment using Mebendazole and albendazole chemotherapy may not always kill the cyst/lesion but can control the growth of the parasites, and could be applied pre- or post surgery or alone if the patient was not operable or refused surgical treatment (Vuitton, 2001, Kern, 2006). Different formulations of albendazole (ABZ) such as tablet (cheapest), liposomal-ABZ or emulsion-ABZ have now been widely used in severe endemic area of China (Wen et al., 1994; Chai et al., 2004, Li et al, 2006). Serological tests can give useful confirmative information to support a clinical diagnosis, and may also indicate exposure at community levels (Rogan and Craig, 1997, 2002). Prevention and control of echinococcosis is quite difficult in many endemic areas due to complex factors including ethnic belief, religion, education level, sanitary habits, husbandries, transmission ecology etc. However, control programmes against CE in 5 ‘island-based’ countries/areas (Iceland, New Zealand, Tasmania, Falkland Islands and Cyprus) have been successful in the eventual elimination of CE as a public health problem, and in some cases even to elimination of transmission of the parasite in dogs and sheep (Craig and Larrieu, 2006).

1.2 Genus and species of *Echinococcus*

Members of the genus *Echinococcus* are small intestinal tapeworms with an adult length of 1.2–7 mm, and a maximum of 7 segments (proglottides). The metacestode of *Echinococcus* spp. develops and settles in the internal organs (mostly the liver and lungs) of a wide range of mammalian intermediate hosts and is a fluid-filled cystic or vesicular structure composed of two main parasite layers with an outer host layer of fibrous capsule. The outer layer of the parasite is the laminated membrane, a carbohydrate-rich, acellular structure that is unique to the genus *Echinococcus*. It supports and also encloses the germinal membrane, which also produces protoscoleces asexually and these are the infective stage for the carnivore definitive host. The asexual production of protoscoleces by *Echinococcus* spp. is the reason for high adult worm burdens in carnivore definitive hosts.

The genus *Echinococcus* includes up to 8 species of tapeworms in the family Taeniidae. Infection with *Echinococcus* results in hydatid disease, also known as hydatidosis and echinococcosis.

Recommended Species (2008):

Echinococcus granulosus
Echinococcus multilocularis
Echinococcus oligarthrus
Echinococcus vogeli
Echinococcus shiquicus
Echinococcus ortleppi
Echinococcus equinus
Echinococcus canadensis

Currently of these, the first five recognized species of cestode are undisputed within the genus *Echinococcus*, which includes *E. Shiquicus* described in China in 2006 (Xiao, et al., 2005; Xiao et al., 2006). Some authorities however, consider that *E. granulosus* is not a single species but rather comprises at least 4 species i.e. *E. granulosus* sensu strictu, *E. ortleppi*, *E. equinus* and *E. canadensis* (McManus, 2002; Nakao et al., 2006). In addition, a ninth species *E. felidis* has very recently been proposed for the parasite that uses lions as a definitive host in sub-Saharan Africa (Huttner et al, 2008)

The species *E. granulosus* has, until recently, been divided into 10 genotypes (G1-G10). The G1 sheep strain of *E. granulosus* is the most widespread and important zoonotic genotype, although cattle, cervid, pig and camel genotypes also show zoonotic potential. The G4 horse strain is recommended to become *E. equinus*, the G5 *E. ortleppi* and G6–G10 (*E. Canadensis*) (Nakao et al, 2006). Human infection with the metacestode (hydatid cyst) of *E. granulosus* is geographically widely distributed, from the sub-arctic to the tropics, with an estimated 2 million cases mostly associated with regions of sheep herding (Craig, Rogan & Allan, 1996). The other three major *Echinococcus* species (*E. multilocularis*, *E. vogeli* and *E. oligarthrus*) are also potential zoonoses. *E. multilocularis* is a species distributed only in the Nearctic and Palearctic regions, but also cause more human infections (probably >100 000 cases) than either *E. vogeli* (approximately 120 cases described) or *E. oligarthrus* (<5 cases described). The latter two species are limited to neotropical forest and wet savannah due to their forest transmission cycles, and only a few epidemiological studies has been reported. Within the species *E. multilocularis*, intraspecific variation appears low in comparison to *E. granulosus*, and based on current assessments nucleic acid analysis can only broadly differentiate *E. multilocularis* regional isolates from Alaska, Eurasia, Japan and US/Canada (Rinder et al. 1997; Nakao et al. 2006).

Table 1.1: Genotypic variation in *Echinococcus*

Species strain / isolate (genotype)	Known intermediate hosts	Infective to humans?	Known definitive hosts	Probable geographical distribution	Proposed taxonomix designation
Echinococcus granulossus					
Sheep strain (G1)	Sheep, cattle, pigs, camels, goats, macropods	Yes	Dog, fox, dingo, jackal and hyena	Australian mainland, Europe, USA, New Zealand, Africa, China, Middle East, South America and Russian	*E. granulosus*
Tasmanian sheep strain (G2)	Sheep, cattle ?	Yes	Dog, fox	Tasmania, Argentina	*E. granulosus*
Buffalo strain (G3)	Buffalo, cattle ?	?	Dog, fox ?	Asia	*E. granulosus*
Horse strain (G4)	Horses and other equines	No	Dog	Europe, Middle East, South Africa,	*Echinococcus equinus*
Cattle strain (G5)	Cattle	Yes	Dog	Europe, South Africa, India, Nepal, Russian, South America ?	Echinoccus ortleppi
Camel strain (G6)	Camel, goats, cattle ?	Yes	Dog	Middle East, Africa, China, Argentina	*E. granulosus (E. canadensis)*
Pig strain (G7)	Pigs	Yes	Dog	Europe, Russian, South America	*E. intermidius (E. canadensis)*
Cervid strain (G8)	Moose, caribou, reindeer	Yes	Wolf, coyote, dog	North America, Eurasia	*E. granulosus (E. canadensis)*
Fennoscandinavian cervid strain (G10)	Reindeer, moose ?	?	Wolf, dog	Eurasia	*E.granulosus ? (E. canadensis)*
Lion strain	Zebra, wildebeest warthog, bushpig buffalo various antelope, giraffe ? Hippopotamus ?	?	Lion	Africa	*E. felidis*
Echinococcus multilocularis					
European isolate	Rodents, domestic and wild pig, dog, monkey	Yes	Fox, dog, cat, raccoon-dog	Europe, China ?	*E. multilocularis*
Alaskan isolate	Rodents	Yes	Fox, dog, cat	Alaska	*E. multilocularis*
North American isolate	Rodents	Yes	Fox, dog, cat, coyote	North America	*E. multilocularis*
Hokkaido isolate	Rodents, pig, mondey, horse	Yes	Fox, dog, cat raccoon-dog	Japan	*E. multilocularis*
Echinoccus vogeli					
(No variants reported)	Rodents	Yes	Bush dog	Central and South America	*E. vogeli*
Echinococcus oligarthrus					
(No variants reported)	Rodents	Yes	Wild felids	Central and south America	*E. oligarthrus*
Echinococcus shiquicus					
(No variants reported)	Lagomorphs	?	Tibetan fox	Tibetan Plateau (China)	*E. shiquicus*

(From Schantz, 2006, modified from Thompson and McManus 2003, Nakao et al. 2006)

1.3 The lifecycle of *Echinococcus* spp.

1.3.1 *Echinococcus granulosus*

E. granulosus is a small tapeworm (approximately 2 to 7 mm in length) with typically three segments and other morphological characteristics (e.g. length of hooks or strobila, position of genital pore, testes number, form of uterus, onset of egg production, etc.) which allow a species diagnosis (WHO/OIE, 2001; Ding and Wen 2000, Shan 2001) (Fig. 1.3). Eggs of *E. granulosus* are difficult to differentiate through morphologic descriptions from those of other tapeworms in the genus *Taenia*. Egg hatches in the stomach after ingested by their intermediate host and release oncospheres in the small intestine. Oncospheres are activated and penetrate the mucosa of the small intestine and enter into the circulatory system and reach its final location and develops into the metacestode stage. A unilocular hydatid cyst develops and several thousands protoscoleces (called 'hydatid sand') may be produced by asexually budding from inner germinal membrane, within a single cyst or within daughter cysts. The protoscoleces evaginates in the upper duodenum after being ingested by a suitable definitive host and develops into the sexually mature adult tapeworm in approximately 45 days (Ding and Wen, 2000; WHO/OIE, 2001) .

In the natural cycle, dogs and other canids are typical definitive hosts and domestic ungulates (sheep, goats, pigs, horses, etc.) intermediate hosts (Fig. 1.1). The cycle mainly occurs as a dog-sheep, so called domestic cycle. A sylvatic cycle also occurs with wolves as definitive host and cervids as intermediate host. In Australia wild dogs (dingo) are also a good definitive host and a sylvatic cycle occurs by predation of dingoes on macropod species (kangaroos and wallabies) (Jenkins 1995; Craig 2000; Torgerson 2003). Both domestic and sylvatic cycles could overlap as has been shown in Australia (Jenkins et al., 2006, WHO/OIE, 2001). The metacestode stage develops in the intermediate host and can be develop in a broad range of mammals, including ungulates, marsupials, lagomorphs, rodents, non-human primates, and humans. These and other hosts may play a role in the transmission cycle (intermediate hosts) or are dead ends of the development (aberrant hosts). Hydatid cysts of *E. granulosus* occur in internal organs (mainly in liver and lung) of humans and other intermediate hosts (Fig. 1.2). The disease is called cystic echinococcosis (CE).

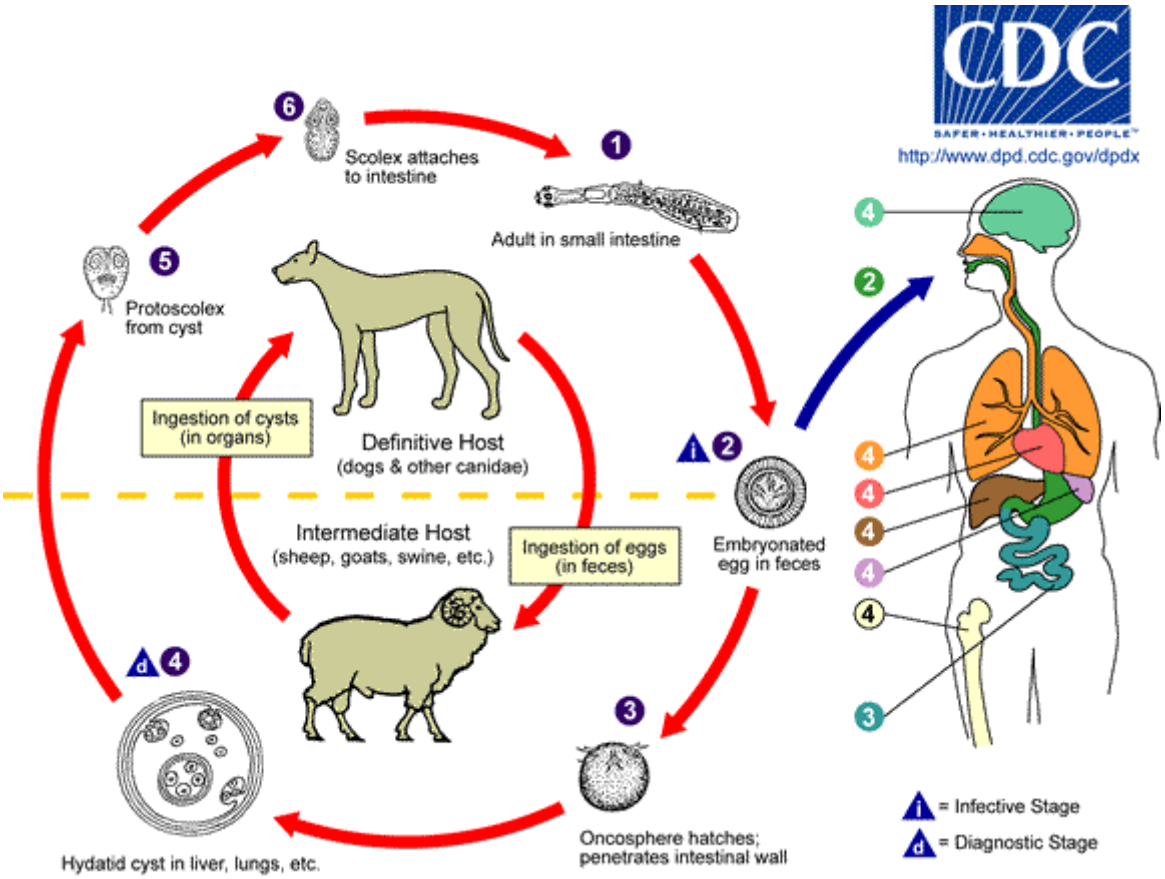


Fig. 1.1: Life cycle of *E. granulosus* (common sheep strain).
(Image adapted from original available at the United States Centres for Disease Control Parasitology Identification Laboratory).
(http://www.dpd.cdc.gov/dpdx/HTML/ImageLibrary/Echinococcosis_il.htm)

The adult *Echinococcus granulosus* (3 to 6 mm long) ❶ resides in the small bowel of the definitive hosts, dogs or other canids. Gravid proglottids release eggs ❷ that are passed in the feces. After ingestion by a suitable intermediate host (under natural conditions: sheep, goat, swine, cattle, horses, camel), the egg hatches in the small bowel and releases an oncosphere ❸ that penetrates the intestinal wall and migrates through the circulatory system into various organs, especially the liver and lungs. In these organs, the oncosphere develops into a cyst ❹ that enlarges gradually, producing protoscolices and daughter cysts that fill the cyst interior. The definitive host becomes infected by ingesting the cyst-containing organs of the infected intermediate host. After ingestion, the protoscolices ❺ evaginate, attach to the intestinal mucosa ❻, and develop into adult stages ❶ in 32 to 80 days. Humans become infected by ingesting eggs ❷, with resulting release of oncospheres ❸ in the intestine and the development of cysts ❹, ❹, ❹, ❹, ❹, ❹ in various organs.

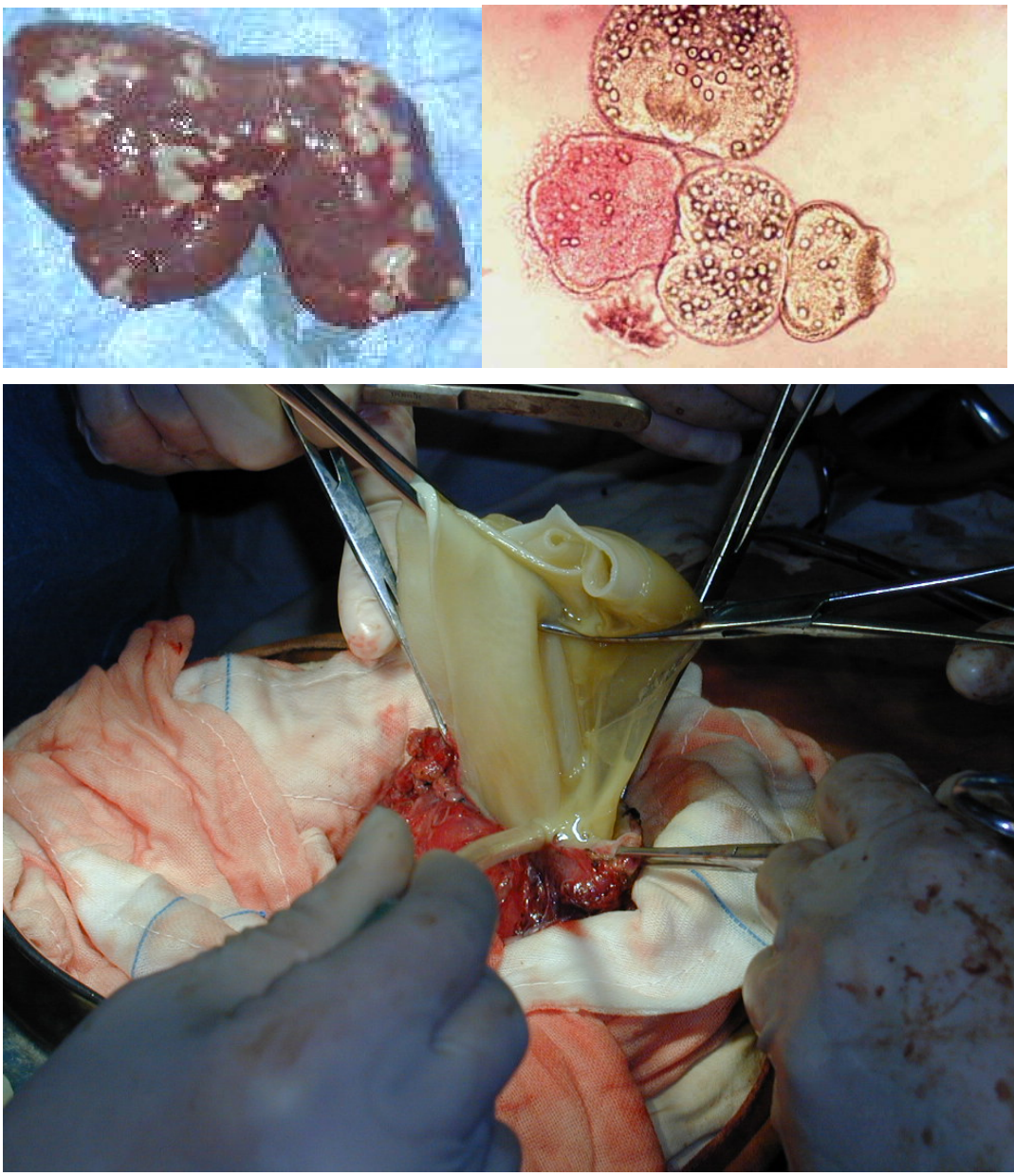


Fig. 1.2: CE cysts and *E. Granulosus* protoscoleces
(Above left, CE cysts in a sheep's liver, Bayinbuluk, Xinjiang, China, 2004; above right, *E. granulosus* protoscoleces from protoscoleces culture in XMUH, made by Dr. Chunfang Zhao; bottom, CE cysts taken during a human operation, XMUH, April 28th, 2001).

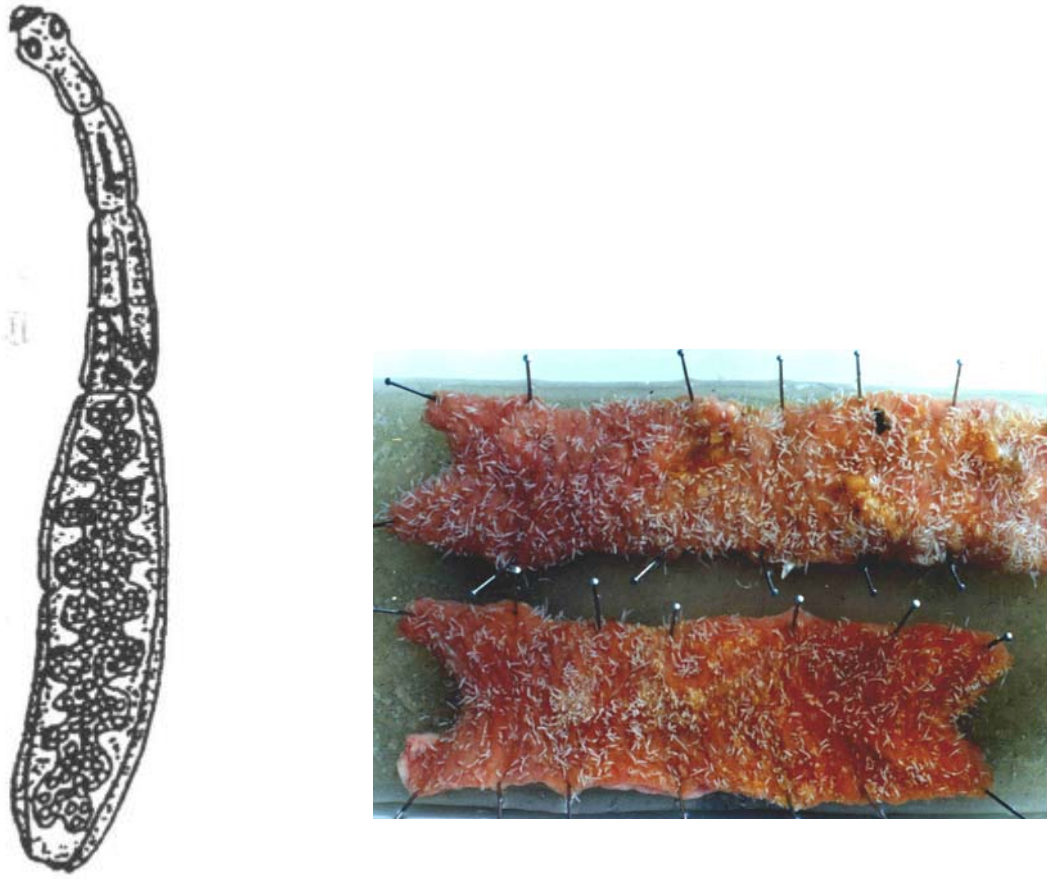


Fig. 1.3: *Echinococcus granulosus* adult worms
(The left diagram is from a book named Human Parasitology, Chinese, Shan XM Eds, 2001, and the right one was a photography of *E. granulosus* infected intestine of a dog, Xinjiang, China, Ding and Wen 2000).

1.3.2 *Echinococcus multilocularis*

The adult stage of *E. multilocularis* is characterized by its small size (length of up to 4.5 mm), a mean number of five segments, a sack-like uterus, and other morphological features, allowing its differentiation from *E. granulosus* and other *Echinococcus* species (WHO/OIE, 2001; Ding and Wen, 2000). The metacestode stage of *E. multilocularis* develops in the intermediate host (99% in liver) by forming aggregates of grape-like lesions composed of many smaller cysts or vehicles (alveolar hydatid) with a jelly-like matrix that enlarges by external budding off of germinal cells or microvesicles. Alveolar hydatid cysts have no host fibrous outer capsule, and they invade and may eventually destroy normal tissue in a host organ. Its development resembles a 'slower' style of malignant tumor, so called "colloid carcinoma", "tumor-like" disease or "parasite tumor" and "alveolar liver" in some endemic areas of China (Ding and Wen, 2000; Wen et al. 2001).

Transmission of *E. multilocularis* occurs in a sylvatic cycle, which is sometimes linked via infected small mammals to domestic dogs (and possibly cats) (Fig. 1.4). In the typical sylvatic cycle, foxes (mainly the Arctic fox [*Alopex lagopus*], and the red fox [*Vulpes vulpes*]) play a key role as definitive hosts and small mammals, mainly microtine rodents, act as intermediate hosts. In some areas, other wild canids, such as coyotes (*Canis latrans*), Tibetan fox (*V. ferrilata*), raccoon dogs (*Nyctereutes procyonoides*), and wolves (*Canis lupus*) can also serve as definitive hosts (WHO/OIE, 2001; Ding and Wen, 2000). Among potential intermediate hosts species of small mammals (more than 40) that are susceptible to *E. multilocularis* under natural conditions, members of the family Arvicolidae (voles and lemmings) and Cricetidae (hamsters, gerbils, and related rodents) are most important as intermediate hosts. Aberrant host animals (including domestic dogs, domestic and wild pigs, horses, monkeys, and large rodents (e.g. *Myocastor coypus)* (Eckert, 1996; Ohbayashi, 1996; Losson and Coignoul, 1997; Deplazes, 2001) and humans can also become infected with the metacestode stage, which has the potential to cause alveolar echinococcosis (AE), one of the most lethal helminthic infection in humans (Fig. 1.5). Although some variation between *E. multilocularis* isolates from North America and Eurasia has been described, there is little evidence so far major for sub-specific genetic differences (Haag et al., 1997, Eckert, 2004, Nakao et al., 2006). This is in accordance with the fact that *E. multilocularis* in various regions, including large areas of the northern hemisphere (Asia, Europe, and North America), appears to be equally infective to humans (Eckert, 1999, 2004; Vuitton 2003; Kimura, 1999; Schantz, 1995, 1996).

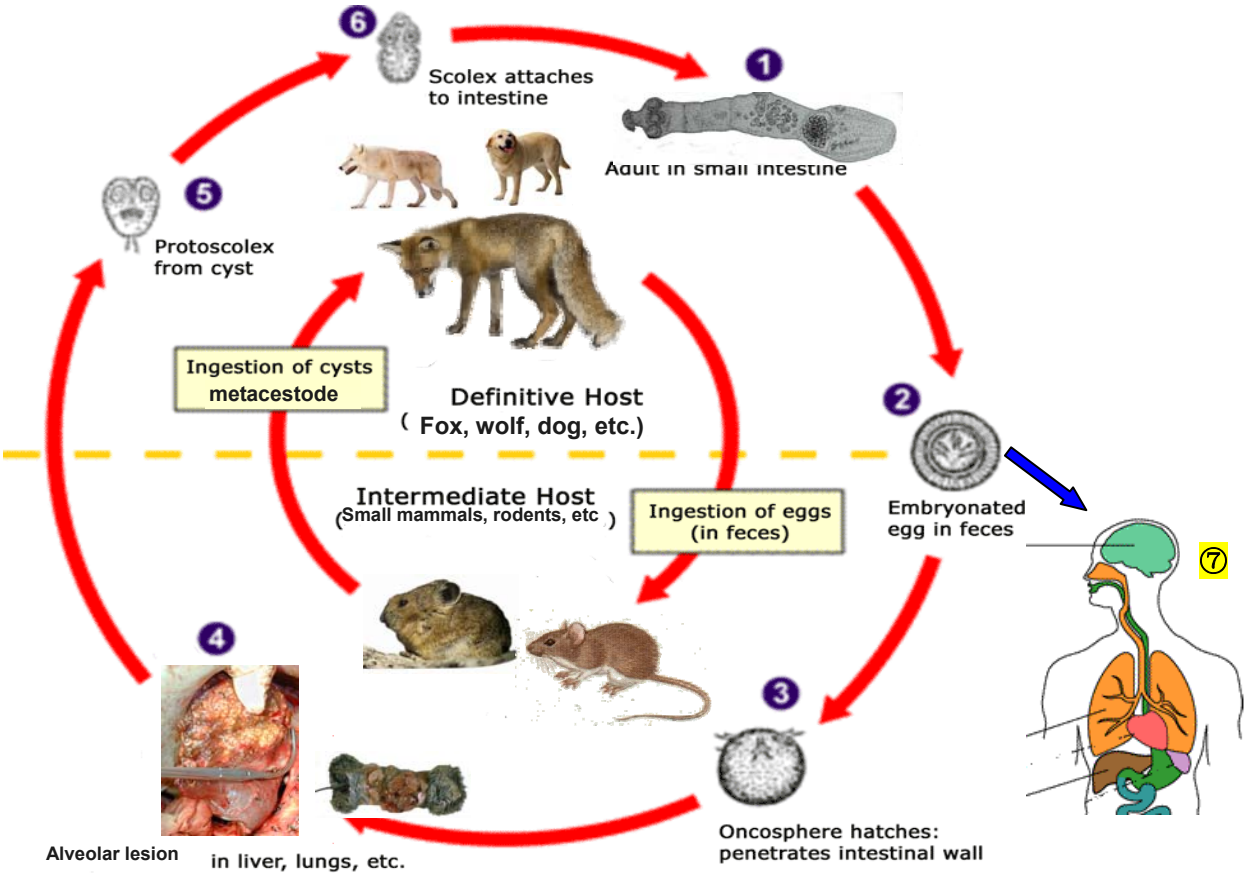


Fig. 1.4: Life cycle of *E. multilocularis.*
Image modified from http://www.dpd.cdc.gov /dpdx /HTML /ImageLibrary /Echinococcosis_il.htm).
(1) *E. multilocularis* is a small tapeworm (1, 2-4, 5 mm in length) that parasites carnivores (fox, dog and wolf) which as definitive hosts. (2) The adult tapeworm, consisting of 2 to 6 proglottids, lives attached to the luminal surface of the small intestine in the definitive hosts. The terminal proglottid contains mature eggs. (3) The embryonated eggs, the infectious stage, are long-lived and highly resistant to high and low temperature (more than 50ºC and down to -40ºC). The mature eggs are shed with faeces and are spread in the environment. It is assumed that the intermediate host acquires the infections through the ingestion of contaminated environment. (4) When the intermediate hosts (predominantly rodents or other small mammals, or, accidentally, humans) ingest eggs, the onchosphere hatches from the egg in the duodenum. (5) The activated oncosphere penetrates the small intestine, enters blood vessels and reaches primarily the liver via the portal vein. In the liver the oncosphere proliferates into the metacestode surrounded by an inner germinal membrane and an outer laminated layer. (6) The lifecycle is completed when an intermediate host, carrier of viable protoscoleces within the cysts, is devoured by a definite host. (7) Humans become infected by ingesting eggs 2, with resulting release of oncospheres 3 in the intestine and the development of lesions 4 in mainly liver (99%), lung and brain.

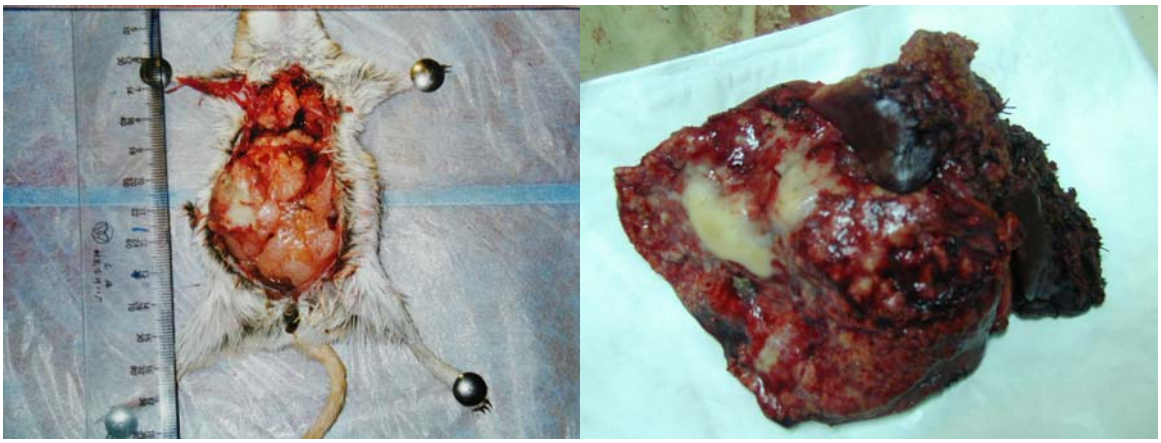


Fig. 1.5: Alveolar echinococcosis (AE) in intermediate host
(Left, experimentally infected gerbil with *E. multilocularis* from the Animal Center of XMUH); right, an AE lesion from human liver (above) after surgery in the XMUH.

1.4. Global distribution of *Echinococcus* species and echinococcosis

1.4.1 *Echinococcus granulosus*

E. granulosus is geographically by distributed worldwide and involves almost all continents. Highest prevalence occurs in parts of Eurasia (especially in China, Kazakhstan, Russian Federation and adjacent Independent States), Mediterranean countries, North and East Africa, Australia, and South America (WHO/OIE, 2001). In the UK, the parasite has a restricted distribution, being found mainly in mid and southern Wales (Williams, 1976; Staullbaumer et al., 1986; Jones and Walters, 1992; Richards et al., 1995). In Europe, zoonotic strains of *E. granulosus* are present in every country with the possible exceptions of Ireland, Iceland and Denmark. It is most intensely endemic in the Mediterranean areas and parts of Eastern Europe such as Bulgaria and Romania (WHO/OIE, 2001). In Asia the parasite is highly endemic in large parts of north and west China and is an important re-emerging zoonosis in the former Soviet Republics in Central Asia (Wang 2000; WHO/OIE, 2001; Craig 2003, Torgerson, 2003). The parasite is also found throughout the Indian Subcontinent and the Middle East. In Africa, *E. granulosus* is widespread and is a particular problem in northern African countries such as Tunisia, Morocco, Libya and Algeria. South of the Sahara the parasite is of specific concern in certain locations such as Turkana District in Kenya. In North America *E. granulosus* is found in Canada and Alaska, but seems to assume mainly a sylvatic cycle involving wolves and cervids. In the continental USA, the parasite is endemic in a few foci such as traditional pastoral Native-American

communities in Utah and California. In South America the parasite is widely distributed, particularly in Argentina, Chile, Uruguay, southern Brazil and the Peruvian Andes (Schantz, 2006). In Australia the parasite is common in domestic sheep-dog and sylvatic dingo-macropod cycles, the latter with over 25% of dingoes and up to 65% of macropod marsupials infected (Jenkins and Morris, 1995; Jenkins, 2002). In some more developed countries, due to the application of successful control programmes, CE has become less prevalent. In Iceland, New Zealand, Tasmania and southern Cyprus the parasite has been effectively eradicated (Economides and Christofi, 2002; Craig and Larrieu, 2006). In many poorer parts of world, however particularly where sheep husbandry is an important agricultural industry, CE remains widespread (Craig et al, 2007).

1.4.2 *Echinococcus multilocularis*

Echinococcus multilocularis, commonly known as the fox tapeworm, can be found in areas of central and northern Europe, northern Asia, and parts of North America. It has also been proposed that *E. multilocularis* may occur in parts of northern Africa, but currently there is not enough information to substantiate this claim (Schantz et al., 1995). The life cycle of *E. multilocularis* is primarily sylvatic. The red fox (*Vulpes vulpes*) is the most well known host but the arctic fox (*Alopex lagopus*), the coyote (*Canis latrans*), the wolf (*Canis lupus*), the raccoon-dog (*Nyctereutes procyanoides*), the sand fox (*Vulpes corsac*), and the Tibetan fox (*Vulpes ferrilata*) are all known definitive hosts, depending on geographic location. Other canids (including domestic dogs), and occasionally felids, can also be definitive hosts if they be come infected through the ingestion of an intermediate host harboring an infective metacestode. The principal intermediate hosts include rodents of the family Arvicolidae, with a number of reports of infection in the Sciuridae, Cricetidae, Dipodidae and Muridae; some of which maybe important locally. Lagomorphs of the family Ochotonidae are frequently infected in parts of China. There have been occasional reports of infections in insectivores such as the Soricidae and Talpidae situation in China (Torgerson and Budke, 2003).

1.4.3 *Echinococcus* and echinococcosis in the People's Republic of China

1.4.3.1 *Echinococcus granulosus* and cystic echinococcosis (CE)

Echinococcus granulosus is endemic in northwest of the People's Republic of

China. Patients with cystic echinococcosis (CE) have been recorded in 21 of People Republic of China's 31 provinces, municipalities and autonomous regions (covered approximately 87% of Chinese territories) and it is a major public health problem in several north-western provinces and autonomous regions (Fig. 1.6 to Fig. 1.8). The prevalence of *Echinococcus granulosus* was showed decrease from west to east and *Echinococcus multilocularis* appeared overlap in some area (Wen 1997, WHO/OIE 2001) (Fig. 1.6, Fig. 1.9).

Fig. 1.6: Initial national survey of human echinococcosis cases (10,790) in P. R. China from 2004-2008 (Ministry of Health, China. 2005 and 2009)
Human echinococcosis cases were indicated in each province/autonomous region involved in this study, and the proportion (%) of all these HD cases (10790) were shown in high endemic areas as well.

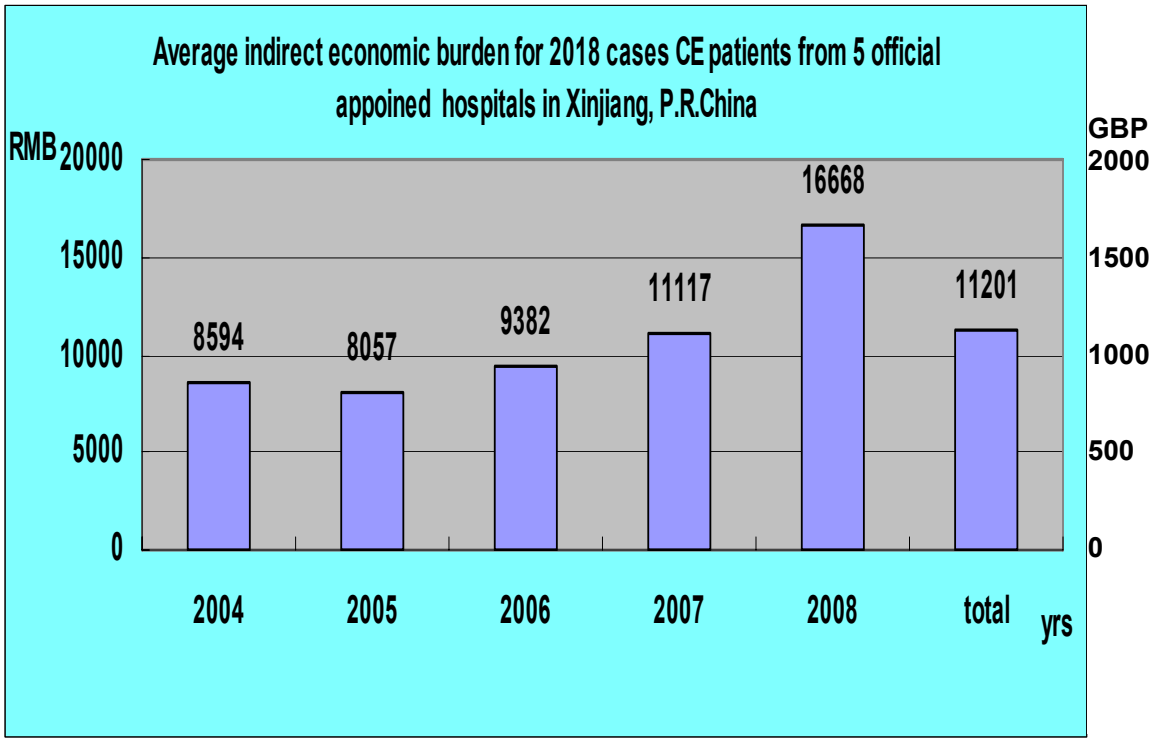


Fig. 1.7: Average indirect economic burden for 2018 cases CE in-hospital patients from 5 officials appointed hospitals in Xinjiang, P. R. China (Wang et al., 2010) (10000RMB would be approximately 800 to 1000 sterling pounds)

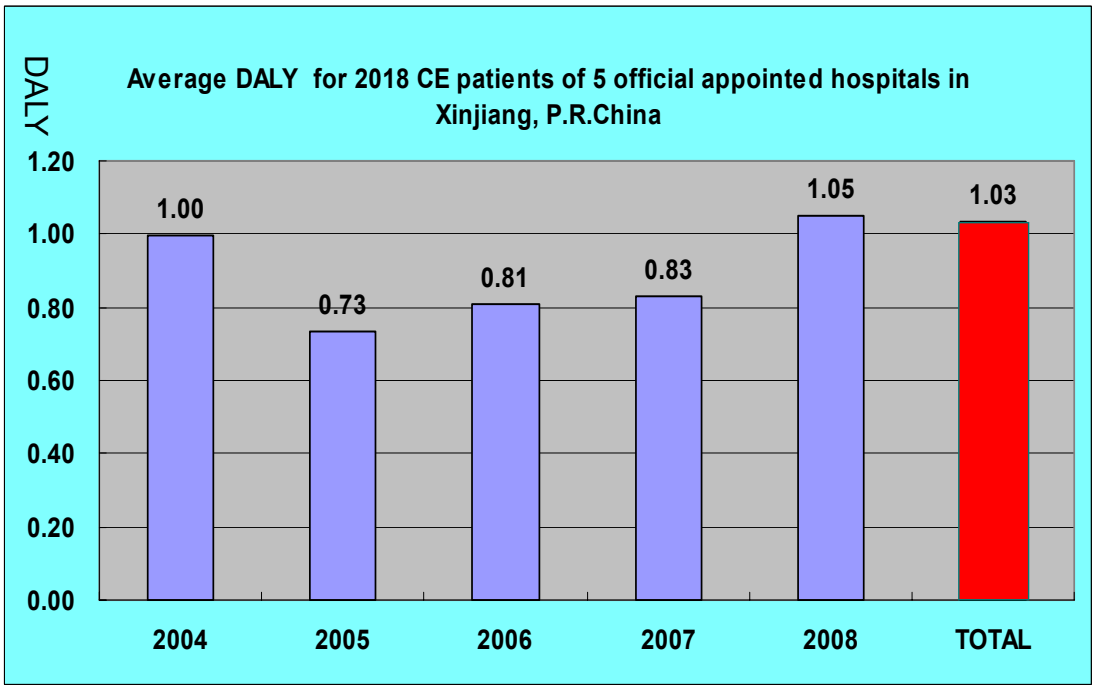


Fig. 1.8: Average DALY for 2018 cases CE in-hospital patients from 5 officials appointed hospitals in Xinjiang, P. R. China (Wang et al., 2010)

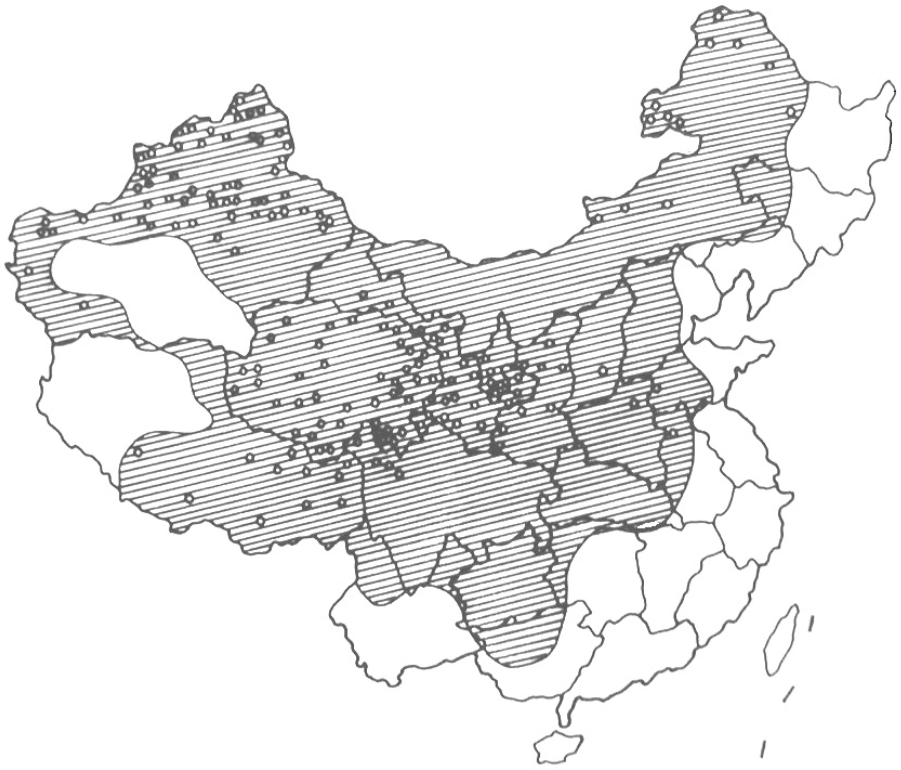


Fig. 1.9: Endemic areas of human cystic echinococcosis (marked with ▨) and alveolar echinococcosis (sporadic dots "∘") in China (Ding and Wen, 2000)

Main lifecycle of *Echinococcus granulosus* in China is mainly domestic dog and herbivores (i.e. Sheep, goat, cattle, yak, horse, camel, pig and farmed red deer (*Cervus elaphus*)) (Schantz et al., 1995; WHO/OIE 2001). Main strain of *Echinococcus granulosus* is the common sheep strain (G1) and reports showed the camel strain (G6) found in Xinjiang (Zhang 1998).

Human cystic echinococcosis (CE) was firstly reported in China in 1905 and since then at least 26,065 cases have been reported in China during four decades between 1951-1990 (Yu et al., 1994; Chai, 1995), with the greatest surgical incidence recorded in Xinjiang, followed by Ningxia, Qinghai, Sichuan, Gansu, Tibet, Inner Mongolia, Yunnan and other provinces. Prevalence baseline investigation organized by Chinese Ministry of Public Health showed 1.08% human CE diagnosed by abdominal ultrasound in 12 provinces or autonomous regions, mean financial burden 2700 RMB (approximately GBP 270) per case and estimated 380,000 human CE cases totally in 2005.

Xinjiang Uygur Autonomous Region (XUAR). XUAR is in the northwest China with 16 million populations. More than half (58.4%) of hospital CE cases in China were recorded in XUAR (Jiang 1991). Human CE cases were recorded all over the region, which consisted of all of 12 prefectures including 5 minority ethnic autonomous prefectures. Main CE endemic area is in north Xinjiang where from Tianshan Mountain to Altai Mountain including Bayinguoleng Mongolian Autonomous Prefecture, Yili Kazak Autonomous Prefecture, Tacheng Prefecture, Altai Prefecture, Changji Prefecture and Hami Prefecture (Fig. 1.10, Fig. 1.11). Hospital records indicated 16,663 cases of CE were surgical treated in Xinjiang during 1951 to 1991 (National Hydatid Disease Center of China, 1993) (Fig. 1.10). Human CE were more endemic in Tianshan Mountain (2.23% 47/2103) and Altai Mountain (2.28% 41/1420) than in Kunlun Mountain (0.6% 6/1000) from a study in 4 counties community screening (Wei 1994). 4407 CE cases were recorded in Xinjiang Medical University Hospital from 1957 to 1997 and annual cases curve showed to be increasing during last decade (Qiu et al., 1999). 1126 hospital CE cases were registered in north 4 counties in Tacheng Prefecture (Qi et al., 1995). 1965 hospital CE cases were reported in Yili Valley which consists of 8 counties and 1 city from 1993 to 2003 (Gao et al., 2005). Communities screening data showed 2.22% (45/2044) CE prevalence in Wulasitai Commune of Nileke County, Yili Valley (Dingmulati et al., 2005); 4.5% (34/755) and 1.91% (17/889) in Habahe County (Song et al., 1999; Zhao et al., 2003), 5.78% (31/536) in Qinghe County (Zhao et al., 2003), Altai Prefecture; 2.4% (49/1844) in Hobuksar County, Tacheng Prefecture (Wang et al., 2001) (Table 1.2).

Table 1.2: Human cystic echinococcosis (CE) cased reviewed in Xinjiang AR

Locations	Duration	Hospital records	Community Prevalence	Refs.
Xinjiang	1951- 1991	16663		NHDC [a] of China, (1993)
Tianshan Mountain Altai Mountain Kunlun Mountain	1994		2.23%, 47/2103; 2.28%, 41/1420 0.6%, 6/1000	Wei, 1994
XMUH [b]	1957-1997	4407		Qiu et al., 1999
Yili Valley	1993-2003	1126		Gao et al., 2005
Yili /Nileke	2004		2.22%, 45/2044	Dingmulati, 2005
Altai / Habahe	1998/2002		4.5%, 34/755 1.91%, 17/889	Song et al., 1999; Zhao et al., 2003
Altai / Qinghe	2002		5.78%, 31/536	Zhao et al., 2003
Tacheng / Hobuksar	1995- 1996		2.4%, 49/1844	Wang et al., 2001

[a] NHFC: National Hydatid Disease Center
[b] XMUH: Xinjiang Medical University Hospital

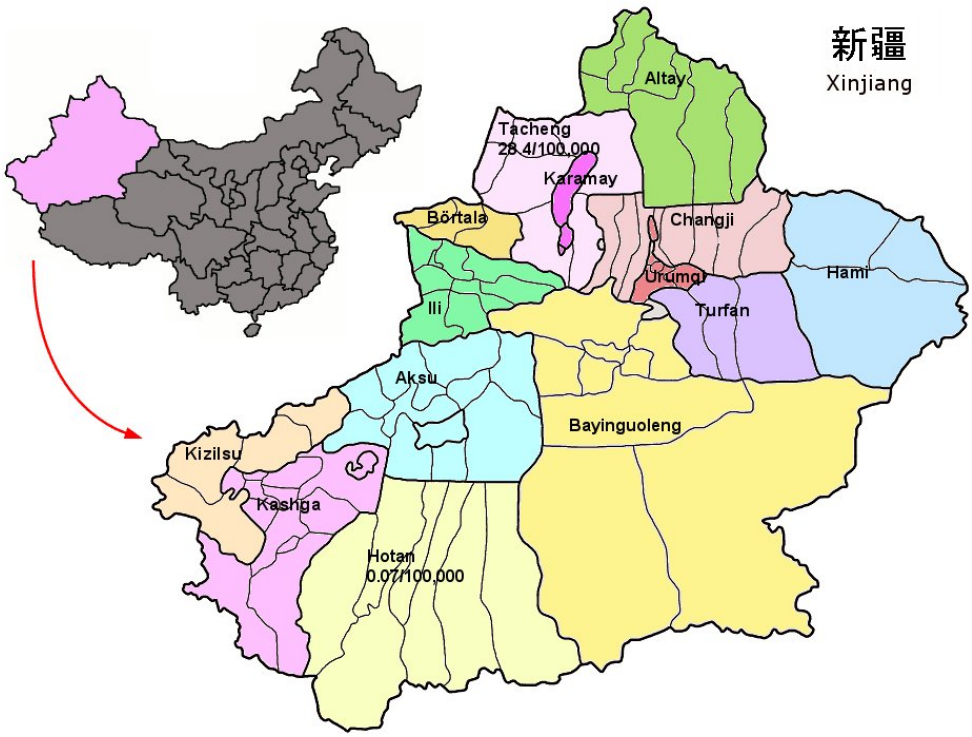


Fig. 1.10: *Echinococcus granulosus* is high endemic in Xinjiang Uygur Autonomous Region. CE cases could be found in all 12 prefectures with higher prevalence in north than in south of Xinjiang. Average annual incidence in 1990 was 8.7 cases per 100,000 populations in Xinjiang, which the lowest was 0.07 in Hotan prefecture and highest 28.4 in Tacheng prefecture (data from Menghebate et al., 1993; WHO/OIE, 2001).

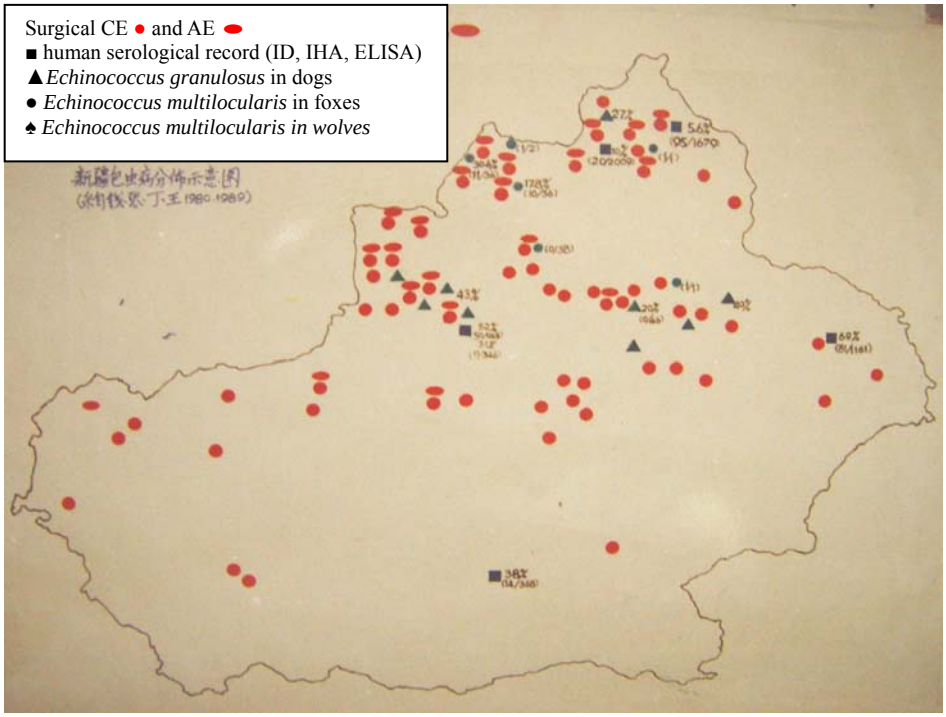


Fig. 1.11: Human or animal infections with *Echinococcus granulosus* and *Echinococcus multilocularis* recorded in Xinjiang in 1980s (Ding, 1986-1989)

Ningxia Hui Autonomous Region. 2134 human CE cases were recorded in all of Ningxia's five prefecture-level cities: Guyuan, Shizuishan, Wuzhong, Yinchuan (capital city), and Zhongwei with higher endemic and overlap with human AE in three counties (Xiji, Guyuan, Haiyuan) of Guyuan Prefecture (Yang et al., 2005, 2006). A community study in Xiji, Guyuan and Longde in Guyuan Prefecture showed 0-7.4% (mean 1.6%) human CE prevalence and 2% in Xiji County (Yang et al.,2006) (Fig. 1.12).

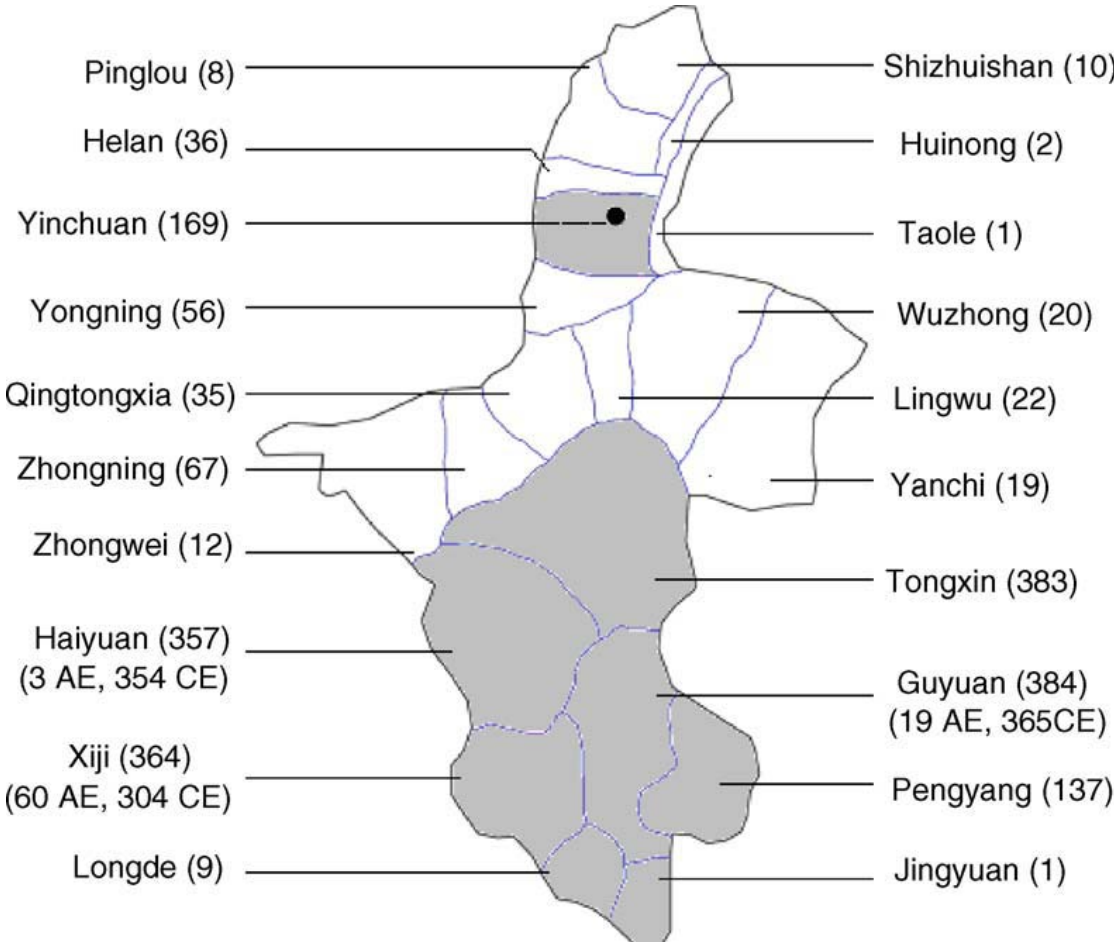


Fig. 1.12: Geographic distribution of confirmed AE and CE cases (numbers in parenthesis) from hospital records (1985–2001) in Ningxia Hui Autonomous Region, China. The shaded areas show the locations of hospitals involved in the survey (Yang et al., 2006).

Gansu Province. 3233 human hospital CE cases were recorded in 13 prefectures and 52 counties (60%) exclude Jiayuguan City in Gansu Province from 1951 to 1990, and 1160 CE cases from 1991 to 1995. Main endemic areas included Jingyuan County (Baiyin prefecture), Huan County (Qingyang Prefecture), Gulang County and Tianzhu Tibetan Autonomous County (Wuwei Prefecture), Jiuquan City (Jiuquan Prefecture), Zhang County (Dingxi Prefecture) and Yongdeng (Lanzhou Prefecture) (Wang et al., 1995; Wang and Zhang, 2000) (Fig. 1.13).

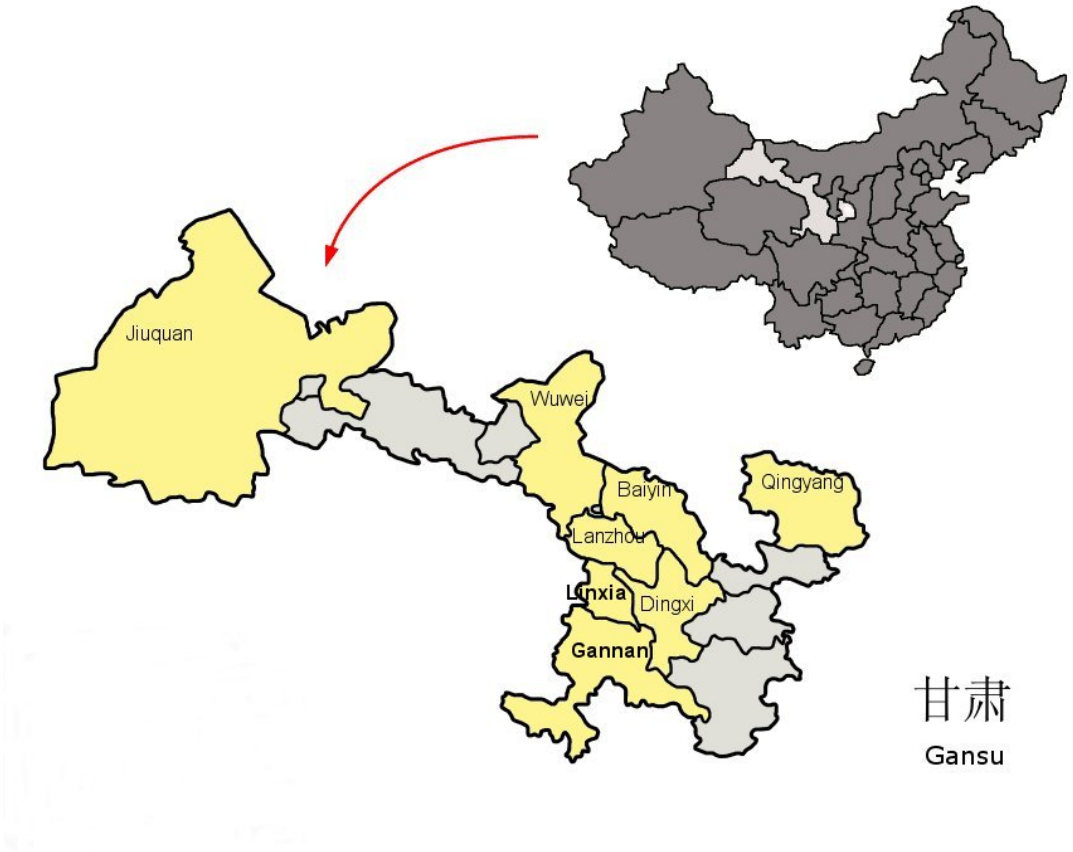


Fig. 1.13: Higher endemic prefectures (yellow areas) for *Echinococcus granulosus* in Gansu Province (data from Wang et al., 1995; Wang and Zhang, 2000).

Qinghai Province. Qinghai is administratively divided into one prefecture-level city Xining, one prefecture Haidong, and six autonomous prefectures: Golog, Haibei, Hainan, Huangnan, Yushu Tibetan Autonomous Prefecture and Haixi Mongolian and Tibetan Autonomous Prefecture. The higher endemic areas for CE in Qinghai were Gangcha County (4.0%) in Haibei AP, Gonghe County (1.8%) in Haihan AP (Wu et al., 2006), Tongren County (1.6%) and Zeku County (7.5%) in Huangnan AR (Liu et al., 2006; Han et al., 2006), Gande County (5.77%), Jiuzhi

County (5.4%) and Banma County (6.1%) in Guoluo AP (Han et al., 2006; Wu et al., 2006), Chengduo County (5% to 9.7%) in Yushu AP (He et al., 2006; Han et al., 2006) (Fig. 1.14).

Fig. 1.14: Human CE endemic areas according community screening data (prevalence) in Qinghai Province (data from Wang et al., 2006, Ma et al., 2006; Wu et al.,2006)

Sichuan Province. Ganzi Tibetan Autonomous Prefecture and Aba Tibetan and Qiang Autonomous Prefecture in west Sichuan are the main endemic area for both CE and AE. Higher endemic area were showed 4.8% (60/1291 in 1998) to 6.8% (216/3199 in 2005) in Shiqu County, 2.04% in Baiyu and Seda County, 0.91% (25/2748 in 1998) in Ganzi County, all in Ganzi Tibetan Autonomous Prefecture (Qiu et al., 2000; Yu et al., 2005; Li et al., 2005). Hospital diagnosed 610 cases of human echinococcosis were recorded in a hospital in Aba Prefecture from 1992 to 2000, which among them 562 cases got surgical treatment including AE 92, CE 347, abscess 93 and calcificated lesion 30 cases (Renzhen et al., 2006). Another report said 35 CE in one year (Oct 2004 – Aug 2005) in Aba Prefecture Hospital (Liu 2006).

Tibet Autonomous Region. Human CE cases were recorded in all of its 7 prefectures and hospital cases showed much higher in Naqu, Lhasa, Shannan and Chamdo (Gong et al., 2001) (Fig. 1.15). An early hospital data showed 94 echinococcosis (not sure CE or AE) in Chamdo Prefecture in 1993 (Luo and Zhao, 1993). And 80 cases of human echinococcosis were reported in a hospital in Shannan ((Zhao et al., 2002). A community study in Naidong County of Shannan Prefecture showed 2.4% (81/3379) prevalence of human CE (Zhou and Xi, 2004). A study using Casoni test as first trial confirmed 48 CE cases by ultrasound in total 20160 populations in Mozugongka and Dangxiong County in Lhasa Prefecture (Hu et al., 1999).

Fig. 1.15: Tibetan Autonomous Region in People's Republic of China.

Inner Mongolia Autonomous Region. 675 CE cases were recorded in main hospitals of Huhehaote (capital) from 1986-1996 (Li et al., 1996). Bayinxile Qi in Xilingele Alliance was regarded with higher prevalence (1.08%) (Zhang et al., 1996)

1.4.3.2 *Echinococcus multilocularis* and Alveolar echinococcosis (AE)

The first five alveolar echinococcosis (AE) cases in China were reported in Xinjiang in 1965 (Yao, 1965, Wen 1997). Following reports showed human AE cases in Qinghai, Ningxia Hui Autonomous Region, Gansu, Aba and Ganzi Tibetan Autonomous Prefecture of Sichuan, and a few cases reported in Inner Mongolian Autonomous Region, Tibet Autonomous Region and Heilongjiang Province (Table 1.3) (Craig, 1992, 2004; Schantz et al., 1995; WHO/OIE, 2001; Vuitton et al., 2003; Ito et al., 2003). *Echinococcus multilocularis* infections were found in fox, Tibetan fox, wolf and even domestic dogs. *Microtus ilaeus* and

Arvicola terrestris Linnaeus in Xinjiang, pika (Tibetan plateau) were involved as an intermediate host role in *Echinococcus multilocularis* lifecycle.

Xinjiang Uygur Autonomous Region. Higher prevalence (3.9/100,000) were observed in Altai Mountain (Zhou et al., 2000) and also 84 hospital AE cases were recorded between 1993-2003 in Ili valley in Tianshan Mountain of Xinjiang Uygur Autonomous Region (Gao et al., 2005). 0.636% (13/2044) human AE were observed in a community survey in Nileke County of Ili valley (Dingmulati et al., 2005). 79 cases of human AE in 4486 hydatid disease records during 1957 and 1997 were treated in Xinjiang Medical University Hospital (Qiu et al., 1999).

Ningxia Hui Autonomous Region. Human AE cases were found in three counties (60 in Xiji, 3 in Haiyuan and 19 in Guyuan) in Guyuan Prefecture through Liupan Mountain area from 1985 to 2001 (Lin and Hong 1991;, Wang et al. 1991, Yang et al., 2006). A community study in 1980s showed 5.9% (141/2389) AE prevalence in Xiji County (Wang et al., 1991). Recent screening in Ningxia in 2001-2003 found 2.4% (88/3629) AE prevalence in Xiji, 0.5% (5/983) in Guyuan and no cases in Longde, all 3 counties are in Guyuan Prefecture (Yang et al., 2006).

Qinghai Province. 90 human AE cases from hospital records were reported from 1960-1991 (Xin, 1994) (another speaking are 111 from 1959-1998 by Wang et al., 2006; 143 from 1980-1992, Vuitton 2003). Several community studies had been reported *Echinococcus multilocularis* was endemic mainly in southeast Qinghai which showed 0.8% (10/1253) in Chengduo County, 2% (8/394) in Yushu County of Yushu Tibetan Autonomous Prefecture; 2.52% (39/1549) in Jiuzhi County, 0.71%(10/1403) in Gande County, 6.11% (65/1277) in Banma County in Guoluo Tibetan Autonomous Prefecture, 0.29% (3/1046) in Zeku County of Huangnan Tibetan Autonomous Prefectures (Wang et al., 2006).

Gansu Province. Human AE endemic area in south Gansu were focus on Zhang and Min County in Dingxi Prefecture from 1980s (Jiang 1981; Craig 1992, 2000; Vuitton 2003). Community study in 1994-1997 showed 3% (84/2482) incidence and overall prevalence of 4.1% (135/3331) in Zhang and Min County. Village (n=31) human AE prevalence rates varied from 0-15.8% which the latter was the highest report in an individual village (Craig et al., 2000).

Sichuan Province. Human cases of AE were found mainly in Shiqu, Ganzi, Seda, Baiyu Kangking County in Ganzi Tibetan Autonomous Prefecture and Nuoergai

County in Aba Tibetan and Qiang Autonomous Prefecture (Lin and Hong 1991; Qiu et al., 1999; Yu et al., 2005; Li et al., 2005). Community study showed highest prevalence of human AE was 2.96% (37/1291 in 1998) and 6.2% (198/3199 in 2005) in Shiqu County, followed by 1.79% in Baiyu and Seda County, 1.42% (39/2748) in Ganzi County (Qiu et al., 1999; Yu et al., 2005; Li et al., 2005).

Tibet AR. First two human AE were reported from Chamdo (Peng, 1988). 12 hospital AE samples were reported in 1993 in main hospitals in Lhasa city of Tibet Autonomous Region (Luo et al., 1993). Cases from Naqu, Lhasa and Chamdo and no more details were published. Two brain AE cases (one was secondary AE) were recorded respectively (Pu 1999; Yixijiacuo et al., 2001). Three human AE cases were reported in Linzhi Prefecture (Duan et al., 2006). So totally 19 human AE cases were reported in Tibet AR. Recently 11 human AE cases were found in a pilot study (n=242) in Dengqing County of Chamdo Prefecture.

Inner Mongolian AR and Heilongjiang. *Echinococcus multilocularis* infection in rodents has been reported in Inner Mongolian Autonomous Region (Tang et al.,2001, 2002, 2006) but just 1 hospital AE case were reported (Li, 1996) without details. 4 human AE cases in Heilongjiang Province were recorded in 1980s and no new report after that (Yu et al.,1994).

Mixed CE and AE individuals were reported in Xinjiang, Ningxia and Sichuan (Wen and Yang1997; Yang et al., 2005; Yu et al., 2005).

Table 1.3: Human alveolar echinococcosis (AE) cased reviewed in Western China

Locations	Duration	Hospital records	Community Prevalence	Refs.
Xinjiang / Altai	2000	3.9/100,00		Zhou et al., 2000
/ Yili	1993-2003	84		Gao et al., 2005
/ Yili/Nileke	2004		0.6%, 13/2044	Dingmulati, 2005
/ XMUH	1957-1997	79		Qiu et al., 1999
Ningxia / Xiji	1985-2001	60		Lin and Hong, 1991, Wang et al., 1991, Yang et al., 2006
/ Haiyuan	1985-2001	3		
/ Guyuan	1985-2001	19		
/ Xiji	1980s		5.9%, 141/2389	Wang et al., 1991
/ Xiji	2001-2003		2.4%, 88/3629	Yang et al., 2006
/ Guyuan	2001-2003		0.5%, 5/983	Yang et al., 2006
Qinghai	1960-1991	90		Xin, 1994
	1959-1998	111		Wang et al., 2006
	1980-1992	143		Vuitton, 2003
Yushu/Chengduo	2006		0.8%, 10/1253	Wang et al., 2006
/ Yushu	2006		2%, 8/394	Wang et al., 2006
Guoluo/ Jiuzhi	2006		2.52%, 39/1549	Wang et al., 2006
/ Gande	2006		0.71%, 10/1403	Wang et al., 2006
/ Banma	2006		6.11%, 65/1277	Wang et al., 2006
Huangman/Zeku	2006		0.29%, 3/1046	Wang et al., 2006
Gansu				
Zhang & Min	1994-1997		4.1%, 135/331	Craig et al., 2000
Sichuan				
Shiqu	1998		2.96%, 37/1291	Qiu et al., 1999
	2005		6.2%, 198/3199	Yu et al., 2005; Li et al., 2005
Baiyu & Seda	2005		1.79%	Yu et al., 2005; Li et al., 2005
Ganzi	2005		1.42, 39/2748	Li et al., 2005
Tibet				
Not sure, Lhasa, Naqu, Shannan and Changdu, Linzhi and Sichuan Origin	1960-2006	19		Peng, 1988; Luo et al., 1993; Pu 1999; Yixijiacuo et al., 2001; Duan et al., 2006
Inner Mongolia	1996	1		Li, 1996
Heilongjiang	1980s	4		Yu et al.,1994

XMUH: Xinjiang Medical University Hospital

1.4.3.3 Risk factors for human CE and AE in China

XUAR. Hospital cases and community studies indicated that the risk factors for human CE might be age over 20s, occupation as farmers and herdsmen, Ethnity as Mongolian, Xibo, Kazak and Han Chinese (National Hydatid Disease Center of China, 1993; Qiu et al., 1999; Gao et al., 2005; Dingmulati et al., 2005; Song et al., 1999; Zhao et al., 2003; Wang et al., 2001). Meanwhile, female, countryside residents, dog ownership, poor disease knowledge, home-slaughter of livestocks, were also involved with higher CE prevalence. As for serological positives, female, different areas (ie, Tacheng Prefecture, Tianshan Mountain), occupation as herdsmen, ethnity as Han, Mongolian were regarded as main risk factors in above studies (National Hydatid Disease Center of China, 1993; Wei 1994; Qi et al., 1995). Human AE was relatively common occurred in the certain areas around Altai, western Junggar, and Tianshan mountain ranges, semi-nomadic groups, Kazakh of Mongol origin, and also to be correlated with aspects of the local climate (Zhou et al., 2000).

Ningxia Hui AR. Hospital based study showed sheep farming, home-slaughter of livestock and lack of piped water were higher risk factors for human CE (Yang et al., 2006). Ages older than 30 yrs, farmers and dog owners were related to higher human CE and AE prevalence, however, Hui ethnic group, female were mainly risk factors for human AE (Yang et al., 2005)

Gansu. Dog and livestock ownership, drinking water contaminatiion were main risk for human CE in Gansu (Wang and Zhang, 2000). Female, age group 20-59 years, a long period dog ownership and close contact were the main risk factors for human AE, meanwhile the ecological features were involved for the risk, such as village surround (\50% ratio scrub:grassland total area, might due to a process of deforestation), density indices of voles, semi-domestic (or synanthropic) cycle of *E. multilocularis* (Craig et al., 2000).

Qinghai. Main risk factors for human CE in Qinghai Plateau from epidemiological studies were analyzed. With the occupation of livestock husbandry, traditional normad lifestyle, lower education level, dogs ownership, increased stray dogs, drinking surface water, unwashed hand and gender as female, were involved with both CE and AE. (Wang, 2004).

Sichuan. In Tibetan areas of western Sichuan, ages below 19 years old, nomadic lifestyle, playing with dogs, hygienic behariors, and yaks or sheep ownership,

were thought to increase the risk of suffering from CE (Wang et al., 2001). But human AE cases showed higher prevalence in ages group over 19 yrs old. Study in Shiqu County showed female, pastoral herdsmen, increased ages, the number of owned dogs, frequency of dog contaction and source of drinking water were risk factors for both CE and AE (Li et al., 2005).

Tibet AR. Just a paper mentioned that the poor hygienic habits were involved with hydatidosis in Tibet AR, such as unwashed hands, eating uncooked meat, drinking surface water, close contact with dogs and dog ownership (Shen et al., 2004).

Inner Mongolia AR. The community studies showed the lower education group, herdsmen, housewife, Mongolian had a relative higher risk for hydatidosis (serology and ultrasound) in Inner Mongolian AR (Zhang et al., 2007). Dog ownership, home slaughter for livestock, suspicious livestock organ to dogs became the main reason for human hydatidosis in Xilinhaote area (Liu et al., 2009).

1.5. Diagnosis of echinococcosis

1.5.1 Human echinococcosis

1.5.1.1 Clinical symptoms

Human CE often occurs as a fluid-filled cyst, single or multiple, with or without daughter cysts in most internal organs of human but especially the liver (around 70%), lung (around 20%), peritoneal cavity, spleen, kidney, brain, bone, and also muscle or subcutis (Ding & Wen, 2000, WHO/OIE, 2001). The initial phase of primary infection is always asymptomatic, and small (<5cm) well-encapsulated cysts located in organ sites, where they do not induce major pathological problem, may remain asymptomatic for many years or even permanently (Ammann & Eckert, 1996; Pawlowski, 1997; WHO/OIE 2001). Disease symptoms arise as the cysts grow bigger and start eroding and/or putting pressure on blood vessels and organs. Hepatomegaly, pain, or with cholestasis and jaundice often occur in human liver CE, symptoms similar to secondary biliary cirrhosis, liver abscess, calcified lesion, portal hypertension, Budd-chiari syndrome, cyst rupture, biliary fistula, etc may occur. Chest pain and chronic cough may happen in lung CE, also with pneumothorax, pleuritis, lung abscess, etc. Pains, tumor-like growth and other symptoms may anaphylactic vary in CE cases within different organ

locations. Large cysts can also cause shock if they happen to rupture (WHO/OIE, 2001).

Cases of human AE are characterized by an initial asymptomatic incubation period of 5 to 15 years duration and a subsequent chronic course. AE mainly occurs as tumor-like lesions mostly in the liver (>99%), with possible lung and/or brain secondary multiple organ involvement in late stages (Ammann et al, 1996, Sato, et al 1993, WHO/OIE, 2001, McManus 2003). AE lesions are typically tumor-like multivesicular, infiltrating structures consisting of numerous small vesicles embedded in stroma of connective tissue; the larval mass usually contains a semisolid matrix rather than clear fluid. The fatality rate of untreated or inadequately treated AE is high, 94% died within 10 years after diagnosis in a patient series described in Germany (Ammann and Eckert, 1995, 1996; WHO/OIE, 2001). Symptoms of AE are primarily cholestatic jaundice (about a third of the cases) and/or epigastric pain (about a third of the cases). In the remaining third of patients, AE may be detected incidentally during medical examination for symptoms such as fatigue, weight loss, hepatomegaly, or abnormal routine laboratory findings (Ammann and Eckert, 1996; Vuitton, 1996; WHO/OLE, 2001).

1.5.1.2 Imaging diagnostic techniques

Individual CE and AE cases are best clinically diagnosed using various imaging techniques such as ultrasonography (US), standard radiology (X-ray), computerized tomography (CT) and/or magnetic resonance imaging (MRI). Aetiological confirmation or support may derive from specific serum antibody detection. Generally, portable US and serological testing has been applied in the diagnosis for human echinococcosis in epidemiological studies because other imaging procedures are often not readily available in resource-poor isolated communities (Bartholomat et al., 2000; Craig et al., 2001).

A recent criteria for ultrasound classification of CE has been published by the WHO Informal Working Group on Echinococcosis (WHO, 2003), which has been suggested for use in both field epidemiological studies as well as for clinical investigators. This classification intends to follow the presumed natural history of CE and starts with undifferentiated simple cysts (CL), as presumably hydatid cysts evolve from these structures. These simple cysts, however, may be due to a number of different aetiologies (parasitic lesions, congenital disorders, biliary cysts or neoplasms) and, therefore, require further diagnostic tests to reveal their

identity. As their origin is uncertain they are not given the designation of a CE type lesion, and, in the proposed classification, should be recorded as cystic lesions (CL). The first clinical group starts with cyst types CE 1 and 2 and such cysts are considered active and usually fertile cysts containing viable protoscoleces and CE2 with daughter cysts. CE Type 3 is a cyst entering a transitional stage where the integrity of the cyst has been compromised either by the host or by chemotherapy and this transitional stage is assigned to the second clinical group. The third clinical group comprises CE Types 4 and 5 which are considered inactive cysts which they have normally lost their fertility and are degenerative. There is a uniform approach and principles of treatment currently recommended for each CE cyst type (Gharbi et al., 1981, WHO/OIE, 2001; Wang et al., 2003). Other classifications have also been considered for example, it was suggested that type-size-number (TSN) of CE types be described according to clinical, epidemiology and follow-up studies (Wang et al, 2003). US classification could also be considered to represent a natural history of hydatid cyst development, and provide the dynamic transmission information in community screening (Table 1.4) (Rogan et al., 2006).

Table 1.4: Comparison of Gharbi, WHO and 'TSN' ultrasound classifications for human cystic echinococcosis cases from community and clinical surveys (Wang, et al, 2003)

Gharbi [a]	WHO [a]	TSN [c]	Description
Type I	Type CL	T0	Univesicular without pathognomonic signs
Type I	Type CE1	T1	Univesicular with pathognomonic signs
Type II	Type CE3	T2	Sagging or floating laminated membrane
Type III	Type CE2	T3	Cysts containing daughter cysts
Type IV	Type CE4	T4	Solid mass or mixed cysts
Type V	Type CE5	T5	Partial or full calcifications

a Gharbi et *al.* (1981). b WHO/OIE (2001).
c TSN, type, size and number (see Results).

In AE patients, the liver is usually enlarged through many years of lesion growth and development. In US and CT imaging, lesions are characterized by heterogenous hypodense masses, often associated with a central necrotic cavity. The lesion contours are irregular and there is no obvious cyst wall to adjoin normal liver tissue. Calcifications are often found inside and/or around the lesion and

exhibit a typical pattern in regard to shape and distribution: clusters of microcalcifications or irregular plaque-like calcified foci are located in the central or peripheral parts of the lesions (Liu 1999; WHO/OIE, 2001; Kern et al 2006). A classification (PNM) for human AE based a parasite location (P), neighbor involvement organ (N) and occurrence of metastases (M) has been recommended (Table 1.5 and 1.6) (Eur Echino Reg, 1998, WHO/OIE, 2001, Kern, 2003, 2006)

Table 1.5: PNM system for classification of human alveolar echinococcosis

Classification of findings	
P:	**Hepatic localisation of the Parasite**
P X:	Primary tumor cannot be assessed
P 0:	No detectable tumor in the liver
P 1:	Peripheral lesions without proximal vascular and/or biliar involvement
P 2:	Central lesions with proximal vascular and/or biliar involvement of one lobe[a]
P 3:	Central lesions with hilar vascular or biliar involvement of both lobes and/ or with involvement of two hepatic veins
P 4:	Any liver lesion with extension along the vessels[b] and the biliary tree
N:	**Extra hepatic involvement of neighbouring organs**
Diaphragm, lung, pleura, pericardium, heart, gastric and duodenal wall, adrenal glands, peritoneum, retroperitoneum, parietal wall(muscles, skin, bone), pancreas, regional lymph nodes, liver ligaments, kidney	
N X:	Not evaluable
N 0:	No regional involvement
N 1:	Regional involvement of contiguous organs or tissues
M :	**The absence or presence of distant Metastasis**
Lung, distant lymph nodes, spleen, CNS, orbital, bone, skin, muscle, kidney, distant peritoneum and retroperitoneum	
M X:	Not completely evaluated
M 0:	No metastasis[c]
M 1;	Metastasis

a For classification, the plane projecting between the bed of the gall bladder and the inferior vena cava divides the liver in two lobes.
b Vessels mean inferior vena cava, portal vein and arteries.
c Chest X-ray and cerebral CT negative.
(Eur Echino Reg, 1998, WHO/OIE, 2001, Kern, 2003, 2006)

Table 1.6: PNM stage grouping of alveolar echinococcosis

Staging of AE	PNM classification		
Stage I	P1	N0	M0
Stage II	P2	N0	M0
Stage IIIa	P3	N0	M0
Stage IIIb	P1– 3	N1	M0
	P4	N0	M0
Stage IV	P4	N1	M0
	Any P	Any N and/or	M1

(Eur Echino Reg, 1998, WHO/OLE, 2001, Kern, 2003, 2006)

1.5.1.3 Laboratory diagnosis for echinococcosis

As a rule, routine laboratory haematology tests show non-specific results. Marked eosinophilia may however occur in cases of cyst rupture. Immunodiagnostic procedures for serum antibody detection are generally used for the aetiological confirmation of imaging structures suggestive for CE or AE for diagnosis or differential diagnosis in cases of uncharacteristic imaging findings. In clinical practice tests for detecting specific serum antibodies are of particular importance in the diagnosis of CE, and the detection of circulating antigens is less relevant (Siles-Lucas and Gottstein, 2001; Craig et al., 2003).

Almost all immunodiagnostic methods have been used for human cystic or alveolar echinococcosis over the past 30 years. An intradermal test (Casoni's test) was the first one used by Casoni from 1912. Casoni's test, indirect haemaglutination assay (IHA), and immunoelectophoresis (IEP) had been used in Xinjiang (China)| from 1960s. The high false positive rate (between 12-67%) of Casoni's test and also risk of allergic hypersensitivity was problematic. In the last 10 years several new techniques have been applied for immunodiagnosis of CE/AE, such as lymphocyte proliferation responses, cytokine analyses, could apparently give more information about post-treatment follow-up studies. The detection of echinococcosis specific antibodies is also important and widely used in clinical and epidemiologic studies. However the sensitivity and specificity of tests are variable, due to the application of different antigens and test methods.

Currently, the ELISA and Western blot (immunoblot) have been the main assays for human CE using cyst fluid antigen and/or antigen B (Craig, 2003). For human AE, Em2 (or $Em2^{plus}$) and Em18 are considered currently best antigens for immunodiagnosis (Gosstein 1993, 1996; Ito 1999). The benefits of serology for human CE/AE has been reviewed by several authors and including: confirmation of imaging/clinical evidence, identification of asymptomatic, infected individuals with no obvious cystic image, provision of long term epidemiological information, and provision of information on the state of the infection and the immune response against the parasites (Rogan and Craig, 2002). Even if the highly specific antigens and detection methods (IgG-ELISA or Western Blot) are used, antibodies may not be detectable in a certain proportion of patients with echinococcosis. For example, CE cyst in brain or eye, calcificated cysts or lesions, cyst with a thick cyst wall, single small cyst, paediatric CE, etc. as they may induce low or no antibody titres. Specific antibody detection is most valuable for human echinococcosis diagnosis and follow-up study compared to circulating antigen detection which is difficult to apply due to its lower sensitivity than other tools (Siles-Lucas and Gottstein, 2001; Craig et al., 2003). However, direct antigen detection in hydatid cyst fluid has been used to confirm the presence of a hydatid cyst at surgery or by fine-needle puncture inspiration (Craig et al., 1986; Wang et al., 2002). Sensitivity and specificity of serologic tests can be quite different in different labs or different areas even when using the same antigen and the same detection method. The main factors affecting a test will be the antigen quality, preparation and whether the detection method is adequately standardized and repeatable.

1.5.2 Antigens in immunodiagnosis of human echinococcosis

For the application of immunodiagnostic in human echinococcosis, diagnostic antigens should be easy to obtain and have relatively stable and higher sensitivity and specificity. Native antigens used for human CE serodiagnosis have been derived from *E. granulosus* hydatid cyst fluid, extracts of protoscoleces (excretory-secretory (ES) or somatic), and *E. granulosus* adult tapeworm, or even oncosphere stages (Carmena et al, 2006). Hydatid cyst fluid antigen of *E. granulosus* from livestock hosts has been the main antigen resource for human CE immunodiagnosis. Crude cyst fluid antigen, and the cyst fluid antigens, antigen B and antigen 5, have been used both in clinical diagnosis or surveys in endemic

areas (Rogan et al., 1991; Shepher et al., 1991; Lightowlers and Gottstein, 1995; Ortona et al., 2000; WHO/OIE 2001; Craig et al., 2003). Crude cyst fluid antigen (EgCF) has been widely used in all immunodiagnostic tools with high sensitivity, and also antigen B (EgB) with its higher specificity (Rogan et al., 1991, 1993, 1997; Liu et al., 1993; Craig et al., 2003; Zhang et al., 2003; Carmena et al., 2006). Antigen 5, which detected by immunoelectrophoresis, is less useful than antigen B for diagnostic purpose due to its lower sensitivity 44%-89% and cross reaction with other cestode, trematode and nematode infections (Yarzabal et al., 1977; Di Felice et al., 1986; Carmena et al, 2006). Crude antigen somatic extracts from protoscoleces (EgP) or adult worms (EgW) have been used for immunodetection in dogs and other carnivores, and also for antibody detection in human sera with 82-90% sensitivity and 48-65% specificity respectively (Allan et al., 1992, Craig et al., 1995; Allan and Craig, 2006; Carmena et al, 2006). Excretory-secretory (ES) antigens from protoscoleces or adult stages also have been used for definitive host coproantigen ELISA tests and for human sera specific antibody detection (Allan et al, 1992; Benito et al., 2005; Carmena et al, 2005, 2006).

Antigens used for human AE serodiagnosis are Em2, Em2 plus, EmP and Em18 which have usually been derived from AE cyst metacestode or protoscoleces taken from experimentally infected rodents. Em2 plus is a mixture of native Em2 and a recombined antigen (Em II/3-10) which has been used commercially with sensitivity 85% and specificity 95% (Gosstein 1993; Rogan and Craig, 2002). Immunoblot tests for identification of Em18 antigen are reported with a sensitivity range of 50-90% and specificities >95% (Ito et al., 1999). Em2 as a native carbohydrate rich laminated layer antigen is relatively easy to obtain, low cost and could be applied in several immunodiagnostic tests (Gosstein, 1993). The antigens EgCF, Ag5, EgP, EgB, Em2 and Em18 which have frequently been used in immunodiagnosis of human echinococcosis are reviewed here.

1.5.2.1 Native crude *E. granulosus* Cyst fluid antigen (EgCF):

Crude *E. granulosus* cyst fluid antigen (EgCF) from livestock hydatid cysts has been used for over 50 years and even now remains most widely used antigen preparation for almost all the specific antibody detection methods. Chordi and Kagan were the first to analyze serum antibody responses in human hydatid infection by gel immunodiffusion with EgCF of sheep origin. Hydatid cyst fluid from human CE cases was not suitable for diagnosis since there are human origin

proteins in cyst fluid such as human IgG. Hydatid cyst fluid from sheep is also the main source for antigen preparation (EgCF, Ag5, AgB) for ELISA and immunoblot for detection of total IgG / IgG subclasses or other Ig isotypes (IgM, IgE, IgA). Crude hydatid cyst fluid, EgCF, is a complex mixture of glycoprotein, lipoproteins, carbohydrates and salts, and contains metabolic products from both the metacestode and the host (mainly albumin and immunoglobulin) (Baveja et al., 1997; Zhang et al., 2003). EgCF has a high sensitivity around 72%-96% for human CE in ELISA (Zhang, et al., 2003), but its specificity is variable with cross-reactions reported against other cestode (89%), nematode (39%) or trematode (30%) species (Eckert and Deplazes, 2004). Due to high sensitivity, EgCF antigen has been recommended by WHO for application of mass screening in endemic areas, especially when used together with ultrasound (WHO/OIE, 2001; Carmena et al., 2006). Partially purified *E. granulosus* cyst fluid antigens were obtained through precipitation at low ionic strength (0.005M acetate buffer, pH 5, Oriol et al. 1971) or eluted after affinity column chromatography (against normal human sera coupled CNBr-activated Sepharose4B) (Zhang, 1999). *E. granulosus* cyst fluid antigens are also often used for the source of antigenic materials for animal intermediate hosts (ungulates, such as sheep and cattle) immunodiagnosis, but cross-reactions were also observed (Lightowlers and Gottstein, 1995; Zhang 2003).

1.5.2.2 *E. granulosus* cyst fluid antigen 5 (Ag5)

Ag5 (Capron et al., 1967) is a lipoprotein complex composed of 57- and 67-kDa components (Di Felice et al., 1986). Under reducing conditions 38 and 22-24 kDa subunits were further found (Lightowlers et al., 1989). Ag5 is thermolabile with high immunogenicity and forms a precipitation line in agar diffusion and immunoelectrophoresis assays known as Arc 5. Ag5 has been used widely in immunodiagnosis of human CE mainly with immunoelectrophoresis (Arc 5) and ELISA. Cross-reaction with other cestode, trematode and nematode infections were however observed using native Ag5 for serodiagnosis of human CE (Yarzabal et al., 1977; Di Felice et al., 1986; Carmena et al., 2006). The sensitivity and specificity of Ag5 ranged between 50-54% and 89-92% respectively (Barbieri et al., 1998; Gonzalez et al., 2000, Carmena et al., 2006). Ag5 have been considered less useful than antigen B for diagnosis of human cystic echinococcosis (Carmena et al., 2006)

Recombinant Ag5 (rAg5) and recombinant Ag5 38 kDa subunit (rAg5-38s) have been evaluated for CE serological diagnosis, but showed less diagnostic value than native preparations (Lorenzo et al., 2005; Camena et al., 2006). Synthetic peptide 89-122 showed variable sensitivity with different panels of patient sera (range 16-85%) with main cross-reaction against sera from alveolar echinococcosis patients (Chamekh et al., 1992; Barbieri et al., 1998, Gonzalez et al., 2000, Camena et al., 2006).

1.5.2.3 *E. granulosus* cyst fluid antigen B (AgB)

AgB is a major heat stable lipoprotein, and a component from hydatid cyst fluid (Oriol et al, 1971). It is a strongly immunogenic polymeric lipoprotein with a molecular weight of 120-160 kDa that dissociates under reducing conditions into 8/12, 16, and 20/24 kDa subunits, suggesting that it consists of polymers of 8 kDa subunits (Lightowlers et al., 1989). The smallest subunit has proved the most useful target in diagnostic studies. A possible new antigen B (AgB) subunit (AgB4) was recently identified (Arend et al., 2004) and shows that AgB is encoded by a multigene family (Haag et al., 2006). Furthermore, AgB has some homology with molecules in *E. multilocularis*, and shares apparent structural similarities with, helix-rich hydrophobic ligand binding proteins (HLBPs) from other cestodes, together with fatty acid binding properties (Chemale et al., 2005). AgB can be detected in CE patient serum as circulating antigen, and used as an immunodiagnostic marker to identify cyst fluid removed from suspected human cases of suspected cystic echinococcosis (Craig et al., 1986; Wang et al., 2002). Antigen B appears to play an important role in the immuno biology of the parasite and probably parasite-host relationship (Shepherd et al., 1991; Rigano et al., 2001, 2002; Zhang, 2003; Carmena et al., 2006). AgB has been regarded as the most specific antigen for human CE (>90%) currently available for immunodiagnosis of human CE. The only important cross reaction that may occur is with other cestode species primarily *E. multilocularis* and to a less extent with *Taenium Solium* (Maddison et al., 1989; Ito et al, 1999). Immunoblot detection of smallest subunit (8kDa) of AgB has proved the most overall useful subunit in diagnostic studies (Ortona et al., 2000). The sensitivity of native AgB for human CE varied between 63-92%. Maddison et al (1989) reported 18% of human CE sera were specific antibodies negative against AgB, and 39% of human AE cases showed cross-reactivity with AgB.

Five major gene clusters named EgAgB1, EgAgB2, EgAgB3, EgAgB4 and EgAgB5 have now been identified in Antigen B (Shepherd et al., 1991; Fernandez et al., 1996; Chemale et al., 2001; Arend et al., 2004; Haag et al., 2004; Carmina et al., 2006).

Recombinant AgB proteins, EgAgB1 and EgAgB2 have been cloned, expressed and assessed for serodiagnosis of human CE to give high specificity but lower sensitivity. Of them, EgAgB2 was shown to have best features in terms of diagnostic efficiency, and significantly higher than native AgB (Virginio et al., 2003), however another evaluation showed different results which had lower sensitivity and specificity (Lorenzo et al.2005). This might due to the different preparation of recombined antigen and the antigen stability might have some influence. Recent research showed antigen B is also expressed in *E. multilocularis*, where 5 cDNAs encoding 8kDa subunit monomers (EmAgB) named as EmAgB/1 to EmAgB/5 (Mamuti et al 2004). The diagnostic value of native and recombinant AgB preparations and synthetic peptides of AgB subunit derived from this molecule in the immunodiagnosis of human CE were compared by Carmena et al (2006) (Table 1.7).

Another interesting finding in human CE is a clear predominance of IgG4 antibody response for both native and recombinant AgB (Wen and Craig, 1994; Mcvie et al, 1997; Daeki et al, 2000). Human IgG4 antibody production appears to be associated with hydatid cyst development, growth and disease progression or active disease (Wen et al., 1994; Roger and Craig, 2002). IgG4 detection with AgB has also been used for follow-up studies of treated CE cases (surgery or chemotherapy) (Rigano et al., 1995). This finding has important implications on the sensitivity of the immunodiagnostic test used in CE patients, the serological profile for IgG and IgG subclass antibodies may vary according to disease stage (e.g., Type 1-5), site and progression (Daeki et al. 2000).

Table 1.7: Main characteristics of antigens used for serodiagnosis of human cystic echinococcosis based on native, recombinant, or synthetic peptides of antigen B (AgB)

Antigen	No. of subjects tested			Test	Sensitivity (%)	Specificity (%)	Cross reaction	Reference
	CE cases	Other disease	Healthy subjects					
Native AgB	210	79	47	IgG4 ELISA	63	81	AE, Cys. Schis.	McVie et al. (1997)
Native AgB	90	88	28	IgG ELISA	77	86	AE	Barbieri et al. (1998)
Native AgB	90	86	28	IgG ELISA	77	86	AE	Gonz´alez-Sapien za et al. (2000)
Native AgB	204	53	90	IgG ELISA	73	100	None	Ortona et al. (2000)
Native AgB	31	87	29	IgG ELISA	77	82	AE, Schis. Tox	Rott et al. (2000)
Native AgB	129	65	203	IgG ELISA	60	93	Cys, Tox	Virginio et al. (2003)
Native AgB	59	55	15	IgG ELISA	80	77	AE, Cys	Lorenzo et al. (2005a)
Native AgB	173	181	29	IgG IB	92	71	AE	Ito et al. (1999)
Native AgB	87	339	200	IgG IB	60-71	98	AE, Cys	Poretti et al. (1999)
Native AgB	204	53	90	IgG IB	73	100	None	Ortona et al. (2000)
rAgB8/1	210	79	47	IgG4 ELISA	65	91	AE	McVie et al. (1997)
rAgB8/1	31	87	29	IgG ELISA	55	80	AE, Schis. Tox	Rott et al. (2000)
rAgB8/1	129	65	203	IgG ELISA	84	91	Cys	Virginio et al. (2003)
rAgB8/1	59	55	15	IgG ELISA	68	88	AE, Cys	Lorenzo et al. (2005a)
rAgB8/1	204	53	90	IgG IB	72	100	None	Ortona et al. (2000)
rAgB8/2	31	87	29	IgG ELISA	84	98	Schis, Tox	Rott et al. (2000)
rAgB8/2	129	65	203	IgG ELISA	93	99	Cys, Tox	Virginio et al. (2003)
rAgB8/2	59	55	15	IgG ELISA	45	86	AE, Cys	Lorenzo et al. (2005a)
p65	90	88	28	IgG ELISA	34-48	80-97	AE, Onch, Schis, Tox	Barbieri et al. (1998)
p65	90	86	28	IgG ELISA	44	96	AE, Schis, Tox	Gonz´alez-Sapien za et al. (2000)
P175	90	86	28	IgG ELISA	49	94	AE, Schis, Tox	Gonz´alez-Sapien za et al. (2000)
P176	90	86	28	IgG ELISA	80	93	AE, Schis, Tox	Gonz´alez-Sapien za et al. (2000)
P176	59	55	15	IgG ELISA	63	83	AE, Cys	Lorenzo et al. (2005a)
P177	90	86	28	IgG ELISA	38	92	AE, Schis, Tox	Gonz´alez-Sapien za et al. (2000)
pGU4	90	88	28	IgG ELISA	12-18	96-100	AE	Barbieri et al. (1998)
pGU4	90	86	28	IgG ELISA	18	98	AE, Schis, Tox	Gonz´alez-Sapien za et al. (2000)

r=recombinant; p=peptide; IB=immunoblot; AE=alveolar echinococcosis, Cys=cysticercosis; Schis=schistosomiasis, Tox=toxoplasmosis; Onch=Onchocerciasis) (Carmena et al, 2006)

1.5.2.4 *E. granulosus* protoscolex extract (EgP)

Antigenic differences have been found in a somatic extract of protoscoleces (EgP) from different species of intermediate hosts (Rafiei and Craig, 2002). Some research showed EgP sensitivity is similar to hydatid cyst fluid antigen (EgCF) and

little specificity (29.6%) in a dot-ELISA immunodiagnostic test of human CE (Qiao et al, 1999). Native EgP generally showed poorer specificity for human CE, some recombinant protoscoleces proteins exhibited higher levels of sensitivity (90-92%) and specificity (95-96%), particularly rEpC1 (Li et al, 2003) and rEgcMDH (Virginio et al., 2003). The real potential of EgP antigen for human immunodiagnosis still remains to be determined.

1.5.2.5 *E. granulosus* adult worm extract (EgW)

E. granulosus adult worm extract (EgW) has also been assessed for serodiagnosis of human CE (Ersfeld et al, 1997). Sensitivity of 82% and specificity 65% in ELISA were observed with EgW. A range of low-molecular-weight antigenic proteins (12-45 kDa), being immunoprecipitated from in vitro-translated *E. granulosus* adult worm mRNA, was recognized by human CE sera (Ersfeld et al., 1997).

1.5.2.6 *E. multilocularis* protoscolex antigen (EmP)

Crude extract from supernatant of *in vitro E. multilocularis* protoscoleces was first demonstrated in 1988 by Auer et al. And an *E. multilocularis* protoscoleces extract (EMP) had been used for community screening and IgG/IgG subclass analysis with better specificity than whole *E. multilocularis* metacestode extract (EmCH). (Craig et al., 1992, 2000; Wen and Craig, 1994; Wen et al., 1995). Main cross-reactivity of EmP was 36% with sera from CE patient and 50% with sera from *T. solium* cysticercosis (Wen et al., 1995). Two low- molecular-weight (16- and 18- kDa) peptides from *E. multilocularis* protoscoleces were regarded as species-specific antigens later (Ito 1993, 1999).

1.5.2.7 *E. multilocularis* metacestode antigen (Em2)

Em2 is a carbohydrate rich, affinity purified, highly species-specific native antigen which is extracted from *E. multilocularis* metacestode derived from experimentally infected animals (Gottstein et al., 1983, 1992). Em2 has been used successfully over a long period for immunodiagnosis of human AE with a sensitivity of 77-92% in ELISA in different endemic areas (Gottstain et al., 1992; Eckert et al., 1992). It has been confirmed as a PAS (periodic acid shifts) staining positive component from the laminated layer of *E. multilocularis*. It appears that Em2 could not differentiate between antibodies in progressive or inactive AE lesions so it may not be suitable for follow-up studies, however it can identify aborted infections in human (Gottstein et al., 1983, 1987, 1993; Bartholomat et al., 2000). Em2 shows

high specificity (95%) but has cross-reaction with some CE cases and other cestode species (Wen et al., 1995). The Em2plus ELISA, that was a combination of native Em2 with a recombinant protein designated II/3-10 (also termed Em10), increased the sensitivity for human AE to 97% (Gottstein et al, 1993). The Em2plus assay exhibited cross-reaction in 25.8% of CE cases) which was higher than the individual Em2 (5.6%) or 11/3-10 (6.5%), but limited cross-reactivity with other diseases (Gottstein et al., 1992).

1.5.2.8 *E. multilocularis* protoscolex antigen (Em18)

An 18-KD antigen from *E. multilocularis* protoscoleces (Em18) was first reported by Ito et al. (1993), as a highly species-specific (96.8%) and sensitive (97%) antigen with potential not only for differentiation of AE from either CE or other helminthes infections, but also for differentiation of active from inactive AE (Ito et al., 1993, 1995, 1997, 1999). A small degree of cross-reaction has been described with CE patient sera (Nirmalan and Craig, 1997; Ito et al., 1999; Ito, 2002). Subsequently, Em18 was shown to be a fragment of the C-terminal of Em10 and recombinant protein (rEm18) was recognized in ELISA and immunoblotting by 87.1% and 90.3% respectively of 31 serum samples from AE patients, respectively (Sako et al, 2002). Recombinant Em18 ELISA and Em18 immunoblot assays have proved very accurate for differentiating AE from CE infection, with Em18 ELISA also being useful for evaluation of the efficacy of treatment in AE patients (Ito et al, 2002). Epitope mapping has indicated that the part of the recombinant Em18 antigen sequence important for detection of AE antibodies occur in the N-terminal half to two thirds of the entire sequence (Jiang, et al., 2004). Em 18 was also shown to be present in *E. granulosus* and recognized by some CE cases (Wen et al., 1995; Nirmalan and Craig, 1997; Ito et al., 2003).

1.5.3 Definitive host diagnosis:

1.5.3.1 Parasitological diagnosis

Canids play the most important role as the definitive host in the transmission and epidemiology of *E. granulosus* and *E. multilocularis*. Necropsy to identify the tapeworms in the small intestine is the best (gold-standard) and traditional way for confirmation of infection in definitive hosts, but is difficult, biohazardous and unethical to use routinely for either domestic dogs or sylvatic hosts (e.g. foxes) (Craig 2001; Zhang 2003, Allan and Craig 2006). Pre-mortem direct microscopic

detection of echinococcus (eggs or proglottides) in faeces may be carried out after purgation with arecoline salts. Purgation, should be 100% specific for E*chinococcus Spp* tapeworms due to direct morphological identification, however, it has several disadvantages: including importantly variable sensitivity (Wachira et al., 1990; Craig 1994), its complicated operation, time consuming, requirement for technical person, is bio-hazardous and may cause distress to some dogs and has a high failure rate (10-20%) (Craig, 1994; 2001; Lahmar et al., 2007). Eggs could be found in faecal samples using routine flotation in saturated saline or using clear adhesive tape to microscopic slide (Deplazes & Eckert 1988). Microscopically detection using canid faeces may also be difficult due to often absence of eggs from faeces, furthermore the morphologic structure under light microscopy of eggs is indistinguishable between *Echinococcus* and *Taenia* species (Euzeby, 1966; Craig et al., 1986; Aluja et al., 1987, Craig, 2001, WHO/OIE, 2001; Allan and Craig, 2006).

1.5.3.2 Serological diagnosis

Serological tests to detect of adult *E. granulosus* tapeworm antibodies using a protoscoleces antigen in definitive host are considered unreliable for due to the lack of specificity and sensitivity. EgP is a crude somatic extract from *E. granulosus* protoscoleces, which has been used for detection of antibodies in the definitive host but lacked sensitivity (Gasser et al., 1988 and Gasser 1994, Carmena et al., 2006). Serological test for dogs was not widely carried due to variable sensitivity (40-90%) (Jenkins et a., 1990; Gasser et al., 1994); lower levels of specific antibody in 25-60% sera from dog with E. granulosus and cross-reactivity with other parasite species (Casser et al., 1988).

Serological diagnosis to detect adult *E. granulosus* tapeworm antibodies with a protoscoleces antigen was assessed using experimental canine echinococcosis in Australia and showed initially 91.8% specificity and 72.7% sensitivity (Gasser et al., 1988). Further studies however exhibited lower sensitivity (40%) as observed in naturally infected dogs in Kenya (Jenkins et al., 1990) and in Uruguay (Craig et al., 1995). The potential application of antibody tests in definitive hosts might supply more information on community studies rather than individual diagnostic value (WHO/OIE, 2001).

1.5.3.3 Coproantigen detection

Sandwich ELISA for detection of parasite specific antigen in faecal samples

(coproantigens) using polyclonal antibodies against somatic or excretory/secretory antigens of adult *E. granulosus* has been developed and used in several specialized laboratories for transmission and epidemiologic studies in endemic areas (Allan et al., 1992; Deplazes et al., 1992; Craig et al., 1995). *Echinococcus* coproantigen has been observed to be very stable during the sampling, storing (at room temperature over several days), and detection. Coproantigen ELISA has been showed to have reasonable sensitivity (70% to 95%) and high genus-specificity (>90%) against other parasites including *Taenia spp* in dogs, dingoes, and foxes, and coproantigen does not depend on the presence of eggs (Allan et al. 1992; Deplazes, 1992; Allan & Craig, 2006). *E. granulosus* coproantigen appeared to be associated with the parasite tegument or glycocalyx due to its components of large molecular weight (around 150 kDa to 670 kDa) with carbohydrate moieties (Casaravilla et al., 2003, 2005; Elayoubi et al., 2003, 2004; Allan and Craig, 2006). Above ELISA coproantigen tests were asked professional laborotary and technitians, which should be carried out in standard reference laborotary. Then a rapid and easy method for using in rural field was needed to apply in endemic area.

1.5.3.4 Copro PCR

PCR amplification of DNA from faeses of foxes infected with *E. multilocularis* was developed by Bretagne et al (1993) and modified for epidemiological studies in Europe (Dinkel et al. 1998; Deplazes et al., 2003; Van der Giessen et al., 1999). Recently PCR (copro PCR) has also been developed and applied for specific detection of *E. granulosus* worm DNA from faeces of definitive hosts (Cabrera et al, 2002; Abbasi et al., 2003, Casulli et al., 2004, 2005; Stefanic et al., 2004, Varcasia et al., 2004; Reiterova et al., 2005). The PCR method should give more information and better chance of species-specific confirmation (Deplazes and Eckert, 2001; Abbasi et al, 2003) and even for environmental detection of *Echinococcus* eggs in soil samples (Shaikenov et al., 2004, Zhang and McManus 2006). Application of coproantigen ELISA and copro-PCR to tests definitive host faecal samples have provided excellent tools for transmission or epidemiology studies, and for monitoring the progress of hydatid control Program (Deplazes and Eckert, 2001; Craig 2003; Craig et al., 2007; Huang et al., 2007).

1.5.3.5 Adult *Echinococcus* spp antigens.

A crude somatic extract from *E. granulosus* adult worms (EgWWE) has been

assessed as the basis coprodiagnosis of echinococcosis. EgWWE was used to boost rabbits to get polyclonal anti-EgWWE antibodies, and then were used for a double antibodies sandwich ELISA to detect coproantigens in definitive host faeces (Allan et al., 1992; Craig et al., 1995; Allan and Craig, 2006). This method considerable improved the sensitivity for detection of coproantigens of adult worm infection in dogs compared to antibody detection in serum (Craig et al., 1995). It has been widely applied in different geographical regions more than 10 years and confirmed its usefulness for epidemiological studies. The EgWWE preparation is a saline extract from adult tapeworm by removal of the non-gravid segments, homogenization and centrifugation. Rabbits were immunized with EgWWE and Freund's complete or incomplete adjuvant after first boost. IgG fraction were purified from rabbit sera using a Protein A sepharose CL 4B column and subsequent elution using a low pH glycine buffer for affinity chromatography. Half of IgG was dialyzed against PBS for following conjugation with horseradish peroxidase and the other half dialyzed against bicarbonate-carbonate buffer to act as capture antibody in a coproantigen sandwich ELISA (Allan et al., 1992). Secretory-excretory (ES) antigens from adult *E. multilocularis* or *E. granulosus* have been investigated for specifically primarily in relation to coproantigen test development (Deplazes, et al., 1992; Allan et al., 1992, Malgor et al., 1997; Casaravilla et al., 2005; Huang et al., 2007). And recently ES antigens from protoscoleces of E. granulosus have been demonstrated for coproantigen ELISA and more effective with modifications (Carmena et al., 2005; Benito and Carmena, 2005).

1.6 Developments in immunodiagnostic assays

There is a long of history using immunodiagnosis tests, and almost all immunodiagnostic methods have been assessed or used at the same time for detection of human echinococcosis (Table 1.8). Various results were observed in both sensitivity and specificity. Non-specific tests, like the Casoni intradermal test, the complement fixation test, the indirect haemagglutination test, and the latex agglutination test, the indirect immunofluorescence antibody test, immunoelectrophoresis (IEP), have now been largely replaced by the ELISA, and immunoblotting (IB) in well equipped laboratories for routine application. The diagnostic sensitivity and specificity of ELISA and IB in comparison to IEP in

detecting IgG antibodies to native or recombinant antigen B and a crude hydatid cyst fluid antigen in CE patient sera were compared. Hydatid cyst fluid fraction-IB gave the highest sensitivity (80%) followed by ELISA (72%) and IEP (31%), the diagnostic sensitivity decreased significantly in relation to hydatid cyst pathology (i.e. active vs. inactive cysts) (Ortona et al., 2000; Zhang et al., 2003). Recombinant and native EgB immunoblots (IB) had similar sensitivity (74%) but gave 20% of CE cases were false negative (Ortona et al., 2000). It has been suggested that combination of several defined antigens (including native or synthetic peptides may obtain more information about serological responses to this infection (Zhang et al., 2003). Antigen detection in the suspected hydatid cyst fluid samples for confirmation of *E granulosus* infection with ELISA or dot-blots has been tried to assess diagnosis of suspected CE during the surgery or PAIR (Craig, 1986; Wang 1997).

In definitive hosts, serological tests were generally of low sensitivity and sometimes difficult to apply. In contrast, the coproantigen sandwich ELISA has been used successfully for mass screening of dogs. Confirmation of coproantigen ELISA positives, copro PCR for species differentiation is the current recommended approach for epidemiological and surveillance studies in canids (WHO/OIE, 2001; Eckert, 2003; Craig et al., 2003; Allan and Craig, 2006).

Table 1.8: Immunodiagnostic Tests used for human cystic echinococcosis

Detection methods	Technique	References
Agglutination, lysis	Complement Fixation Test, CFT	Bradstreet, 1969
	Latex Agglutination Test, LA	Williams and Prezioso, 1970
	Indirect Hemagglutination Test, IHA	Varela-Diaz et al, 1975a
Precipitation	Immunoelectrophoresis, IEP	Varela-Diaz et al, 1975b
	Double Diffusion, DD	Coltori and Varela-Diaz, 1978
	Counter Immunoelectrophoresis, CIEP	Pinon et al, 1979
	Diffusion In Gel-Enzyme-Linked Immunosorbent Assay, DIG-ELISA	Dematteis et al, 1989
Indirect antibody labeled	Radioimmunoassay, RIA	Musiani et al.,1974
	Indirect Fluorescent Antibody Test, IFA	Matossian et al., 1972
	Enzyme-linked Immunosorbent Assay, ELISA	Craig, 1986
	Dot-ELISA	Rogan et al., 1991
	Western Blot	Maddison et al.,1989
	Dot Immuno-Gold Filtration Assay, DIGFA	Fu et al., 2001; Feng et al.,2002; Chen et al.,2005
	Gold Immunochromatographic Assay, GICA	Xue et al., 2005
Skin tests	Casoni intradermal (ID) test	Yarzabal et al., 1975; Gonlugur et al., 2005
	Delayed intradermal reaction	Todorov et al., 1979; Gonlugur et al., 2005
Lymphocyte stimulation,cytokine assays	Lymphocyte Transformation In Vitro ELISA	Siracusano et al, 1988
Coproantigen	ELISA	Allan et al., 1992; Deplazes et al., 1992
DNA probe	DNA hybridization and immunoelectrophoretic assay，Immunoblotting	Rishi and McManus, 1987

1.7 New tools for rapid diagnosis of hydatidosis / echinococcosis

ELISA and immunoblot have confirmed efficacy and use in immunodiagnostic studies. They are relatively easy to set up in any laboratory but have the limitation of long assay-time especially in mass-screening community studies. A rapid

immunological diagnostic method that can be used in both the field and for initial differentiation of CE and AE at clinical level would be very useful (Craig et al., 2000; Bartholomat et al., 2000; Wang et al., 2004). Rapid serological test formats, such as dot-ELISA have been assessed for both human CE and AE, and although useful when associated with mass ultrasound screening, were temperamental and subjective in reading (Rogan et al., 1991).

Dot immuno-gold filtration assay (DIGFA) is a rapid immunodiagnostic test that uses colloidal gold conjugated antibody or antigen instead of enzyme or fluorescence conjugates (Valkirs and Barton, 1985; Beesley 1989; Chun and Chu, 1989; Xiao et al., 1995; Reddy 2006). Antibody or antigen are attached on a nitrocellulose membrane, heparinized blood or serum applied, and colloidal gold conjugated anti-human antibodies give a color change to show positive or negative. The procedure is similar to ELISA, but uses an infiltration system with colloidal gold to give a rapid result.

1.7.1 Colloidal gold preparation

Colloidal gold, also called "nanogold", is a suspension (colloid-like) of sub-micrometre-sized particles of gold in a fluid formation. The liquid shows usually an intense red colour for particles less than 100 nm (Wessling et al, 1996). The gold particles themselves can come in a variety of shapes, e.g. spheres, rods, cubes, and caps are some of those frequently observed.

Known since the ancient Roman times, colloidal gold was originally used as a method of staining glass as an intense red which the process was refined by Andreus Cassius and Johann Kunchel in the 17th century. In 1842, John Hershel found a method that used colloidal gold to record images on paper, called chrysotype (from the Greek word for gold). The first pure sample of colloidal gold was prepared in 1857 by Michael Faraday who used phosphorus to reduce a solution of gold chloride, which he called 'activated gold'. Faraday was also the first to recognize that the colour was due to the size of the gold particles. Modern scientific evaluation of colloidal gold did not begin until Michael Faraday's work of the 1850s (Mulvaney, 2003, Reddy, 2006). Colloidal gold has been widely applied in a wide variety scientific and technological fields, including electronics, nanotechnology due to its unique optical, electronic, and molecular-recognition properties, etc. (Rao et al, 1999, Mulvaney, 2003, Reddy 2006).

Generally, colloidal gold is produced in a liquid formular ("liquid chemical methods") by reduction (usually sodium citrate or sodium borohydride) of hydrogen tetrachloroaurate ($HAuCl_4$). After dissolving $HAuCl_4$ in distilled water, the boiled solution is rapidly stirred while a reducing agent is added. This changes Au^{3+} ions to reduce to un-ionized gold atoms. The solution becomes supersaturated when most gold atoms reduced, and residual gold gradually starts to precipitate as sub-nanometer particles while other rest of the gold atoms stick to the existing particles. The similar size of particles could be obtained if the solution is stirred vigorously enough. To prevent the particles from aggregating, some sort of stabilizing agent that sticks to the nanoparticle surface is usually added. They can be functionalized with various organic ligands to create organic-inorganic hybrids with advanced functionality (Beesley, 1989; Reddy 2006).

Generally used sythenization methods for colloidal gold were sodium citrate reducing (Turkevich et al., 1951; Frens et al., 1970; Beesley 1989, Reddy 2006; Pong et al., 2007); organic reducing (like toluene) using tetraoctylammonium bromide (TOAB) (Brust et al. 1994-1998) and s*onolysis* (Zhang, et al., 2006)

1.7.2 Colloidal gold based immunodiagnostic assays

Colloidal gold conjugated antibody/antigen could be used in immunodiagnostic assays replacing enzyme or fluorescence dyes. Colloidal gold technique was first used in 1970s for immunological purpose in locating special antigen on the surface of cell through electric microscopy (Faulk and Taylor 1971; Horisberger et al., 1975). A dot immunogold infiltration assay (DIGFA) was used for detect HIV in 1989 (Beesley 1989; Chun and Chu, 1989). This technique is basically involves the antigen or antibody attached to a nitrocellulose membrane with a filter tissue underneath with the target antibody / antigen be combined on the membrane (Fig. 1.11). The procedure is similar to ELISA but using an infiltration system and colloidal gold provide a more rapid and reliable result.

Another related technique is the immunogold chromatography assay (IGCA) which has been widely used for example in HCG hormone detection for early diagnosis of pregnancy (May 1991). Gold conjugate and sample are allowed to flow to the capture (antigen or antibody) on the nitrocellulose membrane through chromatography, reaction in precipitation as a line (Millipore corp 1996). Other colloidal dyes such as Palami Red (UK), Samaron Red (Hoechst F.R.G) etc. were

used as an alternative to colloidal gold particles for antigen detection immunoassay with dipsticks (Snowden and Hommel, 1991).

Preparation of antibody-colloidal gold conjugate (Beesley, 1989) was using optimum volume of 1mg/ml antibody for conjugation with colloidal gold; and blocking with 1-10% BSA or gelatin or PEG 20,000 according different design; purification using centrifugation with different speed due to different size of colloidal gold; column chromatography to obtain the evenly size of gold particle.

Dot Immuno-Gold Filtration Assay (DIGFA)

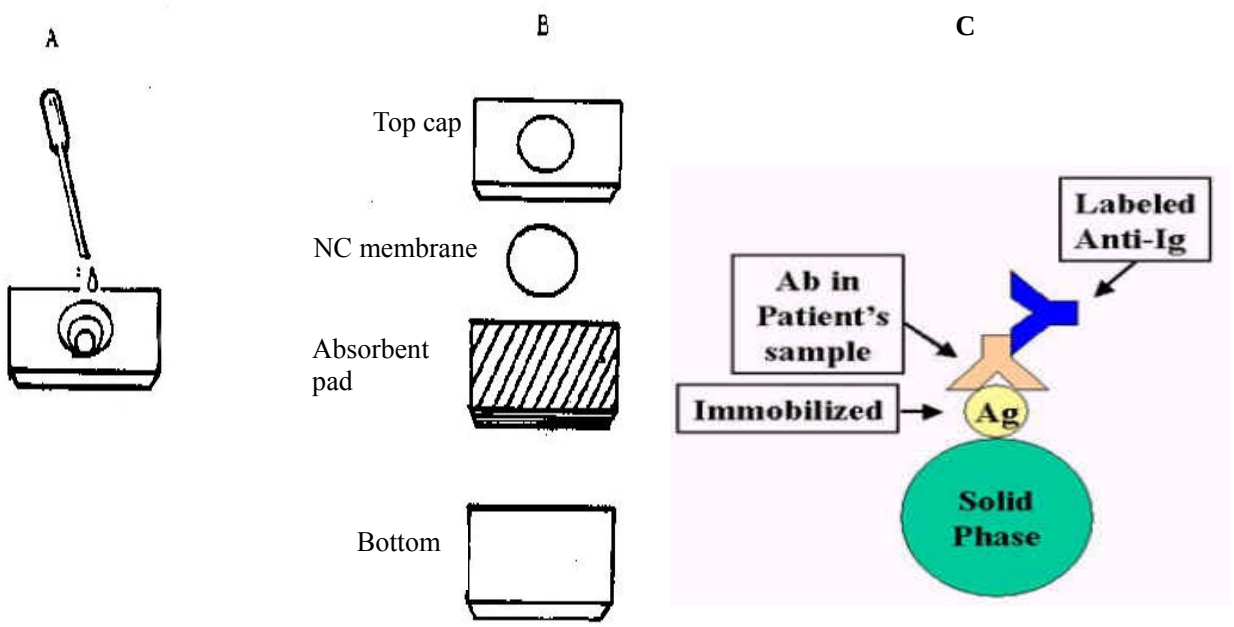


Fig. 1.16: The device for DIGFA. **A**: ready-to-use device; **B**: different components of this device, including a plastic bottom for backup, an absorbent pad for collecting all the liquid, a piece of NC membrane as a reaction barrier, and a top cap with a middle well. **C**: reactions occurred in DIGFA, with specific antigen binded to NC membrane (solid phase), specific antibody in patient's sample would combined with this antigen, and a colloidal gold conjugated anti-Ig could bind to above combination and showed color to judge the reaction.

DIGFA, using nitrocellulose (NC) membrane as a carrier which pre-coated antigen or antibody as a capture, is similar to immunoblot procedure excepted of a substrate step (Fig. 1.16). A sample (including antibody or antigen) dropped on the NC membrane followed by colloidal gold conjugate and washing buffer, antigen-antibody-conjugate would show red dot on the membrane and excessive reagents would filtrate through NC membrane to an absorbent tissue. Antigen or

antibody in sample / conjugate would combined to the antibody/antigen on NC membrane when the solution past through NC membrane and thus was more like a concentration with affinity chromatography. This made the procedure rapid (about 5 minutes), and washing step through filtration became more simple and rapid. DIGFA became one of the point of care test (POCT) due to rapid and simple (no special device needed).

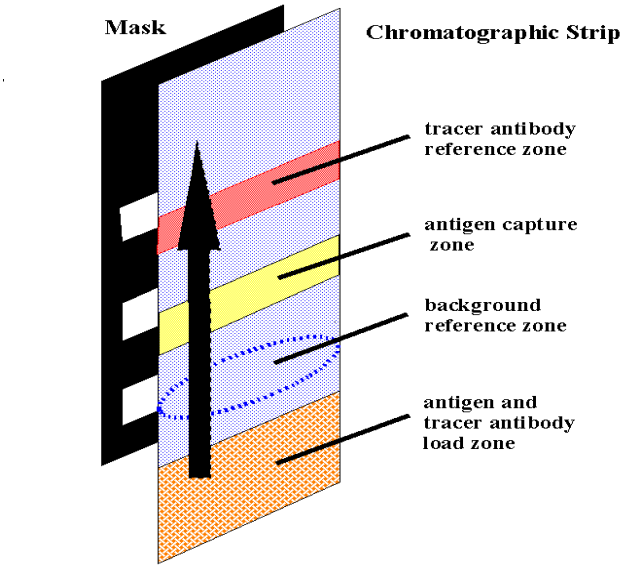


Fig. 1.17: Immunogold chromatography assay strip

Immunogold chromatographic Assays, also called lateral flow assays or simply strip assays, have been developed for some time. This technique is based on an immunochromatographic procedure that utilizes antigen-antibody properties in a novel manner and provides rapid detection of analyte (Fig. 1.17) (Millipore corp 1996). They possess four benefits of user-friendly format, very short time to get test results, long-term stability over a wide range of climates and relatively inexpensive to make. These characteristics render it ideally suited for on site testing by untrained personnel, rapid point of care testing, and testing in the field for various environmental and agricultural analytes. In addition, they provide reliable testing that might not otherwise be available to third world countries. The principle behind the test is straightforward. Basically, any ligand that can be bound to a visually detectable solid support, such as dyed microspheres, can be tested for qualitatively, and in many cases even semi-quantitatively. Some of the more

common lateral flow tests currently on the market are tests for pregnancy (May 1991, Millipore corp 1996), strep throat , and chlamydia (Liu 2005; United States Patent 5393658; European Patent EP0258963; Shim et al., 2006). These are examples of conditions for which a quantitative assay is not necessary.

The main advantages of immunogold techniques are rapid and efficient. The procedure is simple, cheap, and easy to apply and does not require a reader device or machine and therefore cheap. Sensitivity could also be comparable to ELISA if good quality controls have been included. Such test have been used for diagnosis in some parasite diseases, such as antibody detection circulating antigen in toxoplasmasis (Wang et al., 2007; Li et al., 2004; Tang and Zhou, 2004), schistosomiasis (Zhu et al., 2002; Wu et al., 2005), cysticercosis (Liu et al., 2001, 2002), circulating antigen detection for *Plasmodium falciparum* (Moody, 2002; Li et al., 2004; Grobusch et al., 2004) and *Plasmodium vivax*, *Wuchereria bancrofti* and *Leishmania spp* (Yang, 2003, Zhu, 2002; Garcia et al., 2000;).

Colloidal gold antibody conjugate based DIGFA or similar immuno-chromatographic assays have also become an acceptable rapid clinical bed-side detection method for drug screening and diagnosis of severe microbial and parasitic infections (Dar et al., 1994; Xiao et al., 1995; Dylan and Kevin, 1999; Feng et al., 2000; Garcia et al., 2000; Feng et al., 2002; Zhu et al., 2002; Sorell et al., 2002; Yang, 2003; Hujakka et al., 2003; Chen et al., 2005). Initial applications of DIGFA for human echinococcosis in China indicated good potential as a rapid test (Fu et al., 2000; Feng et al., 2002; Zhang et al., 2001).

1.8 Aims and Objectives.

Echinococcosis is highly endemic in northwestern China. In order to improve immunodiagnostic tests in support of community screening and for hospital use, this study tried to develop and standardize a dot immunogold filtration assay (DIGFA) with novel antigens for rapid serodiagnosis of human CE and AE, then compare the performance of DIGFA with a standard ELISA, and the diagnostic value included sensitivity, specificity, accuracy, positive and negative predictive value of DIGFA would be assessed in hospital diagnostic setting and in endemic community screenings in northwest China. This study also tried to develop an immunochromatographic assay (ICA) test that would be initial developed for rapid *E.granulosus* antigen detection in cyst fluid samples to identify / confirm that cysts

are of hydatid origin. Another ICA strip was to be disigned for rapid coproantigen detection in dog faeces. A series of epidemiological surveys using portable ultrasound and DIGFA test during 2002-2007 would be reported and risk factors for human echinococcosis would be analyzed for further understanding the epidemiological features, where in the counties of Habahe, Qinghe, Mulei, Hejing, Wenquan, Xinyuan, Nileke, Hoboksaier in Xinjiang Region; Xiji in Ningxia; Ganzi Tibetan Autonomous Prefecture in Sichuan Province; and Dangxiong and Dingqing in Tibet Autonomous Region (TAR) in northwestern China. This research has implications for further development of rapid tests in support of human and canine echinococcosis diagnosis and for surveillance of transmission in China and elsewhere.

Chapter 2. Materials and methods

2.1 Study sites

Serological studies for human echinococcosis were mainly undertaken in Xinjiang Hydatid Clinical Research Institute, First Teaching Hospital of Xinjiang Medical University, Urumqi, P. R. China. Most rapid test development for *Echinococcus* coproantigen detections and for human hydatid cyst fluid antigen test was carried in Salford University. Community studies in several regions were undertaken in Xinjiang, Ningxia, Sichuan and Tibet.

2.2 Materials and methods for developing a rapid DIGFA test for hydatid disease

2.2.1 Human serum samples

Hospital human hydatid cases serum samples were collected from Xinjiang Medical University Hospital (XMUH) during 1998-2006; Chongqing Medical University, P. R. China. Blind test *s*era from Xinjiang Medical University Hospital (XMUH) Salford University (UK), Besancon University Hospital (France). Non-endemic control sera were collected from Greater Manchester hospital, UK (non-endemic area).

Serum samples for community study were collected from endemic communities in northwest China screened by ultrasound (US). The screened communities were Qinghe, Hobukersaier, Wenquan, Xinyuan County and Bayinbuluk Pasture in Xinjiang; Xiji County in Ningxia, Ganzi County in Sichuan, and Dangxiong, Dingqing County in Tibet AR.

2.2.2 Antigens for human *Echinococcus* antibodies detection

Four native antigens were used for DIGFA test which included *E. granulosus* cyst fluid antigen (EgCF), crude somatic extract of *E. granulosus* protoscoleces (EgP), *E. granulosus* cyst fluid antigen B (EgB) and *E. multilocularis* metacestode antigen Em2. The first three antigens were made from hydatid cyst fluid and *E. granulosus* protoscoleces, which were obtained by aseptic aspiration from naturally infected sheep livers or lungs collected in abattoirs from different endemic areas of Xinjiang, P. R. China. Em2 was made from *E. multilocularis* larval metacestode masses which were obtained from experimentally infected gerbils (*Meriones*

unguiculatus) at XMU. The details for preparation of them were in Appendix I.

The assay followed the standard indirect ELISA procedure for specific IgG detection with slight modification as used in the XMUH laboratory (Zhang et al., 2001). Buffers used are detailed in Appendix 2.

2.2.3 Development of multiple-antigen DIGFA for immunodiagnosis of human echinococcosis

Colloidal gold and conjugate was made following laborotary protocols. Rapid dot immunogold infiltration assay (DIGFA) was then designed for rapd test which was including a test plate and three test buffers: buffer A, B and C. Stability of DIGFA were observed in different temperatures for over then one year. Test procedure was finally standarded.

2.2.4 Comparison between DIGFA and ELISA

Serum samples from a hospital panel were tested by DIGFA and ELISA tests. The difference of sensitivity and specificity was calculated by SPSS 16.0 software (at $p<0.05$ level). The sensitivity and specificity of each antigen in DIGFA and ELISA were assessed.

2.2.5 Evaluation of diagnostic accuracy of DIGFA

The sensitivity, specificity, accuracy, negative predictive value (NPV) and positive predictive value (PPV), Odds Ratio (OR), Youden index, Positive likelihood ratio (PLR) and Negative likelihood ratio (NLR) of DIGFA were assessed for evaluation of this diagnostic test in each serum panel (The TDR Diagnostic Evaluation Expert Panel, 2006). In addition the reproducibility of DIGFA was also assessed for measuring or was test the stability.

True positives and negatives were previously determined by the gold-standard of clinical diagnosis with imaging (which was confirmed by surgery in hospital cases or ultrasound in the community studies).

2.3 Materials and methods for Rapid immunochromatographic assay (ICA) test for direct detection of human *E. granulosus* cyst fluid antigen B (EgB)

Human hydatid cyst fluid samples were collected from the First Teaching Hospital of Xinjiang Medical University.

*E. granulosus a*ntigen B (EgB, from sheep cyst fluid) was prepared as Appendix I. Hyperimmune rabbit serum anti EgB sera was then made and prepared for capture and conjugated antibodies. Half of above rabbit anti-EgB IgG was

conjugated with colloidal gold.

Direct and indirect DIGFA test for antigen detection in human hydatid cyst fluid were tried for the rapid test but did not perform very well.

Immunochromatographic Assay (ICA) was then another better choice for antigen detection using a sandwich assay style approach. Therefore, an ICA test was designed to be composed of a sample pad, a conjugate pad, a NC membrane with test and control lines and an absorbent pad.

Finally the ICA results were initially assessed.

2.4 Dog faeces sampling and preparation for coproantigen test

2.4.1 Study and sampling sites for canine echinococcosis

Most research on diagnosis of canine echinococcosiswas undertaken in Salford University with the main faecal samples came from China.

E. granulosus adult worms used for developing a rapid immunochromatographic assay (ICA) were obtained from experimentally infected dogs in Xinjiang Veterinary Research Institute, China.

A panel of normal dog faeces was gain from a UK non-endemic area and experimental normal control non-parasite infected dogs from XMUH, China.

Screening of owned dog was carried at the same time as the human echinococcosis surveys in northwestern China. Dog sampling occurred mainly in Habahe, Qinghe, Xinyuan, Bayinbuluk, Wenquan and Hoboksar counties in Xinjiang; Yushu County in Qinghai; Ganzi County in Ganzi Tibetan AutonomousPrefecture in Sichuan, and Dangxiong and Dingqing counties in Tibet AR.

2.4.2 Matierials and methods for canine echinococcosis

Matierials.

E. granulosus adult worms used for developing a rapid immunochromatographic assay (ICA) were obtained from 9 experimentally infected dogs at Xinjiang Veterinary Research Institute, China. Required *E. granulosus* protoscoleces were collected freshly from hydatid cysts from sheep liver or lungs in a slaughter house in Xinjiang. The adult worms were collected and stored as described by Allan et al ,1992 and Craig, 1997.

Faecal samples were prepared essentially as described by Allan *et al.* (1992).

E. granulosus whole worm extract antigen (EgWWE) was prepared essentially

according to Allan et al. 1992.

Preparation of hyperimmune rabbit antiserum was done according to the method described by Allan and Craig (1989) with a few small alterations.

Sandwich ELISA test for canine coproantigen detection

Preparation of capture and HRP conjugated rabbit anti-serum IgG was made according to the methods mentioned from Allan and Craig, 1989 and the conjugate was made via the method described by Wilson and Nakane (1978). Coproantigen ELISA procedure was used as described by Allan and Craig, 1989.

Development of a rapid ICA coproantigen test

A rapid sandwich ICA test were developed and optimized. Dog faecal samples were tested by ICA and ELISA for coproantigen detection. Results were compared and assessed with sensitivity and specificity with 95 confidence interval. Positive and negative predictive values were also determined.

2.5 Community studies on echinococcosis in northwest China

2.5.1 Study locations and communities

The study was part of mass screening survey for echinococcosis in highly endemic areas of northwest China during 1998-2007. There included main study sites of Yili, Tacheng, Altai, Boertala and Bayinguoleng Prefectures in Xinjiang Uygur Autonomous Region; also collaborate work with -Guyuan Prefecture in Ningxia Hui Autonomous Region, Ganzi Tibetan Autonomous Prefecture in Sichuan, Lhasa and Chamdo Prefectures of Tibet Autonomous Region.

2.5.2 Human echinococcosis screening

i. Questionnaire including general information and relative risk factors history (see appendix).

ii. Blood samples were collected from volunteers after the questionnaire and serological tests were did with Rapid DIGFA kit

iii. Abdominal ultrasound scanning were did for all the volunteers and Images with CE or AE characters or suspected cases were recorded.

iv. Serology follow-up were did by double check by DIGFA and/or US, or a chest X-ray for suspects.

v. All the CE or AE detected cases that needed treatment were initially given albendazole tablets or surgical treatment.

2.5.3 Canine echinococcosis surveys

Dog surveys by different teams were performed at the same time as community screening for human disease. A questionnaire (see appendix III) for dog owners included their host general information and dog's details. Dog faecal samples were collected.

2.6 Data analysis

Data analysis was used to evaluate the immunodiagnositic tools and determine risk factors in epidemiological studies on echinococcosis.

Chi-square tests were used to compare differences between rapid DIGFA and portable US scanning for human CE/AE; between rapid DIGFA and ELISA for human echinococcosis diagnosis; contribution differences among four different antigens; between rapid ICA and ELISA for coproantigen detection in dogs; and distributions of CE or AE in different locations/ethnic groups/ages/occupations.

Univariate odds ratios were used to determine independent associations of risk factors in different areas and different variables (including dog owner, livestock owner, home slaughter, occupation, education, hygienic habits, etc.)

Multivariate logistic regression was used to find adjusted odds rations to assess the relationship of diseases with different risk factors in different ethnic groups, different areas and find the key control points.

All analyses performed using SAS 9.0, SPSS 16.0 and Epi info version 6.0.

Statistical significance was set at alpha=0.05.

Chapter 3. Development and application of a rapid dot immunogold filtration assay (DIGFA) antibody detection kit for human CE and AE

3.1 Introduction

Echinococcosis is a worldwide zoonosis caused by the larval stages of tapeworms (cestodes) belonging to the genus *Echinococcus* (family Taeniidae). *Echinococcus granulosus* and *Echinococcus multilocularis*, which cause human cystic echinococcosis (CE) and alveolar echinococcosis (AE) respectively, are highly endemic in China (Wen and Yang, 1997; Craig, 2004). Both cause serious and potentially life-threatening diseases, the latter especially with high fatality rates and poor prognosis if not diagnosed and treated in the early stages (Zhou et al., 2000; WHO/OIE 2001; Craig et al., 2003). Mixed CE and AE cases are rare but have also been reported in China (Wen et al., 1992; Yang et al., 2006a; Yang et al., 2006b). Currently, mortality for human CE may vary between 0.2% and 4.5%, and for human AE 10-15% (Wen and Yang, 1997; WHO/OIE 2001; McManus et al., 2003; Zhang et al., 2003).

Early diagnosis of human echinococcosis is difficult because CE and AE patients usually have no signs or symptoms during the first few years of infection. Human echinococcosis commonly comes to the attention of clinicians because of non-specific clinical signs (e.g. upper abdominal pain, jaundice, allergic reactions) or due to incidental image findings of echinococcal cysts or lesions, or after specific mass-screening surveys by ultrasound and/or serology (WHO 1996; WHO/OIE 2001; Zhang and McManus, 2006).

The frequent difficulty in obtaining a definitive diagnosis is one reason why immunological methods have played an important role of diagnosis of human echinococcosis (Wen et al., 1995, Rogan and Craig 1997, 2002; WHO/OIE 2001). Almost all traditional immunodiagnostic methods (e.g. Casoni intradermal test, complement fixation test, indirect haemagglutination test, indirect immunofluorescence antibody test, immunoelectrophoresis (IEP), and latex agglutination test), have now been replaced by the enzyme-linked immunosorbent assay (ELISA) and/or immunoblotting (IB) which are commonly performed in routine laboratory diagnosis of human echinococcosis (Rogan and Craig, 2002;

Craig et al., 2003). Hydatid cyst fluid lipoprotein antigen B (AgB) from *E. granulosus*, and Em2/Em2plus, and/or Em18 antigens from *E. multilocularis*, are currently considered to be the most specific native or recombinant antigens for immunodiagnosis of human CE and AE respectively (Gottstein et al., 1987; Ito, 2002; Zhang et al., 2003; Carmena et al., 2005, 2006).

Although ELISA and immunoblot are very useful laboratory tests for human echinococcosis, a rapid immunological method that can be used for initial diagnosis of clinically suspected CE or AE, and that could be applied in community screening, would be extremely convenient. Rapid serological test formats such as dot-ELISA have been previously assessed for both human CE and AE, and although useful in conjunction with mass ultrasound screening, they were temperamental and difficult to use and interpret (Zheng et al., 1986; Rogan et al., 1991; Eliades et al., 1998; Qiao et al., 1999; Craig et al., 2000). Dot immuno-gold filtration assay (DIGFA) is a rapid immunodiagnostic test similar to a 'pregnancy' test that uses colloidal gold conjugated antibody or antigen instead of enzyme or fluorescence conjugates (Faulk and Taylor 1971; Horisberger et al., 1975; May 1991; Chun and Chu 1989; Xiao et al., 1995). Antigens are attached on a nitrocellulose membrane, and serum or whole blood applied, followed by colloidal gold conjugated anti-human antibodies to give a desired color change to indicate a positive or negative reaction.

In the current study a rapid DIGFA has been developed for human echinococcosis and assessed with four different native antigen-preparations including, *E. granulosus* crude hydatid cyst fluid antigen (EgCF), hydatid cyst fluid native antigen B (AgB), an *E. granulosus* protoscolex antigen extract (EgP), and an *E. multilocularis* metacestode laminated layer extract (Em2). The test was assessed in Xinjiang Medical University Hospital (Urumqi, northwestern China), which has treated over 6000 human echinococcosis cases in the last 40 years (Wen and Yang, 1997). The current study showed that the major advantages of DIGFA were rapidity, convenience, and ability to provide initial diagnosis and even differentiation of cystic and alveolar echinococcosis in approximately 80% of cases either in clinical or community screening settings.

3.2 Methods and Approaches

3.2.1 Serum samples and echinococcosis patients

3.2.1.1. Hospitalized hydatid patients

Archived serum panels used in the initial laboratory development and standardization of the DIGFA, were available from 108 post-operative hepatic CE cases, 34 post-operative hepatic AE cases, and 101 healthy controls collected from Xinjiang Medical University Hospital (XMUH) during 1998–2000. In addition 25 sera from cysticercosis (Taenia solium) patients were a gift from Prof. Y.H. Liu, Chongqing Medical University, P. R. China. (Table 3.1).

A serum panel was also available to assess hospital-based diagnosis of DIGFA and compared with standard ELISA. It consisted of 857 CE sera including 717 hepatic CE cases: among them, 516 ultrasound and/or surgery confirmed patients with less than 2 years post-surgery, 64 lung CE cases (diagnosed by X-ray or computerized tomography (CT)), 11 abdominal CE (diagnosed by ultrasound or CT), 18 multi-organ CE (diagnosed by ultrasound and CT) and 47 non-liver/lung CE cases (diagnosed by ultrasound, CT or magnetic resonance imaging (MRI)) (Table 3.2 and Fig. 3.1). In addition, sera from 42 liver AE cases and 1 mixed AE/CE case were assessed. In total 702 serum samples from non-hydatid disease patients were used as negative controls: non-parasite simple cystic disease 153, carcinoma 85, tuberculosis 28, solid or complicated space-occupying lesions (non-echinococcosis by imaging) 266, cirrhosis 6, abscess 13, cysticercosis 3, cholecystitis/gallstones 12, other patients treated in internal medicine (for hypertension, diabetes, and other clinical conditions) 88 and healthy individuals 5 (Table 3.3). All samples were collected and tested in XMUH during the period 1999–2006. For non-endemic controls, 35 sera from healthy people were collected from a hospital in Greater Manchester, UK, which is a non-endemic area.

Table 3.1: Hospital serum samples for developing and application of DIGFA serodiagnosis of human echinococcosis

Duration	Where	CE	AE	Mixed CE/AE	Cysticercosis (*Taenia solium*)	Normal control	Total
1998-1999	XJMUH[a]	108[b]	34[b]	0	0	101	243
1998	Chongqing Medical University [c]	0	0	0	25	0	25
1999-2006	XJMUH	857	42	1	0	702	1602
Total		965	76	1	25	803	1870

[a]: XJMUH means Xinjiang Medical University Hospital, Urumqi 830000, P. R. China
[b]: hepatic CE or AE cases
[c]: sera from cysticercosis (*Taenia solium*) patients were a gift from Prof. Y.H. Liu in Chongqing Medical University, P. R. China.

Table 3.2: Hospital cystic echinococcosis cases in different organs in XJMUH (1999-2006)

Organs	Cases	Percentage
liver	717	83.7%
lung	64	7.5%
abdominal cavity	11	1.3%
pelvic cavity	17	2.0%
multi-organ	18	2.1%
brain	5	0.6%
spleen	6	0.7%
kidney	5	0.6%
bone	7	0.8%
heart	3	0.4%
thoracic wall	3	0.4%
pancreas	1	0.1%
Total	857	100%

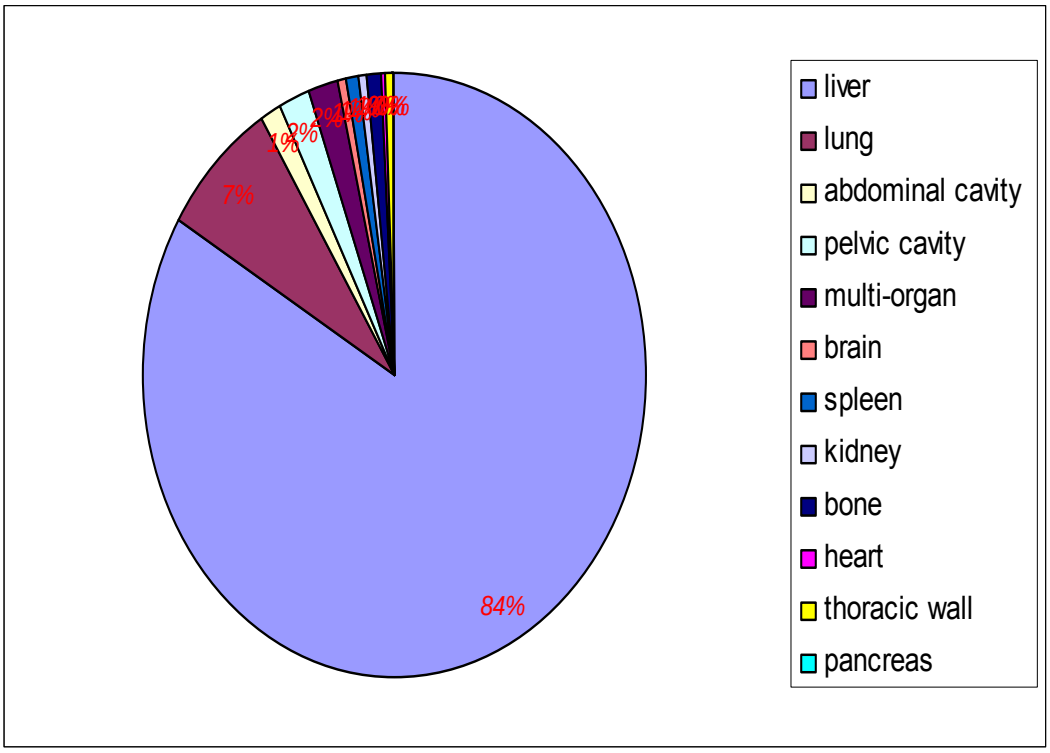


Fig. 3.1: Hospital cystic echinococcosis cases in different organs in XJMUH (1999-2006)

Table 3.3: Hospital non CE/AE control cases in different disease in XJMUH (1999-2006)

Clinical diagnosis	Organs	N	Percentage
Non-parasite cystic disease	Liver	154	21.9%
(n=196)	Lung	4	0.6%
	Brain	5	0.7%
	Kidney	5	0.7%
	Spleen	5	0.7%
	Abdominal cavity	5	0.7%
	Pelvic	8	1.1%
	Multi-organ	5	0.7%
	Others	5	0.7%
Carcinoma	Benign	30	4.3%
(n=85)	Malignant	49	7.0%
	Unknown	6	0.9%
Tuberculosis	Thoracic cavity or lung	22	3.1%
(n=28)	Spine	3	0.4%
	Abdominal cavity	3	0.4%
Solid or complicated space-occupying lesions (non echinococcosis by imaging)	Liver, lung and others	266	37.9%
Cirrhosis		6	0.9%
Abscess	Lung, liver etc.	13	1.8%
Cysticercosis (*T. solium*)	Brain	3	0.4%
Cholecystitis /gallstones	Gallbladder	12	1.7%
Other internal medicine	Diabetes, hypertension, etc.	88	12.5%
Normal	Healthy individuals	5	0.7%
Total		702	100%

Blind test. Sera from 224 CE and 68 AE patients from Xinjiang Medical University Hospital (XMUH) confirmed by surgery and pathology were assessed. In addition, 112 serum samples, including 33 AE, 18 CE, 32 cysticercosis (*T. solium*), 10 schistosomiasis and 19 negative controls were available from Salford University

(UK), and 128 serum samples, comprising 11 CE, 72 AE and 45 negative controls were provided by Besancon University Hospital (France). All sera were labeled by number only and tested in XMUH and Salford University.

Non-endemic control sera. 35 'normal' (non echinococcosis) controls from healthy people were collected from Greater Manchester hospital, UK (non-endemic area).

Serum sample preparation from hospital human hydatid cases

All blood samples were collected through vein injection (3-5mL per person), allowed to clot for 1 hour under the room temperature, or mixed well with procoagulant reagent (Clot activator for quick clot in 5 mins, such as 'increased silica act clot activator, silicone-coated interior', which could be applied in "procoagulant tube" products, Zhejiang Gong-Dong Medical Plastic Company), centrifuged at 2000 rpm for 10 mins and aliquots 0.5ml/tube, stored at 4 °C for test within 2 days or -80 °C for long-term storage.

3.2.1.2 Samples collection from community screening.

Echinococcosis endemic communities in northwest China that were screened by ultrasound and serology were: Qinghe, Hobukersaier, Wenquan, Xinyuan County and Bayinbuluk Pasture in XUAR; Xiji County in Ningxia Hui Autonomous Region (AR); Ganzi County in Sichuan Province; and Dingqing County in Tibet AR (Feng et al., 2002, Chen et al., 2005, Wang et al., 2001, Yang et al., 2006a, 2006b). Based on ultrasound scan as the gold standard, serum samples from 160 CE and 108 AE cases, and 2923 normal persons came from endemic communities in northwest China screened by ultrasound (US). Ultrasound normal cases with a previous history of echinococcosis surgery were not included in the 'negative' group. All persons included gave informed consent for their serum to be collected and assessed in the study. Ethical permission was granted by the Xinjiang Medical University Hospital Ethical Committee.

3.2.2 Preparation of diagnostic antigens

Native extracts of *E. granulosus* and *E. multilocularis* were used because they can be prepared relatively easily by most laboratories. Sheep hydatid cyst fluid and protoscoleces from *E. granulosus* were collected in Xinjiang Uygur Autonomous

Region (XUAR), China. Crude cystic fluid (EgCF) was partially purified by affinity chromatography using a normal human serum coupled to CNBr–Sepharose 4B to remove non-specific host reactive proteins from sheep hydatid cyst fluid (Rogan et al., 1991, Zhang et al., 2000). A crude somatic extract of *E. granulosus* protoscoleces (EgP) with >85% viability were harvested from fertile sheep hepatic hydatid cysts, prepared by ice cold homogenization and centrifugation (13,000 × *g* for 30 min at 4 °C) and partially purified by affinity chromatography as for EgCF (Zhang et al., 2000). *E. granulosus* cyst fluid antigen B (AgB) was purified from fresh sheep hydatid cyst fluid by precipitation, boiling, centrifugation and concentration by dialysis as previously described (Rogan et al., 1991, Rogan and Craig, 1997, Zhang et al., 2000 and Zhang et al., 2001). Experimental infections of gerbils (*Meriones unguiculatus*) after 3 months post-infection with *E. multilocularis* protoscoleces (in a metacestode homogenate suspension) were used to produce metacestode tissue for extraction of a laminated layer enriched antigen (Em2) by homogenization, centrifugation and affinity chromatography using rabbit anti-*E. granulosus* cyst fluid-IgG coupled CNBr–Sepharose 4B column (Gottstein et al., 1983 and Zhang et al., 2001). (Details in appendix I).

3.2.3 Development of a rapid DIGFA system for human echinococcosis

A rapid dot immuno-gold filtration assay (DIGFA) was chosen for development an immunodiagnostic test for detection antibody of human echinococcosis, which could be easily used to replace Casoni test for initial diagnosis combined with ultrasound in the rural area. Several antigens were selected and applied with a DIGFA test for evaluation their sensitivity and specificity for human echinococcosis. This developed DIGFA test for human echinococcosis was applied in the patients from Xinjiang Medical University Hospital and the results were compared with standard ELISA.

3.2.3.1 Diagnostic Antigens selection and preparation

Several native antigens were generally used for antibody detection of human echinococcosis by ELISA or immunoblot, e.g., native *Echinococcus* antigens EgCF, EgP, Arc-5 and antigen B for CE, EmP, Em2 and Em18 for AE (see Chapter 1). Combined different antigens might supply more information for diagnosis of human echinococcosis and obtain better sensitivity and specificity.

Antigens were selected by materials available, easy preparation, sensitive and

specific for both CE and AE. Preparation of *Echinococcus granulosus* cyst fluid antigen (EgCF) and *E. granulosus* protoscoleses abstract (EgP) were studied by ELISA and dot immunoblot assay (DIBA) to obtain better sensitivity of human CE (Zhang et al., 2000). Meanwhile, more specific antigen B for human CE and Em2 for AE were selected and tested by ELISA.

Native extracts of *E. granulosus* and *E. multilocularis* were used because they can be prepared relatively easily by most laboratories. Sheep hydatid cyst fluid and protoscoleces from *E. granulosus* were collected in Xinjiang Uygur Autonomous Region (XUAR), China. Crude cystic fluid (EgCF) was partially purified by affinity chromatography using a normal human serum coupled to CNBr–Sepharose 4B to remove non-specific host reactive proteins from sheep hydatid cyst fluid (Rogan et al., 1991, Zhang et al., 2000). A crude somatic extract of *E. granulosus* protoscoleces (EgP) with >85% viability were harvested from fertile sheep hepatic hydatid cysts, prepared by ice cold homogenization and centrifugation (13,000 × *g* for 30 min at 4 °C) and partially purified by affinity chromatography as for EgCF (Zhang et al., 2000). *E. granulosus* cyst fluid antigen B (AgB) was purified from fresh sheep hydatid cyst fluid by precipitation, boiling, centrifugation and concentration by dialysis as previously described (Rogan et al., 1991, Rogan and Craig, 1997, Zhang et al., 2000 and Zhang et al., 2001). Experimental infections of gerbils (*Meriones unguiculatus*) after 3 months post-infection with *E. multilocularis* protoscoleces (in a metacestode homogenate suspension) were used to produce metacestode tissue for extraction of a laminated layer enriched antigen (Em2) by homogenization, centrifugation and affinity chromatography using rabbit anti-*E. granulosus* cyst fluid-IgG coupled CNBr–Sepharose 4B column (Gottstein et al., 1983 and Zhang et al., 2001). (Details see appendix I).

3.2.3.2 Preparation of colloidal gold and conjugate

Preparation of colloidal gold

Gold chloride ($HAuCl_4$, 10%) was prepared with H_2O using HAuCl4·3H_2O, Gold (III) Chloride trihydrate, ACS reagent (Sigma G4022). Pure water 100mL in a 500mL glass flask was boiled in a microwave oven (around 90 seconds in 750W). 300 µL of 10% $HAuCl_4$ was added until the solution was pale yellow and then boiled again and 7.5 ml 1% sodium citrate (fresh made) was added immediately. The flask was swirled quickly to mix the reagents well. The colour should change into wine red in

1-2 minutes. The colloidal gold was cooled at room temperature and then stored at 4°C with a foil cover until used for conjugate to antibody.

Preparation of colloidal gold conjugate

Goat anti-human IgG (Sigma I1886, USA; Sino-American Biotechnology Co, Luoyang, China) was diluted to 1mg/ml in 20mM Tris-HCl buffer, and 384μL of IgG solution added (determined by colloidal gold conjugate volume, see appendix 3) to 10 mL of colloidal gold with 1 mL of 10mM borate buffer. The conjugate was allowed to combine at 4°C for over 45 minutes. Blocking buffer including 100mM Tris-HCl, 25% sucrose, 5% Gelatin (Sigma-Aldrich, G7765, from cold water fish skin) and 0.03% polyethylene glycol 20,000 (PEG, BDH Chemicals Ltd. Poole, UK) was added 2.95mL and mixed well to block unbound colloidal gold. The conjugate was then filtered through a 0.2μm syringe filter (Millipore, UK) and stored at 4°C within dark bottles (stable for up to 1 year).

3.2.3.3 Building a Rapid dot immunogold infiltration assay (DIGFA)

3.2.3.3.1 Composition of a DIGFA test Plate:

The test components were set up in advance: plastic plate base plus filter tissue plus Nitrocellulose (NC) membrane (0.45μm pore size, Sartorius, Germany) plus top cap (Fig 2-2).

Normal human sera control for test function diluted with pH 8.2 200mM Tris-HCl buffer (1:1 ratio) was added as one drop (1μL) onto the middle of the NC membrane ensuring that the pipette tip did not contact with the NC membrane; Antigen solutions EgCF, EgP, EgB, Em2 (the concentration of each was determined by chess-board check with CE or AE patient sera) were coated as a small dot (0.5 μL per dot) onto the 4 corners of the NC membrane, and allowed to dry completely.

The test kit with antigens was vacuum-packed in an aluminium foil bag and stored at 4°C until used (stable for up to 1 year).

3.2.3.3.2 Test buffers: buffer A, B and C

Buffer A was a sample dilution buffer and also blocking buffer and consisted of 20mM Tris-HCl, 1% Gelatin and 0.05% Tween 20. Washing buffer B was made up of 20mM Tris-HCl, 0.9% NaCl and 0.4% Tween 20. Buffer C was the colloidal gold

conjugated goat anti-human IgG (see above).

3.2.3.3.3 Stability of DIGFA

The test kit and buffers were stored at 37 °C for 2 weeks and at room temperature (20-25°C) for 2 months with no change to test efficacy.

The test result could be kept stable in 24 hours at 4°C and 20 minutes in room temperature.

3.2.3.3.4 Test procedure

Serum (20μL) was diluted with 5-drops (about 200 μL) of Buffer A. Whole blood (heparinized blood) was diluted 40 μL with 5-drops Buffer A and centrifuged at 3000rpm for 3 min and the supernatant retained. Diluted sera or whole blood supernatant was added (100μL) to the well of NC membrane until absorbed. Then the NC membrane was added with 3 drops of washing buffer (Buffer B).

Three drops colloidal gold conjugated goat anti-human IgG (Buffer C) was added until absorbed. Then the NC membrane was washed with 3 drops washing buffer (Buffer B) again.

The membrane was read then (better in 5 minutes). Red or purple colour of an antigen dot means positive.

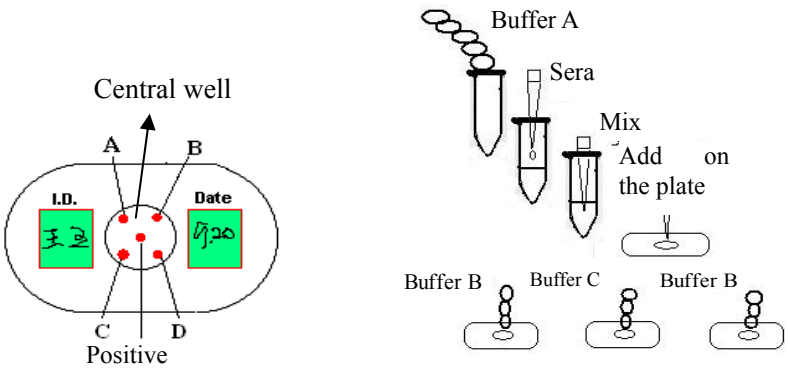


Fig. 3.2: Left: the model of test kit; Right: procedure step by step, 3-5 minutes in total.

3.2.3.4 Optimization of DIGFA

In general, DIGFA is using nitrocellulose (NC) membrane as a carrier which pre-coated antigen or antibody as a capture which is similar to immunoblot without

substrate step (see Chapter 1). An immunoblot nitrocellulose (NC) membrane (pore size 0.45μm, Sartorius, German) was used in this study. Pretreatment with 20% methanol of NC membrane made it operable to put into a frame of DIGFA kit since original NC membrane was crisp and easy to break. Additional treatment with 0.01% SDS and 0.01% Tween 20 were optional when the absorbance of antigens on NC membrane was not good enough due to storing for a longer time (eg. more than one year).

Optimal concentrations of antigens were assessed by coated with different dilutions (1:2, 1:4, 1:8 and 1:16) and tested with serum samples of CE, AE cases and healthy individuals (Table 3.4). Finally EgCF 1:2、EgP 1:2、AgB 1:3、Em2 1:6 were determined. Optimal concentration of colloidal gold conjugate (see Chapter 2) was also determined by the same serum panel.

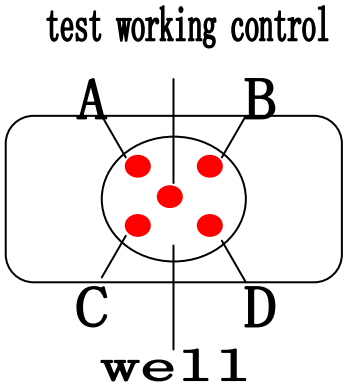
The DIGFA kit was designed with a test working control (mixed sera of healthy individuals) in the central of a test well and four antigens at 4 corners. DIGFA procedure was carried out as described in Appendix. The DIGFA results were assessed usually as positive or negative spots (Fig. 3.3).

Table 3.4: Optimization of concentrations of antigens for DIGFA

Antigens [a]	DIGFA results [b]								
	CE1	CE2	CE3	AE1	AE2	AE3	N1	N2	N3
EgCF original	3+	+	2+	3+	+	2+	±	-	-
EgCF 1:2	2+	±	+	3+	±	-	-	-	-
EgCF 1:4	2+	±	±	2+	-	-	-	-	-
EgCF 1:8	-	-	-	+	-	-	-	-	-
EgP original	3+	+	2+	3+	+	+	±	-	-
EgP 1:2	2+	±	+	3+	+	+	-	-	-
EgP 1:4	±	±	±	2+	-	-	-	-	-
EgP 1:8	-	-	-	+	-	-	-	-	-
AgB original	3+	+	2+	3+	±	+	-	-	-
AgB 1:2	2+	±	+	2+	±	-	-	-	-
AgB 1:4	2+	±	+	+	-	-	-	-	
AgB 1:8	+	-	+	-	-	-	-	-	-
Em2 original	+	+	+	4+	+	3+	±	±	-
Em2 1:2	±	±	±	3+	+	2+	-	-	-
Em2 1:4	±	-	-	2+	+	+	-	-	-
Em2 1:8	-	-	-	2+	±	+	-	-	-

[a] The protein concentration of original antigens were ranged from 2-4mg/ml.
[b] CE1,2 and 3 were serum samples of CE cases which showed strong, weak and middle positives by ELISA; AE1, 2 and 3 were serum samples of AE cases which showed strong, weak and middle positives by ELISA; ‘’+’’ was positives and ‘’-‘’ was negatives.

(i)



(ii)

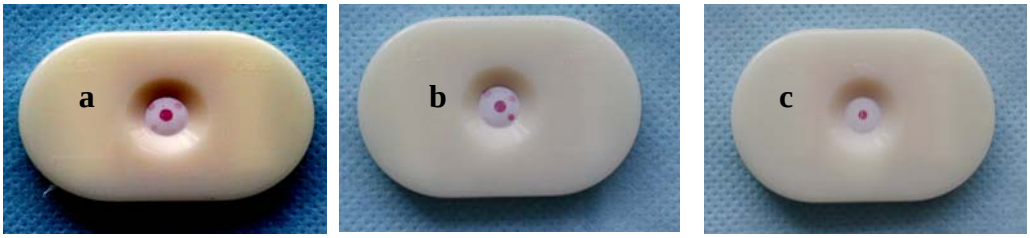


Fig. 3.3: DIGFA test kit
(i) Diagram of antigen dots:
Dot A is for EgCF, Dot B for EgP, Dot C for AgB and Dot D for Em2 antigens
(ii) Actual DIGFA result after serum test (3 minutes)
(a) Serum positive CE case, (b) serum positive AE case, (c) negative control

3.2.4 ELISA tests for detection of human serum antibodies

Optimal dilutions of antigen extracts (5-15µg/ml) were determined by check-board titration using EgCF, EgP, EgB or Em2 antigens. Antigens were coated 100µl/well on to the 96-well microtitre plates (12well *8 line and frame, from Shanghai Kehua Bio Tech Co., China) with carbonated coating buffer (0.01M NaHCO3, pH9.6) and left at 4 ºC overnight.

The microtitre plate was washed 3 times with 0.15M PBS (pH7.2) including 0.1% Tween20 (PBSTW) and then blocked 37ºC for 2 hours with a blocking buffer consisted of 0.15M PBS, 1% BSA (bovine sera albumin), 0.3% Tween20 and 1.6% gelatin added to reduce non-specific binding.

After washing the plate 3 times with PBSTW, sera was added 100µl/well at 1:100 dilution in a sample buffer (0.15M PBS with 0.3% Tween20 and 3% normal sheep sera for 30 minutes at 37 ºC. Plate then washed as above.

Horseradish peroxidase (HRP) conjugated goat anti-human IgG (Sigma A6029) at

100μL/well (1:8000 diluted according to manufactories instruction by a conjugate buffer including 0.15 M PBS, 0.3% Tween20 and 3% normal sheep sera) was added for 20 minutes at 37ºC. Plate then washed as above.

Substrate of tetramethyl benzidine (TMB) (buffer A and B 50 μL/well respectively, TMB powder from BBI, Canada, imported by Shanghai Sangon Co, buffer A and B made according appendix 2) was added and incubated for 15 minutes at 37ºC.

After final substrate incubation, the reaction was stopped after 15-20 minutes by adding 50 μl/well of stop buffer (0.5% sulfuric acid).

Microtitre plate OD values for each well was read at 450nm using a microplate reader (Bio-Rad 550, USA). The positive-negative cut-off was the mean OD of control sera plus 3 SD, or 2.1 times the OD value of normal controls. The latter was used routinely in the XMU hospital ELISA microplate reader's software programme. Buffers details were shown in Appendix II.

3.2.5 Assessment of DIGFA in diagnosis of human CE and AE

The sensitivity, specificity, accuracy, negative predictive value (NPV) and positive predictive value (PPV), Odds Ratio (OR), Youden index, Positive likelihood ratio (PLR) and Negative likelihood ratio (NLR) of DIGFA were assessed for evaluation of this diagnostic test in each serum panel (The TDR Diagnostic Evaluation Expert Panel, 2006). The reproducibility was tested in the same or different batch of DIGFA kit. The storing stability was determined by testing after storing at 37°C 2 for 2 weeks and at room temperature (20-25°C) for 2 months. Comparisons of DIGFA with ELISA were carried out in initial development panel and hospital test panel.

The definitions and formulations for above assessment indexes were as follows.

i) Gold standard or reference standard means the best available test that is used as comparison and provided by professional authorities. Here the confirmed clinical diagnosis by ultrasound, X-ray, computerized tomography (CT), magnetic resonance imaging (MRI and/or surgery was used as gold standard.

ii) Sensitivity (Se) means that the probability of test positives from truly infected individuals (true positive rate). Sensitivity= no. of test positives in infected individuals /no. of infected individuals *100%

iii) Specificity (Sp) means that the probability of test negatives from truly uninfected individuals (true negative rate). Specificity = test negatives in uninfected individuals/ uninfected individuals *100%

iv) Accuracy means that the percentage of correct test results compared with the gold standard. Accuracy = (true positives + true negatives)/(infected + uninfected)*100% (the number of correct results divided by the total number of results)

v) Negative predictive value (NPV) means the probability of a negative result accurately which indicates the absence of infection. NPV=true negatives/(true negatives + false negatives)

vi) Positive predictive value (PPV) means the probability of a positive result accurately which indicates the presence of infection. PPV= true positives / (true positives + false positives)

vii) 95% confidence intervals (CI): $p \pm 1.96\sqrt{\frac{p(1-p)}{n}}$

viii) The diagnostic odds ratio (DOR) is a measure of the effectiveness of a diagnostic test. It is defined as the ratio of the odds of the test being positive if the subject has a disease relative to the odds of the test being positive if the subject does not have the disease. A DOR of exactly one means that the test is equally likely to predict a positive outcome whatever the true condition. Higher DOR are indicative of better test performance.

$$\text{Diagnostic Odds Ratio} = \frac{\text{Sensitivity}}{\text{1 - Sensitivity}} \times \frac{\text{Specificity}}{\text{1 - Specificity}}$$

ix) Youden index is an index for accurate diagnosis, which shows the ability of the test could judge patient or non-patient.

$$\text{Youden index} = \text{Sensitivity} + \text{Specificity} - 1$$

x) Positive likelihood ratio (PLR) indicates the ratio between true positive rate and false positive rate, which explains the multiple between the probability of the test positives in patients and in non-patients. Higher PLR means better validity and valuable for clinical diagnosis.

$$PLR = \frac{\text{True positive rate}}{\text{False positive rate}} = \frac{\text{Sensitivity}}{\text{(1-Specificity)}}$$

xi) Negative likelihood ratio (NLR) is the ratio between false negative rate and true negative rate, which indicates the multiple between the test negative probability in patients and in non-patients. Lower NLR means better validity.

$$NLR = \frac{\text{False negative rate}}{\text{True negative rate}} = \frac{\text{(1-Sensitivity)}}{\text{Specificity}}$$

For the ELISA procedure, positive-negative cutoff value was determined as the mean optical density (OD) value of a panel of negative controls (n=35) plus three standard deviations (Table 3.5). Sensitivity and specificity were calculated using 95% confidence intervals (CI) and significance values were also determined at the 95% probability level.

Table 3.5: Comparison of DIGFA and ELISA with non-echinococcosis human sera from non-endemic area

Antigens	DIGFA (n=35)			ELISA (n=35)		
	Positive*	Negative	Specificity	Positive	Negative	Specificity
EgCF	6	29	82.9%	1	34	97.1%
EgP	6	29	82.9%	0	35	100%
AgB	0	35	100%	1	34	97.1%
Em2	0	35	100%	1	34	97.1%

* These positives in DIGFA all showed just suspectable with very poor positive.
ELISA positives cutoff was mean OD of all samples add 3 times standard deviation.
Cutoff-1 for EgCF: 0.570, EgP: 0.327, AgB: 0.303, Em2: 0.343

3.3 Results

All the human serum samples were tested by the DIGFA and/or ELISA formats prepared in the Xinjiang Hydatid Clinical Research Institute, Xinjiang Medical University Hospital.

3.3.1 Development and Initial validation of multiple Echinococcus antigens (EgCF, EgP, AgB and Em2) in DIGFA for human echinococcosis

In preliminary assessment of archived serum samples the sensitivity of DIGFA for human CE (n=108) was 92.6%, 90.7% and 89.8% with EgCF, EgP and AgB native antigen preparations respectively. For human AE (n=34) the sensitivity of DIGFA was 91.1% for Em2 antigen. The specificity for CE with AgB antigen in DIGFA was 88.1% with 95% confidence intervals (CI) (82.0%--94.2%), and for AE with Em2 antigen was 93.6% with 95% CI (89.4%--97.8%). Cross-reactivity between human CE and AE for AgB antigen was 35.3% for AE, while cross-reactivity for Em2 with human CE was 7.4%. Cross-reaction in DIGFA with serum from cysticercosis patients was observed with all four antigens (60% and 56% with EgCF and EgP; 8%, 16% respectively for AgB and Em2). There was no statistical difference between results observed using DIGFA versus the standard ELISA ($p>0.05$). Of the four different native antigen preparations the highest sensitivity occurred with antigen EgCF (92.6% for CE cases and 88.2% for AE cases) (Table 3.6).

The reproducibility, or called reliability was checked by the same or different batches of DIGFA kits and 95.5% of reproducibility was obtained as 95% (Table 3.7 and 3.8). The stability of DIGFA kits was confirmed by the same results obtained after 2 weeks at 37°C and 2 month at room temperature.

Table 3.6: DIGFA and ELISA results with multiple antigens (EgCF, EgP, AgB and Em2) for detection of confirmed human hepatic cystic (CE) or alveolar (AE) Echinococcosis. (n=268)

Antigens		CE cases [a] (n=108)		AE cases [a] (n=34)		Non-hydatid (n=101)		Cysticercosis (n=25)	
		DIGFA	ELISA	DIGFA	ELISA	DIGFA	ELISA	DIGFA	ELISA
EgCF	Positive	100	101	30	32	7	5	15	16
	Sensitivity (%)	92.6	93.5	88.2	94.1	Cross-reaction (%)		60	26.7
	Specificity (%)					93.7	95.1		
EgP	Positive	98	102	28	32	7	5	14	15
	Sensitivity (%)	90.7	94.4	82.4	94.1	Cross-reaction (%)		56	60
	Specificity (%)					93.7	95.0		
AgB	Positive	97	99	12	10	4	3	2	3
	Sensitivity (%)	89.8	91.7	35.3	29.4	Cross-reaction (%)		8	12
	Specificity (%)					96.0	97.0		
Em2	Positive	8	10	31	32	3	2	4	5
	Sensitivity (%)	7.4	9.3	91.1	94.1	Cross-reaction (%)		16	20
	Specificity (%)					98.02	95.05		
Total		108	108	34	34	101	101	25	25

[a] CE or AE cases were selected surgical confirmed samples – post-surgery, in Xinjiang Medical University Hospital(XMUH)

Table 3.7: Reproducibility in the same batch of DIGFA kits

Sera Group	Antigens	No.	Test positive	Test negative	Reproducibility (%)
Positives (CE or AE)	EgCF	22	21	1	95.5
	EgP	22	21	1	95.5
	AgB	22	21	1	95.5
	Em2	22	21	1	95.5
Controls	EgCF	22	1	21	95.5
	EgP	22	1	21	95.5
	AgB	22	1	21	95.5
	Em2	22	1	21	95.5

Table 3.8: Reproducibility in different batch of DIGFA kits

Sera Group	Antigens	No.	Test positive	Test negative	Reproducibility (%)
Positives (CE or AE)	EgCF2	22	20	2	90.9
	EgP2	22	21	1	95.5
	AgB	22	21	1	95.5
	Em2	22	21	1	95.5
Controls	EgCF2	22	1	21	95.5
	EgP2	22	1	21	95.5
	AgB	22	1	21	95.5
	Em2	22	1	21	95.5

3.3.2 Diagnostic evaluation of the rapid DIGFA in a hospital setting

The DIGFA was applied for immunodiagnosis of clinical echinococcosis using a panel of sera (n=899, CE and AE) taken from patients that were treated in XMUH over the period September 1999 to April 2006. The DIGFA test had a lower sensitivity for CE (80.7%) compared with the initial laboratory based standardization study ($p<0.01$) for all CE patients combined including liver, lung, or other organs (Table 3.9 and 3.10). Specificity of DIGFA for CE was 93.4% (695/744 non-CE cases were negative) (Table 3.11). The sensitivity of DIGFA for human AE showed no statistical difference between the laboratories based study (91.1%) and the main hospital study (90.3%) ($p>0.05$) (Table 3.12). The sensitivity of DIGFA for CE in different organs was, 94.4% in multi-organ CE, 83.4% for hepatic CE, 80.7% for pulmonary CE, 80% for CE in the pelvic cavity, 70% for CE in the abdominal cavity, and 56.7% for CE in other organs (including heart, kidney, brain, spine, bone, subcutaneous). Significant statistical differences were observed between DIGFA and ELISA for serodiagnosis of CE. The ELISA exhibited lower sensitivity for hospitalized human CE cases (75.0%), but higher specificity (97.6%) compared to DIGFA ($p <0.01$). For human AE both ELISA and DIGFA had similar sensitivities (97.6% vs 92.9%) ($p> 0.05$) but different specificities (78.6.1% vs. 90.3%) ($p<0.01$) (Table 3.9, Table 3.12 and 3.12.1). Overall a false positive rate of 10.4% occurred with the DIGFA for sera from persons without echinococcosis.

Table 3.9: Comparison of rapid DIGFA and standard ELISA applications for serodiagnosis of human echinococcosis in hospitalized CE (n=857) or AE cases (n=42) using four native antigen preparations: *E. granulosus* cyst fluid (EgCF, AgB), *E. granulosus* proscoleces (EgP) and *E. multilocularis* (Em2).

Clinical Diagnosis	N	Ag	DIGFA		ELISA		Chi^2	*p* [b]
			Pos	Neg	Pos	Neg		
CE:	857	EgCF	683	174	533	324	63.68	<0.01
		EgP	631	226	437	420	93.5	<0.01
		AgB	586	271	492	365	22.09	<0.01
		Em2	146	711	290	567	63.78	<0.01
		Any dot[a]	692	165	643	214	8.13	<0.01
		Sensitivity		**80.7%**		**75.0%**		
AE:	42	EgCF	39	3	27	15	10.18	<0.01
		EgP	39	3	27	15	11.18	<0.02
		AgB	28	14	21	21	2.4	>0.05
		Em2	35	7	33	9	0.31	>0.05
		Any dot*	39	3	41	1	0.26	>0.05
		Sensitivity		**92.9%**		**97.6%**		
Controls (non CE/AE)	702	EgCF	63	639	27	675	15.39	<0.01
		EgP	55	647	36	666	4.24	<0.05
		AgB	21	681	28	674	1.04	>0.05
		Em2	5	697	20	682	9.16	<0.01
		Any dot*	73	629	72	630	0.01	>0.05
		Specificity		**89.6%**		**89.7%**		
Total	1601[c]							

Ag=Antigen, Pos=positive, Neg=negative

[a]: means any antigen showed positive in positive column, and none antigen showed positive in negative column.

[b]: means p value when compared DIGFA and ELISA, Chi-Square test and correction if one cell had expected count less than 5. (SPSS 16.0)

[c]: Total tests included another mixed CE and AE case, which showed positive with four antigens in DIGFA and all positive exclude Em2 in ELISA.

Table 3.10: Comparison of rapid DIGFA and standard ELISA applications for serodiagnosis of human echinococcosis in hospitalized CE (n=857) or AE cases (n=42) (modified from Table 3-8).

Clinical Diagnosis	N	DIGFA			ELISA			P
		Positive [b]	Negative	Sensitivity (%) (95% CI [c])	Positive	Negative	Sensitivity (%) (95% CI)	
CE	857	692	165	80.7 (78.1-83.3)	643	214	75.0 (73.5-76.5)	<0.01
AE	42	39	3	92.9 (88.9-96.8)	41	1	97.6 (95.2-99.9)	>0.05
AE/CE	1	1	0	100	1	0	100	
Controls [a]	702	73	629	10.4 [d]	72	630	10.4*	>0.05
Total	1602	805	797		757	845		

a. Controls were defined as those free from either CE or AE infection.
b. Positive means any one of the four antigen dots changing color.
c. 95% confidence interval, $p \pm 1.96\sqrt{\frac{p(1-p)}{n}}$, p means sensitivity or specificity, n means number.
d. False positive rate in control group (non CE/AE).

The diagnostic assessments were then analyzed by accuracy, PPV, NPV, diagnostic odds ratio, Youden Index, PLR and NLR. The accuracies of DIGFA were 84.7% ((692+629)/(857+702))for CE and 89.8% for AE ((39+629)/(42+702)), meanwhile the accuracies of ELISA were 81.7% ((643+630)/(857+702)) for CE and 90.2% ((41+630)/(42+702)) for AE. PPV of DIGFA and ELISA for CE (90.5% and 89.9) was better than for AE (34.8% and 36.3%), however, NPV of DIGFA and ELISA for CE (79.2% and 74.6%) was lower than for AE (99.5% and 99.8%). DOR of DIGFA and ELISA for CE (36 and 13) was lower than for AE (112.7 and 354). Youden index of DIGFA and ELISA for CE (0.7, 0.65) was also lower than for AE (0.8, 0.87). And also DIGFA and ELISA for AE showed a better probability for true positives and true negatives than for CE with higher PLR and lower NLR results (Table 3.10.1).

Table 3.10.1: Assessments of rapid DIGFA and standard ELISA applications for serodiagnosis of human echinococcosis in hospitalized CE (n=857) or AE cases (n=42)

	Assessments:	CE		AE	
		DIGFA	ELISA	DIGFA	ELISA
i.	Se (%) (95%CI)	80.7 (78.1-83.3)	75.0 (73.5-76.5)	92.9 (88.9-96.8)	97.6 (95.2-99.9)
ii.	Sp (%) in Non CE or AE controls (95%CI)	89.6 (88.4-90.8)	89.7 (88.6-90.8)	89.6 (88.4-90.8)	89.7 (88.6-90.8)
iii.	Accuracy (%)(95%CI)	84.7 (82.9-86.5)	81.7 (79.8-83.6)	89.8 (88.3-91.3)	90.2 (88.7-91.7)
iv.	PPV (%) (95%CI)	90.5 (89.1-91.9)	89.9 89.1-91.9	34.8 32.4-37.2	36.3 33.9-38.7
v.	NPV (%)(95%CI)	79.2 (77.2-81.2)	74.6 (72.4-76.8)	99.5 (99.2-99.8)	99.8 (99.6-100)
vi	DOR	36.0	13.1	112.7	354.2
vii.	Youden Index	0.703	0.647	0.825	0.873
viii.	PLR	7.76	7.28	8.93	9.48
ix.	NLR	0.22	0.28	0.08	0.03

The diagnostic value was also assessed for DIGFA and ELISA antibody test against a single antigen and parallel combination (Table 3.10.2 and 3.10.2a for EgCF, Table 3.10.3 and 3.10.3a for EgP, Table 3.10.4 and 3.10.4a for AgB, Table 3.11 for parallel combination) for CE. The generally lower Se, Sp, Accuracy, PPV, NPV, DOR, Youden Index and PLR were observed in DIGFA antibody against single antigen (EgCF, EgP and EgB) for CE compared to that against parallel combination, except a higher specificity (93.4%), PPV (92.3%) and PLR (10.36) was occurred in single antigen AgB (Table 3.11).

Table 3.10.2: Sensitivity and specificity of EgCF antigen in DIGFA and ELISA for hospitalized CE cases

Clinical diagnosis	N	DIGFA		ELISA		P value
		Positive	Negative	Positive	Negative	
CE	857	683	174	533	324	<0.01
Non-CE	744	102	642	54	690	>0.05
Total	1601	785	816	587	1014	

Table 3.10.2a: Assessments of EgCF antigen in rapid DIGFA and standard ELISA applications for serodiagnosis of human echinococcosis in hospitalized CE (n=857) or non-CE cases (n=744)

Assessments	EgCF for CE			
	DIGFA		ELISA	
Se (%) (95%CI)	78.1	(76.1-80.1)	62.3	(59.9-64.7)
Sp (%) in Non CE controls (95%CI)	86.3	(85.4-87.2)	92.7	(91.4-94.0)
Accuracy (%) (95%CI)	82.8	(80.9-84.7)	76.4	(74.3-78.5)
PPV (%) (95%CI)	87.0	(85.3-88.7)	90.8	(89.4-92.2)
NPV (%) (95%CI)	78.7	(76.7-80.7)	68.0	(65.7-70.3)
Odds ratio	24.7		21.0	
Youden Index	0.644		0.556	
PLR	5.701		8.534	
NLR	0.254		0.407	

Table 3.10.3: Sensitivity and specificity of EgP antigen in DIGFA and ELISA for hospitalized CE cases

Clinical diagnosis	N	DIGFA		ELISA		P value
		Positive	Negative	Positive	Negative	
CE	857	631	226	437	420	<0.01
Non-CE	744	94	650	63	681	>0.05
Total	1601	725	876	500	1101	

Table 3.10.3a: Assessments of EgP antigen in rapid DIGFA and standard ELISA applications for serodiagnosis of human echinococcosis in hospitalized CE (n=857) or non-CE cases (n=744)

Assessments	EgP for CE			
	DIGFA		ELISA	
Se (%) (95%CI)	73.6	(71.4-75.8)	51.0	(48.5-53.5)
Sp (%) in Non CE controls (95%CI)	87.4	(85.8-89.0)	91.5	(90.1-92.9)
Accuracy (%) (95%CI)	80.0	(78.0-82.0)	69.8	(67.5-72.1)
PPV (%) (95%CI)	87.0	(85.3-88.7)	69.8	(67.5-72.1)
NPV (%) (95%CI)	74.2	(72.0-76.4)	87.4	(85.8-89.0)
Odds ratio	19.3		11.2	
Youden Index	0.61		0.425	
PLR	5.841		6.0	
NLR	0.302		0.536	

Table 3.10.4: Sensitivity and specificity of AgB antigen in DIGFA and ELISA for hospitalized CE cases

Clinical diagnosis	N	DIGFA		ELISA		P value
		Positive	Negative	Positive	Negative	
CE	857	586	271	492	365	<0.01
Non-CE	744	49	695	49	695	>0.05
Total	1601	635	966	541	1060	

Table 3.10.4a: Assessments of AgB antigen in rapid DIGFA and standard ELISA applications for serodiagnosis of human echinococcosis in hospitalized CE (n=857) or non-CE cases (n=744)

	Assessments	AgB for CE			
		DIGFA		ELISA	
i	Se (%) (95%CI)	68.4	(66.8-69.9)	57.4	(54.1-60.7))
ii	Sp (%) in Non CE controls (95%CI)	93.4	(91.6-95.1)	93.4	(91.6-95.1)
iii	Accuracy (%) (95%CI)	80.0	(78.0-82.0)	74.1	(71.9-76.3)
iv	PPV (%) (95%CI)	92.3	(91.0-93.6)	90.9	(89.5-92.3)
v	NPV (%) (95%CI)	71.9	(69.7-74.1)	65.6	(63.2-67.9)
vi	Odds ratio	30.6		19.1	
vii	Youden Index	0.618		0.508	
viii	PLR	10.36		8.69	
ix	NLR	0.338		0.456	

Table 3.11: Assessments of multiple antigens and single antigen in rapid DIGFA applications for serodiagnosis of human echinococcosis in hospitalized CE (n=857) or non-CE cases (n=744)

Assessments	Multiple antigens	EgB	EgCF	EgP
Se (%) (95%CI)	80.7 (78.1-83.3)	68.4 (66.8-69.9)	78.1 (76.1-80.1)	73.6 (71.4-75.8)
Sp (%) in Non CE controls (95%CI)	89.6 (88.4-90.8)	93.4 (91.6-95.1)	86.3 (85.4-87.2)	87.4 (85.8-89.0)
Accuracy (%) (95%CI)	84.7 82.9-86.5	80.0 (78.0-82.0)	82.8 (80.9-84.7)	80.0 (78.0-82.0)
PPV (%) (95%CI)	90.5 89.1-91.9	92.3 (91.0-93.6)	87.0 (85.3-88.7)	87.0 (85.3-88.7)
NPV (%) (95%CI)	79.2 (77.2-81.2)	71.9 (69.7-74.1)	78.7 (76.7-80.7)	74.2 (72.0-76.4)
Odds ratio	36.0	30.6	24.7	19.3
Youden Index	0.703	0.618	0.644	0.61
PLR	7.76	10.36	5.701	5.841
NLR	0.22	0.338	0.254	0.302

The diagnostic value was also assessed for DIGFA and ELISA antibody test against Em2 for human AE. Almost all the assessment parameters by single Em2 were lower than parallel combination in both DIGFA and ELISA, except a higher specificity (90.3%) and accuracy (90.1%) for single Em2 in DIGFA (Table 3.12 and 3.12a).

Table 3.12: Sensitivity and specificity of Em2 antigen in DIGFA and ELISA for hospitalized AE cases

Clinical diagnosis	N	DIGFA		ELISA		P value
		Positive	Negative	Positive	Negative	
AE patients	42	35	7	33	9	>0.05
Non AE	1559	151	1408	310	1249	<0.01
Total	1601	186	1415	343	1258	

Table 3.12a: Assessments of Em2 antigen in rapid DIGFA and standard ELISA applications for serodiagnosis of human echinococcosis in hospitalized AE (n=42) or non-AE cases (n=1559)

Assessments	Em2 for AE	
	DIGFA	ELISA
Sensitivity (%)	83.3	78.6
(95%CI)	(72.1-94.6)	(66.2-91.0))
Specificity (%) in Non AE controls	90.3	78.0%
(95%CI)	(89.6-91.0)	(75.9-80.1)
Accuracy (%)	90.1	80.1
(95%CI)	(88.6-91.6)	(78.1-82.1)
Positive predictive value (%)	18.8	10.6
(95%CI)	(16.9-20.7)	(9.1-12.1).
Negative predictive value (%)	99.5	99.3
(95%CI)	(99.2-99.8)	(98.9-99.7)
Odds ratio	46.4	13.0
Youden Index	0.736	0.566
Positive likelihood ratio	8.59	5.78
Negative likelihood ratio	0.185	0.274

3.3.3 Comparison of DIGFA with different sources of serum samples from China, UK and France

Double-blind test was carried out for DIGFA using serum stored in XMUH (Xinjiang, China), Salford University (UK) and Besanson University (France). A higher sensitivity and specificity was observed for both CE and AE in XMUH compared in Salford and Besancon (Table 3.13). There were cross reactions found in serum of cysticercosis and schistosomiasis (31.3% and 40%).

Table 3.13: Blinding test for evaluation of DIGFA in the sera from China, Britain and France

Countries	Clinical Diagnosis	No.	DIGFA		Sensitivity (%)	Specificity (%)
			Positive	Negative		
China	**CE**	224	216	8	96.4	
	AE	68	62	6	91.2	
	Normal	169	12	157		92.9
Britain	**CE**	18	13	5	86.7	
	AE	33	30	3	90.9	
	Cysticercosis	32	10	22	**Cross-reaction**	31.3%
	Schistosomiasis	10	4	6	**Cross-reaction**	40%
	Normal	19	2	17		89.5
France	**CE**	10	4	6	40	
	AE	73	57	16	78.1	
	Normal	45	2	43		95.6
Total		701	412	289		

3.3.4 Diagnostic evaluation of the DIGFA for endemic community hydatid mass screening in northwest China

When DIGFA was used in conjunction with ultrasound in community mass screening studies, it showed good sensitivity for human AE (90.7%) but lower sensitivity for CE (71.8%). Overall specificity for CE was 78.1% and for AE was 97.6% based on ultrasound abdominal screening as the gold standard. AgB antigen had the lowest sensitivity (51.3%) in DIGFA in comparison to ultrasound confirmed human CE (n=160), while Em2 antigen in DIGFA had a sensitivity of 77.8% for ultrasound confirmed AE cases (n=108) (Table 3.14 to 3.16). Specificity of AgB for community detected human CE was 94.6% and Em2 for AE was 97.1% in this study (Table 3.14 and 3.15). AgB antigen in DIGFA gave high cross reaction 68.6% (74/108) with community detected AE cases, while Em2 antigen cross reacted with 11.3% (18/160) of ultrasound confirmed CE cases.

Table 3.14: Comparison of DIGFA test with abdominal ultrasound imaging in mass screening community studies in western China (data combined from Xinjiang, Ningxia, Sichuan and Tibet AR)

Ultrasound	No.	EgCF Positive	EgP Positive	AgB Positive	Em2 Positive	All Negative
CE	160	112	88	82	18	45
AE	108	95	83	74	84	10
Normal	2923	641	462	91	70	2175
Sensitivity	CE	70%	55%	51.3%	11.3%	71.8%[a]
For each antigen	AE	87.9%	76.9%	68.5%	77.8%	90.7%[b]
Specificity		78.1%	84.2%	96.9%	97.6%	74.4%[c]

a. 95% CI of general sensitivity for CE was 64.9%-78.8%.
b. 95% CI of general sensitivity for AE was 85.2%-96.2%.
c. 95% CI of general specificity for both CE and AE was 72.8%-76.0%.
d. Accuracy of DIGFA for CE was 74.3% ((160-45) + 2175)/(160+2923)) and for AE was 75.0% ((108-10)+2175)/(108+2923).
e. NPV of DIGFA for CE was 98.0% (2175/(2175+45)) and for AE was 99.5% (2175/(2175+10)).
f. PPV of DIGFA for CE was 13.3% ((160-45)/((160-45)+(2923-2175)) and for AE was 11.6% ((108-10)/((108-10)+(2923-2175)).

Evaluations of DIGFA test with abdominal ultrasound in community studies showed a lower sensitivity, specificity, accuracy, PPV, DOR, Youden Index and

PLR compared to hospitalized study, especially PPV (11.9% to 90.5%) (Table 3.14.1 and 3.14.1a).

Table 3.14.1: Assessment cross tab of DIGFA test with abdominal ultrasound CE imaging in mass screening community studies in western China (data combined from Xinjiang, Ningxia, Sichuan and Tibet AR)

Clinical diagnosis	N	DIGFA	
		Positive	Negative
CE	160	115	45
Non-CE / Non HD	3031 / 2923	846 / 748	2185 / 2175
Total	3191 / 3083	961 / 863	2230 / 2220

Table 3.14.1a: Evaluations of DIGFA test with abdominal ultrasound CE imaging in mass screening community studies in western China (data combined from Xinjiang, Ningxia, Sichuan and Tibet AR)

Assessments	Multi-DIGFA for CE	
Se (%) (95%CI)	71.895 / 71.895	(69.7-74.1 / 69.7-74.1)
Sp (%) in Non CE/HD controls (95%CI)	72.088 / 74.410	(69.9-74.3 / 72.2-76.6)
Accuracy (%) (95%CI)	72.078 / 74.278	(69.9-74.3 / 72.1-76.4)
PPV (%) (95%CI)	11.967 / 13.326	(10.4-13.6 / 11.6-15)
NPV (%) (95%CI)	97.982 / 97.973	(97.3-98.7 /97.3-98.7)
DOR	6.612 /7.431	
Youden Index	0.439 /0.463	
PLR	2.577 /2.809	
NLR	0.389 /0.378	

The diagnostic value in community study was also assessed for DIGFA antibody test against a single antigen and parallel combination (Table 3.14.2 and 3.14.2a for EgCF, Table 3.14.3 and 3.14.3a for EgP, Table 3.14.4 and 3.14.4a for AgB, Table 3.15 for parallel combination) for CE. The lower sensitivities were observed in DIGFA antibody against single antigen (EgCF, EgP and EgB) for CE compared to against parallel combination (Table 3.15).

Table 3.14.2: Assessment cross tab of EgCF-DIGFA test with abdominal ultrasound imaging in mass screening community studies in western China (data combined from Xinjiang, Ningxia, Sichuan and Tibet AR)

Ultrasound	EgCF-DIGFA		Total
	Positive	Negative	
CE patients	112	48	160
Non-CE / Non-HD control	735 / 641	2296 / 2282	3031 / 2923
Total-1	847 / 753	2344 / 2330	3191 / 3083

Table 3.14.2a: Evaluations of EgCF-DIGFA test with abdominal ultrasound CE imaging in mass screening community studies in western China (data combined from Xinjiang, Ningxia, Sichuan and Tibet AR)

Assessments	EgCF-DIGFA for CE	
Se (%) (95%CI)	70 /70	(67.7 / 67.7 - 72.3 / 72.3)
Sp (%) in Non-CE/HD controls (95%CI)	75.75 / 78.07	(73.6 / 76.0 - 77.9 / 80.1)
Accuracy (%) (95%CI)	75.46 / 77.65	(73.3 / 75.6 - 77.6 / 79.7)
PPV (%) (95%CI)	13.22 / 14.87	(11.5 / 13.1 - 14.9 / 16.6)
NPV (%) (95%CI)	97.95 / 97.94	(97.3 / 97.2 - 98.6 / 98.6)
Odds ratio	7.29 / 8.307	
Youden Index	0.458 / 0.481	
Positive likelihood ratio	2.886 / 3.192	
Negative likelihood ratio	0.396 / 0.386	

Table 3.14.3: Assessment cross tab of EgP-DIGFA test with abdominal ultrasound CE imaging in mass screening community studies in western China (data combined from Xinjiang, Ningxia, Sichuan and Tibet AR)

Ultrasound	EgP-DIGFA		Total
	Positive	Negative	
CE patients	88	72	160
Non-CE patients	545	2486	3031
Non-HD control	462	2461	2923
Total-1	633	2558	3191
Total-2	550	2533	3083

Table 3.14.3a: Evaluations of EgP-DIGFA test with abdominal ultrasound CE imaging in mass screening community studies in western China (data combined from Xinjiang, Ningxia, Sichuan and Tibet AR).

Assessments	EgP-DIGFA for CE
Se (%) (95%CI)	55 / 55 (52.5– 57.5 / 52.5-57.5)
Sp (%) in Non CE/HD controls (95%CI)	82.0 / 84.2 (80.1– 83.9 / 82.4 - 86.0)
Accuracy (%) (95%CI)	80.7 / 82.7 (78.7-82.6 / 80.8-84.5)
PPV (%) (95%CI)	13.9 / 16 (12.2– 15.6 / 14.2-17.8)
NPV (%) (95%CI)	97.2 / 97.2 (96.4– 98.0 / 96.3-97.8)
Odds ratio	5.575 / 6.510
Youden Index	0.37 / 0.392
Positive likelihood ratio	3.059 / 3.480
Negative likelihood ratio	0.549 / 0.534

Table 3.14.4: DIGFA test using AgB antigen for immunodiagnosis of CE in community mass screening studies (data combined from Xinjiang, Ningxia, Sichuan and Tibet AR) in comparison to abdominal ultrasound

Ultrasound	AgB-DIGFA		Total
	Positive	Negative	
CE patients	82	78	160
Non-CE / Non-HD control	165 / 91	2866 / 2832	3031 / 2923
Total	247 / 173	2944 / 2910	3191 / 3083

Sensitivity 51.3% (82/160), specificity 94.6% (2866/3031); Accuracy of AgB-DIGFA for CE was 92.4% ((82 + 2866)/3191). NPV of AgB-DIGFA was 97.4% (2866/2944). AgB PPV was 33.2% (82/247) in DIGFA.

Table 3.14.4a: Assessments of antigen B by DIGFA for serodiagnosis of human echinococcosis in community CE (n=160) or non-CE/HD cases (n=3031/2923)

Assessments	AgB-DIGFA for CE (95%CI)
Se (%) (95%CI)	51.25/51.25 (0.488-0.5373/0.4878-0.537)
Sp (%) in Non CE/HD controls (95%CI)	94.556/96.887 (0.9344-0.9567/0.960-0.977)
Accuracy (%) (95%CI)	92.385/95.621 (0.9108-0.9369 / 0.946-0.966)
PPV (%) (95%CI)	33.198/47.399 (0.3086-0.3553 / 0.449-0.499)
NPV (%) (95%CI)	97.350/97.319 (0.9656-0.9814 / 0.965-0.981)
Odds ratio	18.26/32.717
Youden Index	0.458/0.481
PLR	9.414/16.462
NLR	0.515/0.503

Table 3.15: Assessments of multiple antigens and single antigen in rapid DIGFA applications for serodiagnosis of human echinococcosis in community CE (n=160) or non-CE/HD cases (n=3031/2923)

Assessments	Multiple antigens	EgB	EgCF	EgP
Se (%)	71.89 /71.89	51.25/51.25	70 /70	55 / 55
Sp (%) in Non CE/HD controls	72.09 /74.41	94.56/96.89	75.75 /78.07	82.0 / 84.2
Accuracy (%)	72.08 /74.28	92.38/95.62	75.46 /77.65	80.7 / 82.7
PPV (%)	11.97 /13.33	33.2/47.4	13.22 /14.87	13.9 / 16
NPV (%)	97.98 /97.97	97.35/97.32	97.95 /97.94	97.2 / 97.2
Odds ratio	6.61 /7.43	18.26/32.72	7.29 /8.31	5.57 / 6.51
Youden Index	0.44 /0.46	0.46/0.48	0.46 /0.48	0.37 / 0.39
PLR	2.58 /2.81	9.41/16.46	2.89/3.192	3.06 / 3.48
NLR	0.39 /0.38	0.51/0.50	0.4 / 0.4	0.55 / 0.53

The diagnostic value in community study was also assessed for DIGFA against Em2 for human AE. The DIGFA sensitivity by single Em2 (77.8%) were lower than parallel combination (90.7%) in community study (Table 3.14) and also lower than Em2 (83.3%) in hospitalized study. Meanwhile the higher specificity (97.1%), accuracy (96.5%) and PPV (48.8%) for single Em2 in DIGFA was found in community study (Table 3.16).

Table 3.16: DIGFA test using Em2 antigen for diagnosis of AE in community mass screening studies (data combined from Xinjiang, Ningxia, Sichuan and Tibet AR) in comparison to abdominal ultrasound

Ultrasound	Em2-DIGFA		Total
	Positive	Negative	
AE patients	84	24	108
Non AE	88	2995	3083
Total	172	3019	3191

Sensitivity 77.8% (84/108) (95% CI 70.0%-85.6%), specificity 97.1% (2995/3083) (95% CI 96.5%-97.6%)
Accuracy of Em2-DIGFA for AE was 96.5% ((84 + 2995)/3191).
NPV of Em2-DIGFA was 99.2% (2995/3019).
Em2 PPV was 48.8% (84/172) in DIGFA.

3.3.5 False positives and negatives

For clinically defined echinococcosis patient sera, there were in total 14.6% (131/899) false negatives in the DIGFA test and these were also negative in the standard ELISA (Table 3.9 and 3.10). Clinical features of false negative CE cases were collapsed, degenerated, necrotic (Type CE4), or consolidated cysts, or calcificated type cysts (CE5), or single, small univesicular cysts (Type CE 1), and also for small single cysts deep in organ locations (WHO/OIE 2001). A false positive rate of 10.4% (73/702) occurred in hospitalized persons in XMUH without echinococcosis, and these cases (including simple non-parasitic cysts, carcinoma or tuberculosis) showed no evidence of echinococcocal cysts or lesions by ultrasound or other imaging methods (X-ray or CT) (Table 3.17). Three *T. solium* cysticercosis cases treated in XMUH were all seronegative in DIGFA and ELISA. In addition, there were other 12 false negatives in the DIGFA test that were also negative in ELISA (Table 3.18). Clinical features of false negative CE cases indicated 12 patients had collapsed cyst, degenerated, necrotic, consolidated or calcified CE5 type cysts, or single, small size (Type CE 1) cysts, or cysts in a deep location in the organ or far away from blood vessels. Another 12 false positive cases from XJMUH appeared as patients with other diseases including 1 cysticercosis, 3 carcinoma, 2 tuberculosis, 5 simple cyst cases and 1 lipoma case (Table 3.19).

Table 3.17: False positives occurred by DIGFA in 702 non echinococcosis sera from XMUH (1999-2006)

Clinical diagnosis	N	DIGFA positives (false positive rate)
Cystic disease	196	16 (8.2%)
Carcinoma	85	11 (12.9%)
Tuberculosis	28	8 (28.6%)
Space-occupying lesions (non echinococcosis by imaging)	266	29 (10.9%)
Cirrhosis	6	1 (16.7%)
Abscess	13	1 (7.7%)
Cysticercosis (*T. solium*)	3	0 (0%)
Cholecystitis /gallstones	12	1 (8.3%)
Other medicinal disease	88	6 (6.8%)
Normal	5	0 (0%)
Total	702	73 (10.4%)

Table 3.18: Analysis influent factors in 12 false negative CE patients

No.	Sex	Age	DIGFA	Results of surgery and pathology	Clinical Remarks
1	Male	50	(-)	Single hydatid cyst in right lobe of liver	Deep in inner liver, 3 cm from surface of liver
2	Male	25	(-)	Single hydatid cyst in Ⅷ segment	Full of yellow, sticky, necrotic fluid
3	Female	27	(-)	Single hydatid cyst in the peak of liver	Adhere to phrenic muscle
4	Male	69	(-)	Single hydatid cyst in low lobe of right lung	Inner cyst had infected, broken and necrosis
5	Female	55	(-)	Multiple hydatid cysts both in liver and lung	Inner cyst had broken and degenerated
6	Female	15	(-)	Hydatid cyst in abdomen, malignant tumor in ovary	Hydatid cyst had calcificated
7	Male	9	(-)	Alveolar hydatid in left lobe of liver	child
8	Male	68	(-)	Single hydatid cyst in left lobe of liver	Necrosis and consolidated
9	Female	34	(-)	hydatid cyst in thyroid gland	Deep in thyroid gland
10	Female	42	(-)	Single hydatid cyst in lung	Single
11	Female	30	(-)	Single hydatid cyst in right lobe of liver	Deep in liver, the wall is thick, protoscleces were found in puncture
12	Male	11	(-)	hydatid cyst in brain	child

Table 3.19: Analysis influent factors in 12 false positive cases

No.	Sex	Age	DIGFA	Results of surgery and pathology	Clinical Remarks
1	Male	41	(+)	cysticercosis in brain	Necrosed
2	Male	41	(+)	malignant tumor in mediastinum	
3	Female	63	(+)	carcinoma of the lung	
4	Male	23	(+)	granuloma in socket of the left eye	
5	Female	30	(+)	peritonitis caused by tuberculosis	
6	Female	60	(+)	cholelithiasis, multiple cysts in left lobe of liver	
7	Female	7	(+)	cyst in mediastinum	
8	Male	66	(+)	multiple cysts in kidney and liver	
9	Female	38	(+)	carcinoma of the lung	
10	Male	32	(+)	lipoma in upper lobe of lung	
11	Male	26	(+)	cyst in mediastinum	
12	Female	30	(+)	pulmonary tuberculosis	

3.4 Discussion

Early diagnosis of human cystic echinococcosis (CE) and especially for alveolar echinococcosis (AE) could provide significant improvement in the quality of clinical management and treatment prognosis of both these zoonotic diseases (WHO/OIE, 2001; Craig et al 2003). Diagnostic laboratory methods that are cheap, rapid and easy to use would be very useful for basic clinics in endemic rural areas and in support of mass screening programmes since most persons in the early stages of CE or AE are asymptomatic (Rogan and Craig, 1997, 2002; Craig et al., 2000, 2003; WHO/OIE, 2001). Specific antibody detection appears most valuable for serodiagnosis of human CE or AE and has also shown promise in some post-treatment follow-up studies (Wen et al, 1995; Ito, 2002; Rogan and Craig, 2002). Gold standard laboratory tests for human echinococcosis are currently based on ELISA or immunoblots using *E. granulosus* hydatid cyst fluid antigen B for CE, and *E. multilocularis* metacestode antigen Em2 or antigen Em18 for AE (Gottstein et al, 1983, 1987; Zhang et al., 2000, 2001; Rogan and Craig, 2002; Ito, 2002; Craig et al., 2003; Carmena et al., 2006).

Dot-ELISA rapid format has been applied in a few community based studies for human CE but has limitations since enzyme-conjugates are difficult to store and apply in field conditions (Zheng et al., 1986; Rogan et al., 1991; Qiao et al., 1999). Colloidal gold labeling techniques were first used in the 1970s to locate specific antigens on the cell surface using electron microscopy (Faulk and Taylor, 1971; Horisberger et al., 1975). “One-step pregnancy test strip” type tests are a good example of a colloidal gold based rapid diagnostic test (May, 1991; Millipore Corp, 1996). A dot-immunogold infiltration assay (DIGFA) was first developed for serodiagnosis of HIV in 1989 (Chun and Chu, 1989; Spielberg et al., 1989). The procedure is similar to dot-ELISA but has the advantage of an infiltration system and use of colloidal gold conjugated IgG to give a more rapid, reliable and clear result. Colloidal gold antibody conjugate based DIGFA or similar immunochromatographic assays have also become an acceptable rapid clinical bed-side detection method for drug screening and diagnosis of several microbial and parasitic infections (Dar et al., 1994; Xiao et al., 1995; Dylan and Kevin, 1999; Feng et al., 2000; Garcia et al., 2000; Feng et al., 2002; Zhu et al., 2002; Sorell et al., 2002; Yang, 2003; Hujakka et al., 2003; Chen et al., 2005). Initial applications of DIGFA for human echinococcosis in China indicated good potential as a rapid

test (Fu et al., 2000; Feng et al., 2002; Zhang et al., 2001).

The current study reports the most comprehensive assessment and application of a rapid DIGFA for quick serodiagnosis of human echinococcosis. We confirm that DIGFA exhibited the following features: (1) the test could give a reliable diagnostic result within 2--3 minutes using only 20μL of serum or 40 μL heparinized blood; (2) the test was able to detect human echinococcosis in approximately 80-93% of cases and differentiate human CE and AE in about 80% of confirmed cases; (3) the DIGFA procedure is simple and no special training was required and therefore it had practical value for support of both community mass-screening in conjunction with ultrasound, and for hospital based diagnostic confirmation of echinococcosis (Fu et al., 2000; Zhang et al., 2001; Feng et al., 2002; Chen et al., 2005).

We developed this DIGFA test which acted as a qualitative immunological diagnostic method for detection antibody of human echinococcosis. Antigens used for DIGFA had been studied from 1980s and the preparation had been partially modified by our research group in Xinjiang Hydatid Clinical Research Institute from 1997 to 2000. Those antigens had been tested by ELISA and dog immunoblot assay (DIBA) and a sensitivity of 91% for EgCF in ELISA and DIBA, 93% in ELISA and 91% in DIBA for EgP had been reported (Zhang et al., 2000). I had tried to apply DIBA for the community study in 1998 but the storing and application of enzyme conjugate became the main problems since no fridge or freezer available. And also the DIBA procedure was similar with ELISA and asked for at least 1 hour test time at room temperature. But the stability of antigens on the NC membrane kept effective for longer time (at least 2 months without special package) indicated that we might find another stable conjugate to develop a test for using in the rural area. Colloidal gold conjugate could be stable at 4°C in working dilution and had been successfully applied in DIGFA and other tests for some infection disease (e.g. HAV IgM for hepatitis, Feng et al., 2000). The four antigens were combined together in one test for obtain better sensitivity and also partially specific for differentiation of CE and AE. Optimization the concentration of four antigens could supply stable and reproductive results and commercial DIGFA kit had been allowed to apply in many hospitals in hydatid endemic area of northwest China for clinical diagnosis and community study. DIGFA kit with 4 antigens was accepted since no special device needed, easy to perform and effective for both CE and AE. Other antigens had been tried to apply as well.

Native *E. multilocularis* crude abstract showed a higher false positive in normal controls in initial trial (not exactly calculated) and was given up. A recombined antigen B (r-AgB) was also regarded as a potential candidate antigen but need more work for that since initial poor sensitivity was observed due to lower concentration and poor stability. Em18 or recombined Em18 might also be optional antigens for future research and application in DIGFA for human AE.

Based on a panel of 1601 serum samples from advanced CE or AE patients confirmed by imaging, pathology and/or surgery in Xinjiang Medical University Hospital (XMUH, Urumqi, China), and control sera, overall DIGFA sensitivity was 80.7% (692/857) for human CE and 92.9% (39/42) for human AE. Specificity for echinococcosis (both CE and AE) was 89.6% (629/702); while *E. granulosus* antigen B specificity for CE was 93.4% (695/744), and *E. multilocularis* Em2 antigen specificity for AE was 90.3%(1408/1559).

When applied to community mass screening studies in western China (i.e. sites in Xinjiang, Ningxia, Sichuan, Tibet, see also Chapter 6), the *Echinococcus* DIGFA showed slightly lower sensitivity (71.8% for CE and 90.7% for AE) and specificity (74.4% for echinococcosis in general, 94.6% with antigen B for CE, 97.1% with Em2 for AE) compared with the hospital based DIGFA assessment. The DIGFA test was nevertheless extremely useful in these resource-poor settings as a combined diagnostic tool with ultrasound. Sera could be tested within 1 hour of ultrasound scan and up to 200 sera tested in one day. Diagnosis of CE or AE was confirmed in more than 80% of community detected cases and therefore facilitated efficient clinical treatment and assisted follow-up recommendations. Reasons for lower sensitivity of DIGFA in community (vs hospital settings) may be due to exposure without a detectable abdominal cyst lesion, involvement of sites not ultrasound detectable, and / or presence of small cysts or lesions, or degenerate, calcified, or necrotic cysts/lesions. The false negative rate for hospitalized CE cases was 19.3% (165/857) compared to 7.1% (3/42) for AE, while false positives occurred in 6.6% of CE (49/744), and in 9.7% (151/1559) of AE cases in XMUH. The DIGFA test could reliably differentiate CE and AE cases from each other around 80% of the time and an Em2 positive reaction appeared in 17.1% (146/857) of CE case sera. The DIGFA results were comparable to those obtained with the standard ELISA (false negative for CE 25.0%, for AE 2.4%, false positive for both 10.3%), and in general the ELISA was less sensitive ($p<0.01$) but

exhibited comparable specificity with DIGFA for human CE. The AgB antigen preparation from *E. granulosus* and Em2 metacestode extract from *E. multilocularis*, showed reliable specificity (90.3% --97.1%) in DIGFA for CE or AE disease, and were comparable to other studies using traditional ELISA formats (Gottstein et al., 1983, 1987; Liu and Zhao, 1993; Poretti et al., 1999; Carmena et al., 2006).

3.5 Summary

In conclusion, a rapid 3 minute eye-read dot immunogold filtration assay (DIGFA) for serodiagnosis of human cystic (CE) and alveolar (AE) echinococcosis was developed in which 4 crude or semi-purified native antigens from *E. granulosus* (EgCF, EgP, AgB) and *E. multilocularis* (Em2) were utilized simultaneously. The overall sensitivity of DIGFA in a hospital diagnostic setting was 80.7% for human cystic echinococcosis (CE) (n=857) and 92.9% for human alveolar echinococcosis (AE) (n=42). The *E. granulosus* protoscoleces (EgP) and crude cyst fluid (EgCF) extracts provided high sensitivity for the test; while *E. granulosus* partially purified antigen B (AgB) and *E. multilocularis* antigen (Em2) ensured specificity comparable to standard ELISA. Highest specificity was 93.4% with AgB extract for CE, and 90.3% with Em2 antigen for AE when CE vs AE cross-reactivity was excluded. Anti-AgB antibodies were present in 35.5% of AE cases and anti-Em2 in 7.4% of CE cases. In endemic communities in northwest China screened for echinococcosis, the sensitivity of DIGFA ranged from 71.8% to 90.7% in comparison to abdominal ultrasound; specificity for CE using AgB was 94.6% and for AE using Em2 was 97.1%. The DIGFA format was used successfully in conjunction with ultrasound for mass screenings to identify or confirm asymptomatic CE and AE cases in co-endemic communities in western China.

Chapter 4. Development and application of a rapid antigen detection method in cyst fluid for human CE

4.1 Introduction

Crude *E.granulosus* cyst fluid antigens (EgCF) and antigen B or are present in *E.granulosus* cyst fluid. Therefore, detection of antigen B or EgCF with immunological methods can be used to identify / confirm that cysts are of hydatid origin.

Generally we did not get cyst fluid from confirmed CE patients before operation is not usually tested or available. Diagnosis of CE is usually already made for most cases using imaging techniques with or without serological confirmation. However testing cyst fluid could be helpful in some special situations. PAIR treatments for those CE cases who were not suitable for surgery (as elder, poor healthy status or unwilling for a surgical operation) and as a less medical alternative to surgery，was developed in Italy and China (Felice et al., 1990, 1997, 2000; Wang 1994; Wen 1997). A cyst fluid antigen test could confirm the diagnosis of CE and following PAIR or albendazole treatment could be useful to those cases. Another requirement can be for the differential diagnosis for non-specific fluid such as the lavage fluid under bronchoscopy, or other cyst fluid from cystic lesions in the liver, eyes or other organs. The use of ELISA and a rapid dot-ELISA have been applied to test cyst fluid for hydatid origin (Craig, et al., 1986; Paul et al., 1997; Wang et al., 2002). The latter just needs 10 minutes and this was really useful for clinical practice. However, shelf-life of enzyme labeled antibody, could be limited as it was easily degraded.

Rapid tests with colloidal gold techniques have been widely applied in clinical laboratory diagnosis for urine HCG, sera HBsAg, and some initial screening for drugs, etc. The advantages of colloidal gold tests is their speed and easy to operate. This kind of ‘bedside’ detection test was designed for rapid identification of hydatid cyst fluid in the current study for potential use during surgery and/or PAIR for CE.

4.2 Methods and approaches

4.2.1 Preparation of antigen, rabbit anti sera and conjugate

i) Antigen preparation: *E. granulosus a*ntigen B (AgB, from sheep cyst fluid) was prepared (details shown in Appendix I).

ii) Preparation of rabbit anti antigen B: Rabbit anti AgB IgG was prepared by boosting rabbit with AgB. AgB 0.5 ml and complete Freund's adjuvant (Sigma F5881) 0.5ml were mixed evenly to emulsify and kept overnight at 4^{o}C. A New Zealand rabbit, approximate 2kg, was injected subcutaneously in multiple sites on the back and intramascular injection in the thigh with this emulsion. After 10-14 days, another emulsion of mixed EgB 0.5ml and incomplete Freund's Adjuvant 0.5ml (Sigma F5506) was injected into the rabbit as above, followed by once a week for 2-3 weeks until antibody titers were sufficiently raised. One week after the last boost, the rabbit was anesthetized and bled from the heart or cervical artery. The blood was stored at room temperature for 1 hour; the clot separated and then at 4 °C overnight, centrifuged 2000 g for 10 minutes and the serum supernatant collected. Approximately 40-50 ml of rabbit anti-sera could be collected finally, and stored at -80°C. An IgG fraction prepared from rabbit anti-sera by Protein A column affinity chromatography. A protein A Sepharose CL 4B column (Pharmacia) was prepared with PBS following the manufactures instructions. 1 ml serum was added to a 10 ml column and subsequent elution of IgG using a low pH glycine buffer (0.2M glycine/HCl + 0.5M NaCl, pH3.0). After dialysis against 500 vol. PBS, the IgG fraction was reconcentrated by an Amicon ultra filtration cell with YM10 membrane (Amicon Corp, MA, USA) to a final concentration of 8.6 mg/ml, which was determined by spectrophotometry at 280nm.

Capture antibody: Half of above rabbit IgG anti-EgB was diluted to 5μg/ml with BCB (pH9.6) for use in ELISA, and diluted to 2mg/ml with 20mM Tris-HCl (pH 8.5) for the colloidal gold rapid test as a capture antibody.

Conjugate: Half of above rabbit anti-EgB IgG was conjugated with colloidal gold.

iii) Conjugate Procedures:

(1) Preparation of colloidal gold and conjugate.

Preparation of colloidal gold was the same as the procedure for DIGFA for human serology test as described in section 3.2.

Colloidal gold conjugated rabbit anti-EgB IgG was prepared similar to section 3.2 but with 5% BSA blocking buffer and purification through the following procedures. The conjugate was centrifuged at 2000g for 30 minutes to remove some bigger particles. The conjugate solution was concentrated in a dialysing tube covered sucrose powder until one third of original volume. The concentrated conjugate was coupled to a Sephadex G200 column (1g for a volume 15-20ml) column, and eluted with 2% BSA 20mM Tris-HCl. Since uneven sizes of colloidal conjugate would have different pass speed, the middle part of the elute with a deep wine red colour was collected to get similar size (40nm) conjugate. The final volume of conjugate was around 4ml. The OD value was checked at a wavelength of 570nm. The conjugate was diluted with conjugate buffer to lower an OD value of 0.7**.**

(2). Determination of the optimized labeling volume of IgG:

The colloidal gold, IgG and 10%NaCl were mixed in order in small eppendor tubes; the optimal volume of IgG was selected according to the better color change point (Table 4.1).

Table 4.1: The trial for optimizing the volume of IgG to conjugate with colloidal gold.

Reagents	**Tube 1#**	**Tube 2#**	**Tube 3#**	**Tube 4#**	**Tube 5#**	**Tube 6#**
Colloidal gold	100 µl	100 µl	100 µl	100 µl	100 µl	100 µl
IgG 1mg/ml	0.4 µl	0.8 µl	1.6 µl	3.2 µl	6.4 µl	12.8 µl
10%NaCl	10 µl	10 µl	10 µl	10 µl	10 µl	10 µl
Results	Grey	Grey	Grey-red	Purple red	Red	Red

The optimized volume of IgG was 3.2 µl in 100 µl colloidal gold in this test. And practical optimized volume of IgG was 3.2*120%=3.84 µl for 100 µl colloidal gold.

(3). Conjugation procedure was same with goat anti human IgG conjugate with colloidal gold which was described in Chapter 2. The conjugate used for DIGFA

was not needed to be purified again but should be done for ICA tests. The blocking buffer for conjugate used in ICA was 5% BSA blocking buffer. Then the conjugate was centrifuged under 2000g for 30 minutes, the suspension was concentrated to 1/3 of original volume by polyethylene glycol (PEG) 4000 and then purified by pass through Sephadex G 200 column. The combined collections were test OD value under 570nm wavelength and the results was 1.3.

4.2.2 Optimizing capture, conjugate, blocking reagents, sample buffer and washing buffer

The optimal capture dilution for ICA was determined by continuous dilution method and final capture was 2mg/ml. The conjugate (non-dried) was 20μl per strip; the sample buffer was PBS with 0.3% Tween-20.

Solution A and B for DIGFA were prepared as described in Appendix III.

4.2.3 Detection methods.

(1). Direct dot immunogold filtration assay:

Preparation of immunogold kit and reagents. The kit with NC membrane was the same as DIGFA used for human serology (in section 3. 2). A control dot was goat anti rabbit IgG (2mg/ml), and a test dot was rabbit anti sheep EgB IgG. Sample dilution buffer A and washing buffer B were prepared as the same as section 3.2.

Add 8~10ul sample to the middle of the hole with NC membrane, then add Solution A 2 drops, Solution B 3 drops, Solution C 4 drops, Solution B 3 drops. The results can be read in 3 minutes. This assay was just used as an initial trial and not for formal test.

(2.) Indirect DIGFA (sandwich method): Coated rabbit anti AgB IgG to NC membrane until dried completely. Add 2 drops Solution A, then 50~100ul sample in the hole, then Solution B 3 drops, Solution C 4 drops, Solution B 3 drops. The appearance of a red colour on the test dot indicated a positive reaction and presence of antigen B in the tested cyst fluid sample.

(3). ICA (immuno chromatography assay) strip:

Because the original hydatid cyst fluid might be mixed with bile or other host contaminants, the DIGFA results did not perform very well due to poor background. Furthermore the DIGFA set up was not good for a sandwich design test. Immunochromatographic Assay (ICA) became the better choice for antigen

detection using a sandwich assay style approach. Therefore, an ICA test was designed to be composed of a sample pad, a conjugate pad, a NC membrane with test and control lines and an absorbent pad.

NC membrane with test and control line: The NC membrane used in the ICA test was different to that used in the DIGFA. General blotting NC membrane with pore size 0.45μm was tried but failed to function due to its smaller pore size. A commercial Whatman Immunopore FP was chosen finally due to a more acceptable flow speed. For a quality control reagent, goat anti-rabbit IgG (affinity purified, from Chemicon International Inc., UK), at a concentration of 2.18mg/ml, was diluted to 0.5-2mg/ml to act as a control line. Final optimal concentration was 2mg/ml. The test line was rabbit anti-EgB IgG diluted to 0.5-4mg/ml with a final optimal concentration of 2mg/ml. Capture buffer for dilution of both control and test IgG captures was 10mM Tris-HCl, 3% ethanol, pH 8.2.

Conjugate Pad. Colloidal gold conjugated rabbit anti-EgB IgG were prepared as above and absorbed to a glass fiber or cellulose based membrane (Whatman rapid 27) (15-20μl per test depending on the width of pad). Left to dry at 37ºC for 2 hours or freezer-dried overnight. Conjugate dilution Buffer for adjusting conjugate to optimal ratio was 20mM Tris-HCl containing 2% BSA, 10% sucrose, 0.1% Tween 20.

Sample pad. A Whatman 3MM sample pad was soaked in 0.1M PBS with 0.6% Tween 20 and 1% gelatin and left to dry at 37ºC for 2 hours or freezer-dried overnight.

Finally, Rabbit anti AgB IgG was coated on the NC membrane (Millipore HF 180) as a capture (protein conc: 2mg/ml) and goat anti rabbit IgG (affinity purified, by Chemicon International, Inc) as a quality control (2mg/ml). The strip was made with the absorbent pad (Whatman 3MM) above, NC membrane in middle and sample pad at the bottom end. In the test tube, 20μl PBS with 0.3% Tween-20, 20μl cyst fluid, and 20μl colloidal gold labeled rabbit anti AgB IgG was added in order. Then the strips were plugged into the test tube, the liquid would go up until the absorbent pad in 5-10 minutes.

4.3 Results

4.3.1 AgB detection trial in cyst fluid with indirect DIGFA

In total of 26 confirmed CE cyst fluid samples from human and livestock or rodents

were tested, just 3.9% (1/26) was false negative, 1 could not be read due to poor situation of cyst fluid sample. The sensitivity was 92.3%. The specificity was not calculated since sample number was too fewer (Table 4.2).

Table 4.2: AgB detection in different hydatid cyst fluid isolates with DIGFA.

CF Origin	No. of samples	Positive	Negative
Human	4	3	1
Sheep	7	0	0
Horse	9	8	1*
Buffalo	2	2	0
Bovine	1	1	0
Cotton rat	2	2	0
Gerbil	1	1	0
Negative(L.C.R)	2	0	2
Total	28	24	4

*: The sample looked yellow; it may be contaminated with bile and looked difficult to filtrate.
Sensitivity=Positive number in positive samples/positive samples*100%
=24/26 *100% =92.3%

4.3.2 ICA for human cyst fluid samples

Different concentration of Tween 20 could reduce the background of the ICA test. The following results showed 0.6% Tween 20 could be better for this method (Table 4.3 and Fig. 4.1).

Table 4.3: First trial of ICA strips with different conc. of Tween 20 for AgB in cyst fluid

No	Sample	Results	
		0.3% Tw20	0.6% Tw20
B1-1	Liver CE CF (female, 12y, 8/00), T3	++	++
B1-2	Liver CE CF (male, 14y, 10/00, Qinghe), T1	+	+
B1-3	Liver CE CF (female, 17y, 10/00), T3	+++	++
B1-4	Liver CE CF (male, 27y, 7/00,), T3,	++	+
B1-5	CE CF (male, 34y, 4/99), T1	+	+
B1-6	Liver CE CF (male, 61y, 5/99, Huang Yuntang), T1	+	++
B1-7	Liver non-parasite CF (male, 56y, 25/7/00)	Suspicious	-
B1-8	Liver non-parasite CF (male, 7/00)	-	-
B1-9	CE CF (male, 10y, 7/00), T1	Poor +	+
B1-10	CE CF (male, 26y, 7/00), T1	+	+
B1-11	CE CF (female, 27y, 7/00), T3	+	++
B1-12	CE CF (female, 12y, 8/00), T3	+	++
B1-13	872, fluid from residual cavity (7/00)	Suspicious	-
B2-N	PBS with 0.6% Tween 20	-	-

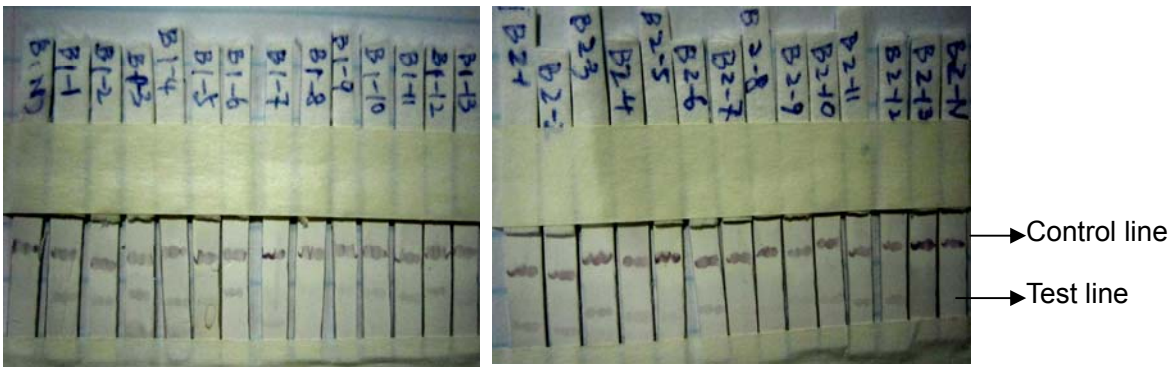


Fig. 4.1: First trial of ICA strips with different conc. of Tween 20 for AgB in cyst fluid (left half with 0.3% Tween 20 and right half with 0.6% Tween 20 in the sample buffer).

A total of 23 cyst fluid samples from Xinjiang, China were used for initial trial of ICA strips. The result showed all 22 confirmed human CE cyst fluid samples were positive, 1 cyst fluid from residual cavity were suspicious (Table 4.4 and Fig. 4.2).

Table 4.4: Trial of ICA strips for AgB in human cyst fluid

No	Sample	Results
B3-1	CE CF (female, 27y, 7/00),	++
B3-2	Human CE CF2 91	+
B3-3	Human CE CF2 91	+
B3-4	Human CE CF2 91	+
B3-5	HU CE CF, X264	+
B3-6	CE CF (female, 27y, 7/00), T3, ②	++
B3-7	CE CF (female, 27y, 7/00), T3, ④	++
B3-8	872, fluid from residual cavity (7/00)	Suspicious
B3-9	HU CE CF, X264	+
B3-10	Human CE CF2 91	+
B3-11	Human CE CF2 91	+
B3-12	Liver CE CF (male, 61y, 5/99), T1④	++
B3-13	Liver CE CF (male, 61y, 5/99, Huang Yuantang), T1②	++
B3-14	Human CE CF2 91	+
B3-15	Liver CE CF (male, 61y, 5/99), T1③	+
B3-16	Liver CE CF (female, 12y, 8/00), T3②	++
B3-17	Liver CE CF (female, 12y, 8/00), T3③	++
B3-18	Liver CE CF (female, 12y, 8/00), T3②	++
B3-19	Chinese human cyst fluid, 2000	++
B3-20	Chinese human cyst fluid, 2000	++
B3-21	Human CF	++
B3-22	Human CF	++
B3-23	Human CF	++
N	Sample buffer	-

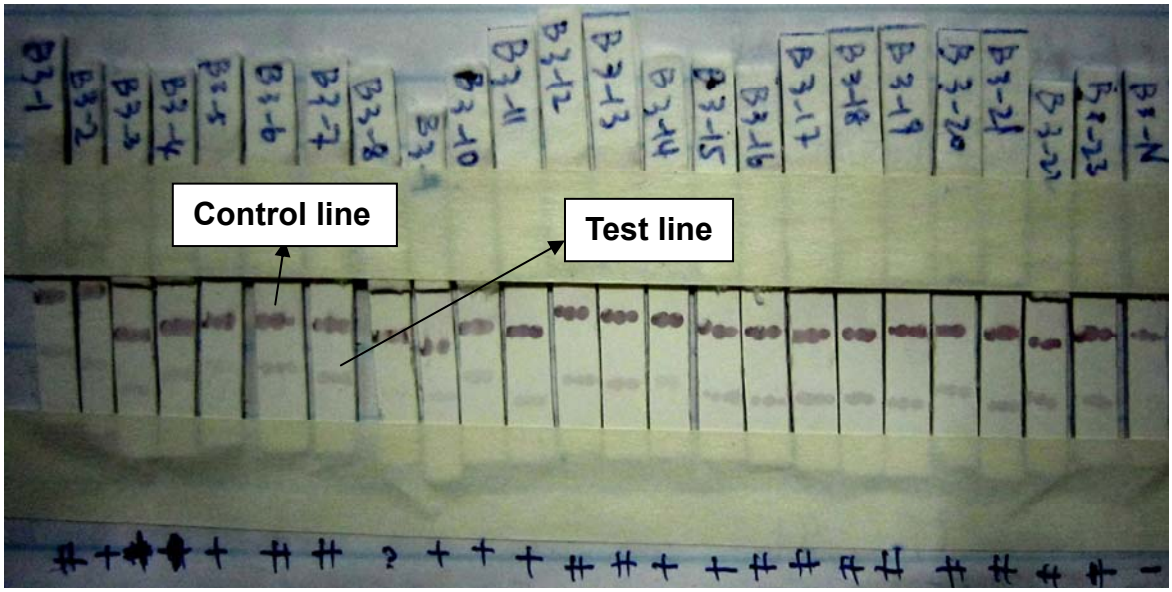


Fig. 4.2: Trial of ICA strips for AgB in human cyst fluid (samples details see Table 4.4.)

A total of 109 human CE cyst fluid samples were collected in Xinjiang Medical University Hospital and 6 fluid samples from non-parasite cysts, were tested with the ICA strips. The results showed the sensitivity was 93.58% (95% CI 91.23-95.93%) and the specificity was 100% (except the samples with color or contamination) (Table 4.5 and 4.5a).

Table 4.5: Sensitivity of ICA strips for AgB in human cyst fluid

Samples	No. of Sample	ICA Positive (%)
CE cyst fluid	109	102 (93.58)
Non-CE sample	6	0
Total	115	102

Table 4.5a: Assessments of ICA strips for AgB in human cyst fluid

Assessments	AgB-ICA for CE cyst fluid (95%CI)	
Sensitivity (%)	93.58	(91.23-95.93)
Specificity (%) in Non CE controls	100	
Accuracy (%)	93.91	(91.68-96.14)
Positive predictive value (%)	100	
Negative predictive value (%)	46.15	(41.51-50.80)
Youden Index	93.58	(91.29-95.86)

4.4 Discussion

Identification of cyst fluid as being of hydatid origin is of use for clinical requirements especially for unconfirmed cyst pathology. The use of immunological methods to detect hydatid antigen B since it could be more useful for cyst fluid identification than other antigens such as crude *E. granulosus* cyst fluid antigen. Antigen 5 has also been assessed by previous research (Paul et al., 1997). ELISA has been a traditional and practical tool for antigen detection, especially with double antibody sandwich methods. AgB ELISA and AgB Dot-ELISA have been confirmed useful and effective (Craig et al., 1986; Wang et al., 2002).

Colloidal gold techniques in this study have been confirmed as a simple rapid and functional method for detecting hydatid antigens in cyst fluid, either was DIGFA or ICA approaches. Results were readable after only several minutes. This then could be useful for hydatid diagnosis during the surgical or percutaneous procedures. Generally the two methods had similar sensitivities but practical aspects were really different. ICA is easy to be made since the strips could be easily coated with an antibody line and cut into strips by a Bio-Dot machine. And DIGFA is more complicated since the NC membrane should be pre-set in the plate to fix its position to apply the dots. A disadvantage of DIGFA in our study was poor background because the cyst fluid might be turbid or contaminated by bile leakage.

The initial trial with the ICA test showed satisfactory sensitivity (93.6%) and accuracy (93.9%) but specificity was not so accurate 100% (6/6) due to less non-parasite cyst fluid or some given up cyst fluid samples with contamination. The advantages of an ICA test for cyst fluid identification were rapid, simple and

effective. It could be used as an instant test during the surgical treatment or PAIR and enable differential diagnosis with other cyst pathologies.

The ICA test however was still in an initial developing stage and not a well rounded final product. For developing this method in the future, some further assessment is required. The purification of rabbit anti AgB IgG could be improved to a better capture and conjugate. Using recombined AgB as a stimulant to boost rabbit, or recombined antibody for AgB due to phage antibody library technique. More samples testing and development of this method to test both cyst fluid and sera will be preceded in the future research.

4.5 Summary

A rapid immuno chromatographic assay (ICA) for hydatid cyst fluid identification was initially developed with a rabbit anti AgB IgG as the capture and colloidal gold labeled rabbit anti AgB IgG as the conjugate. A satisfactory sensitivity (93.6%) and accuracy (93.9%) was observed. The ICA test however was still in an initial developing stage and need to be improved to a well rounded final product.

Chapter 5. Development and application of a rapid sandwich ICA (Immuno Chromatographic Assay) coproantigen detection for canine echinococcosis

5.1 Introduction

Carnivores play the most important role as a definitive host in *Echinococcus* transmission. Therefore, diagnosis and treatment of definitive hosts become a major point for prevention and control for echinococcosis in endemic areas including China (Craig et al., 2000, 2006, 2007; Ito et al., 2003).

Carnivores, especially dogs, are infected in a domestic lifecycle after eating livestock offal infected with *Echinococcus granulosus* cysts that contain protoscoleces. Protoscoleces evaginated and attach to the epithelium wall of dog small intestine with suckers and rostellar hooks (Ding and Wen 2000; WHO/OIE 2001). Protoscoleces develop to the adult tapeworm stage after evagination, elongation (day 1), formation of first proglottid (day 11-14), genital rudiment division and first segment fully formed (day 14-17), rudimentary testes appear and second proglottid begin to form (day 17-20), two-segmented worm (day 20-28), male and female genitalia fully mature (day 28-33), ovulation and fertilization in terminal proglottid, 3-4 segments (day 33-37); gravid with embryonated eggs, 3-5 segments (day 37-45) (WHO/OIE 2001).

The gold standard for detection of adult *Echinococcus* worms in dogs is based on morphological check after necropsy of the small intestine and proglottid check under microscopy. But necropsy could not be accepted as a routine diagnostic approach for domestic dogs, and can only be used for selected sampling in research and possibly dog slaughter (for foods in some areas). Proglottid check could be accepted easily but the sensitivity is generally lower for light infection. Microscopy for eggs can not differentiate *Echinococcus* from other taeniid eggs. Arecoline purgation can be very useful for pre-mortene diagnosis of *Echinococcus* for in dogs, but its time consuming, biohazards and may lack sensitivities (Craig, 1997; WHO/OIE, 2001). Coproantigen ELISA and copro-PCR therefore became more important for general detection assays in epidemiological survey and control projects (Craig et al, 1995, 1996; Sakai et al., 1995; Eckert et al, 1997). The satified sensitivities (84%-95%) and very high specificities (>95%) of coproantigen

ELISA were reported (Allan et al., 1992, Deplazes et al, 1992, 1999; Sakai et al., 1995; Fraser et al, 2002). A disadvantage of these two assays was the professional technical aspects, cost and instruments.

The aim of this research was to develop a rapid test for initial screening of dog fecal samples so that the first check could be carried on dogs in the field and rural endemic areas.

5.2 Methods and approaches

Experimental infected dogs: Protoscoleces of *E. granulosus* were collected from freshly hydatid cysts from sheep liver or lungs in an official slaughter house in Xinjiang. Then the protoscoleces were packed within dog food (sheep intestine) and feed to 9 dogs which had been treated with praziquantel 1 week before infection. Each dog received above 80,000 protoscoleces by oral dosing. Dog faecal samples were collected every 2 day until necropsy. Infection periods were 20-, 34- and 53 days respectively. Three dogs were post-mortem at 20 days after infection and 2 of them were confirmed infected with *E. granulosus* worms in their intestine. Three dogs were post-mortem 34 days, and the remaining 3 were killed 53 days after infection; all were confirmed infected. Intestines of dogs were removed at necropsy as described by Allan et al (1992) and put into 37℃ saline. *E. granulosus* adult worms were washed out from intestinal wall using warm physiological saline, washed by decantation and collected into tubes with saline and frozen at -80 ºC immediately (Craig, 1997) for further developing the IGCA rapid test.

Faecal Samples: A total of 163 faecal samples were collected during the infection from 0 days to 53 days from above *E. granulosus* experimental infected dogs in Xinjiang Veterinary Research Institute, China. And 158 faecal samples among them were extracted for DNA which would be used for Copro PCR test. The normal controls were from Britain non-endemic area and experimental controlled non-parasite dogs in XMUH (n=30). Faeces were stored at -80 ºC for a week and then transferred to -20 ºC.

Faecal samples for coproantigen sandwich ELISA and rapid ICA test were prepared essentially as described by Allan *et al.* (1992). This method involved mixing faecal material (~2gm) with an equal volume of 0.15 M PBS containing

0.3% Tween 20 (Sigma). The samples were then vigorously shaken by hand or shaker to produce a homogeneous mixture. Faecal samples were then centrifuged at 3000rpm for 30 minutes at room temperature and supernatants were aliquoted for later testing. If sample were not tested in 2 days they were frozen at -20ºC/-80ºC.

The application of rapid coproantigen tests were carried on feces samples from communities including Hoberkesaier, Bayanbulake County in Xinjiang.

5.2.1 Preparation of adult worm antigen (EgWWE)

E. granulosus adult worms came from Xinjiang Medical University. They were obtained from experimentally infected dogs with *E. granulosus* protoscoleces from sheep hydatids and necropsied at 20-, 34- and 53- days after infection.

E. granulosus whole worm extract antigen (EgWWE) was prepared essentially according to Allan et al. (1992) by selection of the non-gravid segments (could be done under a microscope), which were then washed three times in sterile 0.15 M phosphate-buffer saline (PBS) (pH 7.2) for at least 30 minutes. The worms in PBS were frozen and thawed at -80ºC / room temperature, then homogenised on ice using a hand held glass homogenizer. To the homogenate 5ml of PBS plus protease inhibitors 0.1M Phenylmethanesulfonyl fluoride (PMSF) was added, and then the preparation was ultrasonicated (60sec, 40W, 80%pulse) on ice. This extract was centrifuged at 12 000g for 30 mins at 4 ºC and the supernatant was removed and aliquot to use as an antigen (EgWWE), frozen at -20°C. Protein concentration of EgWWE was measured by Bradford Assay (Allan and Craig, 1992).

Above EgWWE from worms with 20-, 34- and 53-day infection, were checked with a SDS-PAGE and western blot (Fig. 5.1). The gel and blotting showed EgWWE-34D and -53D owned similar protein bands with standard EgWWE control but EgWWE-20D had stronger reactions. Their protein concentrations were tested by ultraviolet spectrophotometry which checks OD value of 1:30 dilution antigen under 280nm wavelength. The result showed OD value was 0.038, 0.040 and 0.124 respectively and calculated protein concentration was 0.844mg/ml, 0.889mg/ml and 2.756mg/ml using the formulation OD×30/1.35.

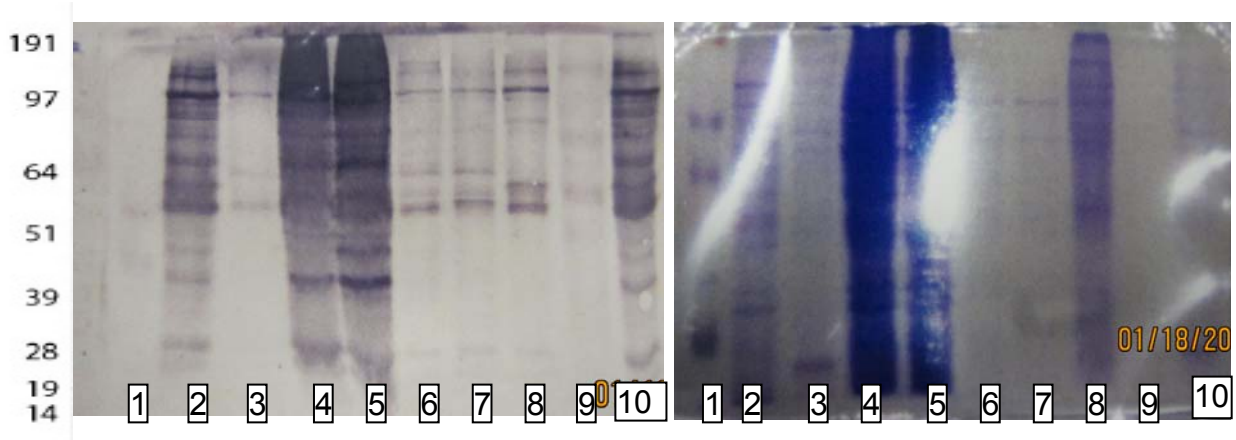


Fig. 5.1: Western-Blot and SDS Page results for EgWWE antigens.
1. Protein marker; 2. EgWWE control; 3. Eg immature worm antigen (20 days after infection); 4. Eg mature worm antigen (34 days after infection); 5. Eg adult worm antigen (53 days after infection); 6. Eg mature worm antigen 1:62.5 (protein conc 0.1mg/ml); 7. Eg adult worm antigen 1:125 (protein conc 0.1mg/ml); 8. EgWWE H (homogenized); 9. EgWWE NH (none-homogenized); 10. EgWWE H;8-10. EgWWE from different sources. The antibody used for blot was HRP conjugated rabbit specific anti-EgWWE IgG.

5.2.2 Preparation and purification of rabbit anti *E. granulosus* (EgWWE) antibodies

The preparation of hyperimmune antiserum was produced according to the method described by Allan and Craig (1989) with a few small alterations.

A hyperimmune rabbit antiserum was obtained by the following steps. First boost was using the injections consisted of intramuscular and subcutaneous of a rabbit with 0.5ml (250µg protein) EgWWE in 0.5ml Freund's complete adjuvant (Sigma). This was followed by a second boost 10-14 days after first one, and then other 2-3 boosts at 7-10 days intervals, using 0.5ml EgWWE in 0.5ml Freund's incomplete adjuvant (Sigma) from second boost. Prior to each injection, approximately 0.5ml blood from an ear vein was separated and serum tested for antibody levels using a standard ELISA (EgWWE as a capture, goat anti-rabbit IgG-HRP as a conjugate). The rabbit was then killed and bled-out 7-10 days after the third or fourth boost when satisfactory antibody level (>1:30,000 titre) occurred. Blood was allowed to clot for 1 hour at room temperature and overnight at 4 °C before being centrifuged at 3000rpm and serum removed. Serum was used within 2 days or stored at -80°C until used.

5.2.3 Preparation of horseradish peroxidase (HRP) conjugates

An IgG fraction was then isolated from above hyperimmune rabbit for use in ELISA. This was achieved by passage of the sera through a protein A sepharose CL 4B column (Pharmacia, Sweden) and subsequent elution using a low pH glycine buffer (0.2M glycine/HCl + 0.5M NaCl, pH3.0). After dialysis against 500 vol. PBS, the IgG solution was re-concentrated by vacuum dialysis to a final concentration of 6-8 mg/ml, which was determined by spectrophotometry at 280nm (Allan and Craig, 1989).

The hyperimmune serum IgG was divided into equal portions, one was dialysed with 0.15M PBS (pH7.2) for use as capture antibodies, and another part dialysed with 0.01M BCB buffer (pH9.6) for conjugation to horseradish peroxidase (HRP, type VI, Sigma P6782), via the method described by Wilson and Nakane (1978).

Conjugation to HRP procedure: HRP (Sigma P6782) was dissolved in pure H_2O with a ratio of 1:2 (w/w) with IgG (e.g. 1ml 8mg/ml IgG would using 4 mg HRP). Fresh prepared 0.1M sodium periodate ($NaIO_4$) was added to the HRP solution with a ratio 200μl per ml and mixed well to become a blue-green solution and covered with foil (to prevent light) for 20 minutes at room temperature. The above solution was dialyzed against 0.001M sodium acetate buffer pH4.4 overnight at 4°C, then the pH was raised to 9.5 by adding 20μl/ml freshly prepared 0.2M sodium carbonate/bicarbonate buffer (BCB) pH 9.6. Additional volume of 0.05M BCB was added to bring the volume of the IgG solution to the same as the HRP solution. Immediately, the HRP and IgG solutions were mixed together and gently stirred for 2 hours at room temperature in a foil covered tube.

Finally, 0.1ml/ml fresh prepared sodium borohydride (NaBH4, 4mg/ml) was added to the conjugation solution and mixed well for 2 hours at 4^{o}C. Finally the conjugate solution was dialyzed against 0.15M PH7.4 PBS, overnight at 4^{o}C and aliquoted and store at -80^{o}C.

Coproantigen ELISA

The optimal dilutions for capture IgG antibody and HRP conjugated antibodies were obtained by checker-board titration. There ranged from 5-10μg/ml for capture antibody.

A 96-well Immulon 4 microtitre plate (Product code M129AIV-50, Dynex Technologies Ltd, UK) was coated with capture antibody -- rabbit anti-EgWWE IgG (diluted with 0.05M NaHCO3 / Na_2CO_3 buffer pH9.6) 100µL/well, 2 wells should be left as blank controls (not antibody, faeces or conjugate solution added) and covered with cling film and left at 4ºC overnight. Plates were washed with PBS (0.1% Tween 20), and blocked with 100 µl/well of PBS with 0.3% Tween 20 for 1 hour at room temperature.

Supernatant from a faecal sample (see section 2.5.2) was diluted 1:1 in PBS 0.3% Tween 20 and 50µL/well added to 50µL/well of heat-inactivated foetal calf sera (FCS, to prevent non-specific reaction) already in each well and incubated at room temperature for 1 hour. Positive and negative control faecal samples were included in each plate and all the samples including controls were tested in duplicate.

Faecal samples were discarded from the plate into 10% bleach. Plates were washed 3 times with PBS (0.1% Tween 20), and then 100 µL/well of conjugate (HRP-IgG anti-EgWWE, diluted with PBS 0.3% Tween 20) added at optimal concentration, and incubated at room temperature for 1 hour. Plates were washed 3 times with PBS (0.1% Tween 20). Then a substrate of tetramethyl benzidine (TMB microwell peroxidase system, Catalog no 50-76-00. Dynex Ltd. UK) was added at 100 µL/well including blank wells for 20 mins. OD values were determined initially at 630nm by microplate reader; after addition of stopping buffer (1M phosphoric acid at 50µL/well) was final ODs were read at 450nm if necessary.

5.2.4 Preparation and purification of colloidal gold conjugate

5.2.4.1. Colloidal gold:

10% $HAuCl_4$, were prepared with H_2O using $HAuCl4{\cdot}3H_2O$, Gold (III) Chloride trihydrate, ACS reagent, Sigma G4022. 100 ml pure H_2O was brought to boil on a heat board or in a microwave. Then 10% $HAuCl_4$ 300 µL was added and back to boil again. Add 7 ml 1% Sodium citrate into above hot solution quickly. Keep boiling for more 10 minutes with stirring evenly on a heat board or just shake the

bottle quickly to mix the reagents well. The colour would be changed into wine red in 1-2 minutes. Cool in room temperature and store at 4°C with foil covered.

5.2.4.2 Optimized antibody concentration for gold conjugate

1 ml of Colloidal gold were first added into each tube, then followed by different volumes antibody (8 µl, 16µl, 32µl, 64µl, 128µl respectively) and 100µl 10% NaCl at last. Observe the colors of each tube after 1 hour. The 3rd one with 32µl antibody showed red-purple which just changed to red from black or dark purple.

A rabbit anti- EgWWE antigen was prepared as described in Section 2.5.2. Sera was taken out and then purified through Protein A column to obtain IgG. A part of IgG was conjugated with HRP as described in general protocol using $NaIO_4$ which have details in 5.2.3, in order to use it as secondary antibody in the sandwich ELISA. Another part of the rabbit IgG was conjugated with colloidal gold.

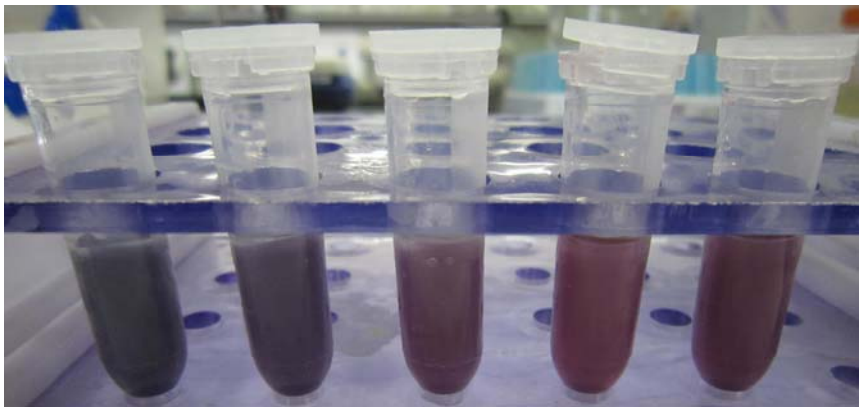


Fig. 5.2. Experiment for optimizing the antibody conc. to be labelled with colloidal gold. Antibody was added into 1ml colloidal gold for conjugate with different volumes (8 µl, 16µl, 32µl, 64µl, and 128µl) from left to right. The tube was selected when the color of liquid was just turned into purple-red from black or purple. Above result showed the third one with 32µl volume was that we needed. Then the optimal concentration of antibody for 1ml colloidal was expected as 32*120=38.4µl for the conjugation.

5.2.4.3 Procedure for Anti-EgW Gold Conjugate:

Mixed 10 ml Colloidal Gold with 1 ml 0.2 M Sodium borate first and then added 0.384ml 1mg/ml rabbit anti-EgW IgG prepared in 20 mM Tris-HCl. Kept above mixture at 4 °C for 45 minutes or 1 hour. Add 440 µL 5% PEG 20,000 and 2.94ml

Blocking buffer (certain concentration of sucrose, Gelatin and BSA in 200mM Tris-HCl). The initial conjugate was then filtered with 0.2 μm pore size syringe filter and stored in dark at 4°C.

5.2.4.4 Conjugate purification:

Above conjugate was centrifuged in 2000rpm for 30 minutes to get rid off some bigger particles. Then the supernatants were concentrated in a dialysing tube with covered PEG 4000 until final one third of original volume left.

A Sephadex G200 column was prepared with 1g Sephadex G200 powder for a volume 15-20ml. Concentrated conjugate was coupled with the column, and eluted with 2% BSA 20mM Tris-HCl. The middle part of conjugate with deep wine red colour was collected due to different sizes of colloidal conjugate which would have different pass speed to get similar size conjugate. The final volume of conjugate was around 4ml. OD value was tested under the wavelength 570nm. The final conjugate was diluted to lower the OD value to the optimal 0.7 with a conjugate buffer.

5.2.5 Development of Immunochromatographic Assay (ICA)

Preparation for ICA test

A rabbit anti- EgWWE antigen was prepared as described (section 5.2.1).

Sera was taken out and then purified through Protein A column to obtain IgG. A part of IgG was conjugated with HRP as described in general protocol using $NaIO_4$ in order to use it as secondary antibody in the sandwich ELISA. Another part of the rabbit IgG was conjugated with colloidal gold.

Colloidal gold was prepared by the reduction of gold chloride with citric acid; protein could be combined were test with rabbit anti-EgWWE IgG and 10% NaCl.

Colloidal gold conjugate anti-EgWWE IgG was prepared using 1mg/ml IgG in 20 mM Tris-HCl buffer, and combined 120% of optimal volume to the suspension of borate buffer balanced colloidal gold (pH>8.0), at 4ºC for 1 hour. Blocking buffer (5%BSA, 100mM Tris-HCl, sucrose, gelatin and PEG 20, 000) was added drop by drop, mixed and filtered through a 0.2μm membrane, then stored at 4ºC in the dark.

The last concentrate could be changed by ultracentrifuge to individual design, the purification methods would be talked in following paragraph.

ICA test kit designed for coproantigen detection

ICA test kit was essentially a kind of dipstick or strip-test. With the NC membrane in the middle, a sample pad, conjugate pad and downstream an absorbent pad, were in proper order and pasted to a commercial plastic back
(Whatman, China) (Fig. 5.3).

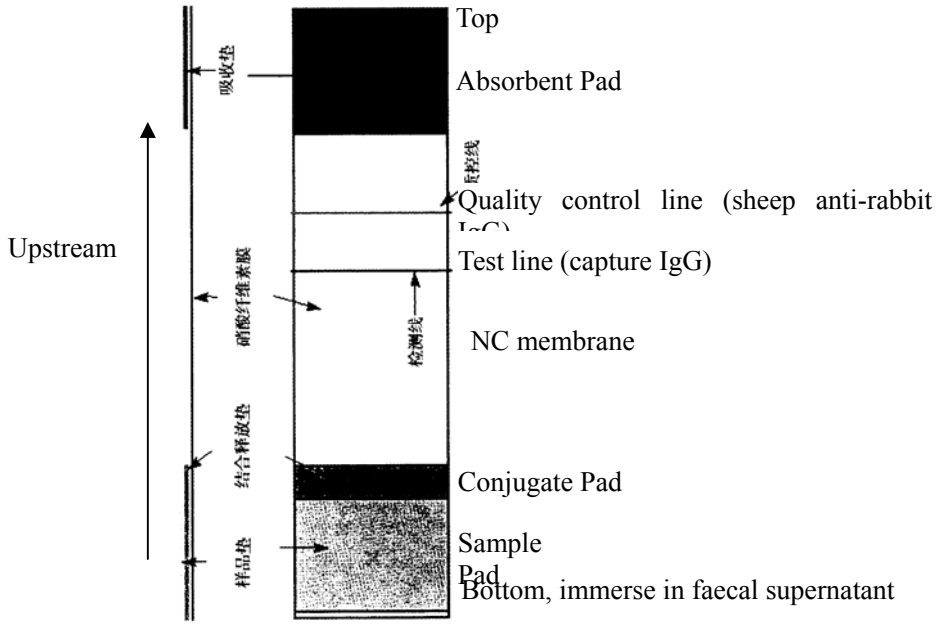


Fig. 5.3: ICA test strip

An ICA test kit consists of the following downstream membrane or pads on a plastic backup: an absorbent pad, a strip of NC membrane with capture, a conjugate pad and a sample pad at the bottom (Fig. 5.4).

The NC membrane used in ICA test was different with that used in the DIGFA approval. General blotting NC membrane with pore size 0.45μm was tried but failed to absorb reagent due to its smaller pore size. A commercial Millipore HiFlow Plus HF 18004 NC membrane was finally chosen due to better flow speed and reaction time.

A quality control line of affinity purified goat anti-rabbit IgG (Chemicon International Inc., UK), at a concentration of 2.18mg/ml, was diluted to 0.5-2mg/ml to act as a control line. Final optimal concentration was 2mg/ml.

Rabbit anti-EgWWE IgG at a concentration of 0.5-4mg/ml (optimal concentration was 2mg/ml) was used as capture antibody diluted in 10mM Tris-HCl, 3% ethanol, pH 8.2.

Colloidal gold conjugated rabbit anti - EgWWE IgG was coated (approximately 32µg/ml, 2µl per test) on the conjugate pad (Millipore, based on glassfibre membrane). The dilution of colloidal gold conjugate was determined by titration with positive control (EgWWE antigen 1:100) and negative dog faecal samples.

A Millipore sample pad made of cellulose was soaked in a Tris-HCl buffer including some surfactants such as Tween 20, PEG 20,000, and 2% BSA to decrease the nonspecific reaction and dried at 37°C for over 2 hours.

A Whatman 3MM chromatographic membrane was used as absortent pad.

The ICA procedure was simple to carry out. The sample pad of the strip was first immersed in the faecal supernatant in a tube. The absorbed faecal supernatant then met with the pre-coated conjugate and antigen-antibody combined together if coproantigen existed in the faecal sample. The mixture was then upstream again on the NC membrane and reacted with capture antibody (double antibodies sandwich). The red color on test line would appear if coproantigen existed. Excess conjugate should go up and combine with the third antibody to act as a quality control line. All the excess liquid would be absorbed into the top absorbent pad to leave the reaction area for clear reading. The procedure could be carried out in 5 to 20 minutes depending on the different type of NC membrane and utilizes.

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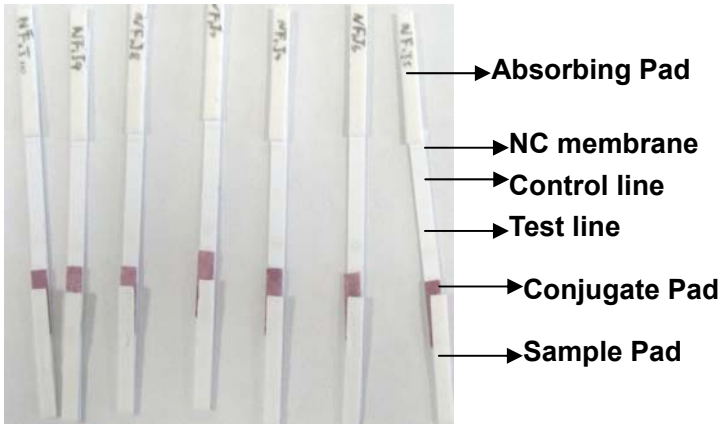

Fig. 5.4: ICA coproantigen strips were prepared to test

5.2.6 Copro PCR for experimental infected dogs

5.2.6.1 Faecal DNA extraction

5.2.6.1.1 Preparation

Enough 2ml & 1.5ml tubes and collecting topless tubes (for DNA collection at end of procedure) were autoclaved before extraction.Tips for loading gels only were autoclave as well. Samples were labeled properly. Plenty of PBS was prepared with 1 tablet PBS adding 200ml H2O. Bleach pot was prepared for used tips and samples. The Sonitech water bath was warm to 70°C--90°C for eggs lysis. PCR sample were stored in ethanol. Medical torgue depnessors were prepared for holding faecal samples.

5.2.6.1.2 Procedures:

Bottles of 12*10ml were labeled and added about 1/3 volume PBS. Tubes (12*50ml) were labeled and added 2.5-3ml percoll per tube, then added PBS until 42.5 ml, put a deposable sieve on the top. 1-2g faeces were weighted and put into the above 10ml-bottle and mixed with PBS. Then the mixture was poured on the sieve on the above tubes. Then the tube top was stewed and the tube was centrifuged at 3600-3660 rpm for 30 minutes. The sieve and faeces were thrown away and the supernatant was collected for next procedure. Operation for stool DNA was then carried out from Step 2 in manufacture's protocol (QIAamp DNA Stool Handbook) and the last DNA eluate stored at -20°C.

5.2.6.2 Copro PCR procedure （Abbasi, 2003; Boufana et al, 2008 ）

The primers was as follows: Eg1121a 5′-GAATGCAAGCAGCAGATG-3′ (upstream) and Eg1122a 5′-GAGATGAGTGAGAAGGAGTG-3′ (downstream). First to calculate how much PCR solution would be needed which 49ul last solution + 1ul DNA for each sample. For 50 µl reaction solution, it was made by 21.25µl H2O, 25µl X2 buffer 9, 0.5µl 1mm dNTP, 0.5µl 1µm Pra, 0.5µl 1µm Prb, 1µl 2% Formamide, 0.25µl 1.25µ Taq, and then 1µl DNA solution. Turn on the UV light before start to work and bring ice back. PCR tubes were put on the rack and then put on the icebox. All the solutions were added one by one to a 2ml tube. Add 1µl DNA to the bottom of PCR tube. Taq could be taken out only the last step.

Mixed them in a rotor and aliquoted to the PCR tubes, the mineral oil could be add on the top. Then all the tubes were capped, marked label on the top. Sample box was taken out from the UV box, and DNA sample did before. PCR machine which need to preheat before starting. The coproPCR was runned with the profile of 5 minutes at 95°C for 1 cycle, 1 minute at 95°C+1minute at 55°C + 10 seconds at 72°C for 35 cycles, then 10 minutes at 72°C for 1 cycle.

PCR Gel preparation:

0.5% TBE buffer 100ml was added agarose 1.5g, heated by a microwave oven for 3 minutes, was cooling down with water bath in 0.5 hours, poured into the gel cassette with 2 drops Ethidium Bromide (0.6mg/ml, 5µl per 100ml).

1 drop Blue one and 10µl DNA sample were mixed and then added to the well, at the same time 5µl DNA marker was added in another line.

5.3 Results

5.3.1 Sandwich ELISA test for canine coproantigen

5.3.1.1 Test dog faecal samples with sandwich ELISA

A total of 163 fecal samples from *E. granulosus* experimental infected dogs were used for copro ELISA. The time-curve of coproantigen ELISA showed the positivity and sensitivity were higher following the infection time. The samples with the infection time fewer than 28 days presented the lower OD value than the cutoff (0.318), and after that time, almost all the samples showed positives. First copropositive occurred by 28dpi (Fig. 5.5).

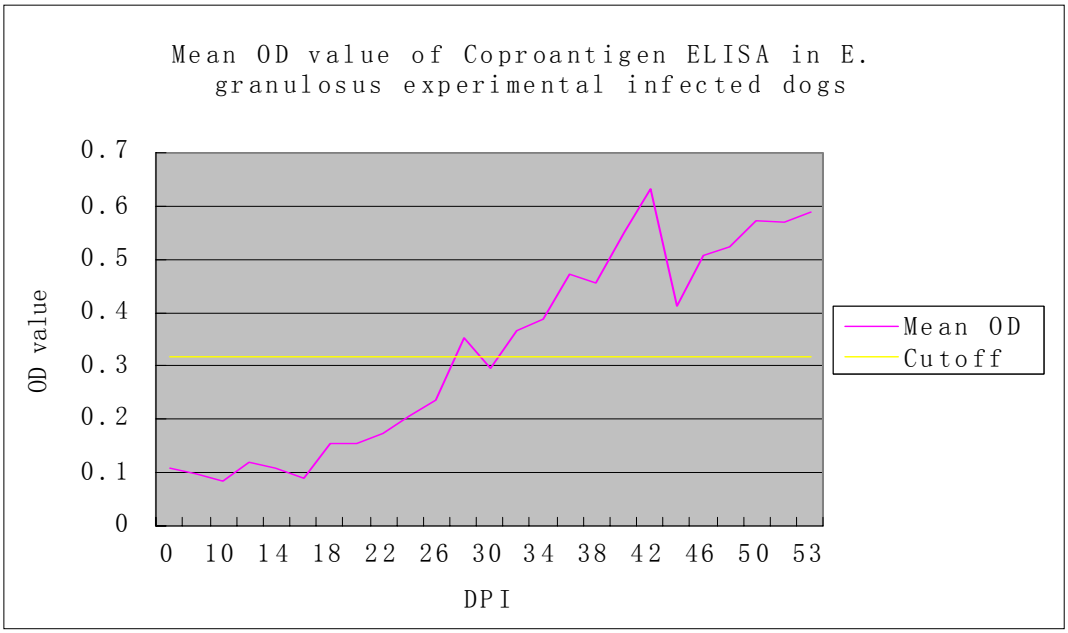

Fig. 5.5: Mean OD value of Coproantigen sandwich ELISA in *E. granulosus* experimental infected dogs. (Cutoff=N+3*SD=0.318)

5.3.1.2 Coproantigen ELISA tests for screening dog-faecal samples from community survey in western China.

The results showed that 3 stronger positive in 21 samples (14.3%) from Habahe and 3 positive in 41 samples (7.3%) from Yushu (Table 5.1). The positive rate in Yushu was lower than we expected. I tested them again with other reagents, but got the similar results with before. I thought that the reason might be relevant with the condition of the faecal samples' collection, dilution and storage. The samples were collected 2 years ago, the diluted ratio looked different in different samples, and they were stored at 4°C for 2 years.

Table 5.1: Coproantigen ELISA tests for screening dog-faecal samples from endemic communities in Xinjiang (X) and Qinghai (Q) of China.

Communities	No.	Positive (%)	Negative
Habahe(X)	21	3 (14.29)	18
Yushu(Q)	41	3 (7.32)	38
Hoboksar(X)	76	36(47.4)	40
Hejing(X)	40	9(22.5%)	31
Total	178	41 (23.03)	127

5.3.2 Diagnostic evaluation of immunogold chromatographyic assay (IGCA) for experimental dogs

96 faecal samples from *E. granulosus* experimental infected dogs were also tested by rapid IGCA assay as for the ELISA test. The results showed the sensitivity was increased followed by infected days as well and had significant difference in four different infection period ($P<0.01$) (Table 5.2(a) and 5.2(b)). The initial IGCA result presented a little lower sensitivity compared with ELISA ($P>0.05$). The specificity was 100% (30/30 for non-infected control group) in ELISA and 93.33% (28/30) in IGCA.

Table 5.2(a): Comparison of coproantigen ELISA and IGCA rapid test for *E. granulosus* experimental infected dogs

Samples	No.	Positives		Negatives		Sensitivity (%)	
		ELISA	IGCA	ELISA	IGCA	ELISA	IGCA
Non-infected	30	0	2	30	28	-	-
1-20days	30	4	2	26	28	13.3	6.7
21-34days	20	10	8	10	12	50	40
35-53days	16	16	15	0	1	100	94
Total	96	30	27	66	69	45.5	42.4

Table 5.2(b): Statistical result for comparison of coproantigen ELISA and IGCA rapid test

	ELISA & ICA	Time courses with ELISA	Time courses with ICA
Chi-square	0.099	56.592	54.696
P	0.753	0.000	0.000

5.3.3 Copro PCR results for experimental infected dogs

A total of 158 DNA samples were extracted from feces from above *E. granulosus* experimental infected dogs. The positive rate was not so good since the fecal sample had stored for near 8 yrs ago. But the interesting result was the clear positive zones occurred in sample No 93 and No. 155 which were fecal samples from two 20-day infected dogs (Fig. 5.6).

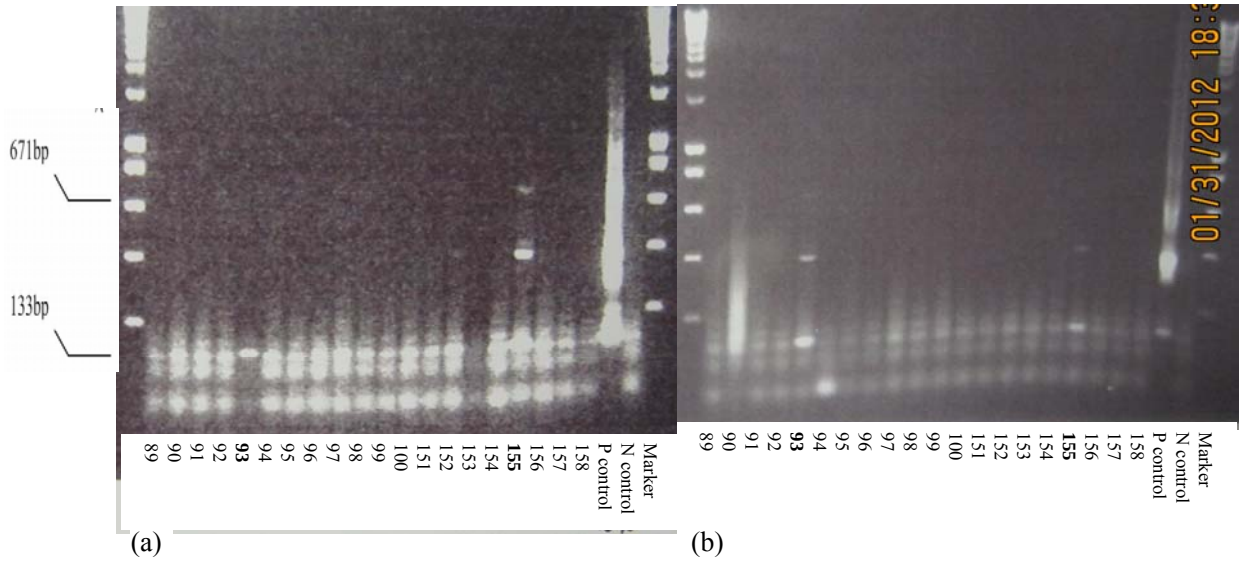

Fig. 5.6: No. 93# and 155# showed all positives within double check with Copro PCR (Abbasi's methods) for experimental infected dogs.
Lanes: fist and last lane were 100-bp molecular DNA ladder, second and third lanes from right were negative and positive control, the other lanes were different DNA samples from dog faeces.
(a) No. 93# (20 days post infection) had one positive lane in 133bp and No. 155# (20 days post infection) had another strong bands.
(b) No. 93# had two obvious positive bands and NO. 155# had a weak positive in its second band.

5.4 Discussion

The dog is a key host in the life cycle of *Echinococcus*, not just for *E. granulosus*, but also for *E. multilocularis* since it has been confirmed to be infected with *E. multilocularis* (Craig et al., 1992, 2000, 2003, 2005; Shi, 1995; Vuitton et al., 2003). Serological tests for dog infection were effective but not so sensitive because serum antibodies may be present from recent infection even if there is no current infection (Craig, 1997). Eggs as the infective source for livestock and human, exist within segments of adult worms and in dog feces. Eggs are however difficult to be differentiated from other taeniid cestode eggs under the microscope. Arecoline purgation is useful to show presence of adult worms after purgation and has been applied in mass screening (Gemmell, 1973; Craig et al., 1995; Budke et al., 2005; Lahmar et al., 2007, 2009). However unsuccessful purgation might cause missed infections and light infections may not be detected (Craig et al., 1995; Schantz et al., 1995; Fraser et al., 2002; Budke et al. 2005). The autopsy of dogs is a gold standard for infection but can not usually be accepted or recommended (Craig et al., 1997; Eckert et al., 1997; WHO/OIE, 2001; Fraser et al, 2002).

Coproantigen sandwich ELISA has been widely accepted as a laboratory detection method for canine echinococcosis (Allan et al., 1992; Craig et al, 1995; Deplazes et al, 1992, 1997; WHO/OIE, 2001; Fraser et al, 2002). One of the most common used antigens for raising antibodies was whole worm extract of *E. granulosus* (EgWWE) and the sensitivity and specificity was generally over 85% (Allan et al, 1992; Deplazes et al., 1992, 1999). The extracts of *E. granulosus* include somatic protoscoleces, excretoary / secretory (E/S) extract of *E. granulosus* protoscoleces, and the FPLC fraction from EgWWE were all used for find a more specific antigen to be used as a boost for coproantigen ELISA (Allan et al., 1992; Craig et al., 1995, 1996; Deplazes 1992, 1999; Sakai et al., 1995; Frazer et al., 2002; Elayoubi et al., 2004). Monoclonal antibody for EgWWE was also another attempt to gain more specific result (Kohno et al., 1991, 1995; Sakai et al., 1995; Malgor et al., 1997; Zhang et al., 2003). Copro-PCR was developed for differential diagnosis of *E. granulosus* and E multilocularis by amphyzation of species specific DNA (Dinkel et al, 1998; Frazer et al., 2002; Abbasi, 2003; Boufana et al, 2008, 2012).

This Chapter reports the first initial development and assessment of a rapid IGCA for coproantigen detection for effective screening of dog infections. The sensitivity of IGCA was not as good (94%) compared with coproantigen ELISA (100%) in 16 experimental 35-53 dpi dogs. This might be due to several possibilities. Polyclonal antibody was used as the capture and detection antibody, but a good monoclonal antibody was difficult to obtain against a purified antigen. Then the detection system was very important, IGCA is a rapid test, based on eye-reading so its sensitivity and specificity were generally lower than for coproantigen ELISA. Thus it might be best used as an initial screening tool in the field.

The *E. granulosus* experimental time-course infection of dogs was subjected to faecal screening. ELISA and IGCA showed that coproantigen level appeared to increase from day 16 after infection and were clearly detected after day 24 dpi by both ELISA and IGCA. This might be thought to correlate when the first segment of worm formed in that period (i.e. day 14-17) and two segmented worm (day 20-28) (WHO/OIE, 2001). And the DNA of *E. granulosus* was amplified from dog faeces from 20 days after infection. Copro DNA has been detected in experimentally infected dogs in other studies pre-patency (Lahmar et al., 2010). DNA might be associated with cell turnover from the worm surface and/or lost /

degraded worms or proglottids from 20-28 dpi.

The copro ELISA was also applied to screen faeces of dog sampled in endemic communities in Xinjiang and Qinghai. The initial trial had been used in some community studies and a mean positive rate of 23% (41/178) was obtained. The samples from Hobokersaier and Hejing showed relative higher positives (47.4% and 22.5%) compared to Habahe and Yushu Counties, and also we confirmed the higher human CE prevalence in epidemiological studies in these two counties in Xinjiang (see Chapter 6).

These results suggested that copro tests (coproantigen ELISA, IGCA, copro-PCR) might be sensitive for canine echinococcosis from 20 days after infection. The use of monoclonal antibody for surface antigen of *E. granulosus* adult worm had been shown to have effective results in other research work (Zhang et al., 2003) and this could be helpful for both coproantigen ELISA and IGCA. And the IGCA might be potential.

5.5 Summary

A rapid IGCA of coproantigen detection for dog infections was initially developed and assessed. The sensitivity of IGCA was 94% in 16 experimental 35-53 dpi dogs. ELISA and IGCA showed that coproantigen level appeared to increase from day 16 after infection and were clearly detected after day 24 dpi by both ELISA and IGCA. The copro ELISA was also applied to screen faeces of dog sampled in endemic communities in Xinjiang and Qinghai. The initial mean positive rate of 23% (41/178) was obtained. The samples from Hobokersaier and Hejing showed relative higher positives (47.4% and 22.5%).The results suggested that copro tests (coproantigen ELISA, IGCA, copro-PCR) might be sensitive for canine echinococcosis from 20 days after infection.

Chapter 6. Epidemiological studies and risk factor analysis for echinococcosis in northwestern China

6.0.1 Introduction

The areas including Wenquan County, Bayinbuluke (Hejing County), Xinyuan County and Hoboksar Mongol Autonomous County in Xinjiang, Xiji County in Ningxia, Ganzi County in Sichuan, Dangxiong County and Dingqing County in Tibet (Fig. 6.0.1) were considered as suspected endemic areas for human echinococcosis (Menghebat et al., 1993; Guo et al., 2001; Bai et al., 2002; Feng et al., 2002; Huang et al., 2002; Bao et al., 2003; Tang et al, 2003; Budke et al., 2005; Li et al., 2005, 2010; Wang et al., 2005, 2006; Yang et al., 2006; Chu et al., 2010; Pang et al., 2010). But the limitations of above studies showed just Casoni test was a serological tool, or just ultrasound result without serology, the relative risk for local people not sure, and ever no general information (ie. Tibet). A comprehensive epidemiology model included ultrasound for abdominal echinococcosis with serology (DIGFA), and questionnaires for risk factor analysis, was nessessary for mass human screening of these endemic areas. Furthermore, the performance evaluation of DIGFA for community study could be assessed for further understanding the epidemiological features.

Fig. 6.0.1: Community studies for echinococcosis in 8 Counties of Northwest China

6.0.2 General methods for community studies

6.0.2.1 Study locations and communities

The study was part of mass screening survey for echinococcosis in highly endemic areas of northwest China during 1998-2007. There included main study sites of Yili, Tacheng, Altai, Boertala and Bayinguoleng Prefectures in Xinjiang Uygur Autonomous Region; also collaborate work with -Guyuan Prefecture in Ningxia Hui Autonomous Region, Ganzi Tibetan Autonomous Prefecture in Sichuan, Lhasa and Chamdo Prefectures of Tibet Autonomous Region. Most populations in these area included Kazakh, Mongolian, Hui, Han and Tibetan ethnic groups, many of them were nomadic or semi-nomadic and a few farmers (Table 6.0.1 and Table 6.0.2).

During each community screening, local administrative support from local hospital, Center of Disease Control, Public Health Office and local government was obtained. All the procedures during screening were explained to individuals through local language translators according to ethical standards under the directions of Xinjiang Medical University Hospital Ethics Committee.

Table 6.0.1: General information of studied communities on echinococcosis in Northwestern China

Prefectures	Counties /Communities	Population[a]	Ethnic[b]	Longitude	Latitude	Altitude[c]
Bayinguoleng	Hejing /Bayinbulak	14310	Mongolian	82°27'-86°17'	42°18'-43°34'	2400m
Boertala	Wenquan /Angelige	10618	Han, Mongolian, Kazak	81°02′--81°08′	44°51′--44°57′	1030-1250m
Yili	Nileke /Wulasitai	9098	Kazak	81°58-84°58′	43°25′-44°17′	800-4590m
	Nileke /Musi	10145	Kazak			700-900m
	Xinyuan /Nalati	27000	Kazak	83°10′-- 84°25′	43°15′-- 43°40′	900-4275m
	Xinyuan /Turgen	12000	Kazak	83.27	43.41	900-1120m
Tacheng	Hoboksaier	50000	Mongolian	84°37´--87°20´	45°20´--47°12´	1292m
Altai	Qinghe	55096	Kazak, Mongolian	90°37	46°71	900-3659m
	Habahe	79050	Kazak	85°31 ～87°19	47°42 ～49°09	440-3248m
Guyuan	Xiji	471000	Hui	105°20′—106°04′	35°35′—36°14′	1688-2633m
Ganzi	Ganzi	56000	Tibetan	99°08′——100°25′	31°24′——32°54′	3325-5688m
Lhasa	Dangxiong	41918	Tibetan	90°45′—91°31′	29°31′—31°04′	4200-7111m
Chamdo	Dingqing	60000	Tibetan	94°39′-96°17′	31°01′-32°21′	3300-6328m
Shannan	Qusong	20000	Tibetan	92°11	29°3'	4200

a. Population got from government statistics (2003 or 2005) and indicated communities or counties if not specialize commune.
b. Main ethnic groups in these areas.
c. Mean altitude or low-highest altitude above sea level.

Table 6.0.2: Community mass screening sites for human echinococcosis by XMUH in China during 1998-2007

Province or Autonomous Region	Prefecture	County	Commune	Survey period	No of screened
Xinjiang	Bayinguoleng	Hejing[1]	Bayanbuluke	2004-8, 2004-10	1398
	Boertala	Wenquan[2]	Angelige	Oct-05	1292
	Yili	Xinyuan[3]	Narati	Oct-03	1815
		Xinyuan	Turgen	May-05	1841
		Nileke[4]	Wulasitai	Apr-04	2044
		Nileke	Musi	May-05	1851
	Changji	Mulei[5]		2002.3	962
	Altai	Habahe[6]		1998-9, 2002-5	1648
		Qinghe[7]		2001-10,2002-5	1134
	Tacheng	Hobuksaier[8]		2007-3,2007-10	1325
Ningxia	Guyuan	Xiji[9]		2002.10	945
Sichuan	Ganzi	Ganzi[10]		2006-4	1685
Tibet	Shannan	Qusong[11]		2003-8	722
	Lhasa	Dangxiong[12]		2006-10	557
	Chamdo	Dingqing[13]		2007-11	232

Ethnic groups were mainly 1. Mongolian; 2. Han, Kazak, Mongolian; 3. Kazak; 4. Kazak; 5. Kazak; 6. Kazak; 7. Mongolian and Kazak; 8. Mongolian; 9. Hui; 10. Tibetan; 11. Tibetan; 12. Tibetan; 13. Tibetan.

All the community studies shown here were conducted from 1998 – 2007 in Xinjiang (Fig. 6.0.2). Also other community studies including assessment at a rapid serology test were carried out in Xiji County, Ningxia Hui Autonomous Region in 2001 and 2002; in Ganzi County, Ganzi Tibetan Autonomous Prefecture of Sichuan Province in 2006, in Qusong (2003, just ultrasound and DIGFA results, no questionnaires), Dangxiong (2006) and Dingqing (2007) Counties, Tibet Autonomous Region.

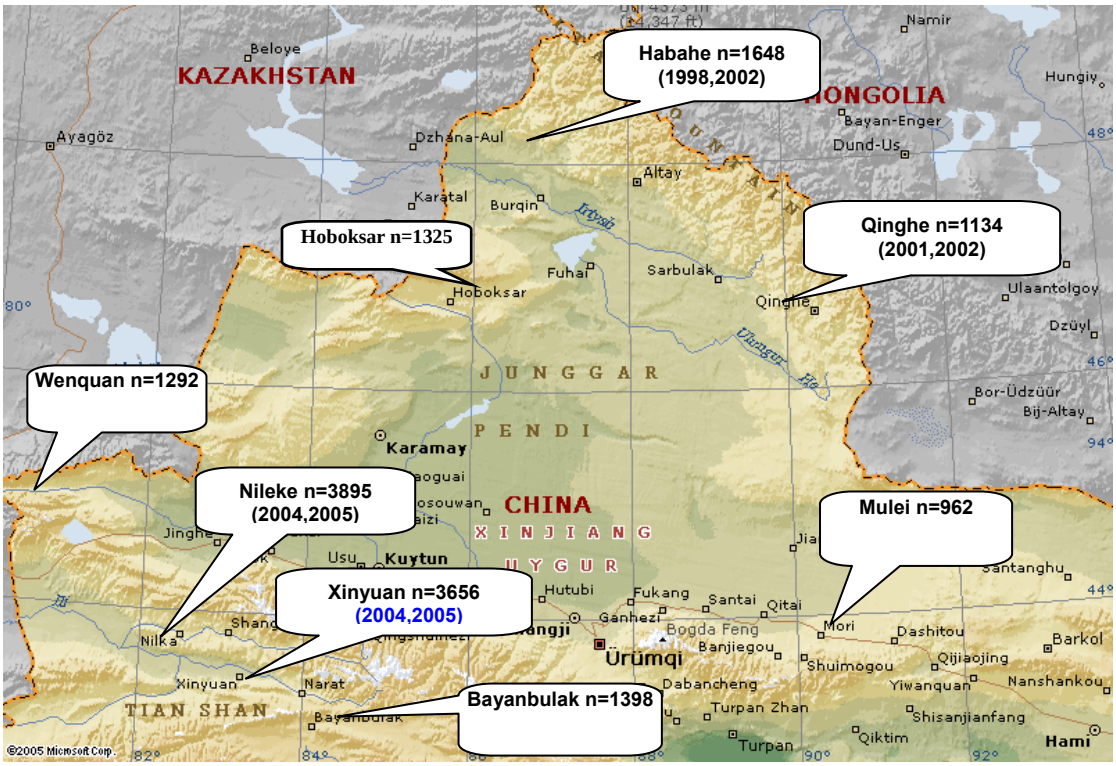

Fig. 6.0.2: Community screening sites for echinococcosis in Xinjiang Uygur Autonomous Region, China

6.0.2.2 Human echinococcosis screening

i. Questionnaire

Each person was asked by the registering person to answer a questionnaire (2 sides) and was given a registration number. Generally questionnaires were completed with the help of local translators. Questionnaire included the following information request:

a. General information including gender, age, ethnic, occupation, income, address

b. Risk factors history: dog owner, fox/wolf contact, livestock ownership, home slaughter, health education (see Appendix VI).

ii. Blood samples and serology

Most people voluntary donated 3-5ml of venous blood for immunological testing. The blood collection procedures were undertaken using strict standard operations by a registered nurse with a single-use disposable syringe or vacuum blood collection tube. The blood samples were centrifuged at 2000g for 10 minutes after

1 hour or 10 minutes if using procoagulant tube. Sera were collected into 1.5ml/0.5ml capped tubes. Rapid DIGFA was carried out in the field and the results provided to participant the same day. Sera were stored at -20°C for ELISA test later in the XMUH laboratory. Sera were random selected for clinically normal and DIGFA negatives, and all ultrasound positives and/or DIGFA positive cases.

iii. Abdominal ultrasound scanning

People were scanned by abdominal ultrasound (US) operated by an experienced registered sonographer (from XMUH) using a portable ultrasound scanner (Sonoline SX Siemens, Germany; or LOGIQ Book XP, USA). Images with CE or AE characters or suspected cases were recorded, and for CE cyst were graded Type I, II, III, IV, V according to WHO classification (WHO/OIE 2001). For AE liver lesions were classified using the PNM system (EurEchinoReg, 1998, WHO/OIE, 2001, Kern, 2003, 2006)

iv. Serology follow-up

Sero-diagnostic results were compared with the US result. Double checks for serology (DIGFA) were performed if one was positive but another showed negative. Sero-negative but US positives were regarded as true positive (US results as ‘gold’ standard). Sero-positive but US negatives were double check by US also arranged to have a chest X-ray. Sero-positives without abdominal or chest cysts or lesions were recorded for follow-up management study.

v. Treatment of CE or AE cases

All the CE or AE detected cases that needed treatment were initially given albendazole tablets for 3-6 months. A few CE cases that were not suitable for surgery were given fine-needle puncture aspiration injection reaspiration (PAIR) treatment under US guidance in a local hospital (e.g. Habahe County in Xinjiang). Cases with cysts or lesions that could be surgically removed, received surgical treatment performed by the professional surgeon group from XMUH that follow-up the community screening. Both medical, PAIR or surgical treatment were free to patients and supported by Chinese governmental funds, cooperation funds (e.g. NIH) and/or the Chinese Song Qingling Charity Fund Committee.

6.0.2.3. Canine echinococcosis surveys

Dog surveys by different teams were performed at the same time as community screening for human disease. A questionnaire (see Appendix VII) for dog owners included general information, the number of dogs owned and history of ownership, hunting practices, health behaviors (washing hands before eating, etc.). Dog faecal samples were collected from the freshest faeces on the ground or taken with a plastic loop from the rectum (Fig. 6.0.3). Faecal sample was divided into one tube with PBS (0.3% Tween 20, 5% formal saline), and another tube with 95% or 100% ethanol (for future copro-PCR test), sealed and stored at room temperature during the field work and then -20ºC in the laboratory later.

Fig. 6.0.3: Left: rapid DIGFA test for human serum in the field; Right: dog faecal sampling by loops.

6.0.2.4. Data analysis

Data analysis was used to evaluate the immunodiagnositic tools and determine risk factors in epidemiological studies on echinococcosis.

Chi-square tests were used to determine the distributions of CE or AE in different locations/ethnic groups/ages/occupations.

Univariate odds ratios were used to determine independent associations of risk factors in different areas and different variables (including dog owner, livestock owner, home slaughter, occupation, education, hygienic habits, etc.)

Multivariate logistic regression was used to find adjusted odds rations to assess the relationship of diseases with different risk factors in different ethnic groups, different areas and find the key control points.

All analyses performed using SAS 9.0, SPSS 16.0 and Epi info version 6.0.

Statistical significance was set at alpha=0.05.

6.1 Community study in Wenquan County, Boertala Mongol Autonomous Prefecture, Xinjiang

6.1.1 Introduction to study site

Wenquan County is located within the west of Boertala Mongol Autonomous Prefecture, west of Xinjiang Uygur Autonomous Region, P.R.China, at longitude 81º02'- 81º08'E and latitude 44º51'- 44º57'N, elevation is 1030-1250 metre above sea level (Fig 6.1.1). It contains an area of 5,900 km^2. According to the 2002 census, it has a population of 70,000.

Wenquan County was reported as a high endemic area for CE which showed 185 CE cases (191.8 cases per 100,000 populations) during 1981-1990 (National Hydatid Disease Center of China, Menghebat et al., 1993). Sheep infection with echinococcosis was 84.6% in Wenquan County (Li et al., 2005).

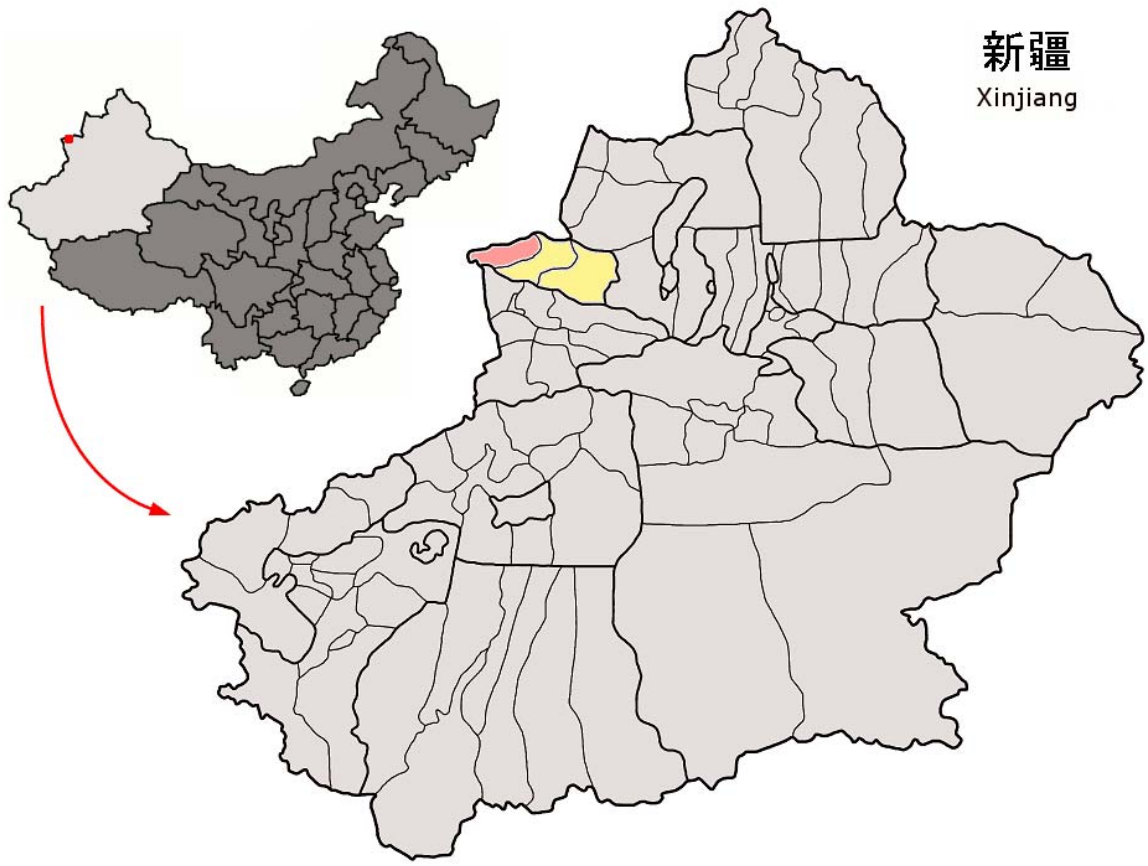

Fig. 6.1.1: Wenquan County in Boertala Mongol Autonomous Prefecture, Xinjiang Uygur Autonomous Region, P. R. China

A community study on echinococcosis was carried out in 2005 in Angelige Commune of Wenquan County, 16 Km from Wenquan County Center (Fig. 6.1.2). Angelige Commune is a semi- agriculture and semi-pastoral area, covered 617 Km^2 of which 120000 acres pasture and 12850 acres agriculture land. The population of 10618 consisted of 3204 households in 16 villages, comprising Mongolian, Han, Uygur, Kazakh, Hui and other 9 ethnic groups. The dominant ethnic group was Mongolian, Kazak and Han.

Fig. 6.1.2: Community study site- Angelige Commune, for human echinococcosis in Wenquan County（44° 59′ 0″ N, 81° 4′ 0″ E; 5663.7 km^2; 1:60,000）

6.1.2 Results

6.1.2.1 Mass screening

In this study 1293 residents (12.2% of the commune population) were screened in four agriculture villages (Bureletunge, Erantuoergan, Tuoli and Angeligebuhu) and two livestock husbandry villages (Benbutu and Tuosihuertu). Each person was registered (plus questionnaire), screened by abdominal ultrasound and serological test with vein blood. The screened people included 604 males and 688 females, with ages from 12 to 81 years (average 36 years), and ethnic groups comprised by Mongolian 189 (14.7%), Kazakh 175, Hui 142, Han 734, Uygur 47 and other ethnics 5. Their occupations were farmers 697, herdsmen 170, and students 330, others 95 (cadres, businessmen, etc.).

6.1.2.2 Ultrasound and Serological prevalences of human echinococcosis

Ultrasonographic scan showed 13 active cystic echinococcosis (CE) cases (1% prevalence in 1292) including 6 that had a previous surgical history. There were another 11 individuals with a previous surgical history of CE but with no recurrence sign on ultrasound scan. No human AE cases were found in this survey. Echinococcus antibodies serological test using DIGFA showed a 15.3% (197/1288) seropositive rate in total. All 24 (1.86%) CE cases were analyzed for distribution and risk factors.

i) Prevalence by gender, ethnicity and occupations. (Table 6.1.1)

More female CE cases (15/24, 2.18%) were found by ultrasound in this study, even no statistical difference in genders ($p>0.05$). But females showed higher seropositives (17.4%) than males (13%) ($p<0.05$)..

The main ethnic groups had a similar ultrasound prevalence of CE from 1.1% to 3.4% ($p>0.05$). And Han and Mongolian ethnic groups had higher seropositives than other ethnic groups ($p<0.05$).

Occupation as farmers or herdsmen, dog or livestock owners appeared as the main risk factors for human ultrasound CE in this study ($p<0.05$). There were also significant differences in seroprevalence between occupation groups ($p<0.05$).

Table 6.1.1: Human ultrasound CE prevalence and seropositivity by gender, ethnic group and occupation in Wenquan County (Xinjiang)

		Ultrasound			Chi-Square	*p*	Serology			Chi-Square	*p*
		No.	CE	CE%			No.	Positives	P%		
Gender	**Male**	604	9	1.5%			602	78	13.0		
	Female	688	15	2.52			686	119	17.4		
	Total	1292	24	1.9%	0.84	0.36	1288	197	15.3	4.770	0.029
Ethnicity	**Han**	734	8	1.1%			730	127	17.4		
	Hui	142	4	2.8%			142	18	12.7		
	Mongolian	189	5	2.7%			189	32	16.9		
	Uygur	47	1	2.1%			47	5	10.6		
	Kazak	175	6	3.4%			175	15	8.6		
	Others	5	0	0			5	0	0		
	Total	1292	24	1.9%	0.00	0.14	1288	197	15.3	11.034	0.026
Occupation	**Farmer**	697	18	2.6%			695	96	13.8		
	Herdsman	170	4	2.4%			170	18	10.6		
	Student	330	1	0.3%			329	64	19.5		
	Others	95	1	1.1%			94	19	20.2		
	Total	1292	24	1.9%	0.00	0.04	1288	197	15.3	10.230	0.017

ii) Prevalence by age and locations.

Human ultrasound CE prevalence (2.03%-3.6%) in the 20-50 years people were higher than other age groups (P<0.05). Seropositivities was observed increase in the over 20 year group (p<0.05) (Table 6.1.2).

Agriculture village residents had a similar ultrasound CE prevalence compare to residents in livestock husbandry villages (p>0.05). Seroprevalences were different with ultrasound prevalence at village level and there were differences between all villages (p<0.05). (Tables 6.1.3).

Table 6.1.2: Human ultrasound CE prevalence and serological positives by DIGFA in different age groups in Wenquan County (Xinjiang)

Age group	Ultrasound			Chi ^{2}	*p*	DIGFA serology			Chi ^{2}	*p*
	No	CE	Prevalence			N	Positives	P（%）		
＜**20**	347	1	0.29%			346	65	18.8		
20-	148	3	2.03%			148	15	10.1		
30-	250	9	3.6%			249	30	12.2		
40-	221	5	2.26%			220	28	12.7		
50-	165	2	1.21%			164	27	16.5		
60-	113	2	1.77%			113	21	18.6		
70-	48	2	4.17%			48	11	22.9		
Total	1292	24	1.86%	0.000	0.034	1288	197	15.3	12.711	0.048

Table 6.1.3: Human CE prevalence and seropositivity in different villages in Wenquan County (Xinjiang)

Villages	Ultrasound			Chi ^{2}	*P*	DIGFA serology			Chi ^{2}	*P*
	No.	CE	Prevalence			No	Positives	P (%)		
Bureletunge	195	1	0.51%			193	25	12.95		
Eranhaergan	245	7	2.86%			245	50	20.41		
Tuoli	201	6	2.98%			201	18	8.96		
Angeligebuhu	270	2	0.74%			270	46	17.04		
Benbutu	57	1	1.75%			57	5	8.77		
Tuosihuertu	128	5	3.91%			128	16	12.50		
Others	112	2	1.79%			111	21	18.92		
Town	84	0	0			83	16	19.28		
Total	1292	24	1.86%	0.000	0.107	1288	197	15.30	17.414	0.015

6.1.2.3. Analysis of risk factors for human CE in Wenquan County

From all the questionnaires, 69.4% (896/1292) of people raised livestock, and 57.8% (747/1292) owned dogs in which 32.8% (245/1292) owned dogs more than 5 yrs. Both females 368 (49.4%) and males 197 (26.4%), and both 181 (24.2%)

reported close contact with dogs. There were 597 (79.9%) people said they fed animal offal to dogs, and 135 (18.1%) people reported that they applied dog faeces to fertilize their gardens. A total of 135 (18.1%) people reported that they used dogs for livestock herds; 617 (47.8%) people raised dogs and livestock at the same time; and 816 (63.2%) people said they had the habit to slaughter livestock at home. Only 295 (22.8%) people thought they knew something about echinococcosis. People who owned dogs had a higher CE prevalence than those without dogs ($p<0.05$) and people who owned livestock showed the same difference ($p<0.05$) (Table 6.1.4)

Table 6.1.4: Relationship between livestock and dog ownership with risk of human CE in Wenquan County

Ownership	CE	Non CE	Total	Ultrasound Prevalence	Chi	*p*
Dog						
Yes	19	728	747	2.54%		
No	5	540	545	0.92%		
Total	24	1268	1292	1.86%	0.0165	0.0366
Livestock						
Yes	22	874	896	2.46%		
No	2	394	396	0.51%		
Total	24	1268	1292	1.86%	0.0079	0.0137

Multivariate analysis of risk factors of human CE was carried out for three variables including ethnic, occupation and dog ownership (Table 6.1.5).

Table 6.1.5: Multivariate analysis of risk factors of human CE in Wenquan County (Analysis of Maximum Likelihood Estimates)

Variable	Parameter					
	DF	Estimate	Standard Error	Chi-Square	Pr > ChiSq	Hazard Ratio
Ethnic	1	-0.80443	0.45509	3.1246	0.0771	0.447
Occupation	1	0.64717	0.51837	1.5587	0.2119	1.910
Dog	1	1.04214	0.51186	4.1452	0.0418	2.835

Analysis of Maximum Likelihood Estimates

Parameter	DF	Estimate	Standard Error	Wald Chi-Square	Pr > ChiSq
Intercept	1	-4.6775	0.3835	148.7668	<.0001
Dog owner	1	1.0605	0.5071	4.3731	0.0365
Occupation	1	-0.6076	0.2527	5.7835	0.0162

ln(P/1-P)=-4.6775+1.0605x1-0.6076x2;

OR＝dog owner/non dog owner＝exp (1.0605) =2.887, and OR for occupation (farmer & herdsman/others) was 1.837. These 2 factors could be the main risk factor for local human echinococcosis.

6.1.3 Discussion (Wenquan County, Xinjiang)

Wenquan County has been known an endemic area of CE from 1950s. Hospital records showed 185 CE cases (19.18 per 100,000 pop in 1990) in Wenquan County from 1951-1990 (National Hydatid Disease Center of China, 1992), with had 67 cases of CE were reported during 1987-1989 (Wu, 1994). An ultrasound survey of students in Wenquan County indicated a prevalence of 0.4% (22/5550) in 2003, which was similar to our study (0.3%, 1/330). Echinococcosis in livestock and dogs were very high in Wenquan County, i.e. 71.5% (1492/2083) in sheep and 54.8% (17/31) in dogs (Dang et al., 1997).

Although the Xinjiang provincial authorities had applied a control programme for echinococcosis since late 1980s, human CE cases still occurred in western Xinjiang. The total prevalence of confirmed human CE (new and existing cases) in Angelige Commune of Wenquan County, in Boertala Mongolian Autonomous

Prefecture (western Xinjiang, China), was 1.86% (24/1292) in the current study. No alveolar echinococcosis (AE) cases were found during this study but a case report of AE existed (from Prefecture Hospital records). The youngest CE case in the current study was 16 yrs old and the average age of all 24 CE cases was 41.3 yrs. A total of 13 new cases were detected. New young case meant the transmission of *echinococcus granulosus* was still active in this area.

Human CE prevalence usually resulting from the presence of an active dog-sheep cycle, close contact with dogs and livestock, and human behavior, which contribute to main risk factors for human CE infection (Craig et al., 1994, 2000, 2004, 2007, Eckert et al., 2000; WHO-OIE, 2001; McManus et al., 2003; Schantz et al., 2003; Budke, 2006, Yang et al., 2006). Occupations as farmers or herdsmen, and the dog or livestock ownership were the main risk factors for human CE in Wenquan County. Farmers had similar prevalence with herdsmen since they also raised livestock and dogs, as well as Herdsmen. The same reasoning applies for different ethnic groups because Han were engaged with livestock husbandry as well as Mongolian and Kazakh groups. Local people's lifestyle around dogs and livestock might be the main reason for active *E. granulosus* life cycle.

Dogs were considered very important for most rural people in Xinjiang. Muslim people did not eat dog meat and Mongolians were forbidden to kill dogs under Buddhism beliefs. Eggs in dog faeces could cause contamination because of faeces in public areas or from faeces fertilization of gardens. Poor hygiences such as non-washed hands after touching dogs or poor contaminated surroundings possibly also contribute to infection. Home slaughter was very common in Wenquan with 79.9% of persons reported to give infected offal to their dogs. In addition, poor knowledge of CE (<25%) might have also contributed to the transmission likelihood over many years.

From above observation, CE infection in humans and an active dog-sheep cycle appeared to be ongoing in Wenquan County. Progress in the National Echinococcosis Control Programme had been carried in northwest China since 2007, based on dog treatment (with praziquantel) and health education in selected endemic areas. Further follow-up after the control programme should be assessed through dog and human prevalence and disease burden.

6.2 Community study in Bayinbuluke Town, Hejing County, Bayinguoleng Mongol Autonomous Prefecture, Xinjiang

6.2.1 Introduction to study site

Bayinbuluke (means 'abundant spring' in Mongolian) Grassland, located at longitude E82°27’-86°17’and latitude N42°18’-43°34’, is a main pasture area (the second largest grassland in China) in Hejing County, Bayinguoleng Mongol Autonomous Prefecture (Fig. 6.2.1). The well-known Swan Lake is located in the prairie. In fact it has many small lakes and is the only nature reserve for swans in China. Bayinbuluke is in northwest of Hejing County and Yanqi Basin, southeastern Yili Valley, south side of Tianshan mountain, with mean elevation 2390-2500 meters (4000-5000 meters for mountains around), and covering 23,868 km2 area. Local populations was 14,310, including 9945 herdsmen and 95% of them were Mongolian. Main livestock were cattle, yak, horses, sheep and camels (760,000 totals).

Bayinguoleng Mongol Autonomous Prefecture was recorded as a high endemic area for cystic echinococcosis which had 1412 hospital CE cases were reported during 1950-1990 (National Hydatid Disease Center of China, by Menghebat et al., 1993). Hejing County was one of counties with high endemic for human CE in Bayinguoleng Mongol AP which showed 334 CE cases and 120.3 cases per 100,000 populations during 1981-1990. A serological and ultrasound survey in other communes/towns showed 1.18% (21/1785) human CE prevalence (Gu et al., 1991). A pilot study by ultrasound indicated 6.6% (20/300) human echinococcosis (unpublished data, personal communicate) in Bayinbuluke Town. And a case of human AE was reported in Hejing County (Zhang et al., 2000).

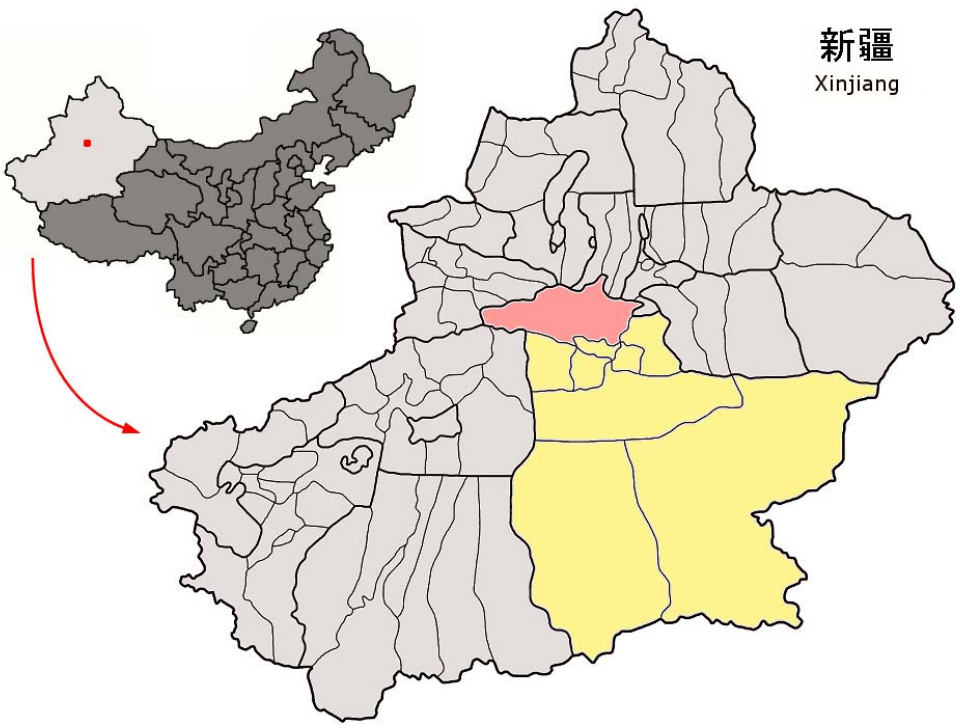

Fig. 6.2.1: Hejing County in Bayinguoleng Mongol Autonomous Prefecture, Xinjiang Uygur Autonomous Region, P.R.China

Fig. 6.2.2: Community study sites in Bayinbuluke Area of Hejing County, Xinjiang, P. R. China （1：1, 550,000）

A community study for echinoccosis by ultrasound and serology was carried on in 2 communes and one pasture of Bayinbuluke Town in 2004 (Fig. 6.2.2). In total 1398 people were screened including 597 (42.7%) males and 801 (57.3%) females; age from 7 to 82 years old (average 29 years); ethnic groups included Mongolian 1377 (98.5%), Uygur 6 (0.43%) and Han 15 (1.07%); occupations included herdsmen 826 (59.1%), farmers 16 (1.14%), cadres 129 (9.2%), students 407 (29.1%) and others 20 (1.43%). And 30 dogs from Bayinbuluke were checked by autopsy.

6.2.2 Results

6.2.2.1 Prevalence of CE and AE by ultrasound in human

A total of 60 CE (including 1 case combined with a confirmed CE cyst and a query AE lesion), 3 AE cases were detected by ultrasound. There were 18 of 45 individuals with a previous history of surgery for CE that they showed normal images in this study. So the overall prevalence of human CE was 5.6% (78/1398), AE was 0.2% (3/1398) in Bayinbuluke in this survey.

i) Ultrasound prevalence by gender and age.

No significant difference was found between male and female for CE, AE or both ($p>0.05$). CE cases occurred in age range 7-68 yrs old with mean age 34.3 yrs and AE cases from 26-82 yrs old with average 45.3 yrs. Differences among age classes showed > 10 yrs had higher prevalence for both CE and AE ($p<0.05$) (Table 6.2.1).

Table 6.2.1: Human echinococcosis prevalence by genders and ages in Bayinbuluke Town

Ages	Male			Female			Total		
(-ys)	No	CE (%)	AE (%)	No	CE (%)	AE (%)	No	CE (%)	AE (%)
<10	27	0(0)	0	26	1(3.9)	0	53	1(1.9)	0
11~20	198	11(5. 6)	0	226	4(1.8)	0	424	15(3.5)	0
21-30	146	13(8.9)	0	220	12(5.5)	2(0.9)	366	25(6.8)	2(0.5%)
31-40	131	10(7.6)	0	159	5(3.1)	0	290	15(5.2)	0
41-50	62	3(4.8)	0	94	3(3.2)	1(1.1)	156	6(3.8)	1(0.6)
51-60	22	2(9.1)	0	51	9(17.6)	0	73	11(15.1)	0
>60	11	2(18.2)	0	25	4(16)	0	36	6(16.7)	0
Total	597	41(6.9)	0	801	38(4.7)	3(0.4)	1398	79(5.7)	3(0.2)

For gender analysis of CE: Pearson Chi-Square Value2.893 Asymp. Sig. (2-sided) 0.089
For gender analysis of AE, Pearson Chi-Square Value2.241 Asymp. Sig. (2-sided) 0.134
Fisher's Exact Test Exact Sig. (1-sided) 0.188
For gender analysis of both CE&AE, Pearson Chi-Square Value1.895 Asymp. Sig. (2-sided) 0.169
For Ages analysis: Fisher's Exact Test, Table Probability (P) 1.112E-06, Pr <= P 0.0341

ii) Ultrasound prevalence by ethnicity.

No significant differences were found between different ethnic groups (p>0.05) for both CE and AE in Bayinbuluke (Table 6.2.2).

Table 6.2.2: Ultrasound CE and AE prevalence by ethnic group in Bayinbuluke Town

Ethnic groups	No.	CE (%)	AE (%)
Mongolian	1377	78(5.7)	3 (0.2%)
Uygur	6	1(16.67)	0
Han	15	0(0)	0
Total	1398	79(5.7)	3 (0.2%)

Fisher's Exact Test: Table Probability Pr <= P 0.3419

iii) Ultrasound prevalence by occupation.

Most people were involved with livestock husbandry and farming was very limited. Herdsmen had a higher prevalence than other occupations (p<0.01) (Table 6.2.3).

Table 6.2.3: Ultrasound CE and AE prevalence by occupation in Bayinbuluke Town

Occupations	No.	CE (%)	AE (%)
Herdsman	826	63(7.6)	3(0.4)
Farmer	16	0	0
Cadre	129	1(0.8)	0
Student	407	14(3.4)	0
Others	20	1(5)	0
Total	1398	78(5.6)	3 (0.2)

Fisher's Exact Test for CE: Table Probability (P) 1.032E-06 Pr <= P 5.516E-04
For both CE and AETable Probability (P) 9.159E-07, Pr <= P 3.945E-04

6.2.2.2 Serological prevalence by DIGFA

Serological assay with DIGFA showed 24.54% positive (293/1194) among people who had the vein blood test. Female showed the higher positive rate (29.2) than male ($p<0.01$) (Table 6.2.4 and Fig. 6.2.3).

Table 6.2.4: Serum screening by DIGFA in Bayinbuluke

Ages	Male			Female			Total		
	No	Positives (%)		Screened	Positives (%)		No	Positives (%)	
≤10	16	2	12.5	17	3	17.7	33	5	15.15
11-	156	17	10.9	186	19	10.2	342	36	10.53
21-	121	23	19.01	199	68	34.2	320	91	28.44
31-	111	23	20.72	143	57	39.9	254	80	31.5
41-	55	16	29.09	86	29	33.7	141	45	31.91
51-	21	3	14.29	49	21	42.9	70	25	35.71
>60	11	3	27.27	23	8	34.8	34	11	32.35
Total	491	87	17.72	703	205	29.2	1194	293	24.54

Chi-Square Tests for gender Pearson Chi-Square Value20.485 Asymp. Sig. (2-sided) 0.000
Chi-Square Tests for ages, Pearson Chi-Square Value57.085 Asymp. Sig. (2-sided) 0.000

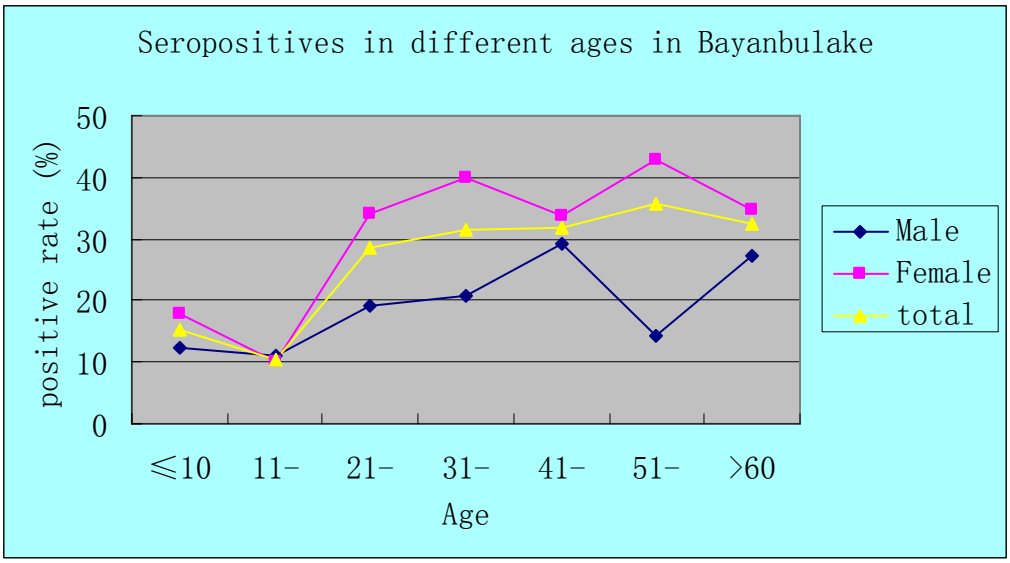

Fig. 6.2.3: Seropositives by gender and age groups in Bayinbuluke (n=1194).

6.2.2.3 Risk factors for human CE

People being a dog owner had a higher ultrasound CE prevalence (6.1%, 76/1245) than those who did not have dogs (2%, 3/153) ($p<0.05$) (Table 6.2.5). The same situation occurred in people who had livestock around. Persons with livestock ownership had a higher CE prevalence (5.7%, 78/1369) than those without livestock (3.5%, 1/29), but no statistical difference was found ($p>0.05$) (Table 6.2.5).

Table 6.2.5: Dog/Livestock ownership and human hydatid disease (CE, AE).

Ultrasound	No. (%)	Dog ownership				Livestock ownership			
		Yes	No	Chi	*p*	Yes	No	Chi	*p*
CE	79 (5.7%)	76 (6.1%)	3 (2.0%)	0.01	0.04	78 (5.7%)	1 (3.5%)	0.31	1.00
AE	3 (0.2%)	2 (0.2%)	1 (0.6)	0.26	0.29	3 (0.2%)	0	0.32	1.00
Non HD	1316 (94.1%)	1167 (93.7%)	149 (97.4%)			1288	28		
Total	1398	1245	153			1369	29		

For hydatid cased including CE and AE, Chi value was 0.0274 and P was 0.0697.

The varieties, which included gender, age, occupation, ethnic groups, dog owner, livestock owner, wildlife contact and home slaughter, were selected for risk factor analysis and valuated as Table 6.2.6. Others in ethnic group included Uygur ethnic (n=6) and Han ethnic (n=15); in occupation group included farmers (n=16), cadres (n=129) and others such as businessmen, housewives (n=20).

Table 6.2.6: Analysis variables and valuation for hydatid disease in Bayinbuluke

Variables	Valuation
Gender	1＝Male, 2＝Female
Ethnic	1＝Mongolian, 2＝others
Occupation	1＝Herdsmen, 2＝Students, 3＝others
Dog ownership	1＝Yes, 2＝No
Wild life contact	1＝Yes, 2＝No
Livestock ownership	1＝Yes, 2＝No
Home slaughter	1＝Yes, 2＝No

The single factor logistic regression analysis showed that the dog ownership was probably the most import risk factor for human ultrasound CE (OR=2. 298) in Bayinbuluke in the current study (Table 6.2.7). The other relative risk factors might be age, wildlife contact and home slaughter.

Table 6.2.7: CE prevalence and univariate logistic regression analysis for Bayinbuluke

Factor		Prevalence (%)	OR value (95%CI)
Age		5.7（79/1389）	1.028（1.013-1.043）
Gender	Male	6.9（41/595）	0.679（0.431-1.070）
	Female	4.8（38/794）	
Ethnic	Mongolian	5.7（78/1368）	0.827（0.110-6.242）
	Others	4.8（1/21）	
Occupation	Herdsmen	7.5（63/839）	0.442（0.245-0.799）
	Student	3.5（14/404）	0.171（0.41-0.707）
	Others	1.4（2/146）	
Dog ownership	Yes	6.0（75/1242）	2.298（0.828-6.376）
	No	2.7（4/147）	
Wild Life contact	Yes	6.3（45/711）	1.280（0.809-2.024）
	No	5.0（34/678）	
Livestock ownership	Yes	4.7（44/927）	0.608（0.384-0.962）
	No	7.6（35/462）	
Home slaughter	Yes	6.1（44/720）	1.179（0.747-1.862）
	No	5.2（35/669）	

The single factor logistic regression analysis was also used for CE seropositive prevalence and suggested more risk factors involves included age, gender, occupation, dog owner, wildlife contact, livestock and home slaughter (Table 6.2.8).

Table 6.2.8: Univariate associated with CE seropositives for hydatid disease in Bayinbuluke

Factor	Prevalence (%)	OR value (95%CI)
Age	14.2(198/1392)	1.033(1.023-1.044)
Gender		
Male	9.9(59/595)	1.919(1.386-2.657)
Female	17.4(139/797)	
Ethnic		
Mongolian	14.2(195/1371)	1.005(0.293-3.444)
Others	14.3(3/21)	
Occupation		
Herdsmen	19.0(160/842)	1.568(0.940-2.616)
Student	4.7(19/404)	0.330(0.169-0.643)
Others	13.0(19/146)	
Dog ownership		
Yes	14.6(182/1245)	1.402(0.815-2.411)
No	10.9(16/147)	
Wildlife contact		
Yes	17.2(123/714)	1.673(1.229-2.279)
No	11.1(75/678)	
Livestock ownership		
Yes	14.8(138/930)	1.167(0.843-1.617)
No	13.0(60/462)	
Home slaughter		
Yes	16.5(119/722)	1.476(1.087-2.005)
No	11.8(79/670)	

Multivariate logistic regression analysis of CE ultrasound prevalence showed students and wildlife contact were main risk factors in Bayinbuluke (Table 6.2.9). And multivariate logistic regression analysis of seropositives showed female and wildlife contact were risk factors (Table 6.2.10).

Table 6.2.9: Multivariate logistic regression analysis for CE ultrasound prevalence in Bayinbuluke

Variable	B	S. E.	Wald value	P value	OR value (95%CI)
Constant	-4.965	0.819	36.765	0.000	0.007
Age	0.026	0.01	6.893	0.009	1.026（1.007-1.046）
Occupation					
Herdsmen			5.897	0.053	
Students	1.784	0.736	5.871	0.015	5.951（1.406-25.188）
Others	1.753	0.811	4.674	0.031	5.771（1.178-28.273）
Livestock ownership	-2.954	0.781	14.293	0.000	0.052（0.011-0.241）
Wildlife contact	2.628	0.783	11.251	0.001	13.841（2.981-64.266）

Table 6.2.10: Multivariate logistic regression analysis of possible risk factors for CE seropositive in Bayinbuluke

Variable	B	S. E.	Wald value	P value	OR value (95%CI)
Constant	-2.921	0.379	59.427	0.000	0.054
Age	0.013	0.007	3.652	0.056	1.013(1.000-1.026)
Gender	0.586	0.17	11.863	0.001	1.796(1.287-2.507)
Occupation					
Herdsmen			17.813	0.000	
Students	-1.221	0.301	16.494	0.000	0.295(0.164-0.532)
Others	-0.426	0.264	2.614	0.106	0.653(0.390-1.095)
Livestock ownership	-0.859	0.459	3.498	0.061	0.424(0.172-1.042)
Wildlife contact	0.937	0.463	4.097	0.043	2.551(1.030-6.320)

6.2.3 Discussion (Bayinbuluke, Hejing County, Xinjiang)

The Mongolian population in Xinjiang mostly lives in two Mongolian Autonomous Prefectures (Bayinguoleng and Boertala) and one Mongolian Autonomous County (Hoboksaier). But Bayinbuluke in Bayinguoleng AP has the highest proportion of 95% population is Mongolian and this relatively rare since the ethnic group move

widely and commonly (Zhang, 1995). Mongolian lifestyle is more close to Tibetan with communities at higher altitude (2400 meters), Buddhism religion, mainly work as herdsmen. Both CE and AE were all endemic in Tibetan areas such as western Sichuan, north Qinghai and Tibet AR (Qiu et al, 1995, 2000; Wang et al., 2000; Budke et al, 2005; Li et al., 2006, 2010). Human CE prevalence was also higher in Inner Mongolian AR (Zhang et al., 1996; Tao et al, 2011). In Xinjiang, higher CE prevalence in Mongolian communities in Hoboksar has been reported (Wang et al., 2005, 2006; Chu et al., 2007, 2010). We now show that human CE was also very serious in Bayinbuluke. This could be an active area for transmission of *E. granulosus* since younger people (<10 years) were found to have a higher ultrasound prevalence (1.9%) and seropositive rate (15.2%). Poor health education and low socioeconomic level were a limitation for local people to go outside and get medical attention.

The domestic dog as a definitive host of *E. granulosus* appears to be the main risk factor for local people for both CE and AE. We found most dogs were not tied during day and night, and the dogs ran around tents even the open area for cheese dried in the sun (Fig. 6.2.4). Dog faeces could be found anywhere even near the stream used for drinking and washing. Livestock slaughter in the street is common and small cysts in sheep liver are thrown to any nearby dogs (Fig. 6.2.5 and Fig. 6.2.6).

Twenty dogs (66.7%) were found infected with *E. granulosus* after necropsy from 30 dogs in Bayinbuluke. The high prevalence in dogs suggested that the active transmission of *E. granulosus* in Bayinbuluke. People in this area, whatever gender, age, occupation, inevitably lived in an area of high endemically for *E. granulosus*. Unfortunately they did not understand the risk since health education was very limited.

Human AE cases were reported rarely but existed in Hejing County (Hu 1993; Zhang et al., 2000). In the current study 3 AE cases and a suspicious CE and AE mixed infection were found by ultrasound in Bayinbuluke and also a dog was reported to have a mixed infection with *E. granulosus* and *E. multilocularis* (Zhang et al., 2006). This suggested that the transmission of *E. multilocularis* existed in Bayinbuluke and the domestic dog was involved in the life-cycle of *E. multilocularis*. The sylva lifecycle of *E. multilocularis* in Bayinbuluke is probably

similar to the Tianshan Mountains because human AE cases were previously reported in Ili Valley (Zhou et al., 2000; Gao et al, 2005; Dingmulati et al., 2005). The suggestion from this survey, health education around hydatid disease should be given to local people as soon as we can. Local dogs should be registered and treated with praziquentel twice a year. People should prohibit their dogs close to their water source and foods, and do not give a suspicious livestock organ to dogs. These suggestions had been reported to local government and they now have a policy to have parts of herdsmen moving to other area under the high land and training for other occupation such as agriculture, worker, etc.; so that to escape of overgraze, poor education and bad medical concern.

Fig. 6.2.4: A dog close to a sheet with sun-dried cheese (August, Bayinbuluke).

Fig. 6.2.5: Untied dogs in a yard, they came to scavenge after home slaughter (October, Bayinbuluke).

Fig. 6.2.6: A sheep slaughtered in the town center of Bayinbuluke, infected liver was thrown to dogs nearby.

6.3 Community study in Xinyuan County, Yili Kazakh Autonomous Prefecture, Xinjiang

6.3.1 Introduction to study site

Xinyuan County, also called Künes County (43° 27′ 4″ N, 83° 8′ 52″ E), is a county situated within Yili Kazakh Autonomous Prefecture (AP) and located in the east of Ili Valley in the north part of West Tianshan Mountains in Xinjiang (Fig. 6.3.1). It contains an area of 7,581 km^2. According to the 2002 census, it has a population of 290,000 Most Kazakh people in China live in Yili Kazakh AP. Narati Town located in the east end of Xinyuan County, 60 km and local population was 25,986. As one of the world's four largest grasslands, Narati is located in the hinterlands of the Tianshan Mountains, to the east of the Ili River Valley, covers a total area of 400 square kilometers, with an altitude of 1,800 meters. Turgen Commune is 42 km from Narati with 8,490 populations. Surveys for human echinococcosis were planned and carried out during 2003 to 2005 (Fig. 6.3.2).

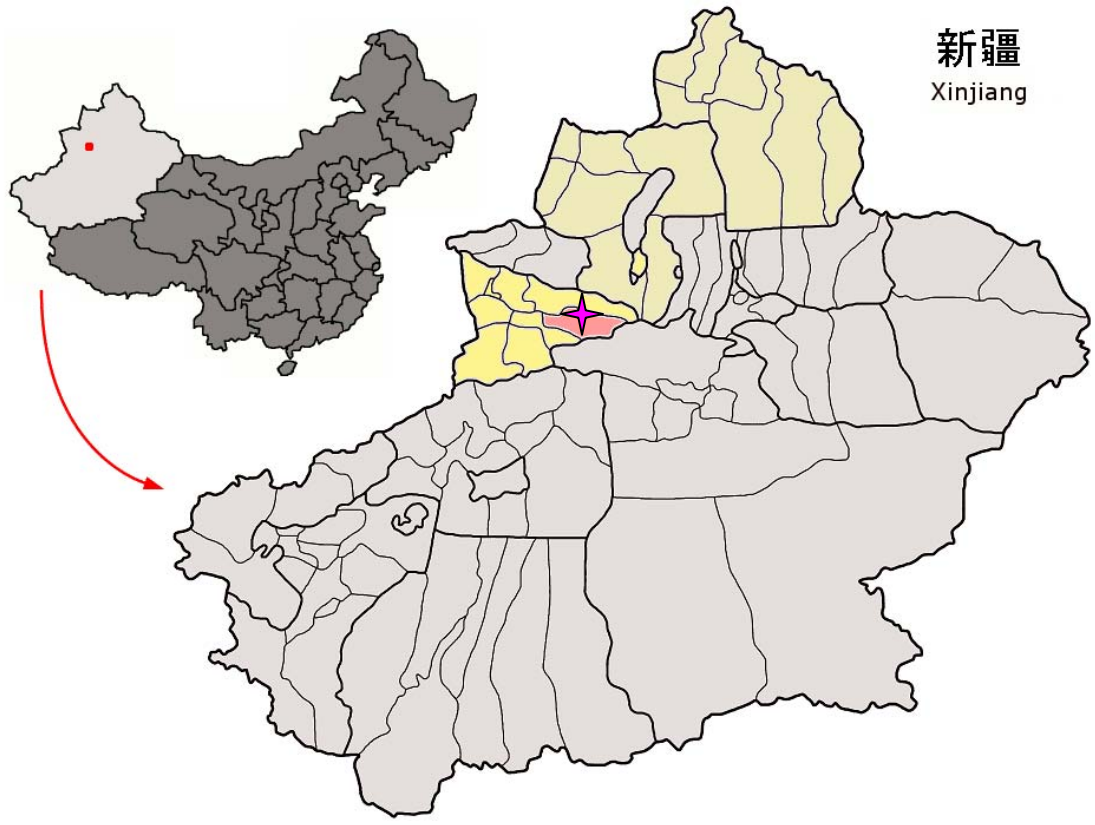

Fig. 6.3.1: Xinyuan County (pink ✦) in Ili Kazakh AP of Xinjiang, China

Fig. 6.3.2: Narati Town and Turgen Commune in Xinyuan County (1:650000).

6.3.2 Results

A total of 3691 residents accepted the questionaire survey, a portable ultrasound check and sera test. This included 1838 persons in Narati Town and 1853 persons in Turgen Commune. The mean age was 33 yrs old with youngest 8 yrs and oldest 90 yrs old. The ratio of male to female was 0.93: 1. In total 7 ethnic groups were screened of which Kazak was 69.06%. The proportion of people whose occupation was herdsman was 39.1% and agricultural peasant was 33.89%.

6.3.2.1 Ultrasound prevalence of human echinococcosis and seropositives in Xinyuan

56 CE and 11 AE cases were diagnosed by abdominal ultrasound which gave 1.52% CE prevalence (56/3691) and 0.3% AE prevalence (11/3691). The youngest CE case was 11 yrs old and oldest was 72 yrs old. Kazakh CE cases were 85.71%, herdsmen and peasants were 85.71%. The CE prevalence by ultrasound was 1.74% in Narati Town and 1.29% in Turgen Commune, which no significant difference was found ($p>0.05$). The human AE prevalence was 0.32% and 0.27% in Narati and Turgen respectively.

A total of 933 people (25.28%) were seropositives by DIGFA The youngest was 11 yrs old and oldest was 86 yr old; and Kazak proportion was 71.81%, herdsmen and peasants were 74.38%. Seropositive rate was 19.15% in Narati Town and 31.33% in Turgen Commune, the later was higher ($p<0.05$).

i.) Ultrasound and seropositive CE prevalence by genders and age

No significant differences was found in different genders on ultrasound prevalence of CE ($p>0.05$) which the CE prevalence was 1.69% in male and 1.36% in female. The seropositive rate was 23.31% in males and 27.11% in females, which was significant higher in females ($p<0.05$) (Table 6.3.1).

There was no difference of CE ultrasound prevalence in different age groups ($P>0.05$), the highest prevalence was 2.56% in 40-50 yrs old group. No obvious difference was found in different age groups ($p>0.05$). Seropositive rate increased with age and the highest was 30.08% in over 60 yrs old group (Table 6.3.1, Fig. 6.3.3, Fig 6.3.4).

Table 6.3.1: Ultrasound and seropositive prevalence of human CE by genders and age in Xinyuan County

Distribution	No.	US CE		χ^2	*P*	Serology		χ^2	*P*
		Positives (%)				Positives (%)			
Gender									
Male	1780	30	(1.69)	0.65	0.42	415	(23.31)	7.02	0.01
Female	1911	26	(1.36)			518	(27.11)		
Ages									
<20	942	9	(0.96)	10.69	0.06	227	(24.10)	8.42	0.14
20～	696	16	(2.30)			168	(24.14)		
30～	917	10	(1.09)			221	(24.10)		
40～	546	14	(2.56)			141	(25.82)		
50～	354	5	(1.41)			105	(29.66)		
60～	236	2	(0.85)			71	(30.08)		
Subtotal	3691	56	(1.52)	—	—	933	(25.28)	—	—

US: ultrasound

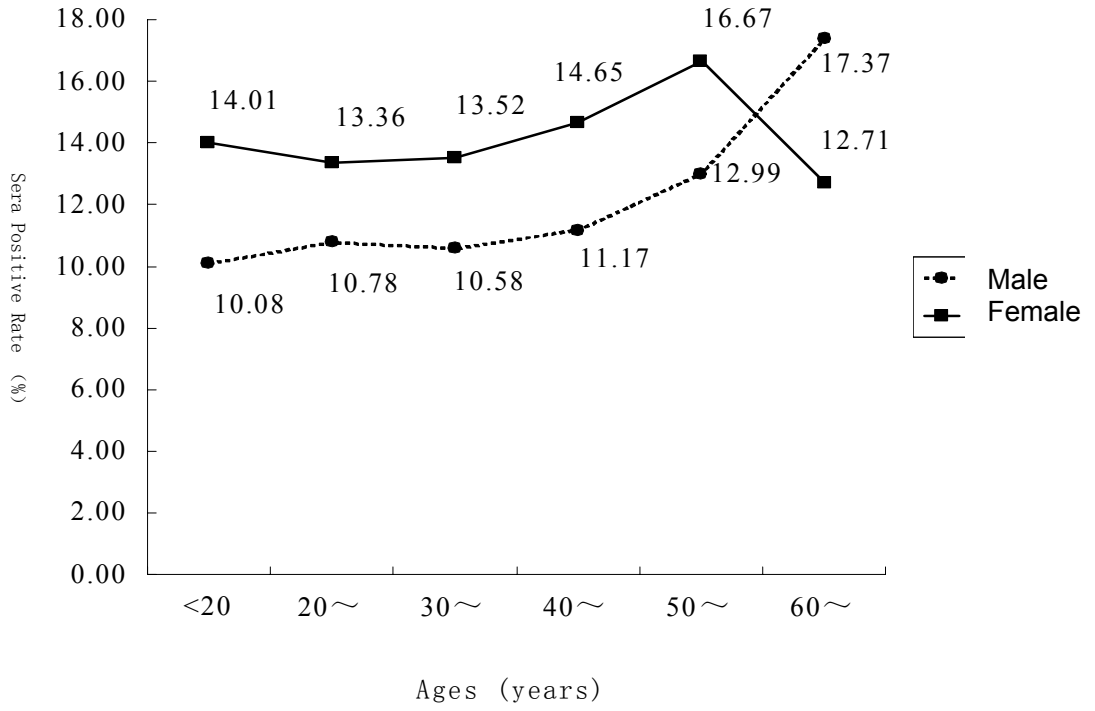

Fig. 6.3.3: Comparison of seropositive rate in different ages and genders in Xinyuan County

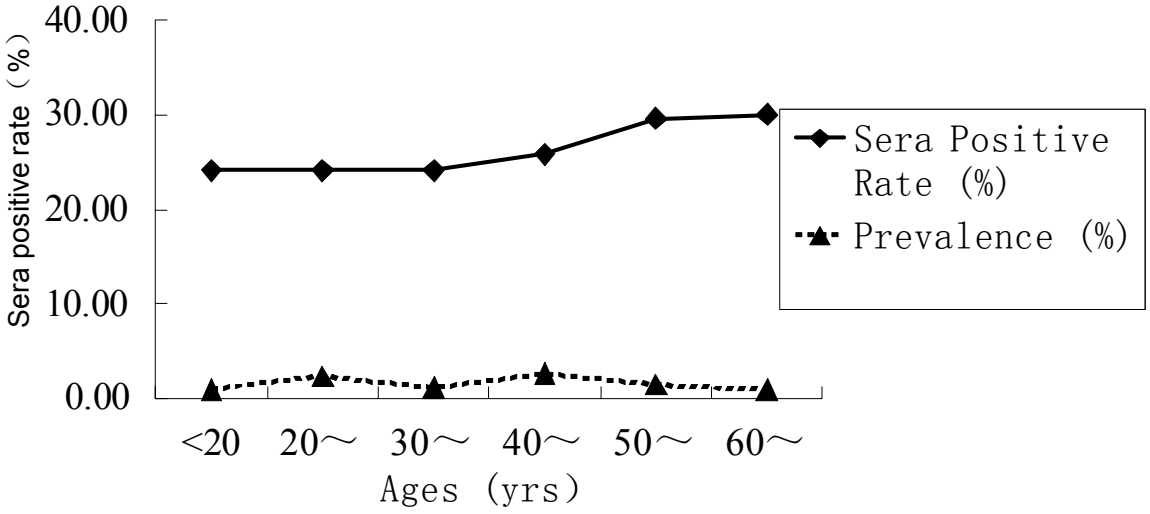

Fig. 6.3.4: Ultrasound and seropositive prevalence for human CE in different ages in Xinyuan County

ii.) Ultrasound CE and seropositive prevalence by ethnic group and occupations

The prevalence of CE by ultrasound was 1.60% in Han, 1.72% in Mongolian, 1.57% in Kazak echnic group and which no difference was found (p>0.05). The seropositive rate was 19.65% in Han, 21.26% in Mongolian, 26.28% in Kazak. Significant difference was found (p<0.05) in seropositives among different ethnic groups (Table 6.3.2).

The highest seropositive rate was 27.60% in farmers and herdsmen, then 25.03% in students, but no statistical difference (p>0.05). Cadres had the highest CE prevalence of 5.45%, and then was herdsmen 1.80%. Statistical difference was showed in this study. The highest CE prevalence by occupation was cadre, herdsmen, students, peasants and others (including worker, businessmen, soldier, housewife and slaughterers) (Table 6.3.2 and Fig. 6.3.5).

Table 6.3.2: Seropositives and prevalence (by US) of human CE in different ethnic groups/ occupations / Townships in Xinyuan

Distribution	No.	Ultrasound		χ^2	*P*	Serology		χ^2	*P*
		CE (%)				Positives (%)			
Ethnic groups									
Han	626	10	1.60	1.06	0.79	123	19.65	17.59	0.00
Mongolian	174	3	1.72			37	21.26		
Kazakh	2549	40	1.57			670	26.28		
Others	342	3	0.88			103	30.12		
Occupations									
Herdsmen	1443	26	1.80	21.73	0.00	352	24.26	5.96	0.20
Farmer	1251	12	0.96			342	27.60		
Students	812	9	1.11			201	25.03		
Cadres	165	9	5.45			33	21.15		
Others	20	0	0.00			5	25.00		
Towns									
Narati	1838	32	1.74	1.23	0.28	352	19.15	72.75	0.00
Turgen	1853	24	1.29			581	31.35		
Subtotal	3691	56	1.52	—	—	933	25.28	—	—

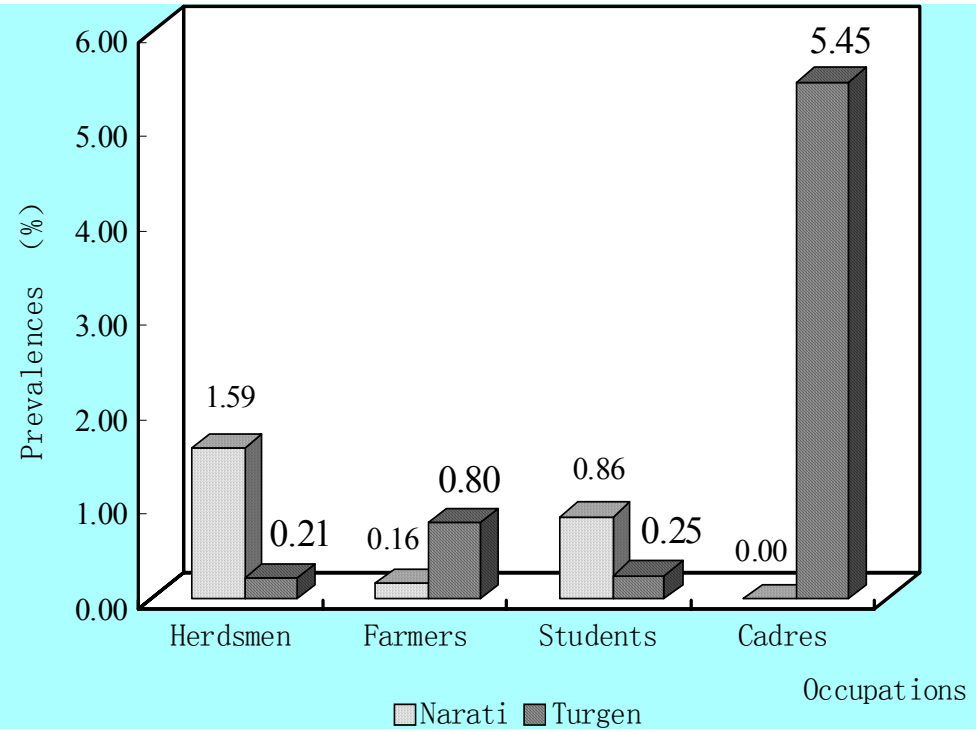

Fig. 6.3.5: The comparison of human CE prevalence by occupations in Narati and Turgen of Xinyuan

iii.) Dog and livestock ownership

The proportion of residents who owned dogs and livestock was higher in Narati Town (76.85%, 1412/1838 for dog and 92.9%, 1707/1838 for livestock) than Turgen Commune (71.2%, 1320/1853 for dog and 67.1%, 1244/1853 for livestock) ($p<0.01$) (Table 6.3.3). The highest proportion of dog ownership was occurred in the Mongolian ethnicity which was 81.61% compared tp 75.56% in Kazakh ($p<0.01$) (Table 6.3.3). Herdsmen and students had higher seropositive prevalence (14.8% and 13.2%) compared to farmers, cadres and other occupations in Narati Town. Meanwhile the highest seropostive prevalence occurred in farmers (25%) and other occupations (businessmen, housewife, etc.) in Turgen Commune (Fig. 6.3.6).

Table 6.3.3: Dogs and livestock owning by different ethnic residents in Narati and Turgen in Xinyuan

	No.	People with dogs/livestock ownership（%）			
		Dogs ownership	Livestock ownership	Both owner	Non-owner
Town					
Narati	1838	1412(76.82)	1707(92.87)	1381(75.14)	135(7.34)
Turgen	1853	1320(71.24)	1642(88.61)	1244(67.13)	100(5.40)
Chi		14.77	19.90		
p		0.000	0.000		
Ethnic groups					
Han	626	419(66.93)	527(84.19)	390(62.30)	60(9.58)
Mongolian	174	142(81.61)	159(91.38)	141(81.03)	11(6.32)
Kazakh	2549	1926(75.56)	2346(92.04)	1885(73.95)	147(5.77)
Others	342	245(71.64)	317(92.69)	242(70.76)	17(4.97)
Chi		25.71	38.72		
p		0.000	0.000		
Total	3691	2732(74.02)	3349(90.73)	2625(71.12)	235(6.37)

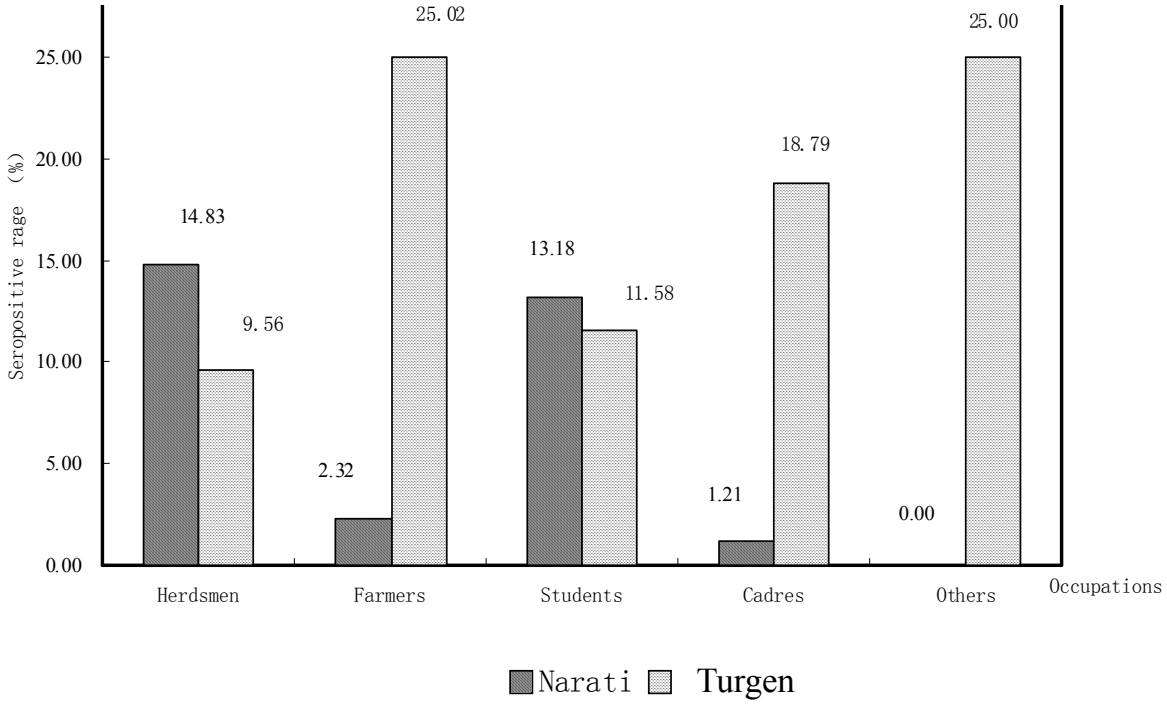

Fig. 6.3.6: The comparison of seropositives for human CE by occupation in Narati and Turgen of Xinyuan

6.3.2.2 Risk factors for human CE in Xinyuan County

i) Risk factors and their evaluation

In this study, dependant variable for risk factors of human CE infection was designated by seropositives, and independant variable was risk factors. Positive and negative were assigned as 1 and 0 respectively. The assignment of risk factors is shown in Table 6.3.4, Univariate and multivariate logistic regressive analysis was calculated (Table 6.3.5).

Table 6.3.4: Assignment of risk factors for human CE in this study

Variable	Assignments
Towns	0=Narati Town，1= Turgen Town
Genders	0=Male，1=Female
Ethnic groups	0=Others，1=Han，2=Mongolian，3=Kazakh
Occupations	0=Others，1=Herdsmen，2=Farmers，3=Students，4=Cadres
Dogs owner	0=No，1=Yes
Livestock owner	0=No，1=Yes
Home slaughter	0=No，1=Yes

Analysis of Univariable Logistic regression of risk factors related with human CE, which showed four risk factors had statistical differences ($p<0.05$) (Table 6.3.5). These risk factors were areas, genders, ages and ethnic groups (Regression coefficient>0, OR>1).

Table 6.3.5: Analysis of Univariate Logistic regression of risk factors related with human CE

Variables	Regression coefficient	Standard Error	χ^2	*P*	*OR*	95% confidence interval of OR
Township	0.66	0.08	71.61	0.00	1.93	1.66-2.25
Genders	0.20	0.08	7.00	0.01	1.22	1.05-1.42
Ages	0.01	0.00	6.57	0.01	1.01	1.00-1.01
Ethnic groups	—	—	17.44	0.00	—	—
Han	0.10	0.21	0.22	0.64	1.10	0.73-1.67
Mongolian	0.38	0.11	11.72	0.00	1.46	1.18-1.81
Kazakh	-0.19	0.13	13.37	0.00	1.76	1.30-2.39
Occupations	—	—	5.94	0.20	—	—
Herdsmen	-0.03	0.52	0.00	0.95	0.97	0.35-2.68
Farmers	0.12	0.52	0.05	0.82	1.13	0.41-3.13
Students	-0.01	0.52	0.00	0.98	0.99	0.35-2.75
Cadres	-0.29	0.55	0.27	0.60	0.75	0.25-2.21
Dog owners	0.03	0.09	0.10	0.76	1.03	0.87-1.22
Livestock owners	-0.06	0.13	0.20	0.65	0.94	0.73-1.22
Home slaughers	0.05	0.21	0.06	0.81	1.05	0.70-1.57

The logistic regression analysis of the multiple factors for risk factors related with human CE showed that five variable which came into the multiple factors regressive model. In this model, different areas, genders, ages, ethnic and occupation were risk factors (Table 6.3.6).

Table 6.3.6: The multivariate logistic regression analysis for risk factors related with human CE

Variables	Regression coefficient	Standard Error	χ^2	*P*	*OR*	95% CI[a] of OR
Township	0.79	0.11	54.24	0.00	2.20	1.78-2.71
Genders	0.20	0.08	6.11	0.01	1.22	1.04-1.42
Ages	0.01	0.00	8.74	0.00	1.01	1.00-1.02
Ethnic groups	—	—	9.63	0.02	—	—
Han	-0.20	0.22	0.84	0.36	0.82	0.53-1.26
Mongolian	0.05	0.12	0.20	0.66	1.06	0.83-1.35
Kazakh	0.39	0.16	5.91	0.02	1.48	1.08-2.03
Occupations	—	—	18.07	0.00	—	—
Herdsmen	0.55	0.54	1.07	0.30	1.74	0.61-4.97
Farmers	0.28	0.53	0.28	0.59	1.33	0.47-3.75
Students	0.67	0.54	1.54	0.21	1.96	0.68-5.69
Cadres	-0.13	0.56	0.06	0.81	0.88	0.29-2.62
Dog owners	0.05	0.10	0.27	0.60	1.05	0.87-1.28
Livestock owners	-0.08	0.14	0.34	0.56	0.92	0.69-1.22
Home slaughers	-0.02	0.22	0.01	0.93	0.98	0.64-1.51

[a]. CI means confidence interval.

6.3.3 Discussion (Xinyuan, Xinjiang)

Xinyuan County located between N 43°03′ to 43°41′ and E82°28′ to 84°56, 196km from Yining City, 495km from Urumqi. It is geographically surrounded by mountains in east, north and south, with the west opened as Yili Valley crossing the border to Kazakstan. Its altitude above sea level is between 792 to 4261 meters where east part is higher and west part is lower. The climate in Xinyuan belongs to continental semi-drought area, so it is easy to be affected by humid airstream from west to east and become the character of relatively warm winter and cool summer in Xinyuan. Annual amount of precipitation is 270 to 800 mm, fewer flatlands and more mountain areas, and difference between east and west is higher than it between north and south. Annual sun period is 2400 to 2700

hours, from a total of 8144 km^2 of Xinyuan, 470 km^2 is natural pasture and 535 km^2 agriculture land. Productive of grass had good quantities and the number of livestock was in the second position in county level in Xinjiang with average population 1 million annually (http://www.xinyuan.gov.cn).

The ultrasound and seropositives prevalence of human CE in Xinyuan County which was presented here, was close to Gongliu County, Yining County and Nileke County where also belongs to Yili Valley region. Those counties had similar geographical characters, which show highland pasture surrounded by forest; and with ethnic Kazak population, similar life styles and business structure. In addition, from hospital records we could find human CE cases had increased during last 50 years (Gao et al., 2005; Dingmulati et al., 2005). So that we could suggest that the transmission of *E. granulosus* is still in an active life cycle and was not well controlled.

A total of 1.52% ultrasound CE prevalence (56/3691), 0.3% AE prevalence (11/3691) and 25.28% seropositives by DIGFA were reported in the current study. 85.71% CE cases were Kazakh, herdsmen and peasants. Seropositive rate was 19.15% in Narati Town and 31.33% in Turgen Commune, the later was higher ($p<0.05$). No significant differences were found on ultrasound prevalence of CE between males and females. However the seropositive rate in males (23.31%) was significant higher than in females (27.11%) ($p<0.05$). There was no difference of ultrasound CE prevalence and seropositive rate in different age groups ($p>0.05$). Significant difference was found in seropositives among different ethnic groups with the highest 26.28% in Kazak ($p<0.05$). Herdsmen and students had higher seropositive prevalence (14.8% and 13.2%) compared to farmers, cadres and other occupations in Narati Town. Meanwhile the highest seropostive prevalence occurred in farmers (25%) and other occupations (businessmen, housewife, etc.) in Turgen Commune.

The proportion of residents who owned dogs and livestock was higher in Narati Town than Turgen Commune ($p<0.01$). The highest proportion of dog ownership was occurred in the Mongolian ethnicity which was 81.61% compared tp 75.56% in Kazakh ($p<0.01$). Different areas, genders, ages, ethnic and occupation were risk factors for human CE in current study.

This high CE endemic status might due to poor veferinary public health. The check and treatment for dogs seemed not to work and no dogs were registered

officially. As the most important definite host of *E. granulosus*, dogs undoubtedly became the main risk factor and other factors such as home slaughter, livestock and poor hygiene habits etc. were also related to dog infection and transmission. Traditional husbandry was practiced by most people and they relied on dogs for herding livestock and guarding their yards. Generally people did not have the dog tied all of the time. The local climate of wet, cold weather appears suitable for *Echinococcus* eggs surviving in pasture or around the village. Occupations which were more close to dogs had more chance to be infected, but farmers were herding livestock as well in many villages and generally they had same risk of CE as herdsmen. Even mostly Kazakh populations in Xinyuan, but other ethnic groups such as Han, Hui, Uygur, had a similar chance to be infected since they had same life-style and habits.

Human AE was relatively sporadic in Xinjiang compared to CE. Higher prevalence (3.9/100,000) was observed in Altai Mountain (Zhou et al., 2000). 79 cases of human AE in 4486 hydatid disease records during 1957 and 1997 were treated in Xinjiang Medical University Hospital (Qiu et al., 1999). A total of 84 hospital AE cases were recorded between 1993-2003 in Ili valley (Gao et al., 2005) and 0.636% (13/2044) human AE prevalence were observed in a community survey in Nileke County of Ili valley (Zhumabai et al., 2005). Human AE prevalence (0.3%) of Xinyuan County in this study showed no significant statistical difference ($p>0.05$) compared to 0.64% in the nearby Nileke County (Zhumabai et al., 2005). The transmission of E. multilocularis might be similar in these two counties because they all located in the Ili Valley of Tianshan Mountains. A sylvatic cycle is maintained in Xinjiang with red fox (*Vulpes vulpes*) as the main definitive host, microtines (voles) as main intermediate host (WHO/OIE, 2001; Ding and Wen, 2000). Domestic dogs were involved in *E. multilocularis* transmission in nearby Bayinbuluke pasture in Xinjiang. So the close contact with dog might be one of the main risk factors for human AE in this case.

The knowledge of how to prevent and control of *E. granulosus* and *E. multilocularis* infection seems very poor for local people. So public health education through TV programme, broadcast, handbook etc, might be helpful to local people to understand the transmission and get to prevent by better hygiene, correct methods for livestock offal and dog feces, and they could accepted the test and prazquentel treatment for dogs.

6.4 Community study in Hoboksar Mongol Autonomous County, Tacheng Prefecture, Xinjiang

6.4.1 Introduction to Study Site

Hobukesaier (Hoboksar) Mongol Autonomous County is a county situated in the Xinjiang Uyghur Autonomous Region and is under the administrative jurisdiction of the Tacheng Prefecture (Fig. 6.4.1). It has an area of 28,799 km² with a population of 50,000. Hoboksar is located northwest board of Jungger Basin, 495 km from Urumqi, with low mountain ranges (altitude above 1000 metre) in the north, Gurbantunggut Desert in the south and piedmont alluvial plain in the middle. Its climate belongs to north temperate zone continental drought weather, with average annual temperature 3.0ºC and annual rainfall 142 mm.

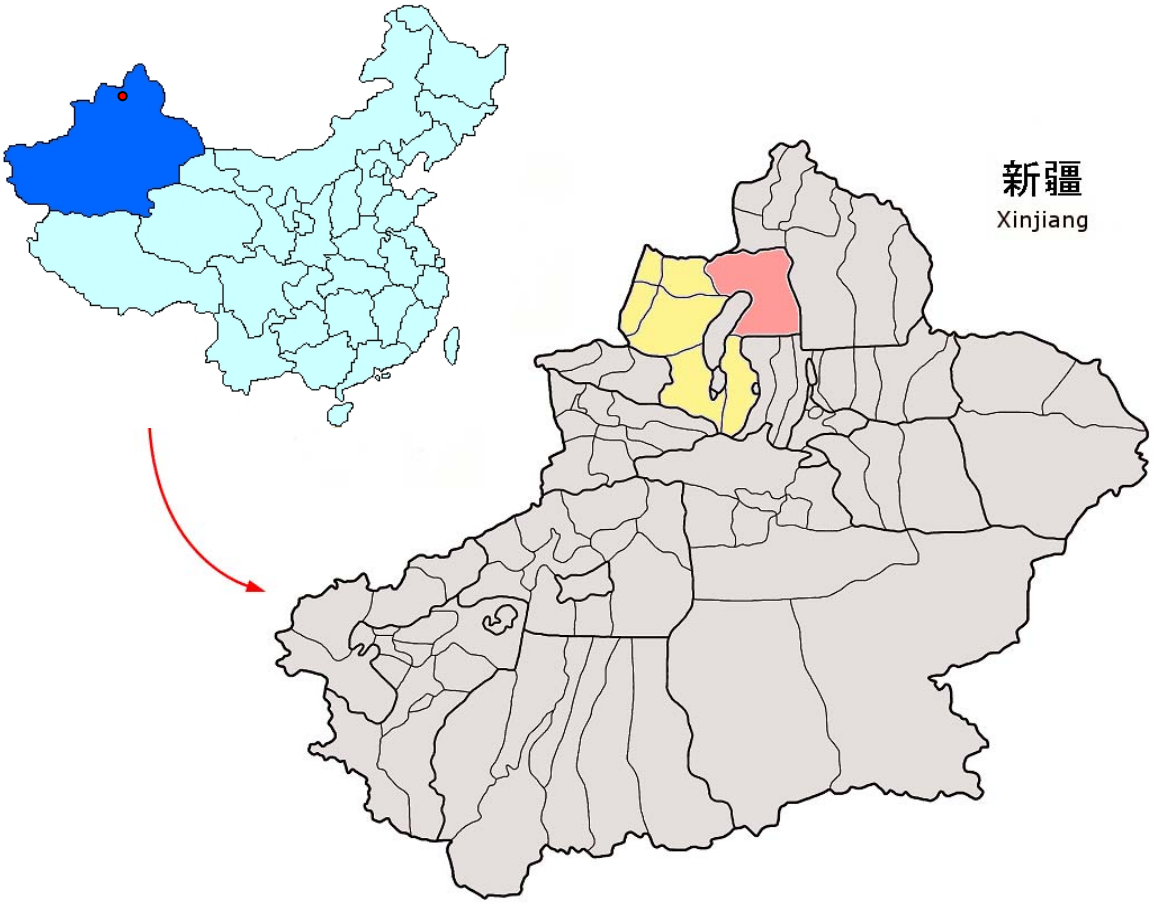

Fig. 6.4.1: Hoboksar Mongol Autonomous County in Tacheng Prefecture, Xinjiang Uygur Autonomous Region, P.R.China

A community study was carried out in five communes/pastures ie. Narenhebuk and Bustunge Pastures, Tiebukenwusan, Chagankuke and Bayinaowa Communes) in Hoboksar Mongol Autonomous County in 2007 (Fig. 6.4.2).

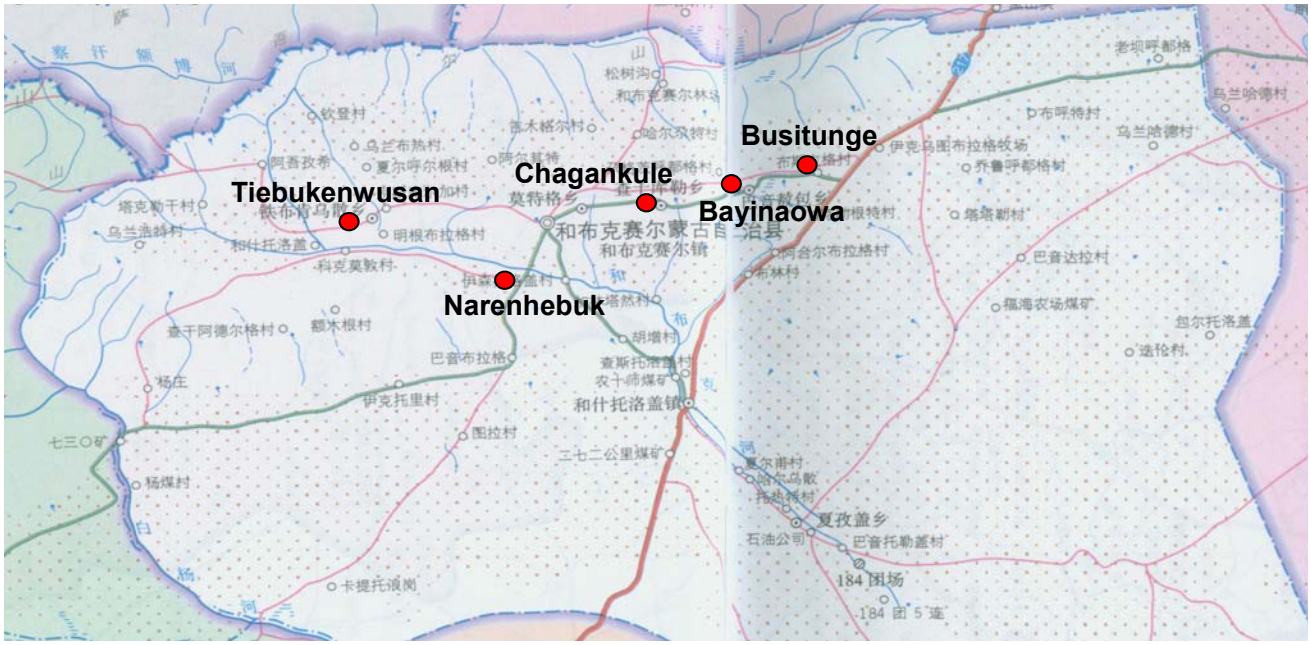

Fig. 6.4.2: Community study sites in Hoboksar Mongol Autonomous County, Tacheng Prefecture, Xinjiang Uygur AR (1:1,200,000)

6.4.2 Results

A total of 1339 residents accepted questionnaire, abdominal ultrasound and serological test by DIGFA. Population sample (age ranged from 6 to 77 years, median 31years) comprised 42.57% (593)male and 53.55% (746) female, with ethnic groups as Han 121 (9%), Mongolian 574 (42.9%), Kazakh 620 (46.3%), and others 24 (1.8%). Their occupations included farmers 491(36.7%), herdsmen 260 (19.4%), students 477 (35.6%), Cadres 76 (5.7%), and others 35 (2.6%). Communes screened were Tiebukenwusan (n=460), Narenhebuke (n=248), Chagankuke (n=268), Bayinaowa (n=240), Busitunge (n=119) and others (n=4).

6.4.2.1 Ultrasound Prevalence

Of 1339 volunteers who were checked by abdominal ultrasound, 68 (5.1%) were confirmed to have CE, 2 (0.1%) to have AE, and 12 (0.9%) to have isolated calcification lesions.

i) Prevalence by communes:

There were significant differences of CE prevalence among different communes ($p<0.01$). Narenhebuke Pasture showed the highest CE prevalence of 9.3%, and

followed by Tiebukenwusan and Chagankuke (Table 6.4.1).

Table 6.4.1 Human CE or AE prevalence by abdominal ultrasound in Hoboksar Mongol Autonomous County, Tacheng Prefecture, Xinjiang Uygur Autonomous Region, P.R.China

Communes	Populations	Screened	CE(%)	AE(%)	Calcification(%)	Normal
Tiebukenwusan	3524	460	23(5)	1(0.2)	7(1.5)	429
Narenhebuke	2374	248	23(9.3)	0	5(2)	220
Chagankuke	4320	268	13(4.9)	0	0	255
Bayinaowa	2460	240	4(1.7)	0	0	236
Busitunge	2268	119	2(1.7)	1(0.8)	0	116
Others		4	3	0	0	1
Total		1339	68(5.1)	2(0.1)	12(0.9)	1257

For CE prevalence in 5 different communes: Chi-Square Value18.3328 Prob 0.0011

ii) Prevalence by gender, ethnic and occupation

Females showed similar CE prevalence to males. Just 2 AE cases were identified both females. Mongolian and Han ethnic people had higher CE prevalence compared to Kazakh ($p<0.01$). Farmers and herdsmen presented higher CE prevalence than other occupations ($p<0.01$) (Table 6.4.2).

Table 6.4.2: Human CE or AE prevalence in three communes in northwest Hoboksar

		Ultrasound			
		N	**CE(%)**	**AE(%)**	**Calcification**
Gender	Male	593	29(4.9)	0	7(1.2)
	Female	746	39(5.2)	2(0.3)	5(0.7)
	Total	**1339**	**68(5.1)**	**2(0.1)**	**12(0.9)**
Ethnic	Han	121	11(9.0)	1(0.8)	1(0.8)
groups	Mongolian	574	34(5.9)	1(0.2)	7(1.2)
	Kazakh	620	20(3.2)	0	4(0.6)
	Others	24	3(12.5)	0	0
	Total	**1339**	**68(5.1)**	**2(0.1)**	**12(0.9)**
Occupation	Farmers	491	30(6.1)	1(0.2)	3(0.6)
	Herdsmen	260	22(8.5)	1(0.4)	7(2.7)
	Students	477	8(1.7)	0	0
	Cadres	76	4(5.3)	0	2(2.6)
	Others	35	4(11.4)	0	0
	Total	**1339**	**68(5.1)**	**2(0.1)**	**12(0.9)**

For genders, Pearson Chi-Square Value.078, Asymp. Sig. (2-sided) 0.780
For ethnic, Pearson Chi-Square Value12.048 Asymp. Sig. (2-sided) 0.007
For occupations, Pearson Chi-Square Value19.748[a] Asymp. Sig. (2-sided) 0.0002

iii) Prevalence by age group

Higher CE prevalence occurred in the > 20 age groups ($p<0.01$) (Table 6.4.3)

Table 6.4.3: Human CE or AE prevalence in different age groups in community study in north Hoboksar Mongol Autonomous County

Ages	N	CE (%)		AE (%)		Calcification (%)		Normal
<20	502	10	(1.99)	0	(0.00)	1	(0.20)	491
20-	353	28	(7.93)	1	(0.28)	6	(1.70)	318
40-	374	25	(6.68)	0	(0.00)	3	(0.80)	346
60-	110	5	(4.55)	1	(0.91)	2	(1.82)	102
Total	**1339**	**68**	**(5.08)**	**2**	**(0.15)**	**12**	**(0.90)**	**1257**

CE prevalence: Chi-Square Value was 17.9491 Prob was 0.0005

6.4.2.2 Serological prevalence by DIGFA

The rapid DIGFA test results showed females had a higher seropositive rate (22.56%) than males (17.41%, $p<0.05$). Han and Mongolian ethnic group had higher seropositives than Kazakh and other ethnic groups ($p<0.01$). Herdsmen and farmers showed higher seropositive rate than students, cadres and other occupations ($p<0.01$) (Table 6.4.4).

Table 6.4.4: Serological prevalence by gender /ethnic group /occupation in Hoboksar.

	No. test	Positive	Positive %	Negative	Chi	P
Genders						
Male	580	101	17.41	479		
Female	727	164	22.56	563	5.2825	0.0215
Ethnic groups						
Han	118	42	35.59	76		
Mongolian	559	129	23.08	430		
Kazakh	606	89	14.69	517		
Others	24	5	20.83	19	31.5574	<0.0001
Occupation						
Farmers	484	139	28.72	345		
Herdsmen	254	66	25.98	188		
Students	463	33	7.13	430		
Cadres &others	106	27	25.47	79	77.7538	<.0001
Age (yrs)						
<20	488	33	6.76	455		
20-39	348	101	29.02	247		
40-59	363	106	29.20	257		
60-	108	25	23.15	83	90.043	0000
Total	**1307**	**265**	**20.28**	**1042**		

Table 6.4.5: Human ultrasound CE and AE prevalence and seropositive prevalence by education level in Hoboksar

Education	Ultrasound			DIGFA	
	N	CE (%)	AE (%)	N	Positives (%)
Illiteracy	58	6	0	58	21
Primary school	595	24	1	580	112
Middle school	510	27	0	499	92
High school	140	7	1	135	35
College and over	36	4	0	35	5
Total	**1339**	**68**	**2**	**1307**	**265**

(Education level: χ^2_{b}=1.692, $P_{\text{b}} > 0.05$; $\chi^2_{\text{血}}$=13.462, $P_{\text{血}}$=0.009)
For CE prevalence Pearson Chi-Square Value7.453 Asymp. Sig. (2-sided) .114
For seropositives Pearson Chi-Square Value13.928 Asymp. Sig. (2-sided) .008

6.4.2.3 Risk factors for human CE

Human CE related risk factors, including ethnicity, age, sex, occupation and personal status on hygiene were analyzed under multifactorial logistic regression. The main risk factors that were significantly associated with CE were ages (OR=7.6, 95%CI: 2.481-23.579) and home slaughtering of livestock (OR=3.2, 95%CI: 1.297-7.809). Herdsmen had the highest prevalence of CE in all of the occupations in this study (Table 6.4.6).

Table 6.4.6 Univariable Logistic regression analysis for risk factors of human CE in Hoboksar

Variables		Prevalence (%)		OR (95%CI)
Gender	Male	29/593	(4.9)	1.073（0.655- 1.757）
	Female	39/746	(5.2)	
Ethnic	Han	11/121	(9.0)	
	Mongol	34/574	(5.9)	1.429(.367-5.562)
	Kazakh	20/620	(3.2)	5.562(0.645-7.986)
	Others	3/24	(12.5)	4.286 (1.181-15.556)*
Occupation	Herdsmen	22/260	(8.5)	
	Farmer	30/491	(6.1)	1.396 (0.451-4.318)
	Students	8/477	(1.7)	1.983 (0.657-5.986)
	Cadres	4/76	(5.3)	7.565 (2.158-26.510)*
	Others	4/35	(11.4)	2.323 (0.546-9.886)
Age (years)	～20	10/502	(1.99)	
	20～	28/353	(7.93)	2.343 (0.785-6.996)
	40～	25/374	(6.68)	0.553 (0.208-1.468)
	60～	5/110	(4.55)	0.665 (0.248 1.779)
Dog owner	Yes	51/942	(5.41)	0.782 (0.446-1.371)
	No	17/397	(4.28)	
Livestock owner	Yes	63/1276	(4.94)	1.660 (0.643-4.283)
	No	5/63	(7.94)	
Home slaughter	Yes	42/885	(4.75)	1.219 (0.738-2.016)
	No	26/454	(5.73)	
Stray dogs	Yes	15/315	(4.76)	1.092 (0.607-1.965)
	No	53/1024	(5.18)	
Drinking water	Tap water	34/533	(6.38)	
	Deep well	1/96	(1.04)	1.129 (0.582-2.190)
	Shallow well	20/528	(3.79)	7.308 (0.941-56.733)
	River or stream	13/182	(7.14)	1.954 (0 .951-4.013)
Wash hand before eating	Yes	65/1298	(5.01)	1.498 (0.450-4.980)
	No	3/41	(7.32)	
Drink unboiled water	Yes	35/822	4.26	1.533 (0.940 -2.500)
	No	33/517	6.38	
Eat uncooked vegetables	Yes	21/617	3.40	1.976 (1.168 - 3.344)*
	No	47/722	6.51	

Three variables were chosen since they seemed to have significant differences for CE. There were ethnicity, occupation and eating of uncooked vegetables, which were selected for multivariable logistic regression. The analyzed results showed that Han, Mongolian and Kazak groups had a lower CE prevalence risk than other ethnic group included Uygur, Hui and Xibo ethnic. Farmers and students had a lower risk than other occupations but herdsmen and cadre had a slighting higher

risk, eating uncooked vegetables did not show any significant differences (Table 6.4.7).

Table 6.4.7: Multivariable Logistic regression analysis for risk factors of human CE in Hoboksar

Risk Factor		B	S.E.	Wald	Sig.	Exp(B)	95.0% C.I.for EXP(B)	
							Lower	Upper
Step 1[a]	Ethnic			15.690	0.001			
	ethnic(1)	-1.368	0.700	3.821	0.051	0.255	0.065	1.004
	ethnic(2)	-2.119	0.650	10.638	0.001	0.120	0.034	0.429
	ethnic(3)	-2.195	0.645	11.583	0.001	0.111	0.031	0.394
	Occupation			12.929	0.012			
	Occupation(1)	-.159	0.659	0.058	0.809	0.853	0.234	3.101
	Occupation(2)	0.076	0.681	0.012	0.912	1.079	0.284	4.100
	Occupation(3)	0.279	0.747	0.140	0.709	1.322	0.306	5.717
	occupation(4)	-1.535	0.745	4.244	0.039	0.215	0.050	0.928
	uncookveg(1)	-0.474	0.287	2.740	0.098	0.622	0.355	1.091
	Constant	-0.418	0.828	0.255	0.614	0.658		

6.4.3 Discussion (Hoboksar, Tacheng Prefecture, Xinjiang)

Geographical CE distribution of human CE in Hobokesar showed that three southwest communes had higher prevalence than others in the north. CE prevalence by ultrasound in Narenhebuke (23/248, 9.3%) was higher than a previous report in same area which was 49/1844, 2.7% (Wang et al., 2001). Similarly in the current study CE prevalence in Mongolians was 5.9% (34/574), which was higher than the 2.7% (34/1267) recorded by Wang et al (2001). This difference might be caused a smaller sample for both Narenhebuke and Mongolian populations in the current survey.

In Hoboksar females had a significant higher seropositive rate than males ($P<0.05$). That might due to close contact with dogs since female had more responsibility to feed dogs and to clean the yards where dogs and livestock are

kept. Generally however females and males had no difference for CE prevalence in other areas of Xinjiang, and also in a previous study in Hobokesar (Wang, 2001). Persons younger than 20 years old had a significantlly lower CE prevalence and seropositives rate than other age groups and seropositives increased with ages. Students, which for most part were younger than 20 yrs old had the lowest CE ultrasound prevalence and sero prevalence compared to other occupation groups. Education was also analyzed in this study with higher CE seropositive rate in persons who just had a primary school level. This might indicate that lower education level had more risk of exposure, not definitely. This could be caused by different health education levels or different level of knowledge about echinococcosis and its transmission, or different (closer) behavior of young children with dogs.

Mongolian and Han ethnic groups showed higher CE ultrasound and sero prevalence compared to Kazakhs in the current study. Wang et al. (2001) found no significant difference for CE among Mongolian and Kazak groups, but higher for Han. That might be because in this study we had more Kazakhs and fewer Mongolian registered. As for Han residents, they have a similar life-style with local Mongolians and Kazakhs and also kept close contact with dogs as well.

Risk factors for human CE were not so obvious in this study in Hoboksar County. That may be due to local people having similar life-styles and living in similar circumstances with dogs and livestock. The overall CE ultrasound prevalence in Hoboksar was high (5.1%) for Xinjiang, and similar with that we obtained in Bayinbuluke (5.7%), but much higher than in for Wenquan (1.86%) and Xinyuan (1.57%) areas.

The youngest CE patient in the Hoboksar study was a 6-year old child. This suggested that E. granulosus was still in higher transmission status in this area since new cases occurred in young children (age group <20).

The main activity in Hoboksar County is livestock production and 93.5% families raised livestock. Furthermore, 75.4% of families slaughtered their livestock in their yards. Dogs as guards or for herding livestock are essential for the local people, and 63% of people owned dogs (average 2 dogs per family). Untied dogs could go anywhere. People have more chance to be contacted with the skin, fur and feces of dogs and livestock. This suggested that people might have specific sera antibodies of *E. granulosus* since they kept contact with tools or materials and

surrounding infected by *E. granulosus* eggs.

The distribution of human CE in southwest Hoboksar was high, but two human AE cases were also found in this study. Transmission of E. multilocularis may occur between the red fox and small mammals in this area (Lin et al., 1993; Zhou et al., 2000). The high CE prevalence could affect local economic development due to livestock loses and human health loses. Prevention could be focus on dog management including routine testing and anthelmintic treatment, strengthen slaughter hygiene supervision, also to strengthen health education especially for children and students, and improve the general hygiene situation. At the same time, survey and treatment of cases and routine prevalence monitoring, which had been confirmed effective for prevention, are important for any effort to reduce human echinococcosis especially human CE.

6.4.4 Discussion ---Community studies on human echinococcosis in XUAR, China

Echinococcosis is a zoonosis with a worldwide distribution caused by adult or larval stages of tapeworms (cestodes) belonging to the genus *Echinococcus* (family Taeniidae) (Zhou et al., 2000; WHO/OIE 2001; Craig et al., 2003). The two major species of medical and public health importance in northwestern China are *Echinococcus granulosus* (E.g) and *Echinococcus multilocularis* (E.m), which result in cystic echinococcosis (CE) and alveolar echinococcosis (AE) in humans (Wen and Yang, 1997; Craig 2004, Xu et al., 2007; Bud). Both can be considered as life-threatening diseases, the latter especially with high fatality rates and poor prognosis if careful clinical treatment is not available in early stages. Mortality for human CE varied between 0.5% and 4.5%, and for human AE between 10-15% (Wen and Yang, 1997; WHO/OIE 2001; McManus et al., 2003; Zhang et al., 2003).

Xinjiang Uygur Autonomous Region (XUAR) is in the northwestern China with a population of 22 million people. A total of 21,560 hospitalized CE cases in China were recorded in Xinjiang from 1950-1990 (Jiang, 1991), over 1965 CE cased from 1993 to 2004 (Chai et al. 2004; Dinmurati et al.2005; Gao et al. 2005. Wang et al., 2008). Human CE cases have been recorded all over the region, which consisted of all of 12 prefectures including 5 minority ethnic autonomous prefectures. Main CE endemic area may focus on the North of Xinjiang from the

Tianshan Mountain to Altai Mountain including Bayinguoleng Mongolian Autonomous Prefecture, Yili Kazak Autonomous Prefecture, Tacheng Prefecture, Altai Prefecture, Changji Prefecture and Hami Prefecture. Hospital records indicated 16,663 cases of CE were treated surgically in Xinjiang from 1951 to 1991 (National Hydatid Disease Center of China, 1993). Wei et al reported that human CE was more endemic in Tianshan Mountain (2.23%, 47/2103) and Altai Mountain (2.28%, 41/1420) than in Kunlun Mountain (0.6%, 6/1000) based on a cross –sectional survey including 4 community populations from 4 counties (Wei 1994). Community screening data showed 2.22% (45/2044) CE prevalence in Wulasitai Commune of Nileke County, Yili Valley (Zhumabai et al., 2005); 4.5% (34/755) and 1.91% (17/889) in Habahe County (Song et al., 1999; Zhao et al., 2003), 5.78% (31/536) in Qinghe County (Zhao et al., 2003), Altai Prefecture; 2.4% (49/1844) in Hobukesar County, Tacheng Prefecture (Wang et al., 2001). Seven thousand two hundred and fifty-five CE cases have been treated in Xinjiang Medical University Hospital since 1957 and the annual cases curve showed a remarkable increase during the last decade (hospital record). 1126 hospitalized CE cases were registered in north 4 counties in Tacheng Prefecture (Qi et al., 1995). 1965 CE cases were reported in Yili Valley which consists of 8 counties and 1 city from 1993 to 2003 (Gao et al., 2005).

To further understand the geographic and ethnic distribution of human *echinococcus* infection (CE and AE) in Xinjiang, our team conducted series fine designed epidemiological studies in 7 counties including 6 natural villages in 5 prefectures. A questionnaire including demographic and economic information and risk factors (dog owner, fox/wolf contact, homeslaughter, health education etc.) need to be completed at first, followed by ultrasound plus serologic tests with a rapid DIGFA and/or ELISA tests randomly selected subjects from each fields. Human hydatid survey and control model can be modified as an system which including basically population data, initial trial in a small group, formal survey (ultrasound plus serological tests), chemotherapy and/or surgical treatment, health education and continuous control programme. The overall results showed that CE were high endemic and AE were sporatic in all these counties of Xinjiang (Table 6.4.11 and Fig. 6.4.3).

Table 6.4.11: The ultrasound prevalence of human CE and AE in field screening studies in Xinjiang (1998-2007)

Counties	No	CE cases	CE prevalence	AE	AE prevalence
Wenquan	1292	24	1.86%	0	0.00%
Hejing	1398	78	5.58%	3	0.21%
Xinyuan	3691	56	1.52%	11	0.30%
Hoboksaier	1339	68	5.08%	2	0.15%
Nileke	3908	94	2.41%	16	0.41%
Habahe	1644	51	3.10%	0	0.00%
Qinghe	536	31	5.78%	0	0.00%
Subtotal	13808	402	2.91%	28	0.20%

For CE, Pearson Chi-Square Value1.073E2 Asymp. Sig. (2-sided) 0.000;
For AE, Fisher's Exact Test Value3.852 Sig. 0.262, 99% Confidence Interval (0.250, 0.273)

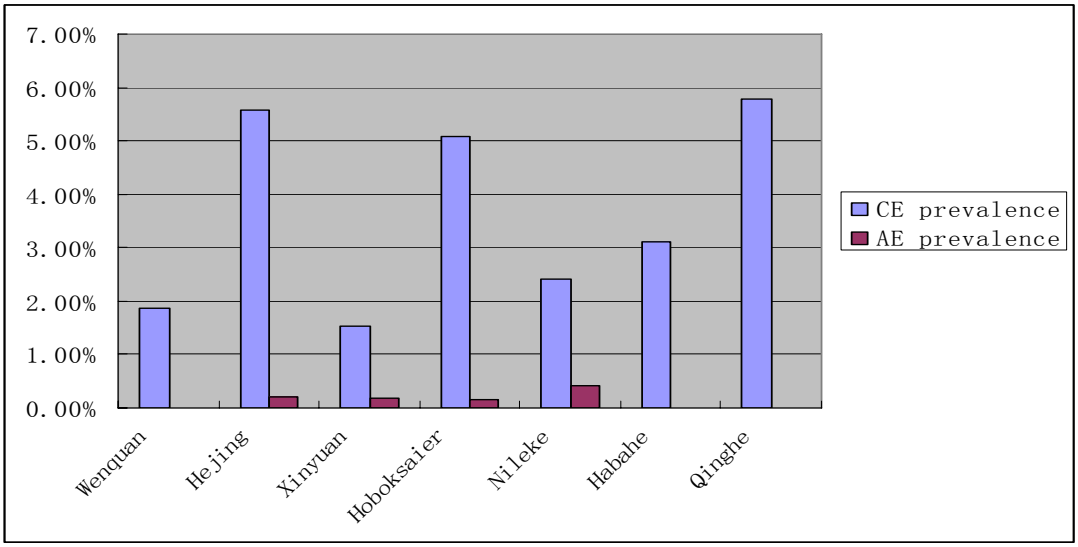

Fig. 6.4.3: The ultrasound prevalence of human CE and AE in field screening studies in Xinjiang (1998-2007)

Field works in above four counties in Section 6.1 to 6.4, which included Wenquan, Hejing, Xinyuan and Hoboksar, were put together to find the differences between them. The main findings appeared as follows. The ultrasound CE prevalences were different in different counties, which were higher in Hejing and Hoboksar counties (Table 6.4.12-6.4.13). There was no gender difference in human CE prevalence in Xinjiang (Table 6.4.14). In contrast females were 1-2 times higher CE than males on Tibet Plateau (Wang et al, 2000, Li et al., 2005, 2010). Mongolians were the main ethnic group with *Echinococcus* infection, with prevalence as high as 5.19% (Table 6.4.15). Persons with occupation as herdsmen and farmers, aged 20-60 years old should have greater risk than other occupation groups (Table 6.4.15 - 6.4.17). Dog and livestock ownership had given more risk for human CE. A total of 23 human AE cases were reported in above four counties including 5 cases in three Mongolian dominated communities (Hejing, Wenquan and Hoboksar). There were no statistical differences for ultrasound AE between three Mongolian dominated counties (Table 6.4.12-6.4.13).

Table 6.4.12: Echinococcosis prevalence by ultrasound in 3 Mongolian dominated communities of Xinjiang

County/ Commune	No.	HD (%)	AE (%)	CE(%)
Hejing / Bayanbuluke	1398	82 (5.87)	3 (0.21)	79(5.6)
Wenquan / Angelige	1292	24 (1.86)	0	24(1.86)
Hoboksar	1339	70 (5.23)	2 (0.15)	68 (5.08)
Total	4029	176(4.27)	5 (0.12)	171(4.24)

For HD, Pearson Chi-Square Value29.365 Asymp. Sig. (2-sided) 0.000
For CE, Pearson Chi-Square Value27.208 Asymp. Sig. (2-sided) 0 .000
For AE, Fisher's Exact Test Exact Sig. (2-sided) 1.000

Table 6.4.13: Ultrasound Echinococcosis prevalence of Mongolian in 3 Mongolian dominated communities in Xinjiang

Communities	No.	HD(%)	AE(%)	CE(%)
Hejing/Bayinbuluke	1377	81(5.88%)	3 (0.21)	78(5.6)
Wenquan/Angelige	189	5(2.65%)	0(0)	5(2.65)
Hoboksar	574	34(5.92)	1 (0.17)	33(5.75)
Total	2140	120(5.61)	4(0.19)	116(5.42)

For HD, Pearson Chi-Square Value3.437 Asymp. Sig. (2-sided) 0.179
For CE, Pearson Chi-Square Value3.120 Asymp. Sig. (2-sided) 0.210
For AE, Fisher's Exact Test Value0.242 Sig1.000

Table 6.4.14: CE prevalence comparision of different genders in 4 counties of Xinjiang

Counties	Male			Female			Subtotal		
	N	CE (%)		N	CE (%)		N	CE (%)	
Wenquan	604	9	(1.49)	688	15	(2.18)	1292	24	(1.86)
Hejing	597	41	(6.87)	801	38	(4.74)	1398	79	(5.65)
Xinyuan	1780	30	(1.69)	1911	26	(1.36)	3691	56	(1.52)
Hoboksar	593	29	(4.89)	746	39	(5.23)	1339	68	(5.08)
Subtotal	**3574**	**109**	**(3.05)**	**4146**	**118**	**(2.85)**	**7720**	**227**	**(2.94)**

Male CE* counties Pearson Chi-Square 53.98, Asymp. Sig. (2-sided) 0.000
Female CE * counties Pearson Chi-Square Value 42.09, Asymp. Sig. (2-sided) 0.000
County * CE Pearson Chi-squarevalue90.11, Asymp. Sig. (2-sided) 2.08E-19
Gender * AE Pearson Chi-squarevalue0.21,Asymp. Sig. (2-sided) 0.647925

Table 6.4.15: Ultrasound CE prevalence by ethnic group in four counties in Xinjiang

Ethnic	N	CE (%)	
Han	1496	29	1.94
Kazak	3344	66	1.97
Mongolian	2314	120	5.19
Others	566	1 2	2.12
Subtotal	**7720**	**227**	**2.94**

Pearson Chi-Square Value58.426 Asymp. Sig. (2-sided) 0.000

Table 6.4.16: Ultrasound CE prevalence by age group in four counties in Xinjiang

Age	N	CE	CE%
＜20	2268	36	1.59
20-	3020	106	3.51
40-	1889	68	3.60
60-	543	17	3.13
Total	7720	227	2.94

Pearson Chi-Square Value20.929 Asymp. Sig. (2-sided) 0.000

Table 6.4.17: Ultrasound CE prevalence by occupation in four counties in Xinjiang

Occupation	No.	CE	Prevalence
Farmer	2455	60	2.44
Herdsman	2699	115	4.26
Student	2026	32	1.58
Others	540	20	3.70
Total	7720	227	2.94

Pearson Chi-Square Value32.859 Asymp. Sig. (2-sided) 0.000

The development of Dot Immuno-Gold Filtration Assay (DIGFA) for serological studies in hospitals and communities shared promise (Fu et al, 2000, Feng et al., 2002, Chen et al, 2005, Feng et al., 2010). Compared with standard ELISA test using the same antigens, the DIGFA test showed higher sensitivity especially for

detection of CE T1, T2, T3 types (Chapter 3).

A network for detection, treatment and control of human echinococcosis has been established in Xinjiang in the period of 2005-2006, which consists of the first 3 sentry hospitals. A total of 29 hospitals were included in the network by the end of 2008. Training, related scientific research, regular surveillance data collection have been done through the network. Official Appointed Hospitals for Human Echinococcosis were defined as two parts: one as Surgical Instruction Hospitals including the 1st Affiliated Hospital of Xinjiang Medical University and the 1st Affiliated Hospital of Medical College in Shihezi University, the other as Official Appointed Surgery Treatment Hospitals in Xinjiang such as Yili Friendship Hospital, Changji Prefecture Hospital and Wusu City Hospital et al., (see details in map). Distant tele-medical consultation for hydatid patients by National surgical aid programme was located in the 1st Affiliated Hospital of Xinjiang Medical University in April 2008 and well done for hydatid patients all over Xinjiang. National Training Programme for hydatid has been activated for mainstay staff of those Official Appointed Hospitals of Xinjiang since 2008 and continued in 2009, which included in serological diagnosis and imaging, surgical treatment if needed. Training video of hydatid intervention financially supported on the surgical aid programme for hydatid control and free charged for education and training in the 7 endemic provinces and Autonomous Regions under the financial aids from Chinese Government. Consultations for the Official Appointed Hospitals in China were performed by MOH (Ministry of Health). In addition, Surgical Consultation Group for hydatid patients had been worked in Guyuan Hospital in Ningxia, Tianzhu Tibetan Autonomous County Hospital in Gansu, Ganzi Prefecture Hospital in Sichuan, Xilinguole Prefecture Hospital in Inner Mongolia, Xining CDC Centre in Qinghai, Wuzhong City Hospital in Ningxia and Shannan Prefecture Hospital in Tibet between 2008~2009 (see Fig. 6.4.4).

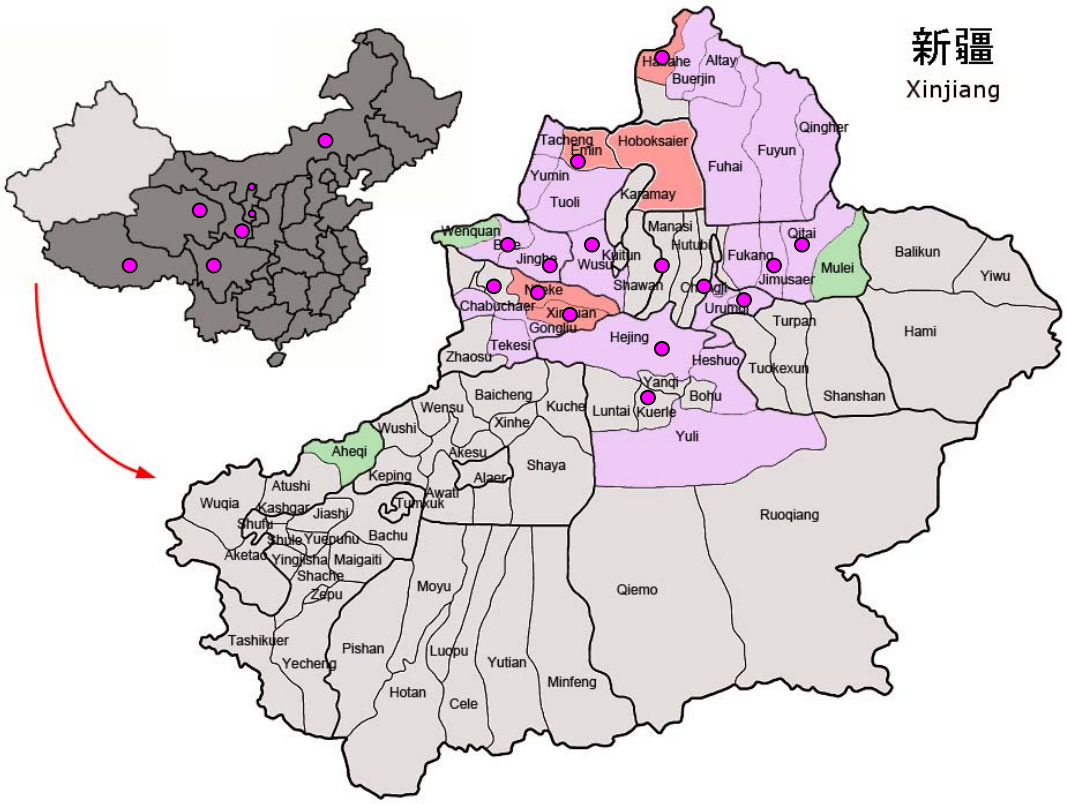

Fig. 6.4.4: Map of the sentry county hospitals all over Xinjiang Uygur Autonomous Region, P.R.China

3 counties in 2005/2006

5 counties in 2007

21 counties in 2008

- 7 Official appointed hospitals in other endemic provinces or autonomous regions and 15 official appointed hospitals in Xinjiang.

6.5 Community study in Xiji County, Guyuan Prefecture, Ningxia Hui Autonomous Region (2002)

6.5.1 Introduction to study site

Xiji County in southern Ningxia Hui Autonomous Region (Fig. 6.5.1), is situated to the west Liupanshan Mountains, with longitude E105°20'-106 °04', latitude N 35°35'-36°14', elevation 1688~2633 metres, and part of the hinterland of Loess Plateau in the middle reaches of the Yellow River. Xiji County is located 63 kilometers from east of Guyuan City, 391 kilometers from the capital Yinchuan City in Ningxia, 459 kilometers (km) from Xian and 270 km from Lanzhou, and about 2200 km from Urumqi. The Region covers 3143.85 square kilometers. The climate is a typical continental climate being sub-humid and semi-arid. It has an annual sunshine time of 2322 hours, with an average annual temperature of 5.3°C and 100-150 days frost-free period, an average annual rainfall around 400 mm. There are owns 3 towns, 16 communes, 306 villages, and the total population of 471,000, of which 255,000 Hui Moslem ethnic group are biggest munorities (54.3%) (http://www.nxxj.gov.cn).

Fig. 6.5.1 Xiji County of Guyuan Prefecture, Ningxia Hui Autonomous Region, P.R.China

6.5.2 Results

6.5.2.1 Ultrasound Prevalence

Following an ultrasound based survey a total 36 (3.81%) CE cases and 57 (6.04%) AEcased were detected in addition another 14 (1.48%) with queried and 40 persons (4.23) showed isolated calcifications on portable ultrasound (Tabel 6.5.1). This region therefore had a high prevalence of human AE as well as CE.

i) Prevalence by gender

No difference between males and females was found in human CE prevalence by ultrasound in this study in Xiji (P>0.05). But as for AE prevalence in Xiji, the situation looked quite different since females had significantly higher prevalence (7.7% vs. 4.4.9%, P<0.05)

Table 6.5.1: Prevalence comparison of human CE and AE in different genders in Xiji County, Ningxia

Gender	N	CE (%)	AE (%)	Query (%)	Calcification (%)	Normal
Male	490	19 (3.88)	22 (4.49)	4 (0.82)	20 (4.08)	425
Female	455	17 (3.74)	35 (7.70)	10 (2.20)	20 (4.39)	373
Subtotal	**945**	**36 (3.81)**	**57 (6.04)**	**14 (1.48)**	**40 (4.23)**	**798**

For CE, Chi-Square Tests Pearson Chi-Square Value.013 Asymp. Sig. (2-sided) 0.910
For AE, Chi-Square Tests Pearson Chi-Square Value4.269[a] Asymp. Sig. (2-sided) 0.039

ii) Prevalence by age

There were significant differences for human CE and AE among different age groups (P<0.01).No CE or AE cases detected by ultrasound occurred in ages younger than 10 yrs old. The ultrasound prevalence of both CE and AE increased with age (Table 6.5.2).

Table 6.5.2: Ultrasound prevalence of human CE and AE by age in Xiji County

Age	N	CE (%)	AE (%)	Query (%)	Calcification (%)	Normal (%)
<10	33	0 (0)	0 (0)	0 (0)	1 (3.03)	32 (96.97)
10-	372	1 (0.27)	0 (0)	1 (0.27)	7 (1.88)	363 (97.58)
20-	127	3 (2.36)	1 (0.79)	0 (0)	9 (7.09)	114 (89.76)
30-	149	6 (4.03)	6 (4.03)	3 (2.01)	7 (4.70)	127 (85.23)
40-	114	5 (4.39)	20 (17.54)	3 (2.63)	8 (7.02)	78 (68.42)
50-	95	12 (12.63)	16 (16.84)	3 (3.16)	5 (5.26)	59 (62.11)
60-	55	9 (16.36)	14 (25.45)	4 (7.27)	3 (5.45)	25 (45.45)
Total	**945**	**36 (3.81)**	**57 (6.03)**	**14 (1.48)**	**40 (4.23)**	**798 (84.44)**

Chi-Square Tests For CE Pearson Chi-Square Value58.715 Asymp. Sig. (2-sided) 0.000
Chi-Square Tests for AE Pearson Chi-Square Value1.161E2 Asymp. Sig. (2-sided) 0.000

iii) Prevalence by ethnic groups

No statistics differences were found between Han and Hui ethnic groups for CE or AE ultrasound prevalence ($p>0.05$) (Table 6.5.3).

Table 6.5.3: Ultrasound prevalence of human CE and AE by ethnicity in Xiji County

Ethnicity	N	AE	AE(%)	Chi	*p*	CE	CE(%)	Chi	*p*
HAN	428	27	6.31%			20	4.67%		
HUI	517	30	5.8%	1.591	0.207	16	3.09%	0.106	0.745
Total	945	57	6.05%			36	3.81%		

iv) Prevalence by occupation

Agriculturalist showed higher prevalence of CE (5.59%) and AE (10.06%) compared to students ($p<0.01$). Other occupations which included cadres, businessmen and housewife, etc., showed high CE and AE prevalence (10% and 6% respectively) (Table 6.5.4).

Table 6.5.4: Ultrasound prevalence of human CE and AE by occupation in Xiji County, Ningxia

Occupation	N	CE (%)	AE (%)	Query (%)	Calcification (%)	Normal (%)
Peasant	537	30 (5.59)	54 (10.06)	12 (2.23)	31 (5.77)	410 (76.35)
Student	358	1 (0.28)	0 (0.00)	1 (0.28)	6 (1.68)	350 (97.77)
Others	50	5 (10.00)	3 (6.00)	1 (2.00)	3 (6.00)	38 (76.00)
Subtotal	945	36 (3.81)	57 (6.03)	14 (1.48)	40 (4.23)	798 (84.44)

For CE Pearson Chi-Square Value22.032 Asymp. Sig. (2-sided) 0.000
For AE Pearson Chi-Square Value38.322 Asymp. Sig. (2-sided) 0.000

6.5.2.3 Serological prevalence by DIGFA

Females showed higher seropositivity (21.32%, 97/455) than males (12.65%, 62/490) for DIGFA test (p<0.01). Seropositive rate increased with age (p<0.01) and was over 30% seropositives in people older than 50 yr old (Table 6.6.5).

Table 6.5.5: Seropositive comparison for human echinococcosis antibodies in different genders/ages in Xiji County, Ningxia

	N	Positives	(%)	Negatives	(%)	Chi	*p*
Gender							
Male	490	62	12.65	428	87.35		
Female	455	97	21.32	358	78.68	12.660	0.000
Age							
<10	33	0	0.00	33	100.00		
10-	372	42	11.29	330	88.71		
20-	127	15	11.81	112	88.19		
30-	149	20	13.42	129	86.58		
40-	114	33	28.95	81	71.05		
50-	95	31	32.63	64	67.37		
60-	55	18	32.73	37	67.27	57.202	0.000
Subtotal	945	159	16.83	786	83.17		

There was no significant statistical difference between Han and Hui echnic group for human serology prevalence (p>0.05) (Table 6.5.5). Peasants had a higher sero prevalence (20.9%, 112/537) compared to students (10.3%, 37/358) (p<0.01) (Table 6.5.6), however the 'other' occupation group included cadre, businessmen

and housewife had a higher seropositive prevalence of 20% (10/50).

Table 6.5.6: Seropositive comparison of human echinococcosis antibodies in different ethnic groups in Xiji County, Ningxia

	N	Positives	(%)	Negatives	(%)	Chi	*p*
Ethnic							
Han	428	72	16.82	356	83.18		
Hui	517	87	16.83	430	83.17	0.000	0.998
Occupation							
Peasant	537	112	20.86	425	79.14		
Student	358	37	10.34	321	89.66		
Others	50	10	20.00	40	80.00	17.37	0 .000
Subtotal	945	159	16.83	786	83.17		

6.5.3 Discussion (Xiji County, Ningxia)

Xiji County is located in the south mountainous area of Ningxia Hui AR and whose hospital records showed the hydatid patients incidence (combined cased of CE and AE) to be 4.62 per 100,000 from 1994-2001 (Zhao et al., 2001; Yang et al., 2006). The CE prevalence in Xiji by mass screening using ultrasound was reported 1.61% (18/1078) (Li et al., 2005), 1.96% (71/3629) (Yang et al., 2006), and 3.81% (36/945) in the current study. The CE prevalence in females showed no difference with males in some studies (Yang, 2006, and this study), or more female CE cases in other reports (Yang et al., 2006; Zhao et al., 2003). Furthermore, a higher seropositive rate in females was found in the current study. This suggested that females had the same or higher risk than males for CE infection. Other reports showed that the Hui ethnic group had a higher hydatid prevalence than the Han group (Yang, et al., 2006; Zhao et al, 2003; Li et al., 2005), but no difference was found in our study. That might be due to local people living in the same endemic environment and having similar hygienic habits, and there were more people from Han- only areas in others reports. Peasants as agriculturalists had the highest CE prevalence of all occupations in all studies here. This suggested that peasants had more direct contact with the dog-livestock cycle of *E. granulosus* compared with other occupations.

Human AE prevalence has been known to be high in Xiji County since the late 1980s. Local red fox population peaks occurred in that time and might have increaded *E. multilocularis* transmission together with increased dog population in 1980s. Human AE prevalence was higher than CE in Xiji in this study and Yang's report (Yang, et al., 2006). All AE cases in the current study were over 20 year old age. Most of them were provided albendazole treatment since they were late stage cases and possibly too late to be treated by surgical resection.

Suggestions for prevention and control of echinococcosis in Xiji County should be focus on improving public health education level and dog management. A new policy had been carried out in poor mountainous areas in Xiji which moved people to relative rich flatlands in recent two years (from 2011). It would be interesting to carry out assessment of *Echinococcus* transmission in those translocated communities.

6.6 Community study in Ganzi County, Ganzi Tibetan Autonomous Prefecture, Sichuan

6.6.1 Introduction of Study Site

Ganzi County (also called Garze), is one of 18 counties in Ganzi Tibetan Autonomous Prefecture (TAP), Sichuan Province, P.R.China (Fig. 6.6.1). Ganzi is located northwest of Ganzi TAP, upper reaches of Yalong River, with longitude E99°08′-100°25′, latitude N31°24′——32°54′, elevation 3325-5688 metres, covers 7303 square kilometers. Ganzi County had a population of 57,557 in 2006 which are mostly Tibetans (54867, 95.3%) involved with livestock husbandry. About 48.5% of the county is pasture area with agriculture in 31.9% of valley area (mainly in Laima and Tuoba Communes), and 19.6% of area classed as semi-pasture and semi-agriculture areas. Ganzi County owns comprising 1 town and 21 communes (villages). The weather is dry and cold, with an average annual temperature of 5.6°C, ranging from -4.4°C in January and 14.4°C in July. The frost-free period is 35-75 days, annual rainfall 636.5 mm and annual sun time 2640.8 hours. An epidemiological survey in Ganzi County was carried out in May 2006 (Fig. 6.6.2).

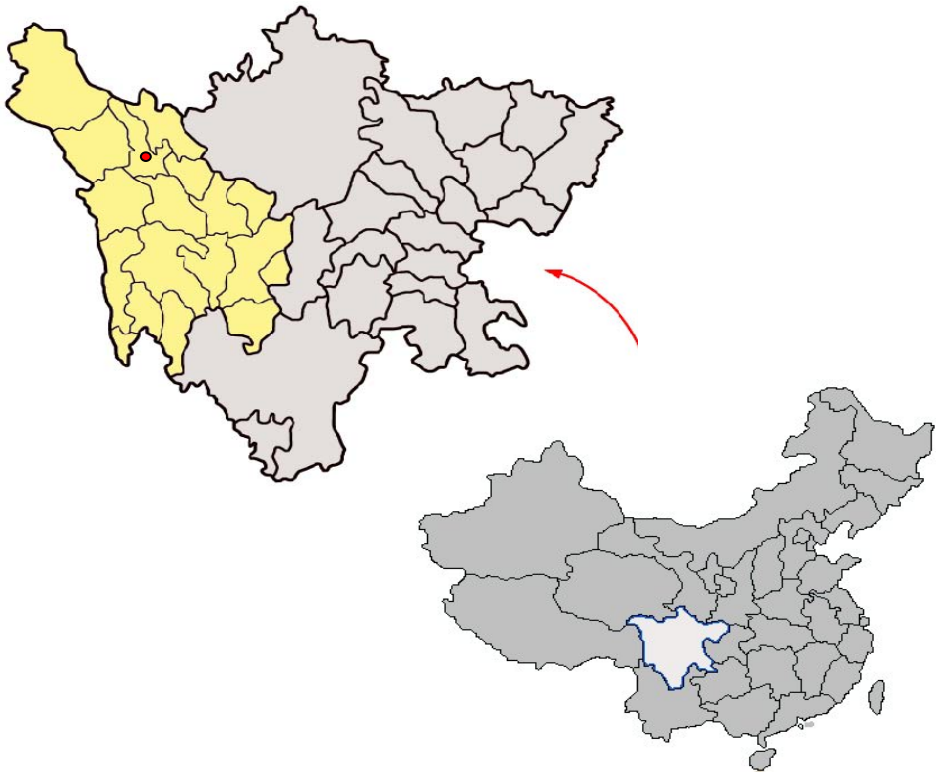

Fig. 6.6.1: Ganzi County (red dot) in Ganzi Tibetan Autonomous Prefecture (yellow) in Sichuan Province, southwestern China.

Fig. 6.6.2: Map of Ganzi County (1:350000)

6.6.2 Results

In total 1655 individuals were screened by portable ultrasound, which including 723 males and 942 females, ages ranged from 1 to 86 years old and average age was 38 yrs old. Among them, a total of 1578 individuals were also screened by serological DIGFA test (see Section 6.0).

A total of 13 CE and 30 AE cases were found by ultrasound and 39 isolated calcification cases were detected as well. Among them, 10 CE and 26 AE cases came from 7 and 10 communes in Ganzi County and other 3 CE and 4 AE cases came from Dege and Shiqu Counties (Table 6.6.1).

Table 6.6.1: Human CE or AE cases were detected in the community study in Ganzi County, Ganzi Tibetan Autonomous Prefecture, Sichuan Province, P. R. China

Area	Communes	Populations	Screened	CE	AE	Calcifications	Normal
Chengguan	Ganzi	10602	519	4	8	7	500
(Yalong)	Gala	1989	214	0	0	2	212
Shengkang	Nanduo	1336	24	0	1	0	23
	Shengkang	2068	78	1	1	2	74
	Gonglong	1505	121	0	0	1	120
	Zhake	3019	136	1	3	3	129
Rongbacha	Laima	3279	65	1	0	3	61
	Xise	2233	85	0	1	2	82
	Kagong	1510	32	0	0	1	31
	Renguo	1685	35	0	0	1	34
Tuoba	Sexidi	2114	21	0	1	0	20
	Tuoba	2610	74	0	0	0	74
	Si'e	2802	19	0	0	1	18
	Tingka	1936	65	0	1	2	62
	Xiaxiong	2237	110	1	2	10	97
Donggu	Sitongda	2566	1	0	0	0	1
	Duoduo	1309	0	0	0	0	0
	Nike	1501	0	0	0	0	0
Datongma	Chazha	2687	5	1	2	1	1
	Dade	2597	0	0	0	0	0
	Kalong	2358	0	0	0	0	0
	Chalong	1760	16	1	6	1	8
Dege County			29	2	4	1	22
Shiqu County			4	1	0	0	3
Baiyu County			2	0	0	0	2
Xinlong County			2	0	0	0	2
Others			8	0	1[a]	1	6
Total			1665	13	30	39	1582

[a] : query

i) Ultrasound and seropositive prevalence by region.

There were no significant differences for human CE or AE ultrasound prevalence in different areas of Ganzi County (P>0.05) (Table 6.6.2). But lower sero prevalence was found in Tuoba Commune compared to other areas (P<0.01) (Table 6.6.3).

Table 6.6.2: Human CE or AE prevalence by abdominal ultrasound in three different areas (excluding Datongma) of Ganzi County

Areas	CE	AE	Query	Calcification	Normal	Total
Chengguan	4(0.5%)	9(1.2%)	0(0)	9(1.2%)	735	757
Rongbacha	3(0.5%)	5(0.9%)	0(0)	13(2.4%)	531	552
Tuoba	1(0.3%)	4(1.4%)	0(0)	13((4.5%)	271	289
Total	8(0.5%)	18 (1.1%)	0(0)	35 (2.2%)	1537	1598

For CE Pearson Chi-Square Value0.171 Asymp. Sig. (2-sided) 0.918, Fisher's Exact Test Table Probability (P) 0.1055 , Pr <= P 1.0000
For AE Pearson Chi-Square Value 0.440 Asymp. Sig. (2-sided) 0.802, Fisher's Exact TestTable Probability (P) 0.0391 Pr <= P 0.7791

Table 6.6.3: Human serological positives by DIGFA in three different areas (excluding Datongma) of Ganzi County.

Areas	Positives (%)		Normal	Total
Chengguan	164	(23.10)	546	710
Rongbacha	149	(28.22)	379	528
Tuoba	105	(8.46)	168	273
Others	23	(35.38)	42	65
Total	441	(27.98)	1135	1576

Pearson Chi-Square Value23.382 Asymp. Sig. (2-sided) 0.000

ii) Relationship of ultrasound with serology.

Using ultrasound as gold standard, the sensitivity of DIGFA in this study was 80% for CE and 92.8% for AE. Also 37.8% (14/37) of calcification lesions showed a seropositive reaction. The specificity of DIGFA for echinococcosis was 72% in this study (Table 6.6.4).

Table 6.6.4: Human serological positives by DIGFA in CE, AE, query, calcification and normal cases of Ganzi County, Ganzi Tibetan Autonomous Prefecture, Sichuan Province, P.R.China

Ultrasound	Positives					Negative	Total
	EgCF	EgP	EgB	Em2	Any dot		
CE	8 (80%)	8 (80%)	7 (70%)	1 (10%)	8 (80%)	2 (20%)	10
AE	24 (85.7%)	24 (85.7%)	25 (89.3%)	25 (89.3%)	26 (92.8%)	2 (7.1%)	28
Query	1 (100%)	1 (100%)	1 (100%)	1 (100%)	1 (100%)	0	1
Calcification	12 (32.4%)	14 (37.8%)	5 (13.5%)	4 (10.8%)	14 (37.8%)	23 (62.2%)	37
Normal	325 (21.7%)	379 (25.3%)	90 (6%)	42 (2.8%)	392 (26.1%)	1108 (73.9%)	1500
Total	370 (23.5%)	426 (27.0%)	128 (8.8%)	73 (4.6%)	441 (28.0%)	1135 (72.0%)	1576

iii) Prevalence and seropositives by gender

Females had a higher CE ultrasound prevalence, 1.2% (11/942) than males (0.3%, 2/723, $p<0.05$) but there was no significant difference between females and males for AE prevalence (both 1.8%) (Table 6.6.5). Also females had a seroprevalence of 31.38% (279/889) which was significantly higher than males (23.58%, 162/687) ($p<0.01$) (Table 6.6.6).

Table 6.6.5: Human CE or AE prevalence in different genders by abdominal ultrasound in community study in Ganzi County, Ganzi Tibetan Autonomous Prefecture, Sichuan Province, P.R.China

Gender	CE	AE	Query	Calcification	Total
Male	2(0.3%)	13(1.8%)	1(0.1%)	15(2.1%)	723
Female	11(1.2%)	17(1.8%)	0	24(2.5%)	942
Total	13(0.8%)	30(1.8%)	1(0.1%)	39(2.3%)	1665

CE: Pearson Chi-Square Value4.193 Asymp. Sig. (2-sided) 0.041
Ae&gendersPearson Chi-Square Value0.000 Asymp. Sig. (2-sided) 0.992

Table 6.6.6a: Serological positives in different genders by DIGFA test in community study in Ganzi County, Ganzi Tibetan Autonomous Prefecture, Sichuan Province, P. R. China

Areas	Seropositiveagainst antigen					Negative	Total
	EgCF	EgP	EgB	Em2	Any dot		
Male	130(18.9%)	157(22.8%)	51(7.4%)	30(4.4%)	162(23.6%)	525(76.4%)	687
Female	240(27.0%)	269(30.2%)	77(8.7%)	43(4.8%)	279(31.4%)	610(68.6%)	889
Total	370(23.5%)	426(27.0%)	128(8.8%)	73(4.6%)	441(28.0%)	1135(72.0%)	1576

Table 6.6.6b: Serological positives in different genders by DIGFA test in community study in Ganzi County, Ganzi Tibetan Autonomous Prefecture, Sichuan Province, P.R.China

DIGFA	Male	Female	Total
Positives	162 (23.58%)	279 (31.38%)	441 (27.98%)
Negatives	525	610	1135
Total	687	889	1576

Pearson Chi-Square Value11.708 Asymp. Sig. (2-sided) 0.001

iv) Ultrasound prevalence and seroprevalence by age.

Human CE ultrasound cases were 0.78% (13/1665) in Ganzi County which occurred in >20 years old group in the current study. Human AE ultrasound cases were 1.8% (30/1665) and happened in all age groups. There were no significant difference by age groups for both CE and AE (p>0.05) (Table 6.6.7).

Table 6.6.7: Human CE or AE prevalence in different age groups by abdominal ultrasound in community study in Ganzi County, Ganzi Tibetan Autonomous Prefecture, Sichuan Province, P.R.China

Age	CE(%)		AE(%)		Query		Calcification		Normal	Total
0-19	0	0.00	1	0.35	0	0.00	5	1.74	282	288
20-39	5	0.81	9	1.45	0	0.00	11	1.77	595	620
40-59	4	0.71	16	2.83	0	0.00	13	2.30	533	566
60-	4	2.09	4	2.09	1	0.52	10	5.24	172	191
Total	13	0.78	30	1.80	1	0.06	39	2.34	1582	1665

Fisher's Exact Test for CE prevalence, Monte Carlo Sig. (2-sided) 0.082
Pearson Chi-Square for AE prevalence Value7.327 Asymp. Sig. (2-sided) 0.062

There was significant difference for seroprevalence between age groups ($p<0.05$). Age >40 years old groups had a higher seropositive rate (30.9%-31.8%) compared to other age groups (24.2%-26.8%) (Table 6.6.8).

Table 6.6.8: Serological positives in different age groups by DIGFA test in community study in Ganzi County, Ganzi Tibetan Autonomous Prefecture, Sichuan Province, P. R. China

Age	Positives (%)		Negative	Total
0-19	72	(26.77)	197	269
20-39	144	(24.20)	451	595
40-59	170	(31.84)	364	534
60-	55	(30.90)	123	178
Total	441(28.0%)		1135(72.0%)	1576

Chi-Square Tests Pearson Chi-Square Value9.103 Asymp. Sig. (2-sided) 0.028

v) Ultrasound prevalence by ethnic group

All CE cases in the current study were Tibetan ethnic group. Meanwhile 27 AE cases were Tibetan (1.8%) and other 3 AE cases were Han ethnic (1.6%). There was no significant differences between ethnic groups for both CE and AE ($p>0.05$) (Table 6.6.9).

Table 6.6.9: Human CE or AE prevalence in different ethnic groups by abdominal ultrasound in community study in Ganzi County, Ganzi Tibetan Autonomous Prefecture, Sichuan Province, P. R. China

Ethnic	Normal	CE	AE	Query	Calcification	Total
Tibetan	1393	13(0.9%)	27(1.8%)	1(0.1%)	38(2.6%)	1472
Han	185	0	3(1.6%)	0	1(0.5%)	189
Hui	1	0	0	0	0	1
Qiang	2	0	0	0	0	2
Yi	1	0	0	0	0	1
Total	1582	13(0.8%)	30(1.8%)	1(0.1%)	39(2.3%)	1665

For CE, Chi-Square TestsContinuity Correction Value.767 Asymp. Si g. (2-sided) 0.381
For AE, Chi-Square TestsContinuity Correction Value.000 Asymp. Sig. (2-sided) 1.000

vi) Ultrasound prevalence by occupation

Other occupations (businessmen, housewife, etc.) had the highest ultrasound CE prevalence (3.7%, 3/73) and followed by herdsmen (1.4%, 2/115), farmers (0.9%,

7/750) and cadres (0.3%, 1/300) ($p<0.05$). However, Herdsmen were found to be the highest ultrasound AE prevalence (6.4%, 9/115) ($p<0.01$) (Table 6.6.10).

Table 6.6.10: Human CE or AE prevalence in different occupations by abdominal ultrasound in community study in Ganzi County, Ganzi Tibetan Autonomous Prefecture, Sichuan Province, P.R.China

Occupations	Normal	CE	AE	Query	Calcification	Total
Farmers	750	7(0.9%)	11(1.4%)	0(0)	14(1.8%)	782
Herdsmen	115	2(1.4%)	9(6.4%)	0	14(10%)	140
Farmer & Herdsmen	75	0	1(1.3%)	0	3(3.8%)	79
Cadres	300	1(0.3%)	5(1.6%)	0	3(1.0%)	309
Students	269	0	1(0.4%)	0	3(1.1%)	273
Others	73	3(3.7%)	3(3.7%)	1(1.2%)	2(2.4%)	82
Total	1582	13(0.8%)	30(1.8%)	1(0.1%)	39(2.3%)	1665

For CE, Fisher's Exact Test Value9.690 Monte Carlo Sig. (2-sided) .041
For AE, Fisher's Exact Test Value17.221 Monte Carlo Sig. (2-sided)0 .002

6.6.3 Discussion (Ganzi County, Ganzi Tibetan Autonomous Prefecture, Sichuan)

Ganzi County in Tibetan Sichuan was known as a high endemic area for both human CE and AE (Qiu et al., 2000). The ultrasound prevalence of CE was reported as 0.91% (25/2748) and AE 1.42% (39/2748) (Qiu et al., 2000). More extensive survey in Ganzi and Aba TAP during 2001-2008 showed a human ultrasound prevalence of 3.2% for CE, 3.1% for AE and 0.04% for dual infection (both CE and AE) (Li et al., 2010), By district that study obtained a prevalence of CE 0.91%(5/549), AE 3.83% (21/549), dual infection 0% in Kalong; CE 2.61% (16/614), AE 6.35%(39/614) ie, total prevalence 8.96% in Chalong; CE 2.59% (3/116), AE 2.59% (3/116) and total 5.17% in Chaza; CE 1.63% (2/123), AE 3.25% (4/123) and total 4.88% in Dade (Li et al., 2010). Dual infection (CE & AE) occurred in Shiqu and Seda Counties in that study (Li et al., 2010).

The current study showed that the ultrasound prevalence in Ganzi County was 0.8% (13/1665) for CE and 1.8% (30/1665) for AE which is similar to the previous study of Qiu et al. (2000). The distribution of human CE indicated that females, persons older than 20 years and occupation as herdsmen were risk factors. This

was similar to previous studies (REF). The highest prevalence of AE occurred in the 40-59 years age group in the current survey.

Echinococcosis in the Tibetan population had been confirmed to have high endemicity in northeast Tibet AR, southeast Qinghai and western Sichuan. The reason could be variable since both CE and AE were all in very high prevalence in same area. *E. granulosus* which is transmitted through a dog-livestock cycle exists in most pastoral area in western China. The higher endemic areas for CE included Xinjiang, Inner Mongolian, Gansu, Ningxia, Qinghai, Tibet and west Sichuan. Furthermore, *E. multilocularis* which is transmitted primarily in wildlife cycles such as rodents - fox, also has high transmission in western Sichuan. A similar situation of co-endemic CE and AE areas was also in Ningxia but the later changes in dog ownership and expansion of agriculture has reduced numbers of new AE cases after that period (Pleydell et al., 2010). Such high AE prevalences might suggest the *E. multilocularis* transmission was very common. Dogs infected with *E. multilocularis*, which might occurr through dogs predating small mammals such as plateau pika, become the key role for human infection. Dog ownership is very common in Tibetan populations since household guard and livestock herding are needed for their pastoral lifestyle. Stray dogs were also very common in these areas because Tibetan monks and local people would like to feed them according their religious beliefs. Poor hygiene habit may also contribute to the high infection rate. Unwashed hands, eating with hands, living close to dogs, are all risk factors commonly in Tibetan areas.

Using ultrasound as gold standard, the sensitivity of DIGFA in the curren study was 80% for CE and 92.8% for AE; meanwhile the specificity of DIGFA for echinococcosis was 72%. Also 37.8% (14/37) of calcification lesions showed a seropositive reaction. Lower sensitivity (70%-80%) and fewer false positives (6% for AgB and 2.8% for Em2) occurred when using single antigen for diagnosis in the current study. The paralleled combination of four antigens could supply more information for immunodiagnosis than single antigen test.

Ultrasound and the DIGFA serological test were easy to carry out in rural areas of Ganzi County since the local hospital owned a portable ultrasound and had a clinical laboratory. But basic training should be a regular work for local hospital since their staff was fewer and always moved to other areas. Residents' health education was also a big problem since not all parents sent their children to school.

Traditional behavior of local people might request in their asking help from the local temple but not the hospital. Higher prevalence in local residents might suggest that develop the health education programme in Tibetan teaching systems so that the students could tell people to get medical check and treatment in time.

6.7 Community study on echinococcosis in Dangxiong County, Lhasa Prefecture, Tibet Autonomous Region, P.R.China

6.7.1 Introduction

Dangxiong County (also called Damxung) is one of seven counties of Lhasa Prefecture (Fig. 6.7.1), Tibet Autonomous Region (TAR), P.R. China. Dangxiong is located 90°45' -- 91°31' E and 29°31'--31°04' N, in the middle of Tibet AR and by the side of the Lhamo Namco Holy Lake, 170 kilometres north of Lhasa City, and borders with Naqu Prefecture (Fig. 6.7.2). The elevation of Dangxiong is over 4200m and the top peak of Nyainqentanglha Mountain is 7111m. The county covers an area of 10,036 square kilometres with a population of 41,918 (2003). (http://www.lasa.gov.cn/gb1/diqu/dangxiong/index.htm).

Fig. 6.7.1: Lhasa Prefecture in Tibet Autonomous Region, P.R. China

Fig. 6.7.2. Damxung (Dangxiong) County located in north of Lhasa Prefecture, Tibet AR

Dangxiong County has 98.8% Tibetan population in its 6 communes and 2 towns, including 7442 house-holds in total of which 6312 house-holds (37,616, 92.73% of total population) owned livestock. Damxung means "selected pasture" in the Tibetan language. The county depends on livestock breeding, and the many domestic animals include goats, sheep, yaks, cattle and horses. Average annual temperature is 1.3°C, the mean temperature in the coldest month (January) is -10.4°C and the warmest in July l0.7°C; lowest temperature -32.5°C (16th of January, 1981) and highest 26.5°C (8th of June, 1995). The annual amount of sun is around 2880 hours, and annual rainfall is 456.8mm. In the area, the Tibetan fox (*Vulpes ferrilata*), wild yak (*Bos grunniens*), wild ass (*Equus*), Blue Sheep (*Pseudois nayaur*), and small mammals such as plateau pikas (*Ochotona curzoniae*) and marmot (*Marmota himalayanus*) are common in Dangxiong County. ‘’Winter-worm’’ or Chinese caterpillar fungus, Xuelian, Fritillaria, and other valuable traditional Chinese/Tibetan medicines are commercially important and part of local production.

Tibet AR is a known endemic area for human CE (Gong, 2001; Jiang 2003; Moh, 2005). A skin test survey (Casoni’s test) carried out in Dangxiong and Mozugongka (Maizhokunggar) Counties, identified 734/20160 (3.65%) Casoni positives of which 48 CE cases were confirmed by ultrasound; 33/48 CE cases were given surgical treatment (Hu et al., 1999).

In the current study, a community survey including questionnaire registration, ultrasound scanning and rapid serological test (DIGFA, see Chapter 3) were carried out in two communes (Wuma and Ningzhong) and one town (Yangbajin) in Dangxiong County in October 2006 (Fig. 6.7.3). Self-selected volunteers from the county (n=557) included 212 males and 345 females, 532 came from above areas, and 25 from other communes excluding Namucuo (Namco) Commune. In total 488 people agreed to provide 2-3ml venous blood per person for rapid antibody DIGFA test, and 165 among those were also tested by standard ELISA in Urumqi. Among the ultrasound confirmed CE cases, 11 cases were given surgical treatment and albendazole post-surgery in Lhasa City Hospital by a surgical group comprising the First Teaching Hospital of Xinjiang Medical University and Lhasa City Hospital. In total 39 cases with active CE cysts were give albendazole tablets for 6 month treatment courses. Surgical and chemotheraphy treatment were financially supported by China Soong Ching Ling Foundation (Beijing, China).

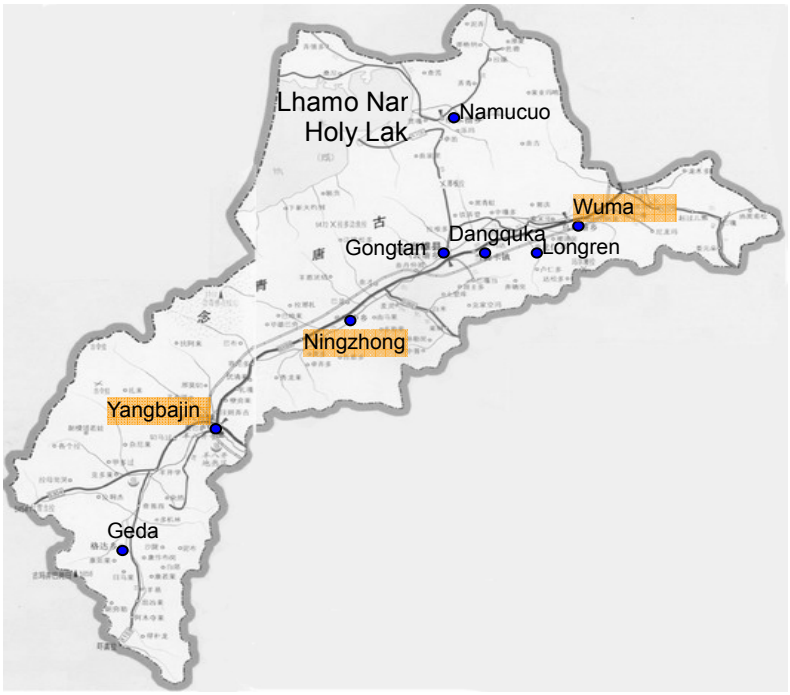

Fig. 6.7.3: Main screened communes and town in Dangxiong County, Lhasa Prefecture, TAR

6.7.2 Results

6.7.2.1 Prevalence of of human CE in Dangxiong County, Lhasa Prefecture, Tibet AR

In total 55 (9.87%) human CE cases were detected in 557 people from Dangxiong County. More CE cases was identified in Wuma (10/89, 11.2%) and Ningzhong (29/295, 9.83%) communes than Yangbajin (8/148, 4.8%) (Table 6.7.1). Of the cases, 33 CE cases were first identified in a previous screening by another study in 2003 (unpublished) and those persons came over for further check and treatment (5 of them did not have an ultrasound CE image this time and they did not accept any treatment during 2003-2006, they were considered as non CE cases). Our study also showed 26 (5.6%) cases of human CE in 463 screened people, were not included all the follow-up of CE cases found in 2003. In total 13 CE cases had evidence of effective for surgical treatment (without active cystic lesion by ultrasound) among 27 CE cases that had a historic CE surgery in Lhasa or other hospitals (local county hospital could not do CE surgery). More CE cases occurred in females 11.6% (40/345) than in males 7.1% (15/212) (Table 6.7.2 and Fig. 6.7.4). The youngest (7 yrs old) and oldest (85 yrs old) CE cases were females; the 7-yrs old had an operation during our survey, the 85-yrs old was too ill to be moved and presented with many large CE cysts (max cyst size 14.2 cm, full of her whole abdominal cavity and only part of right liver lobe could be seen under ultrasound). This latter case was given oral albendazole for a 6-month course.

No human AE cases were identified during the current survey in Dangxiong County.

Table 6.7.1: Dangxiong population and ultrasound CE prevalence by the community/town.

Commune/Town	Population [a]	No	CE	CE surgery history	Prevalence (%)
Dangquka Town	4182	5	3	3	-
Yangbajin Town	4348	148	8	4	4.8
Wuma Commune	5793	89	10	4	11.2
Gongtang Commune	5147	7	2	1	-
Ningzhong Commune	7879	295	29	13	9.8
Geda Commune	3719	1	1	ABZ	-
Longren Commune	3810	12	2	2	-
Namucuo Commune	4291	0	0	0	-
Total	39169	557	55	27	9.6

[a] Commune/town population data was based on local data in 2000.

Table 6.7.1a: Ultrasound CE prevalence by communes/town Crosstabulation

Ultrasound	Communes/Town				Total
	Wuma	Ningzhong	Yangbajin	Others	
CE	10	29	8	8	55
Non CE	79	266	140	17	502
Total	89	295	148	25	557

Pearson Chi-Square Value17.260 Asymp. Sig. (2-sided) 0.001

Table 6.7.2: Ultrasound CE prevalence by age and gender

Ages (yrs)	No	Male			Female		
		No	CE	Normal	No	CE	Normal
<20	74	31	1(3.2%)	30	43	4(9.3%)	39
20-39	233	78	9(11.5%)	69	155	17(11.0%	138
40-59	169	68	4(5.9%)	64	101	12(11.9%)	88
≥ 60	81	35	1(2.8%)	34	46	7(15.2%)	39
Total	557	212	15(7.1%)	197	345	40(11.6%)	305

Table 6.7.2a: Ultrasound CE prevalence by gender Crosstabulation

Ultrasound	Gender		Total
	Male	Female	
CE	15	40	55
Non CE	197	305	502
Total	212	345	557

Pearson Chi-Square Value3.013, Asymp. Sig. (2-sided) 0.083 Continuity Correction 2.526, p 0.112 Fisher's Exact Test Exact Sig. (2-sided) 0.107

Table 6.7.2b: Ultrasound CE prevalence by age group crosstabulation

Ultrasound	Ages				Total
	0-19	20-39	40-59	>=60	
CE	5	26	16	8	55
Non CE	69	207	153	73	502
Total	74	233	169	81	557

Chi-Square Tests Pearson Chi-Square Value 1.272 Asymp. Sig. (2-sided) 0.736

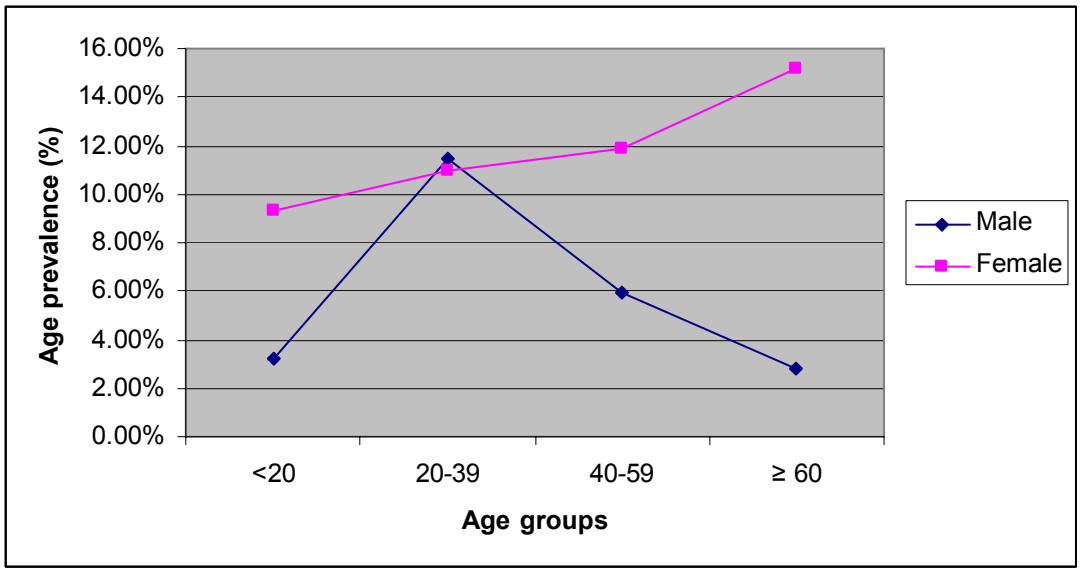

Fig. 6.7.4: Ultrasound CE prevalence by age- and gender in Dangxiong County, Lhasa Prefecture, Tibet AR. (n=55)

6.7.2.2. Serological prevalence by DIGFA

In total 488 serum samples were tested by rapid DIGFA during the survey within one hour of ultrasound scanning. The results showed 17 (32.7%) sero-positives in 52 CE cases, including 12 positives in 23 ‘’active’’ CE type 1 to type 3, 1 positive in 16 ‘’non active’’ CE type 4-5, and 4 positives in 13 post operative cases (4 positives were provided surgical treatment in 2003 and 2005) (Table 6.7.3 to 6.7.4). An ELISA test was carried out in Urumqi which had higher sensitivity than DIGFA (Chapter 3), and showed 43 (86%) seropositives of 50 CE cases (Table 6.7.5). However the specificity of ELISA 78.3% (90/115) was lower than DIGFA (96.8%, 422/436) (Table 6.7.3 and Table 6.7.5). There was a single Em2 positive in DIGFA (0.2%, 1/488) but in ELISA12.1% (20/165). Em2 seroprevalence by DIGFA in Dangxiong (0.2%) was lower than in Dingqing County of Changdu Prefecture (11.2%, 22/195) in all screened people. However Em2 positive responses occurred in 4.2% (7/165) of ultrasound negative CE/AE persons in Dingqing County.

Table 6.7.3: Rapid DIGFA serological test result in Dangxiong County, Lhasa Prefecture, Tibet AR

Antigens	DIGFA results	CE (n=52)	Non CE (n=436)	Total
EgCF	Positive	16	13	29
	Negative	36	423	459
EgP	Positive	16	11	27
	Negative	36	425	461
EgB	Positive	15	6	21
	Negative	37	430	467
Em2	Positive	1	0	1
	Negative	51	436	487
Any antigen	Positive	17	14	31
	Negative	35	422	457
Total		52	436	488

Table 6.7.4: Serological positives in different CE types in Dangxiong County, Lhasa Prefecture, Tibet AR

CE types [a]	No	Serological test by DIGFA and positives					
		No	EgCF Positive	EgP Positive	EgB Positive	Em2 Positives	Anyone positives
T1	8	7	4	5	4	1	5
T2	12	12	4	4	4	0	4
T3	4	4	3	3	2	0	3
T4	12	12	1	1	1	0	1
T5	5	4	0	0	0	0	0
Others [b]	14	13	4	3	4	0	4
Total	55	52	16	16	15	1	17

[a.] CE ultrasound classification according WHO/OIE, 2001
[b.] ultrasound imagines including normal or residual cavity after human CE surgery, 4 cases showed positives in this group were all got surgery treatment in 2003 and 2005.

Table 6.7.5: Serological test by ELISA in Dangxiong County, Lhasa Prefecture, Tibet AR

Antigens	ELISA results	CE (n=50)	Non CE (n=115)	Total
EgCF	Positive	18	2	20
	Negative	32	113	145
EgP	Positive	39	14	53
	Negative	11	101	112
EgB	Positive	25	0	25
	Negative	25	115	140
Em2	Positive	15	5	20
	Negative	35	110	145
Any antigen	Positive	43(86%)	25	63
	Negative	7	90(78.3%)	97
Total		50	115	165

Table 6.7.6: Serological positives by ELISA in different human CE types in Dangxiong County, Lhasa Prefecture, Tibet AR

CE types [a]	No	Serological test by ELISA and positives						
		No	EgCF Positive	EgP Positive	EgB Positive	Em2 positives	Anyone positives	Positive rate (%)
T1	8	6	3	5	5	3	5	62.5%
T2	12	11	6	11	8	5	11	91.7%
T3	4	4	3	4	4	2	4	100%
T4	12	12	4	8	5	4	11	91.7%
T5	5	4	1	1	0	0	1	20%
Others [b]	14	13	1	10	3	1	10	71.4%
Total	55	50	16	16	15	1	42	76.4%

[a.] CE ultrasound classification according WHO/OIE, 2001
[b.] ultrasound imagines including normal or residual cavity after human CE surgery, 4 cases showed positives in this group were all got surgery treatment in 2003 and 2005.

6.7.2.3. Risk factors for human CE in Dangxiong County, Lhasa Prefecture, Tibet AR

Several potential risk factors were analysed for human CE including sex, age, occupation, dog and livestock ownership, home-slaughter, education and income levels, knowledge about CE, and hygiene habits (Table 6.7.2, Tables 6.7.7 to 6.7.11). For occupation, 49 CE cases (9.7%) occurred in herdsmen (504, 90.5% in total 557 surveyed people) and 6 cases (11.3%) in 53 other occupations but with no statistical differences between them ($P>0.05$) (Table 6.7.7). The results also showed no statistical difference between CE prevalence and age, income, education level, or water source (Table 6.7.2, Table 6.7.7 to 6.7.9, Table 6.7.11). However, dog ownership was a significant risk factor ($p<0.01$) (Table 6.7.10). Individual hygiene habits reported was not show the true situation, such as just 110/557 person said they did not washing hand before eating; generally it was higher by observation. Drinking unboiled water was another risk factor, 47 (12.2%) CE cases found in 380 people who drunk unboilded water ($P<0.01\%$) (Table 6.7.10). Eating uncooked vegetables did not show a significant risk since most vegetables were in fact transported from other areas.

Table 6.7.7: Ultrasound CE prevalence by occupation in Dangxiong County

Occupations	CE	Non CE	Total
Herdsmen	49	457	506
Farmer	1	3	4
Cadre	1	7	8
Students	2	23	25
Others	2	12	14
Total	55	502	557

Table 6.7.7a: Ultrasound CE prevalence by occupation Crosstabulation

Ultrasound	Occupation		Total
	Herdsmen	Others	
CE	49	6	55
Non CE	457	45	502
Total	506	51	557

Chi-Square Tests Pearson Chi-Square Value 0.225 Asymp. Sig. (2-sided) 0.635
Continuity Correction 0.052.819 Fisher's Exact Test Exact Sig. (2-sided) 0.622

Table 6.7.8: Ultrasound CE prevalence by education level

Education level	CE	Non CE	Total
Illiteracy	33(9.1%)	330	363(65.2%)
Pre-school children	1	1	2(0.4%)
Primary school	20(11.5%)	154	174(31.2%)
Middle school and over	1(5.5%)	17	18(3.2)
Total	55(9.7%)	502	557

Chi-Square Tests Pearson Chi-Square Value 0.372 Asymp. Sig. (2-sided) 0.542

Table 6.7.9: Ultrasound CE prevalence by income

Income level	CE	Non CE	Total
<2000	12	123	135
2000-5000	17	191	208
>5000	26	188	214
Total	55(9.7%)	502	557

Chi-Square Tests Pearson Chi-Square Value 2.069 Asymp. Sig. (2-sided) 0.355

Table 6.7.10: Ultrasound CE prevalence correlation with dog ownership

	Total	CE	Non CE	Chi	*p*
Dog ownership					
Yes	422	52(12.4%)	370		
No	135	3(2.2%)	132	11.724	0.001
Livestock ownership					
Yes	531	55(10.4%)	476		
No	26	0(0%)	26	1.938	0 .097
Home slaughter					
Yes	514	54(10.5%)	460		
No	43	1(2.3%)	42	2.135	0 .108
Water source					
Tap	4	0	4		
Well	38	3	35		
River and stream	515	52	463	0.121*	0.728
Total	557	55(9.7%)	502		

Chi-Square Tests did by combined Tap and well water as cleaner water source and compared with river and stream.

Table 6.7.11: Ultrasound CE prevalence by drinking unboiled water crosses tabulation

US	Drink unboiled water		Total
	Yes	No	
CE	47(12.2%)	8(4.6%)	55(9.7%)
Non CE	337	165	502
Total	384	173	557

Pearson Chi-Square Value7.772[a] Asymp. Sig. (2-sided) 0.005

6.7.3 Discussion (Dangxiong County, Lhasa Prefecture, Tibet AR)

A human CE ultrasound prevalence of 5.6%-9.7% was obtained in Dangxiong County, which is quite high even for northwest China. CE is also endemic in other Tibetan areas around Tibet AR, for example in adjoining Qinghai, Gansu and Sichuan Provinces (Craig, 2003; Schantz et al., 2003; Wang et al., 2006; Moh, 2005; Li et al., 2005, 2010). A mass screening study in three Tibetan Autonomous Prefectures of Qinghai province showed 6.5% (243/3703) human CE prevalence (Schantz et al., 2003). Prevalence of CE from 1.8% to 4.0% was reported in three Tibetan prefectures around Qinghai Lake (Wang et al., 2006) and higher human CE prevalence (7.55%) occurred in the south high plateau of Qinghai Province (Wang et al., 2000). High CE endemic areas in Sichuan Province showed 4.8% (60/1291 in 1998) to 6.8% (216/3199 in 2005) in Shiqu County, 2.04% in Baiyu and Seda County, 0.91% (25/2748 in 1998) in Ganzi County, all in Ganzi Tibetan Autonomous Prefecture of Sichuan Province (Qiu et al., 2000; Yu et al., 2005; Li et al., 2005, 2010). Hospital cases in Aba Tibetan and Qiang Autonomous Prefecture in Sichuan showed human CE endemic in this eastern Tibet plateau area including counties of Aba, Rangtang, Hongyuan, Ruoergai and Xiaojin (Chen and Qiu, 1996; Renzhen et al., 2006; Liu 2006). Human CE was also shown endemic in all 7 prefectures in Tibet AR, especially higher in ‘’livestock husbandry’’ areas, such as Naqu, Changdu, Lhasa, Shannan (recorded CE cases near or over 100). Naqu Prefecture is a high CE endemic prefecture in TAR with the most hospital CE cases reported (n=709) for the Tibet AR (Gong et al., 2001).

Interestingly no human AE cases were confirmed in this community study in Dangxiong County. The one sero-positive for Em2 by DIGFA 0.2% (1/488) was confirmed ultrasound image with CE not AE. Furthermore, in 12.1% (20/165) Em2 positives by ELISA, 14 were CE cases, 1 had a CE surgery history and 5 were ‘normal’. Thus it would appear that human AE is rare or not present in Dangxiong County of TAR. Tibetan foxes (*Vulpes ferrilata*) and small mammals however appeared common around Dangxiong County, but despite this transmission of *Echinococcus multilocularis* was not found in the current study since no human AE cases and no hospital records proven. The Em2 sero positive rate was low (0.2%) in the DIGFA and is probably due to false positives and cross-reaction with CE cases. Hunting rodents or small mammals by dogs was not observed in this study but was quite common in known AE endemic areas of the eastern Tibetan plateau

eg. Qinghai and Sichuan (Qiu et al., 1995, 2000; Wang et al., Wang et al., 2000; Li et al., 2005, 2010).

Possible risk factors for CE included gender (female), dog and livestock ownership, practise of home-slaughter, using stream/river water and drinking un-boiled water. Most Tibetan people were involved with livestock husbandry in Dangxiong since most areas were not suitable for agriculture at an elevation over 4200 metres. Dog-livestock cycle for transmission of *E. granulosus* exists and is probably maintained since an average 1-3 dogs are owned per household and 14.5 livestock owned per person (total of 232,996 yak, 302,505 sheep, 145,223 goats and 8,063 horses in Dangxiong in 2004). This study showed 422/557 (74.8%) people owned at least 1-3 dogs and some with a long history of dog owning (at least 371 people more than 10 years). Home slaughter was popular and feeding of offal to dogs was a normal practice for most people. Most owned dogs were not tied and stray dogs were common. Most people in rural areas of Dangxiong were still drinking stream or river water (515/527, 92.5%) rather than tap water or from wells. Drinking un-boiled water was common (68.9%), and actually boiling water is difficult due to the high elevation and lack of fuel. Dog ownership and drinking un-boiled water were main risk factors in this study in Dangxiong ($p<0.01$). More CE cases in females might be due to their being the main dog care person (414/421, 98.3%) in most families, and also their special role in routine life (general around the house). Male CE prevalence in the 20-40 years old group was however similar with females, but females exhibited a higher prevalence in all age groups.

6.8 Community screening in Dingqing County, Chamdo Prefecture, Tibet Autonomous Region, P.R. China

6.8.1 Introduction

Dingqing, also called Dengqen, Temgchen, is one of 11 counties in Changdu (Chamdo, Qamdo) Prefecture (one of six prefectures) of Tibet Autonomous Region (TAR), People's Republic of China (Fig. 6.8.1 and 6.8.2). Its location is in west of Changdu Prefecture, northeast of Tibet AR, 800 kilometers from Lhasa, boundary with Leiwuqi (Riwoqe) County of Changdu Prefecture from east; with Baqing, Suo County of Naqu Prefecture from west; with Luolong (Lhorong), Bianba (Banbar) County of Changdu Prefecture from south; with Zaduo and Nangqian County in Qinghai Province from north. The average elevation of Dingqing is 4000 meters (lowest 3500m in southeast (Luohe Village in Nujiang River valley) and highest Bujagangri mountain top is 6328m) above sea level, with longitude 94°39' - 96°17', latitude 31°01' - 32°21', 11,365 km^2 including most grazing lands, just 110,000 acres agriculture land and 6.7% under forest covering. The annual amount of sun time is around 2450 hours, annual rainfall is 641mm, average temperature annually is 3.1 C; the highest recorded temperature in summer (July and August) is 27 C and the lowest is minus 25 C in winter (January and Febrary). (http://www.xzdingqing.com)

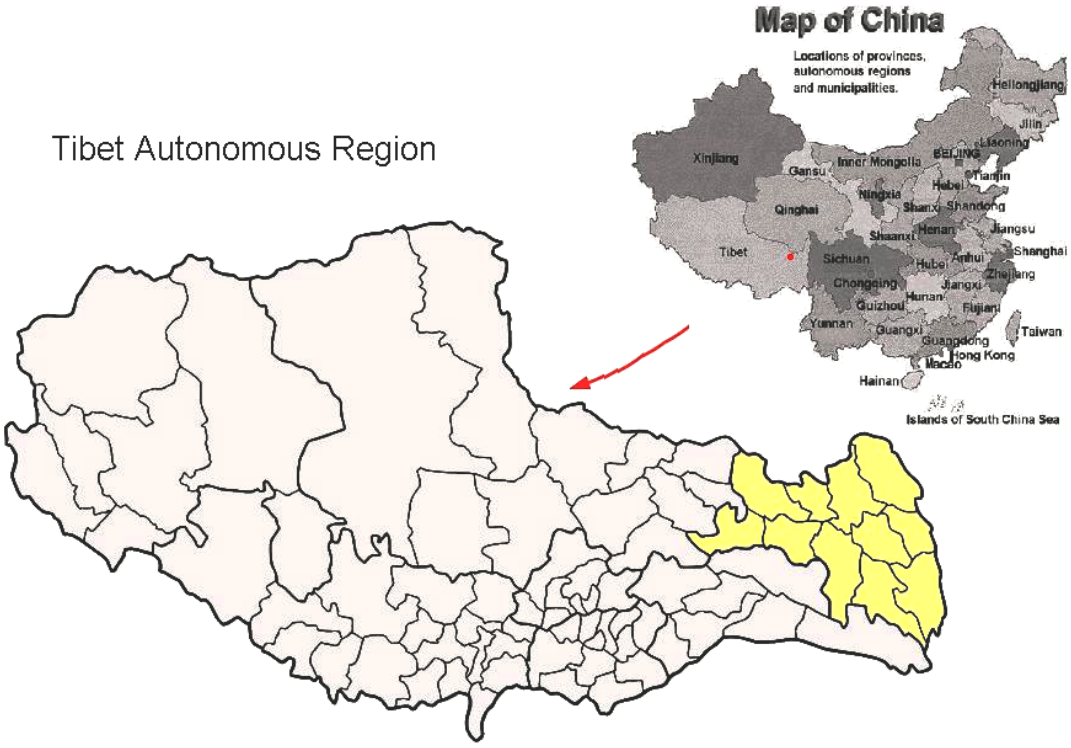

Fig. Fig. 6.8.1: Changdu (Chamdo, Qamdo) Prefecture in Tibet Autonomou Region, P.R. China

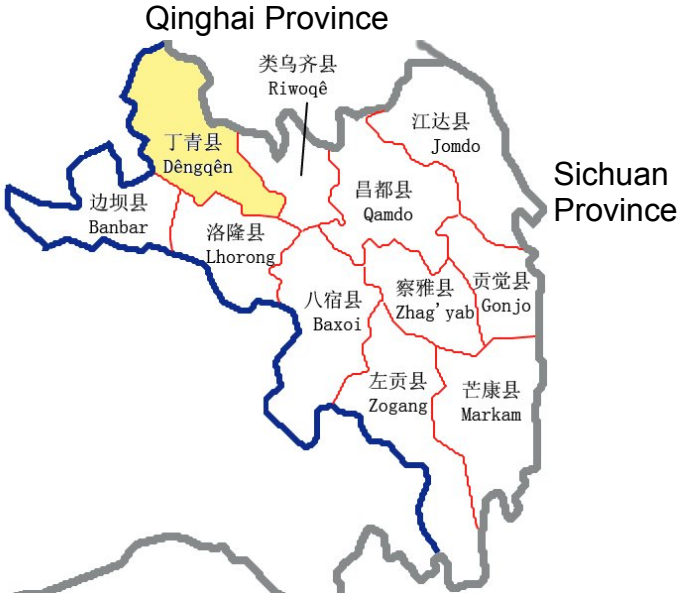

Fig. 6.8.2: Dingqing (Dengqen, Temgchen) County in Changdu Prefecture, Tibetan AR, P.R.China

Population of Dingqing County was 60,000 in 2003 and 62440 in 2006, over 99% Tibetan. Most residents (62143) worked as herdsmen and farmers in 2 towns and 11 communes, 65 villages of Dingqing County. Main kinds of livestock in northwest Dingqing County are yak, cattle, yak-cattle cross breeds, sheep, goats, horses, donkeys, and mules. Main agriculture products are barley, wheat and peas from southeast of Dingqing. About 60% population had been involved with traditional herb ‘’winter worm’’- yartsa gunbu (*Cordyceps sinensis; caterpillar fungus*) collection in the last few years. Domestic dogs and stray dogs are very numerous around towns or villages (approximate 1-3 per household). Among the widespread small mammals are: plateau pikas (*Ochotona curzoniae*), Tibetan fox (*Vulpes ferrilata*), red fox (*Vulpes vulpes*), Himalayan marmots (*Marmota himalayana*), and Tibetan woolly hares (*Lepus oiostolus*).

In total 94 human cystic echinococcosis (CE) cases have been recorded in Changdu Prefecture before 1994 (Luo and Zhao, 1994) and 709 cases all over Tibet AR including 623 from Naqu Prefecture from 1996-2000 (Ze, 2001). 12 cases of alveolar echinococcosis (AE) were first reported in 1993 (Peng, 1988; Yixi, 1992; Luo, 1993; Pu, 1999) in patients involved Lhasa, Naqu and Changdu Prefectures. Community screening in Naqu Prefecture and Dangxiong County in Lhasa Prefecture however did not find evidence for new AE patients. But during a visit to Changdu Prefecture Hospital in 2006, CT scan images with typical AE lesions were found by a hydatid research team from Xinjiang Medical University Hospital. A pilot screening in a village of Jiangda County in Changdu Prefecture found CE cases but no evidence for AE.

6.8.2 Materials and Methods

A pilot community study was performed in Dingqing County of Changdu Prefecture located between Naqu and Changdu (both AE case reported area) for estimate the prevalence of AE in humans in November 2007 by Xinjiang Hydatid Clinical Research Institute (XHCRI) and Tibet AR CDC. From five villages of Seza Commune, one village of Buta Commune and one village of Dingqing town (see Fig. 6.8.3), 232 volunteers came for screening by a questionnaire, serology and ultrasound scanning. *Seza Commune*, locates about 40 km from west of Dingqing Town, 31°32’32.3’’ N, 90°16’05.1’’ E. Its mean elevation over 3927m where has 969 household and population 7761 in 5 villages who work for agriculture, ‘winter-worm’ collection and livestock production. 122 people were screened in all

5 villages. *Buta Commune*, locates in northwest of Seza, 1704km^2, 1928 population in 2 villages called Ruta and Buta. 61 people (all herdsmen) from Ruta Village (20km from Seza, N 31°47'47.5", E 095°21'21.5", mean elevation 4390m) were screened. *Dingqing Town* is the center of Dingqing County, where has 8665 population in 7 villages and engaged in agriculture and livestock production. 49 people (all herdsmen) from Butuo Village (20km from Dingqing Town, N 31°32'10.4", E 95°35'4.2", mean elevation 4273m) were screened.

Fig. 6.8.3: Screened communes/town in Dingqing County, Chamdo Prefecture, Tibet AR

6.8.3 Results

6.8.3.1 Ultrasound prevalence of human CE and AE.

Totally 232 people were registered by questionnaire in Dingqing County and screened by ultrasound and 195 persons accepted serology testing. In total, 11 CE (4.7%), 11 AE (4.7%), 1 mixed CE and AE (0.1%) and 1 suspected AE (0.1%) were diagnosed by ultrasound. In total of 67 (34.3%) were seropositive in 195 individuals. Both CE and AE prevalence in females (6.5% and 7.5%) were higher than in males (3.2% and 2.4%) however no statistical difference were showed between males and females for both CE and AE ($p>0.05$, Table 6.8.1). All CE and

most AE (10/11) cases were occurred in the age groups 20 to 60 years old though this was no statistical different (p>0.05, Table 6.8.2 and Table 6.8.2a). Most cases of CE (10/11), all AE (11) cases, 1 mixed CE/AE, and the suspected AE occurred in farmers and herdsman. However there was no statistical differences between occupations (p>0.05) (Table 6.8.3).

Table 6.8.1: Prevalence of CE or AE by sex by ultrasound diagnosis in Dingqing County, Chamdo Prefecture, Tibet AR

Gender	Ultrasound diagnosis					Total
	Normal	CE	AE	Mixed	Query	
Male	116	4(3.2%)	3(2.4%)	1(0.8%)	1(0.8)	125
Female	92	7(6.5%)	8(7.5%)	0	0	107
Total	208	11(4.7%)	11(4.7%)	1(0.4%)	1(0.4%)	232

Pearson Chi-Square for CE=0.759, *p* value=0.383; Pearson Chi-Square for AE= 2.150, *p* value= 0.143.

Table 6.8.2: Prevalence of CE or AE by sex and age by ultrasound diagnosis in Dingqing County, Chamdo Prefecture, Tibet AR

Ages	Male					Female					Total
	Total	CE	AE	Others	Normal	Total	CE	AE	Others	Normal	
0-10	3	0	0	0	3	7	0	0	0	7	10
11-20	11	0	0	0	11	6	0	0	0	6	17
21-30	33	2	1	0	30	18	0	1	0	17	51
31-40	27	1	0	2[a]	24	29	4	3	0	22	56
41-50	25	0	2	0	23	23	2	2	0	19	48
51-60	9	1	0	0	8	11	1	1	0	9	20
61-70	10	0	0	0	10	11	0	1	0	10	21
71-80	3	0	0	0	3	4	0	0	0	4	7
81-	0	0	0	0	0	2	0	0	0	2	2
Total	121	4	3	2	112	111	7	8	0	96	232

a. including 1 mixed CE/AE,1 suspected AE

Table 6.8.2a: Age- CE Cross tabulation

Age	US result		Total
	CE	Non CE	
0-25	1	50	51
26-50	9	122	131
51-	2	48	50
Total	12	220	232

The SAS: 33% of the cells have expected counts less than 5. Chi-Square may not be a valid test. Fisher's Exact Test, Table Probability (P)=0.0389, Pr <= P 0.4824

Table 6.8.3: Prevalence of CE or AE by occupation with ultrasound diagnosis

Occupation	Normal	CE	AE	Mixed CE/AE	Suspected	Total
Farmer	103	5	4	1	1	114
Herdsman	87	5	7	0	0	99
Cadre	3	0	0	0	0	3
Student	12	0	0	0	0	12
Others	3	1	0	0	0	4
Total	208	11	11	1	1	232

For CE: The SAS System: 60% of the cells have expected counts less than 5. Chi-Square may not be a valid test. Fisher's Exact Test: Table Probability (P)=0.0204, Pr <= P 0.4416
For AE: The SAS System: 60% of the cells have expected counts less than 5. Chi-Square may not be a valid test. Fisher's Exact Test: Table Probability (P)= 0.0481; Pr <= P 0.6334

The CE cases mainly came from Seza and Buta communes and one case from Dingqing town. All the AE cases and the mixed CE/AE, came from Seza and Buta communes. (Table 6.8.4) No statistical differences were found for human CE prevalence between the three communes/town ($p>0.05$) but the ultrasound prevalence of human AE in Buta (7/61, 11.5%) was significantly higher than in Seza (4/122, 1.6%) and Dingqing (0%) ($p<0.05$).

Table 6.8.4: Prevalence of CE and AE in 3 communes/town of Dingqing County, Changdu Prefecture, Tibet AR

Communes /Town	Normal	CE	AE	Mixed CE/AE	Suspected AE	Total
Seza	110	6(4.9%)	4(1.6%)	1(0.8%)	1(0.8%)	122
Buta	50	4(6.6%)	7(11.5%)	0	0	61
Dingqing	48	1(2.0%)	0	0	0	49
Total	209	11(4.7%)	11(4.7%)	1(0.4%)	1(0.4%)	232

Chi-Square Tests for CE 2 cells (33.3%) have expected count less than 5. The minimum expected count is 2.53.Pearson Chi-Square Value1.298 Asymp. Sig. (2-sided) 0.523,

6.8.3.2 Serological (DIGFA) test results

DIGFA test gave 9/10 seropositives in CE cases of which 7/10 seropositive against antigen B. Seropositive AE cases were 10/11 versus antigen Em2. However in abdominal ultrasound negative individuals, 9/172 was seropositive against antigen B and 7/172 seropositive against antigen Em2 (Table 6.8.5). The serological positive prevalence was higher than the abdominal ultrasound CE or AE prevalence ($p<0.01$) (Table 6.8.5a and 6.8.5b).

Table 6.8.5: Serological (rapid DIGFA) results in Dingqing County

Antigens	DIGFA results	CE (n=10)	AE (n=11)	Mixed CE / AE (n=1)	Query (n=1)	Normal (n=172)	Total
EgCF	P	9	10	1	0	47	67
	N	1	1	0	1	125	128
EgP	P	8	10	1	0	38	57
	N	2	1	0	1	134	138
EgB	P	7	7	1	1	9	25
	N	3	4	0	0	163	170
Em2	P	4	10	1	0	7	22
	N	6	1	0	1	165	173
Any one	P	9	10	1	1	48	69
	N	1	1	0	0	124	126
Total		10	11	1	1	172	195

Table 6.8.5a: Ultrasound (CE) and serology crosstabulation

US	Serology (DIGFA)		Total
	positive	negative	
CE	10	1	11
Non CE	59	125	184
Total	69	126	195

Chi-Square Tests1 cells (25.0%) have expected count less than 5. The minimum expected count is 3.89. Pearson Chi-Square Continuity Correction[b] Value13.251 Asymp. Sig. (2-sided) 0.000 Fisher's Exact Test Exact Sig. (2-sided) 0.000

Table 6.8.5b: Ultrasound (AE) prevalence and DIGFA crosstabulation

Ultrasound	DIGFA		Total
	Positive	Negative	
AE	11	1	12
Non AE	58	125	183
Total	69	126	195

Chi-Square Tests. 1 cells (25.0%) have expected count less than 5. The minimum expected count is 4.25 Pearson Chi-Square Continuity Correction[b] Value15.190 Asymp. Sig. (2-sided) .000 Fisher's Exact Test Exact Sig. (2-sided) .000

6.8.3.3 Risk factors for CE or AE

Occupation, education level, income level, dog and livestock ownership, and home slaughter were analyzed by independent test for risk factors vesus CE or AE. No statistical differences were found ($p>0.05$) by occupation, dog and livestock ownership, and home slaughter groups (Table 6.8.3, Table 6.8.6 to Table 6.8.10). Most CE (10/11) and AE (9/11), mixed CE/AE and the suspected AE cases occurred in only 189 dog owners and 194 livestock owners (Table 6.8.7, 6.8.8). Illiteracy and low income were the main risk factors especially for AE ($p<0.05$) (Table 6.8.6 and 6.8.7).

Table 6.8.6: Ultrasound prevalence of human CE or AE by different education level in Dingqing County

Educations	Ultrasound diagnosis					
	Normal	CE	AE	Mixed CE/AE	Suspected	Total
Children under school	5	0	0	0	0	5
Illiteracy	110	7	10	1	1	129
Primary school	83	4	1	0	0	88
Middle school	5	0	0	0	0	5
High school	2	0	0	0	0	2
College and above	1	0	0	0	0	1
Unknown	2	0	0	0	0	2
Total	208	11	11	1	1	232

Table 6.8.6a: Ultrasound CE/AE prevalence by education level

Education	Total	CE	AE	HD	Nomal
Non educated	134	7(6.0%)	11(8.2%)	18	116
Primary school and over	98	4(4.1%)	1(1.0%)	5	93
Chi		0.4116	5.9634	4.3986	
p		0.5212	0.0146	0.0360	
Total	232	11	12	23	209

Table 6.8.7: Ultrasound prevalence of human CE or AE by different income

Income	Ultrasound diagnosis					
	Normal	CE	AE	Mixed CE/AE	Suspected	Total
<2000 RMB	144	9	11	1	1	166
2000-5000 RMB	55	2	0	0	0	57
>5000 RMB	9	0	0	0	0	9
Total	208	11	11	1	1	232

For CE, The SAS System: 33% of the cells have expected counts less than 5. Chi-Square may not be a valid test. Fisher's Exact Test, Table Probability (P) =0.1395, Pr <= P 0.8372. For AE, Fisher's Exact Test: Table Probability (P)=0.0160; Pr <= P 0.0835

Table 6.8.7a: Ultrasound hydatid disease (HD) by income (1)

Income	HD	Non HD	Total
<2000	21	145	166
2000-5000	2	55	57
>5000	0	9	9
Total	23	209	232

The SAS System Chi-Square = 5.0009 *p*=0.0820; Fisher's Exact Test Table Probability (P)=0.0109; Pr <= P 0.1107

Table 6.8.7b: Ultrasound hydatid disease (HD) by income (2)

Income	HD	Non HD	Total
<2000	21	145	166
>2000	2	64	66
Total	23	209	232

The SAS System Chi-Square =4.8938 *p* =0.0270

Table 6.8.8: Independent test for dog /livestock ownership with ultrasound prevalence of human CE or AE

	Total	Normal	CE	AE	Mixed CE/AE	Suspected
Dog ownership						
Yes	189	168	10	9	1	1
No	43	40	1	2	0	0
Chi			0.2316	0.2989		
P			0.7011	1.0000		
Livestock ownership						
Yes	194	173	10	9	1	1
No	38	35	1	2	0	0
Chi			0.139	0.000		
P			0.709	1.000		
Total	232	208	11	11	1	1

For HD, Fisher's Exact Test, Table Probability (P)=0.1897; Two-sided Pr <= P 0.5835

Table 6.8.9: Ultrasound CE /AE prevalence by home slaughter crosstabulation

Ultrasound	Home slaughter		Total	Chi	p
	Yes	No			
CE	11	169	180	0.715	0.398
Non CE	1	51	52		
Subtotal	12	220	232		
AE	8	172	180	0.332	0.565
Non AE	4	48	52		
Subtotal	12	220	232		

Table 6.8.10: Ultrasound CE/AE prevalence by dog & livestock ownership

US	Dog & livestock ownership		Total	Chi	*p*
	Yes	No			
CE	11	160	171		
Non CE	1	60	61	1.242	0.265
Subtotal	12	220	232		
AE	9	162	171		
non AE	3	57	60	0.000	1.000
Subtotal	12	219	231		

6.8.4 Discussion (Dingqing County, Changdu Prefecture, TAR)

Human CE is probably endemic throughout Tibet AR, but especially in Naqu, Lhasa, Shannan and Chamdo Prefectures (Guo et al., 1994; Luo and Zhao, 1994; Gong et al., 2001; Jiang, 2002; Zhou and Xi, 2004; Yin and Wang, 2007). In 1990s, 94 cases of human echinococcosis were reported in Chamdo hospital and 68 human CE cases were specifically recorded recently (Yin and Wang, 1997). In the present study, an initial investigation of clinical echinococcosis cases was carried out in the hospitals in Changdu, and in total 68 cases of hydatid disease and 4 AE was found originating from Changdu Prefecture (Fig. 6.8.4).

Current pilot study confirmed the ultrasound prevalence was 4.7% (11/232) for CE and 4.7% (11/232) for AE in Dingqing County. Seza and Buta Communes are close to Yushu Prefecture of Qinghai Province where both human CE and AE were known to be endemic (Schantz et al., 2003). Our study showed most human CE and AE cases came from these two communes, except one CE case which originated from a village of Dingqing town. Most CE (7/11) or AE (8/11) cases were females with ages from 21 to 60 although there was no statistical difference probably because the screened population was limited.

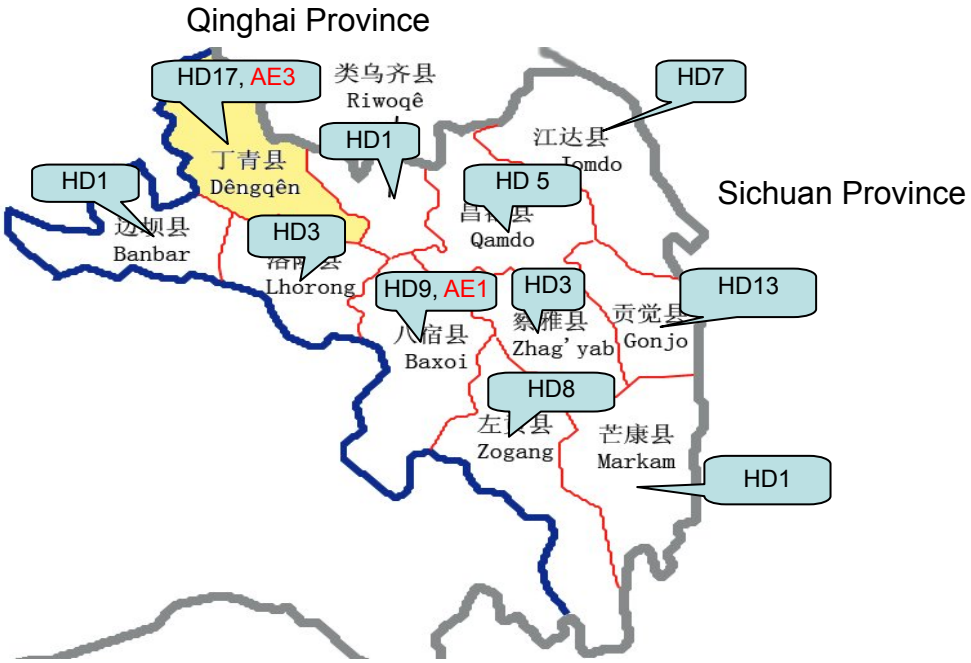

Fig. 6.8.4: Human echinococcosis distribution in Changdu Prefecture (including Dingqing County), Tibet AR.
HD meant hydatid cases (recorded not clear CE or AE);
Four AE cases were diagnosed retrospectively from CT images by Xinjiang Clinical Research Institute.

One case of mixed CE/AE was found in this study, who was male, 33 years old, and a herdsman, non-educated with an annual income less than 2000RMB (～£200). Ultrasound images showed one 10.5cm x 10cm solid AE lesion, one 8.5cm x 8cm CE cyst and 2 smaller CE cysts in his liver. The rapid DIGFA test gave a strong positive against all the four antigens (EgCF, EgP, AgB and Em2).

Possibel risk factors which might related to human CE in Dingqing County of Changdu Prefecture (TAR), included occupation, education level, income, dog or livestock ownership, and home slaughter, however no statistical differences were found (P>0.05). The main reasons might due to the following points: (1) screened population limitation; (2) transmission (dog-livestock) existed and the environment for human life might be contaminated. The main risk of CE for local people might due to several reasons. (1) Dog and livestock ownership were common and stray dogs everywhere, that life cycle for *Echinococcus granulosus* might take eggs all around those area. Feeding offals of livestock to dogs (68.5%, 159/232) and home

slaughter (77.6%, 180/232) were common in Dingqing County. (2) Their water source was mainly from streams (207/232, 89.2%) which might give more chance of egg dispersion. (3) Unhealthy hygienic habits, for example 81.9% (190/232) screened people said they did not have the habit of washing hands before eating. (4) Lower education level and income level: 57.8% (134/232) people never had any education, and 71.5% (166/232) had a lower annual income less than 2000 RMB (280 US$).

General putative risk factors for human AE suggested that education and income level had some influence ($p<0.05$). The life-cycle of *Echinococcus multilocularis* could be maintained between theTibetan fox (*Vulpes ferrilata*) and plateau pikas (*Ochotona curzoniae*) as definitive host and intermediate host respectively in Dingqing County. The landscape is like dry steppe and treeless in Dingqing County and this was typical Tibetan fox habitat and dietary analysis showed 95% pika (*O. curzoniae*) and small rodents (*Pitymus, Alticola, Cricetelus*) in 113 fox scat samples from north west Tibet (Schaller 1998). The traditional 'winter-worm' collection in Spring might be another chance to bring people to rural areas where foxes lived and contact with *E. multilocularis* eggs from fox faeces. Furthermore hunting foxes was restricted in recent years. Dogs as a potential definitive host of *E. multilocularis* might exist due to owned and stray dogs hunting small mammals but the questionnaire responses showed no evidence (copro PCR would performed in future to identify dog's infections). Same risk factors as water source, hygienic habits for CE might relate to AE infections as well.

The curren study had confirmed both CE and AE were endemic in Dingqing County, Changdu Prefecture, Tibet AR. Prevention and control for CE and AE might be difficult due to lower education level and income and poor hygienic habits. Treatment of dogs is also difficult since lots of stray dogs existed. Possibly more health education about CE or AE given to school students, dog treatment with governmental support could be helpful for improving this situation.

6.9 Discussion --- Community studies on human echinococcosis in Tibet Autonomous Region, P.R.China

The Tibet Autonomous Region (TAR) is situated in south-western area of the People's Republic of China, bordered to the north by Qinghai Province and Xinjiang Uygur Autonomous Region from northwest, Sichuan and Yunnan Province to the east, and neighbouring with Myanmar, India, Bhutan and Nepal to the south. Known as the "roof of the world", the Tibet Autonomous Region forms the major part (47%) of the Qinghai-Tibetan Plateau (2.5 million square kilometres). Its varied topography is composed of three different natural areas: the northern Tibet Plateau (two-thirds of the autonomous region) is situated among the Kunlun, Tanggula, Kangdese and Nyainqentanglha mountains; the southern Tibet Valley is located between the Kangdese and Himalayan Mountains, and is drained by the Yarlung Zangbo River and its tributaries; the eastern Tibet Canyon is formed by mountains extending east-west before turning south-north that belongs to the Hengduan Mountain Range.

TAR has an average elevation over 4000 metres above sea level, 1.22 million square kilometres and a population of 2.81 million (lowest population density, 2.3 inhabitants per square kilometre) (China Statistical Yearbook 2007). The regional capital is Lhasa. Tibet AR is the largest Tibetan region in China. Tibetans account for 96% of the region's population. Other groups are Han, Hui, Mongolian, Monba, Lhoba, and others. The total number of Tibetan in China (TAR, Qinghai, Sichuan Gansu and Yunnan) is approximate 6 million.

Tibet Autonomous Region is divided into one prefecture-level city and six prefectures. The prefecture-level city is Lhasa, and the prefectures are Naqu (Nagqu), Ali (Ngari), Linzhi (Nyinchi), Changdu (Qamdo), Shannan and Rikaze (Xigazê).

Human cystic echinococcosis (CE) has been known to be endemic in the TAR since 1987 (Hu *et al.*, 1987), but has continuously been present among of the predominant nomadic pastoral population on the Tibetan plateau for hundred of years. Mass screening programme for echinococcosis, principally using portable ultrasound for abdominal CE or AE, have been undertaken in several Tibetan Autonomous Counties and Prefectures outside the TAR in Qinghai and Gansu Province and in western Sichuan Province (Schantz *et al.*, 2003; Bai 2002; Li *et al.*, 2005, 2010). These studies revealed a significant very high prevalence of both CE

and AE in Tibetan communities in Sichuan and Qinghai with mean prevalence of 4-6% and ranges 1-15% for CE or AE. In 2004, the Chinese Ministry of Health (MoH) carried out a nation-wide assessment of 8 parasitic diseases including echinococcosis (MoH, 2005). The overall ultrasound prevalence (2.4%) by ethnics was in Tibetan communities of TAR, northwest Sichuan and Qinghai province.

Hospital records in TAR showed 709 cases of human CE from three hospitals (Naqu Prefecture Hospital, 1st and 2nd Peoples Hospital of TAR) from 1996-2000 (Gong *et al.*, 2001). In total 623 CE cases were originally from Naqu Prefecture and 76 from Lhasa (35), Shannan (22), Changdu (10), Linzhi (8), Rikaze (4), Ali (2) and other areas (5). 94 cases of echinococcosis (not differentiated as CE or AE) were reported in Changdu Prefecture (Luo and Zhao, 1994), and 116 (1995-2004), 155 (to 2007) or 268 (1988-2002) cases in Shannan (Liao *et al.*, 2003; Peng *et al.*, 2005, Cai *et al.*, 2007).

Several community studies have been carried out in different areas of TAR. Immunodiagnostic positive (Casoni) rate in Tibet AR was 34.9% in a survey in 1988-1991 (Guo *et al.*, 1994). One survey in Dangxiong and Mozugongka County of Lhasa Prefecture in 1999 showed 48 cases of cystic echinococcosis from 734 Casoni's test positive individuals in a test population of 20160 (Hu *et al.*, 1999). In Naidong County of Shannan Prefecture 81 cases of CE from 3379 individuals were detected by ultrasound, IHA and clinical features (Zhou and Xi, 2005).

It appears that a hot spot of human AE occurs in northwest Sichuan and southwest Qinghai which burden eastern TAR (Wang *et al.*, 2005; Li *et al.*, 2005) although there is very little information on human AE in TAR itself. Human AE cases in Tibet AR were first reported in 1987 (1 AE and 174 CE in 1960-1983 in Tibet AR Peoples Hospital) (Hu *et al.*, 1987). Then 2 AE cases (history identified in 1977) were found from Changdu Prefecture (Peng, 1988). Since then, 12 cases of human AE were reported in hospitals in Lhasa and originally from Lhasa, Naqu and Changdu. After that, 2 cranial AE were reported (Pu, 1999; Yixi Jiacuo, 2001) and 3 hepatic AE cases were appeared in a CT image study in Linzhi (Duan *et al.*, 2006).

The current studies of records in the People's Hospital of Changdu Prefecture (TAR), and a pilot community study in Dangxiong County of Lhasa Prefecture, and Dingqing County of Changdu Prefecture (eastern TAR) (2006, 2007), was undertaken by Xinjiang Hydatid Clinical Research Institute with cooperation with

the Centre of Disease Prevention and Control (CDC) of Tibet AR. Community surveys including questionnaire registration, ultrasound scanning and rapid serological test were performed in Dangxiong County in October 2006 and in Dingqing County in November 2007. Volunteered people from Dangxiong were 557 and form Dingqing County were 232, they came for screening by a questionnaire, serology and ultrasound scanning. In total 55 (9.87%) human CE cases were detected in 557 people, and no human AE cases were identified during the current survey in Dangxiong County. Meanwhile, 11 CE (4.7%), 11 AE (4.7%), 1 mixed CE and AE (0.1%) and 1 suspected AE (0.1%) were diagnosed by ultrasound from 232 people in Dingqing County.

Human CE and AE cases were recorded in all of 7 prefectures in Tibet Autonomous Region (Table 1, 2). First reported hospital echinococcosis cases in the People's Hospital of Tibet AR (in Lhasa city) were 174 CE and 1 AE through 1960-1983 but without original patients address (Hu *et al.*, 1987). And serological survey (not mentioned, but main possibility was by Casoni at that period) in Tibet AR showed average 34.9% positive (Guo *et al.*, 1994). In the past 20 years, human CE cases recorded in hospital were over 100 in Naqu (623), Shannan (258), Changdu (94+10+68=172) and not sure in Lhasa (because hospital cases in Lhasa might came from other prefectures due to Lhasa as capital in Tibet AR, which showed 174 CE cases from 1960-1988 and 162 CE (35 from Lhasa) in 2 regional hospitals, 80 CE cases from an Armed Police Hospital) (Hu *et al.*, 1987; Cirendawa and Wang, 2000; Gong *et al.*, 2001; Zhou *et al.*, 2002; Liao *et al.*, 2003; Peng *et al.*, 2005; Yu, 2006; Cai *et al.*, 2007). Cases reported in Rikaze, Linzhi an Ali were very limited but might not really rare in those areas since 9 vagina CE cases (7 cases had hepatic CE and 2 had uterus CE) were reported in Rikaze, and 18 hepatic CE in a CT scan imagine analysis in Linzhi Prefecture Hospital (Hou et al., 2005; Duan et al., 2006).

The human CE cases in above reports and papers in Tibet AR were reviewed in Table 6.9.1.

Table 6.9.1: Human cystic echinococcosis recorded in Tibet Autonomous Region, P.R.China

	Hospital CE			Community		
Prefecture	**Duration**	**cases**	**Refs**	**County**	**CE case**	**Refs**
Naqu	1996-2000	623	Gong *et al.*, 2001			
Lhasa	1996-2000	35	Gong *et al.*, 2001	Mozugongka /Dangxiong Dangxiong	48/20160 (0.2%) 55/557 (9.9%)	Hu *et al.*, 1999 2006
Changdu		94	Luo and Zhao, 1993	Dingqing	11/232 (4.7%)	2007
	1988-1995	70	Lan *et al.*,1999			
	1996-2000	10	Gong *et al.*, 2001			
		68	Yin *et al.*, 2007			
Shannan	1996-2000	22	Gong *et al.*, 2001	Naidong	81/3379(2.4%)	Zhou *et al.*, 2004
	1995-2001	80	Zhou *et al.*, 2002		7/1631(0.4%), 16 query	Shen *et al.*, 2004
	1995-2004	116	Peng *et al.*, 2005	Qusong	11/722(1.5%)	2003
	1988-2002	268	Liao *et al.*, 2003			
	1999-	59	Yu, 2006			
		155	Cai *et al.*, 2007			
Linzhi	1996-2000	8	Gong *et al.*, 2001			
		18	Duan *et al.*, 2006			
Rikaze	1996-2000	4	Gong *et al.*, 2001			
		9	Hou et al, 2005			
Ali	1996-2000	2	Gong *et al.*, 2001			
Not sure	1960-1983	174	Hu et al, 1987			

Human AE prevalence was not as high as CE in Tibet AR. After first reported in 1987, other reports showed 2 cases from Changdo (Peng 1988), 12 cases (3 from Lhasa, others from Naqu and Changdu) (Yixijiacuo, 1992; Luo *et al.*, 1993), 2 cases cranial AE (Pu 1999; Yixijiacuo *et al.*, 2001), and 3 AE cases diagnosed by CT images in Linzhi (Duan *et al.*, 2006).

The human AE cases in above reports and papers in Tibet AR were reviewed in Table 6.9.2.

Table 6.9.2: Human alveolar echinococcosis recorded in Tibet Autonomous Region, P.R.China

Prefectures	Hospital AE		References	Community		References
	Duration	cases		County	AE case	
Not sure		1	Hu, 1987			
Lhasa, Naqu, Shannan and Changdu	1960-1992	12	Luo *et al.*,1993			
Not sure		1	Pu, 1999			
Sichuan Origin		1	Yixijiacuo, 2001			
Changdu	1977	2	Peng, 1988	Dingqing	11/232	2007
	2006	4	Hospital CT imagines			
Linzhi		3	Duan *et al.*, 2006			
Tibet AR					2.76%	MoH, 2005
Total		23			11	

Several community study using ultrasound and serology also found 48 CE cases in Lhasa, 81 echinococcosis cases in Naidong County and 11 CE cases in Qusong County, of Shannan (Table 6.9.1) (Hu *et al.*, 1999; Zhou *et al.*, 2005). No AE cases were found in community studies which have been published. Prevalence of human CE by ultrasound were 9/722 (1.2%), 55/575 (9.9%) and 11/232 (4.7%) in Qusong, Dangxiong and Dingqing and highest was in Dangxiong ($p<0.01$) (Table 6.9.3). Prevalence review of human CE and AE in Tibet AR showed higher CE prevalences occurred in Naqu, Shannan, Changdu and Lhasa; and AE cases were mainly from Changdu, Linzhi and Lhasa (Table 6.9.4).

Table 6.9.3: Community studies for human echinococcosis by ultrasound in Qusong, Dangxiong and Dingqing Counties (2003-2007)

Counties	Population (2003)[a]	No. of screened	CE	AE	mixed CE/AE	Query	Serological positives
Qusong	20,000	722	9(1.2%)	0	0	2(0.3%)	55/722(7.6%)
Dangxiong	41,918	557	55(9.9%)	0	0	0	31/488(6.4%)
Dingqing	60,000	232	11(4.7%)	11(4.7)	1(0.4%)	1(0.4%)	69/195(35.4%)
Total	121,918	1511	75(4.96%)	11	1	3	155/1405(11.0%)

a. Prevalence of human CE in local population was 45/100,000in Qusong, 131.2/100,000 in Dangxiong, 18.3/100,000 in Dingqing and AE prevalence was 18.3/100,000 in Dingqing just from this study.
Chi-Square Tests Pearson Chi-Square Value49.646[a] Asymp. Sig. (2-sided) 0.000

Table 6.9.4: Prevalence review of human CE and AE in Tibet AR

Prefecture	Population (2005) [a]	CE		AE	
		Confirmed cases [b]	Prevalence (1/100,000)	Confirmed cases	Prevalence (1/100,000)
Lhasa	540,500	138	25.5	3	0.6
Naqu	401,871	623	155.0	?	
Ali	86,277	2	2.3		
Rikaze	662,146	13	1.96		
Linzhi	158,167	26	16.4	3	1.9
Shannan	325,063	268 [c]	82.4	?	
Changdu	606,444	182	30.0	17	2.8
Tibet AR	2,770,000	1242	44.8	34	1.2

[a.] population from http://www.xizang.gov.cn until the end of 2005.
[b.] Hospital cases and ultrasound confirmed community cases excluding some cases surgical treated in the recorded hospital in the same period if community studies showed exactly.
[c.] The actual human CE cases in Shannan might be more than 268 (at least other 20 were recorded in Lhasa's hospital), but the data from same hospital were overlapped in different papers, and community study's data did not published on formal magazines, so just choose the most records as a reference.

Human CE cases were found in all seven prefectures of Tibet AR (Gong, *et al.*, 2001, Table 6.9.1 and 6.9.4) with most hospital cases in Naqu, Shannan, Changdu, Lhasa and Linzhi Prefectures. Community studies in Naqu, Lhasa, Changdu and Shannan Prefectures showed highest prevalences by ultrasound (Moh, 2005 and Table 6.9.1). Distribution of human CE cases was mainly located in southeast, east, north and northwest part of Tibet AR, where were located close to the areas

known as high endemic areas, such as southwest of Qinghai, northwest Sichuan Provinces. Previous reports showed that the highest ultrasound prevalence rate for human CE in China were occurred in Tibetan pastoral communities of eastern Tibetan plateau including southwest Qinghai and northwest Sichuan Provinces (Qiu *et al.*, 1999; Schantz *et al.,* 2003; Craig *et al.*, 2004; Moh, 2005).

Transmission of *E. granulosus* in Tibet AR might be due to the distribution of numbers of its major definitive host (dog) and intermediate hosts (domestic livestock mammals, such as sheep, yak, goats, cattle, etc.) (WHO/OIE, 2001, Craig, 2004). Numerous dogs were found in Tibet AR, especially in pastoral areas, which had many uses, such as guard, herd, farm dog, temple dog and also are a suitable companion dog. And adult worms of *E. granulosus* were found in the intestine of dogs in Naqu, Tibet AR (*E. granulosus* infections were reported common in yak, cattle, sheep and goat in Tibet AR (Liu et al., 1994; Zhang et al., 1994; Nimaciren, 1998, Lan et al., 1999).

Chapter 7. General discussion

Echinococcosis is listed as one of the neglected diseases in the world (WHO, 2006). It is difficult to control in many countries due to poor efficacy and prolonged policies or lack of financial support. The vague definition which called hydatid disease for both CE and AE, was widely used in general discussion and scientific papers in western China for many years, although the first AE case was reported in China in 1965. In fact, the transmission lifecycle, clinical diagnosis and treatment are really different between CE and AE. Exact distribution of CE and AE are necessary for differentiating the epidemiological characters between these two kinds of disease and implement efficient prevention and control measures.

A series of epidemiological surveys in this thesis showed there was different prevalence of human CE and AE at regional, prefectural and county level, even local town or commune level. The overall ultrasound prevalence of human echinococcosis in western China was 3.3% (619/18766), with CE 2.73% (513/18766) and AE 0.56% (106/18766) respectively (Table 7.1). Over 80% of human echinococcosis we detected was CE in this study, and CE cases were more than AE cases in most of our study sites. This means CE was endemic in all the study sites, and some counties had relatively higher CE prevalence over 5%. These high endemic areas were: Hejing and Hoboksar in Xinjiang, Xiji in Ningxia, Dangxiong and Dingqing in Tibet AR.

The prevalence of human AE was however rather different. There was large variation in AE prevalence among different counties, for example sporadic AE cases in Xinjiang and high prevalence (>3%) in some counties in Ningxia, Sichuan and Tibet AR. The remarkable data was a relatively centralized focus of human AE cases reported in Shiqu County of Ganzi Tibetan Autonomous Prefecture in Sichuan in recent few years (Li et al., 2006, 2010). Dingqing County in Tibet AR is now reported from the current study as an AE endemic area for the first time. The reason might due to its close location and similar transmission ecological landscape with other AE endemic area such as Yushu Tibetan AP in Qinghai and Shiqu County in Sichuan.

We also found the prevalence in one location or place might change from one

period to another time. The temporal change of echinococcosis transmission has been considered by ecologists and they found landscape changes might cause increase in small mammal populations and eventually outbreak of AE since the life-cycle of *E. multilocularis* will be favoured by suitable small mammal and fox habitats (Giraudaux, et al, 2006).

Table 7.1: Echinococcosis prevalence by ultrasound in northwestern China (current study)

Province/ AR	Prefecture	County	Town/ Commune	Survey Year	Number	HD (%)		AE (%)		CE (%)	
Xinjiang	Altai	Habahe		2002	1644	51	3.10	0	0.00	51	3.10
		Qinghe		2002	1134	31	2.73	0	0.00	31	2.73
	Changji	Mulei		2002	991	3	0.30	0	0.00	3	0.30
	Bayinguoleng	Hejing	Bayinbuluke	2004	1398	82	5.87	3	0.21	79	5.65
	Boertala	Wenquan	Angelige	2005	1292	24	1.86	0	0.00	24	1.86
	Yili	Xinyuan	Narati	2003	1838	38	2.06	6	0.33	32	1.74
			Turgen	2005	1853	29	1.35	5	0.32	24	1.29
		Nileke	Wulasitai	2004	2044	58	2.84	13	0.64	45	2.20
			Musi	2005	1851	20	1.08	0	0.00	20	1.08
	Tacheng	Hoboksar		2007	1339	70	5.23	2	0.15	68	5.08
Ningxia	Guyuan	Xiji		2002	945	93	9.84	36	3.81	57	6.03
Sichuan	Ganzi	Ganzi		2006	1665	43	2.58	30	1.80	13	0.78
Tibet	Dangxiong			2006	557	55	9.87	0	0.00	55	9.87
	Dingqing			2007	208	22	10.58	11	5.29	11	5.29
Total					18766	619	3.30	106	0.56	513	2.73

HD = hydatid diseases cases in total (CE and AE)

For CE, human behavior was more focused on since the transmission of *E. granulosus* usually depends on dog-livestock cycle which is more affected by human themselves. CE was found to be endemic in husbandry or pastoral dominated areas, such as in western China. On the other hand, the environmental factors such as cold climate and grassland landscape might be suitable for *Echinococcus* egg survival (Ding et al., 2000; Wen et al., 2010; WHO/OIE, 2001). This natural dog-livestock ecology balance for *E. granulosus* also has human interference such as feeding dogs with the infected offal of livestock. Human

infections were determined by local parasite burden in dogs which could result in environmental contamination with eggs. As a consequence CE also occurred in persons who did not have close contact with dogs or livestock. People who owned dogs and livestock however might have more risk due to their actions and close proximities to the transmission system of CE. This occupation or gender risk might not come from herding itself. Some studies found human or dog infection risk were not really highest in pastoral areas versus settled communities (Wang et al, 2004; Wang et al, 2001, 2005). The limitation of livestock and dogs might give more chance to keep them close to each other and obtain high density parasite burden. Poor hygienic habits are another aspect of human infection with CE. Washing hands before eating was not common in children and also many adults since they did not feel the danger of unseen eggs existing in the surrounding areas. Uncooked or even unwashed vegetables are another potential egg source due to some people fertilizes their gardens with dog faeces (WHO/OIE, 2001). Another contamination came from drinking water. Streams became the main water source in the wild field for herds and this could be contaminated by dog faeces.

Some general government policies have asked the herdsman to settle down rather than have a traditional nomadic life. The fencing for livestock might not decrease the incidence of CE since the close contact might cause higher parasite density in the limited space. But it could be resolved if routine dog administration could be carried out seriously, such as copro-detection and regular helminthicide treatment (Wang et al., 2004; Wang et al., 2001, 2005).

Risk factors for human CE could be related with three directions including genetic susceptibility, environment and lifestyle-related. Genetic factors are difficult to show e.g. family cases, though some studies suggest familial clustering (Musui et al., 1989). So the other two aspects became very important. For example of environment, farm with livestock husbandry and pastures (where have dog-sheep cycle), home slaughter is popular, relative cold weather, open water sources, etc. Lifestyle-related risk factors for CE from the current study suggests occupation as a herdsman or farmer, owning dogs, poor hygiene habits, ethnic as nomadic people (i.e. Mongol, Kazakh, Tibetan, etc.) (Table 7.2)

For human AE in Xinjiang, patients occasionally appeared in Tianshan and Altay Mountains areas. With red fox (*Vulpes vulpes*) as the main definitive host,

microtines (voles) as main intermediate host, a sylvatic cycle is maintained in Xinjiang (Ding et al., 2000; Wen et al., 2010; WHO/OIE 2001). Other endemic areas, the Tibetan fox *(Vulpes ferrilata*) and Plateau Pika (*Ochotona curzoniae*) might act as two important hotst in *E. multilocularis* transmission. Domestic dogs were involved in *E. multilocularis* transmission in many areas (Ganzi in Sichuan, Bayinbuluke in Xinjiang), which their roles might be a reason for relative centralized AE cases in those areas. So the close contact with dog might be one of the main risk factors for human AE in this case. Human behaviour could be also involved in the AE transmission such as fox hunting, fox skin handling, playing with dog and poor hygiene habits (WHO/OIE 2001). However there were very significant in current study.

Rapid serological colloidal techniques have been widely applied in clinical practice. We developed a DIGFA colloidal gold test for hospital and epidemiological investigations on human echinococcosis. This real time bedside detection was easy to be operated by technicians. Comparable assessments for these developed potential commercial diagnostic tools have been done in many infectious diseases (Dar et al., 1994; Xiao et al., 1995; Dylan and Kevin, 1999; Feng et al., 2000; Garcia et al., 2000; Feng et al., 2002; Zhu et al., 2002; Sorell et al., 2002; Yang, 2003; Hujakka et al., 2003; Chen et al., 2005). Generally these rapid tests were more recommended for mass screening since they had the easy-to-use trait. The limitation of these tests was mainly for quality check rather than quantities measure.

Two basic types of colloidal gold assays developed in the current study were DIGFA and ICA, which have been applied in commercial products for human echinococcosis in China. They have similar detection principle, which has antigen coated on the nitrocellulose membrane, then sera and colloidal gold labeled second antibody was reacted with antigen. They are similar to an indirect ELISA. The sensitivity and specificity was influenced by antigens, second antibody, and the size of colloidal gold and level of labeling. A purified *E. granulosus* cyst fluid antigen was used in ICA kit made in China and another recombined Em18 in another ICA product in Japan. We used a crude extract of *E. granulosus* cyst fluid, a crude antigen from *E. granulosus* protoscoleces, antigen B and a metacetode extract from *E. multilocularis* tissue and protoscoleces Em2 in our DIGFA kits. This kind of combination could give different reactive strengthen with parallel

multiple antigens and supply more information for same patient (Feng et al., 2010). The overall sensitivity of DIGFA was 80.7% for human CE (n=857) and 92.9% for human AE (n=42) in a hospital diagnostic setting, and ranged from 71.8% to 90.7% in comparison to abdominal ultrasound in the community screening. However the higher specificity occurred in both settings which were 93.4% and 94.6% with AgB for CE, and 90.3% and 97.1% with Em2 for AE (Feng et al., 2010).

New techniques and detection tools were developed through many years hard work. Molecular and immunological diagnostic tools has been applied in research laboratory and partially supplied to hospitals and survey group for both human and dog (or wild canids). An initial rapid ICA for coproantigen detection had been carried out in the current study. The 94% sensitivity of ICA in 16 experimental 35-53 dpi dogs was reported. ELISA and IGCA showed that coproantigen level appeared to increase from day 16 after infection and were clearly detected after day 24 dpi by both ELISA and IGCA. The current results suggested that copro tests (coproantigen ELISA, IGCA, copro-PCR) might be sensitive for canine echinococcosis from 20 days after infection. And that would be also helpful to evaluate the efficacy of control policy.

The epidemiological studies showed *E. granulosus* with dog-sheep lifecycle was high endemic in seven northwest provinces/autonomous regions including Xinjiang, Gansu, Ningxia, Qinghai, Tibet and Inner Mongol where livestock husbandry were prevalent and dog owning were popular. The prevalence of CE was different at county-level and generally pastures areas higher than agriculture areas. The distribution of AE was really complicated since higher prevalence occurred in Zhang and Min Counties of Gansu, Xiji County of Ningxia, Guoluo and Yushu Tibetan Prefectures of southeastern Qinghai, and Ganzi Tibetan Prefecture of Sichuan. The transmission of *E. multilocularis* would be relative with populations of wild carnivores and density of rodents which could form the lifecycle. The landscapes of endemic areas should be suitable for those wild animals to survive. But the population of wild animals and land usage might be dynamic and change through different time period. So the distribution of human AE cases was usually as a sporadic status in other areas such as Tianshan Mountain and Altay Mountain in Xinjiang.

Table 7.2: Echinococcosis and risk factors for CE in northwestern China (current study)

Counties	Year	No.	US prevalence						Sero-DIGFA Positives (%)			Major Risk factors	Genders Distribution		Ethinic Distribution		Occupation Distribution	
			HD (%)		AE (%)		CE (%)						US	DIGFA	US	DIGFA	US	DIGFA
Xinjiang Hejing	2004	1398	82	5.87	3	0.21	79	5.65	1288	197	15.3	Dog owner	p>0.05	Female	p>0.05	p>0.05	Herdsmen, Students	Herdsmen, Students
Xinjiang Wenquan	2005	1292	24	1.86	0	0.00	24	1.86	1194	293	24.5	Dog owner, Livestock, Occupation	p>0.05	Female	p>0.05	Han, Mongolian	Farmer& Herdsmen	Students, Cadres
Xinjiang Xinyuan	2003/ 2005	3691	67	1.82	11	0.30	56	1.52	3691	933	25.3	Township, Occupation, Kazak	p>0.05	Female	p>0.05	Mongolian, Kazak	Herdsmen, Cadre	p>0.05
Xinjiang Hoboksar	2007	1339	70	5.23	2	0.15	68	5.08	1307	265	20.3	Occupation, Homeslaughter	p>0.05	Female	Mongolian, Han	Han, Mongolian	Farmer, Herdsmen, Cadre	Farmer, Herdsmen, Cadre
Ningxia Xiji	2002	945	93	9.84	57	6.03	36	3.81	945	159	16.8	Occupation	p>0.05 (CE) Female (AE)	Female (CE & AE)	p>0.05	p>0.05	Farmer	Farmers
Sichuan Ganzi	2006	1665	43	2.58	30	1.80	13	0.78	1576	441	28.0	Female, Occupation, Ages	Female (CE), p>0.05 (AE)	Female (CE & AE)	p>0.05	--	Herdsmen	--
Tibet Dangxiong	2006	557	55	9.87	0	0.00	55	9.87	488	31	6.8	Dog owner, drinking water	p>0.05	--	All Tibetans	All Tibetans	p>0.05	--
Tibet Dingqing	2007	208	22	10.58	11	5.29	11	5.29	195	69	35.4	None	p>0.05	--	All Tibetans	All Tibetans	p>0.05	--
Total		11095	456	4.11	114	1.03	342	3.08	10684	2388	22.4		p>0.05 (CE), Female (AE)					

The control programme for echinococcosis was first started in Xinjiang from end of 1980s. But non-continued financial support did not provide long term intervention. The next focus on echinococcosis was an initial prevention plan for echinococcosis since a serious endemic report of national parasite disease investigation was sent to the National Ministry of Health in 2005. Echinococcosis was not a neglected disease in China at that time. Hydatid disease was internalized into the range of national major infectious diseases with free treatment in highly endemic counties. Most endemic counties were internalized as ‘’prevention and control programme county’’. For example, 30 counties became ‘’prevention and control programme county’’ and 24 hospitals became the appointed hospitals for surgical treatment of echinococcosis in Xinjiang by the end of 2011. Hydatid patients in those counties could obtain medicine treatment free of charge and RMB 8000 yuan (£800) for their surgical treatment from Special Funds for Echinococcosis. And medical staff and CDC staff in those counties and hospitals could receive professional training and technique direction free of charge through this control plan. On the other hand, financial support was supplied to those counties including health education for residents, treatment plan for dogs and management for livestock slaughter. All those policies could be very helpful for practical prevention and control in high endemic areas even the complicated administration system existed.

New techniques and detection tools were developed through many years hard work. Molecular and immunological diagnostic tools has been applied in research laboratory and partially supplied to hospitals and survey group for both human and dog (or fox and wolf). And that would be also helpful to evaluate the efficacy of control policy. Improvement of operation path has been successfully studied and carried out in clinical practice such as ‘’complete resection of hydatid cyst ‘’. New formulations of albendazole and other antihelminth drugs were developed and commercial available for assistant medicine treatment of patient. And the fundamental research on mechanism of transmission, infection model, immunological defense etc., had been evolved in research centers or institutes in Shanghai, Sichuan, Qinghai, Ningxia, Gansu and Xinjiang.

The aetiology and infection route of human echinococcosis are clear but the transmission path or model needs to be established based on GIS technique or

mathematic model from dog infection. The diagnostic tool and vaccination for sheep are also available although the applications of them are still limited due to personal monetary outcome needed. In order to eliminate the preventable disease in northwest China, more effort is expected to be paid on developing comprehensive, effective, feasible policy, strategies and measures, as well as effective implementation and evaluation systems.

Conclusions and recommendations

Rapid diagnostic tests including DIGFA for human echinococcosis, ICA for cyst fluid identification and for coproantigen detection had been developed and evaluated through this study. DIGFA for human echinococcosis has been formally registered in National FDA of China and commercially used for hospitals and community studies from 2010. Results showed accepted sensitivity for above rapid test in hospital setting and also community studies. The rapid tests had been confirmed effective, and valuable for clinical diagnosis and community screening. But the rapid tests had the limitied diagnostic accuracy which the clinical diagnosis must combined imaging results. The false negative and false positive needed to be follow-up with same rapid test or other tests (ie. ELISA, western blot). Furthermore, new tools or techniques could be applied to improving the diagnostic accuracy and validity based on the above assays.

A series of epidemiological surveys including the current study showed the overall ultrasound prevalence of human echinococcosis in western China was 3.3%, with CE 2.73% and AE 0.56% respectively. The transmission of *E. granulosus* and *E. multilocularis* might be involved with dog-livestock, fox or domestic dog and small mammal lifecycle in Xinjiang, Ningxia and Tibetan Plateau (Ganzi of Sichuan and Tibet AR). The risk factors for human CE could be relative with ethnic group, occupation, dog ownership and homeslaughter in the current study.

However, we found that the control of echinococcosis was still difficult in above survey areas. In order to eliminate hydatid disease in Xinjiang, or extending to the whole country, more efforts can be expected for the payment of the development of comprehensive, effective, feasible policy, prevention and control strategies and evaluative system, as well as effective implementation.

It would be very important to establish an administration system for control of human echinococcosis. This system should have several fields to be free from

existing risk factors. Dog control would be firstly responsible for registering all the information for local dogs including their birth/death, drug taken and faecal test and follow-up. Livestock control would be in charge of regular control of possible infected organs both in city slaughter and countryside. Thirdly, human control including hospital, CDC and public health education branch would be focused on prevention, diagnosis, treatment and follow-up observation for local population. Additionally, good hygiene habits and friendly home education formulation would be helpful to control of Echinococcus infections in humans and animals in Xinjiang, and gradually extended in northwestern China, and further more in all over the country.

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Appendix

I. Preparation of antigens for human *Echinococcus* antibodies detection

I.1 Native *E. granulosus* cyst fluid antigen (EgCF)

Hydatid cyst fluid was obtained by aseptic aspiration from naturally infected sheep livers or lungs collected in abattoirs from different endemic areas of Xinjiang, P. R. China. The cyst fluid was centrifuged at 3000rpm for 30 minutes, and then supernatant was dialyzed against 0.15m PBS (pH 7.2) overnight at 4ºC. Dialyzed cyst fluid supernatant was then affinity purified against normal human IgG chromatographic column (DEAE, diethylaminoethyl) coupled cyanogens bromide activated Sepharose 4B (CNBr-4B, Sigma). The affinity absorbed cyst fluid supernatant was concentrated with an Amicon ultra filtration cell using YM10 membrane (Amicon Corp, MA, USA).

The protein concentration of EgCF antigen was estimated by spectrophotometric absorption at 280nm and 260nm (2.493mg/ml, from a calculation for the concentration of protein (1.55*A280-0.76*A260), Stoscheck et al., 1990)).

Hydatid cyst fluid (EgCF) purification procedure with immunoabsorbent chromatography:

- Human immunoglobulin was prepared from normal human serum samples from XJMU (laboratory confirmed without any other infection, e.g. HIV, HAV, HBV, HCV, TB) by precipitating with 40% saturated ammonium sulfate in normal saline for 3 hours at 4ºC, centrifuged 3000rpm for 20 minutes; centrifuged pellets was re-dissolved in saline and the saturated ammonium sulfate precipitation repeated; last pellets were dissolved with original volume of 0.02M PBS and dialyzed against 0.02M PBS, with a change of dialyzing buffer at least 3 times and left overnight at 4ºC.

  Preparation of a DEAE column used the appropriate amount of DEAE cellulose and in a 200ml glass beaker; washed DEAE three to four times with large volume pure water, the DEAE was mixed with water by stirring and then put aside for 1 hour. Water were poured off and DEAE immersed in 0.5N NaOH (15ml per germ of DEAE), mixed well and equilibrated 30 minutes, filtered and washed with water until pH to about 7. DEAE immersed in 0.5N HCl and treated the same as above; repeated immersion in 0.5N NaOH again

and treated the same way. Finally DEAE was immersed in 0.1 M pH 7.4 PB, over night. A quarter volume of 0.1 M pH 7.4 PB added in a column tube, and DEAE was poured into until suitable volume; equilibrated with PB to get same pH 7.4.

Dialyzed normal human Ig with 0.1M pH 7.4 PB, overnight at 4 ºC; added IgG on the DEAE column, eluted with 0.1M pH 7.4 PBS, collected the flow through to get ion-exchange purified human Ig. Dialyzed against with 0.1M pH 8.0 PBS at 4ºC overnight before the coupling.

- Coupling human Ig to CNBr-4B sepharose

  One gram of CNBr-4B was swollen in 10^{-5} HCl solution on a glass filter and washed for 15 minutes; approximately 200ml solution was added.

  Immediately after washing, a solution of normal human Ig which was adjusted pH to 9.0 was added to be coupled at room temperature for 2 hours. Blocked with 0.2M glycine for 2 hours. Washed with 5 volumes 0.2Mol/L pH 9.0 $NaHCO_3$ (0.1Mol/L NaCl). The column was ready for use.
- Crude centrifuged EgCF was passed through the Ig-CNBr sepharose column slowly (1ml/min flow speed), washed through with 0.01Mol/L pH7.4 PBS; and eluted with 0.1 M glycine-HCl buffer (pH 2.4). Partially purified EgCF was collected in tubes containing 1Mol/L $NaHCO_3$ for neutralizing the solution; dialyzing against 0.01M pH 7.4 PBS. This EgCF preparation absorbed against normal human serum was aliquoted (0.5ml/tube) and stored at -80°C until used.

Anti-EgCF IgG preparation:

- A rabbit was hyperimminised with native *E. granulosus* cyst fluid antigen (EgCF) as described by Gottstein 1983.

- Rabbit serum was taken when the specific antibody titre in rabbit was high enough (>10,000, tested with ELISA: capture was native antigen EgCF to the microtitre plate, conjugate was HRP conjugated goat anti – rabbit IgG and TMB as substrate)

- IgG from rabbit sera was purified by precipitation with saturated ammonium sulfate and DEAE ion-exchange chromatography column as described above.

I.2 Native crude somatic extract of *E. granulosus* protoscoleces (EgP)

E. granulosus protoscoleces were harvested by aseptic aspiration from fertile *E. granulosus* hydatid cysts from naturally infected sheep in Xinjiang. Protoscoleces / brood capsule suspension was centrifuged and washed by pH7.2 PBS 3 times. A crude somatic extract was prepared by freeze / thaw twice at -70ºC and room temperature followed by ultrasonic treatment at 70w, 5ms (JY92-II sonicator, Ningbo Xinzhi Bio-Tech Co, China), allowed to stand for 1 hour at 4 °C and then centrifuged at 10000 rpm for 30 min. The supernatant was partially purified by affinity immunosorbent chromatography - affinity purified with normal human Ig coupled CNBr-activated Sepharose4B (Sigma) column as described above. Supernatants were concentrated with an Amicon ultra filtration cell with YM10 membrane (Amicon Corp, MA, USA). The protein concentration of the EgP extract was estimated by spectropherometric absorbance OD value at 280nm/260nm wavelengths.

I.3 Native *E. granulosus* cyst fluid antigen B (EgB)

E. granulosus hydatid cyst fluid was obtained from naturally infected sheep in Xinjiang, P. R. China and EgB antigen prepared as described by Oriol et al. (1971) and Rogan et al (1989). Approximately 200mL of sheep hydatid cyst fluid was centrifuged at 3000 rpm for 10 min at 4°C, the supernatant was then dialyzed against H_2O, followed by 0.005M, pH5.0 acetate buffer overnight at 4 °C. The dialyzed cyst fluid was centrifuged at 15000 rpm at 4 ^{0}C for 60 minutes and the supernatant discarded. The precipitate was re-suspended in 20mL 0.2M pH 8.0 PBS and 40% saturate ammonium sulfate added and left for 1 hour to cause precipitation. The precipitate was centrifuged and the supernatant boiled in a water bath for 15 min, cooled then centrifuged at 15000 rpm at 4 ^{0}C for 60 minutes and the pellet discarded. This supernatant should be enriched for antigen B and was further concentrated with an Amicon ultra filtration cell with YM10 membrane (Amicon Corp, MA, USA) (Rogan et al., 1993, Zhang 1999). The protein concentration of antigen B was estimated by spectrophotometric absorbance at 280nm and 260 nm wavelengths. The final concentration of stock EgB was 0.522mg/ml. Aliquots of EgB were stored at -80°C until used.

I.4 Native *E. multilocularis* metacestode antigen Em2

E. multilocularis larval metacestode masses were obtained from experimentally infected gerbils (*Meriones unguiculatus)* at XMU. The larval mass was broken completely by homogenization (food processor) and mixed with 0.15M pH7.2 PBS. This crude homogenizate was freeze/thawed at -80ºC for 3 times, then centrifuged at 10,000 rpm for 30 minutes at 4°C and the supernatant dialyzed against 0.15M pH 7.2 PBS to get crude *E. multilocularis* (Em) antigen. Crude antigen was passed through a CNBr-Sepharose4B column containing rabbit anti-EgCF IgG and eluted a buffer of 5M $MgCl_2$. The eluate was enriched with Em2 antigen from the laminated membrane. The concentration of Em2 extract was estimated by OD value at 280/260 nm wavelengths (Gottstein et al., 1983; Zhang et al., 2000).

II. Main buffers used for human serodiagnostical ELISA

Coating buffer: pH 9.6

Na_2CO_3	1.85 g
$NaHCO_3$	2.73 g
$MgCl_2.6H_2O$	3.04 g
ddH_2O	add to 1000 mL

20×PBS storing buffer:

NaCl	160 g
$Na_2HPO_4 \cdot 12H_2O$	58 g
KH_2PO_4	4 g
KCl	4 g
ddH_2O	add up to 1000 mL

PBS buffer:

20×PBS	50 mL
ddH2O	950 mL

20×PBST buffer

20×PBS	300 mL
Tween-20	3 mL

Washing buffer

20×PBST buffer	25 mL
ddH_2O	add up to 500 mL

Blocking buffer

20×PBST	20 mL
BSA	4 g
Gelatine	8 mL
ddH_2O	add up to 500 mL

Sample dilution buffer

20×PBST		20 mL
Normal Sheep sera		15 mL
Thiomersal		0.04 g ($C_9H_9HgNaO_2S$)
ddH_2O	add up to	400 mL

Conjugate dilution buffer

PBST	480 mL
Enzyme stable reagent	0.25 g (a production from a Chinese Company)
Normal sheep sera	15 mL(3%) (from healthy sheep)
Proclin 300	0.25 mL (a stable reagent for antigen/antibody)

Substrate buffer A :

Citric acid	7.3 g
EDTA	0.15 g
Mannitol	30 g
ddH_2O	475 mL

Wait for 1hr, then

TMB／DMSO	150 mg / 25 mL

Substrate buffer B

$Na_2HPO_4 \cdot 12H_2O$		11.86 g
Urea peroxide (carbamide peroxide)		0.5 g
ddH_2O	add up to	500 mL

Stopping buffer

Sulfuric acid (98%)		11.2mL
ddH_2O	add up to	2000mL

III. Main buffers used for human serodiagnostical DIGFA

Tris-HCl storing buffer: 200Mm PH8.5 400ml

Tris	9.7 g
NaCl	35 g
NaN_3	3.0 g
Concentrated HCl	2.5ml

Buffer A: Sample buffer

Tris-HCl storing buffer:	50 mL
Tween-20	250 μL
Gelatin	5 mL (Sigma-Aldrich, G7765, from cold water fish skin)
Pure H_2O	445 mL

Buffer B: Washing buffer

Tris-HCl storing buffer:	50 mL
Tween-20	2.0 mL
NaCl	4.5 g
Pure H_2O	448.5 mL

Buffer C: Colloidal conjugate

Test for antibody volume to be conjugate:

Antibody (goat anti-human IgG (γ-chain) diluted to 1mg/ml with 20 mM Tris-HCl (200 mM Tris-HCl storing buffer 5mL and pure H_2O 45 mL).

6 of 1.5 mL tubes were added as follows:

Tube No.	1	2	3	4	5	6
Colloidal gold	1mL	1mL	1mL	1mL	1mL	1mL
1mg/ml antibody	4μL	8μL	16μL	32μL	64μL	128μL
10% NaCl	100μL per tube					

The colour would be purple to wine red in order, choose the one just changed to wine red as the conjugate concentration and added 120% of selected conc. for fully conjugated (eg. 32 μL selected and 32*120%=38.4μL IgG for 1 mL colloidal gold)

Colloidal conjugate:

Colloidal gold	100 mL
0.2 M Sodium borate	10 ml
1mg/ml goat anti-human IgG (γ-chain)	3.84ml
4 °C for 45 minutes or 1 hour	
5% PEG 20,000	440 µL
Blocking buffer	29.4ml

Blocking buffer

200mM Tris-HCl	20 mL
Over saturated sucrose	20 mL (made from 350g sucrose in 150mL pure H_2O)
Gelatin	2 mL
5% PEG 20,000	0.22 mL)

Filtered with 0.2 µm pore size syringe filter and stored in dark at 4°C.

IV. Main buffers used for rapid ICA test for coproantigen in dogs

Conjugate:

Colloidal Gold	10 ml
0.2 M Sodium borate	1 ml
1mg/ml rabbit anti-EgB IgG	0.384ml
4 °C for 45 minutes or 1 hour	
5% PEG 20,000	440 µL
Blocking buffer	2.94ml

Blocking buffer

200mM Tris-HCl	5 mL
Over saturated sucrose	5 mL (made from 350g sucrose in 150mL pure H_2O)
Gelatine	0.5 mL
BSA	0.58 g)

Filtered with 0.2 µm pore size syringe filter and stored in dark at 4°C.

Conjugate purification:

Centrifuged conjugate in 2000rpm for 30 minutes to get rid off some bigger particles.

Concentrated above conjugate in a dialysing tube with covered sucrose powder until one third of original volume.

Preparing a Sephadex G200 column (1g for a volume 15-20ml). Concentrated conjugate coupled with the column, elution with 2% BSA 20mM Tris-HCl. Collected the middle part of deep wine red part of conjugate according that different sizes of colloidal conjugate would have different pass speed to get similar size conjugate. The final volume of conjugate was around 4ml. Test OD value under the wavelength 570nm. Diluted the conjugate to lower the OD value to 0.7 with conjugate buffer (the details would be talked in the following **2.7.3.**

V. QIAamp DNA Stool Handbook

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2. Add 1.6 ml Buffer ASL to each stool sample. Vortex continuously for 1 min or until the stool sample is thoroughly homogenized.

Note: It is important to vortex the samples thoroughly. This helps ensure maximum DNA concentration in the final eluate.

3. Centrifuge sample at full speed for 1 min to pellet stool particles.

4. Pipet 1.4 ml of the supernatant into a new 2 ml microcentrifuge tube (not provided) and discard the pellet.

Note: The 2 ml tubes used should be wide enough to accommodate an InhibitEX Tablet. Transferring small quantities of pelleted material will not affect the procedure.

5. Add 1 InhibitEX Tablet to each sample and vortex immediately and continuously for 1 min or until the tablet is completely suspended. Incubate suspension for 1 min at room temperature to allow inhibitors to adsorb to the InhibitEX matrix.

6. Centrifuge sample at full speed for 3 min to pellet stool particles and inhibitors bound to InhibitEX matrix.

Note: For most samples, 3 min centrifugation is sufficient. With some samples, however, centrifugation for 3 min may result in a pellet that is not sufficiently compact. Therefore it may be difficult to remove enough supernatant to transfer 600 µl supernatant after the next centrifugation step (step 9). In these cases, we recommend to centrifuge for 6 min.

Note: When processing more than 12 samples, for this step and step 7 we recommend processing batches of no more than 12 samples each. This is because the pellets formed after centrifugation will break up quickly if the supernatant is not removed immediately.

7. Immediately after the centrifuge stops, pipet all of the supernatant into a new 1.5 ml microcentrifuge tube (not provided) and discard the pellet. Centrifuge the sample at full speed for 3 min.

Transferring small quantities of pelleted material from step 6 will not affect the procedure.

8. Pipet 25 µl proteinase K into a new 2 ml microcentrifuge tube (not

provided).

9. Pipet 600 µl supernatant from step 7 to the 2 ml microcentrifuge tube containing proteinase K.

10. Add 600 µl Buffer AL and vortex for 15 s.

Note: Do not add proteinase K directly to Buffer AL.

It is essential that the sample and Buffer AL are thoroughly mixed to form a homogeneous solution.

11. Incubate at 70°C for 10 min.

Centrifuge briefly to remove drops from the inside of the tube lid (optional).

12. Add 600 µl of ethanol (96–100%) to the lysate, and mix by vortexing.

Centrifuge briefly to remove drops from the inside of the tube lid (optional).

13. Label the lid of a new QIAamp spin column provided in a 2 ml collection tube. Carefully apply 600 µl lysate from step 12 to the QIAamp spin column without moistening the rim. Close the cap and centrifuge at full speed for 1 min. Place the QIAamp spin column in a new 2 ml collection tube, and discard the tube containing the filtrate.

Close each spin column in order to avoid aerosol formation during centrifugation.

If the lysate has not completely passed through the column after centrifugation, centrifuge again until the QIAamp spin column is empty.

14. Carefully open the QIAamp spin column, apply a second aliquot of 600 µl lysate and centrifuge at full speed for 1 min. Place the QIAamp spin column in a new 2 ml collection tube, and discard the tube containing the filtrate.

Close each spin column in order to avoid aerosol formation during centrifugation.

If the lysate has not completely passed through the column after centrifugation, centrifuge again until the QIAamp spin column is empty.

15. Repeat step 14 to load the third aliquot of the lysate onto the spin column.

16. Carefully open the QIAamp spin column and add 500 µl Buffer AW1. Close the cap and centrifuge at full speed for 1 min. Place the QIAamp spin column in a new 2 ml collection tube, and discard the collection tube containing the filtrate.

17. Carefully open the QIAamp spin column and add 500 µl Buffer AW2. Close the cap and centrifuge at full speed for 3 min. Discard the collection tube containing the filtrate.

Note: Residual Buffer AW2 in the eluate may cause problems in downstream applications. Some centrifuge rotors may vibrate upon deceleration, resulting in the flow-through, which contains Buffer AW2, contacting the QIAamp spin column. Removing the QIAamp spin column and collection tube from the rotor may also cause flow-through to come into contact with the QIAamp spin column.

18. Recommended: Place the QIAamp spin column in a new 2 ml collection tube (not provided) and discard the old collection tube with the filtrate. Centrifuge at full speed for 1 min.

This step helps to eliminate the chance of possible Buffer AW2 carryover.

19. Transfer the QIAamp spin column into a new, labeled 1.5 ml microcentrifuge tube (not provided). Carefully open the QIAamp spin column and pipet 200 µl Buffer AE directly onto the QIAamp membrane. Close the cap and incubate for 1 min at room temperature, then centrifuge at full speed for 1 min to elute DNA.

Note: When using eluates in PCR, for maximum PCR robustness we highly recommend adding BSA to a final concentration of 0.1 µg/µl to the PCR mixture.

For maximum PCR specificity we recommend using QIAGEN HotStarTaq Plus DNA Polymerase (see ordering information on page 39). For best results in downstream PCR, use the minimum amount of eluate possible in PCR; the volume of eluate used as template should not exceed 10% of the final volume of the PCR mixture. Also, note that high amounts of template DNA may inhibit the PCR.

DNA yield is typically 15–60 µg but, depending on the individual stool sample and the way it was stored, may range from 5 to 100 µg. DNA concentration is typically 75–300 ng/µl.

For more information about elution and how to determine DNA yield, purity, and length, see the Appendix, page 36.

For long-term storage, we recommend keeping the eluate at –20°C.

VI. Questionnaire for human screening on hydatid disease in Xinjiang Uygur Autonomous Region, P.R,China

中国新疆地区包虫病调查表

1．登记号：_____姓名：_____性别：男□　女□　年龄：_____
Reg.no. Name Sex: Male Female Age
民族：汉 □　回 □　蒙 □　维 □　哈 □　其他 □
Ethnic: Han Hui Mongolian Uygur Kazak Others
职业：(1)农民□(2)牧民□(3)干部□(4)工人□(5)商人□(6)学生□(7)士兵□(8)家庭主妇□(9)其他□Occupation: Farmer Herdsman Cadre Worker Businessman Student Soldier Housewife Others
2．住址：_____县_____乡_____村_____队
Domicile: County Commune Village Group
曾居住地：_____
Lived before:
3．上一年年收入：(1)＜2000 元　(2)2000-5000 元　(3) ＞5000 元……□
Income of last year (RMB):
4．听说过包虫病吗？　是□ 否 □
Have you heard of hydatid disease? Yes No
5．过去得过包虫病吗？是□ 否□ ；如果是，囊型□ 泡型□ ；做过何种治疗：手术□药物□穿刺□
Have you been a hydatid patient: Yes No; If yes, CE or AE; Treatment: Operation Drugs PAIR
6．家里有人得过包虫病吗：是□ 否□ ； 如果是，是：父□ 母□ 爱人□ 兄弟□ 姐妹□ 孩子□ 其他□
Has someone in your family been a hydatid patient? Yes No; if yes, he is: father mother partner brother sister children others
7．家里养过狗吗？ 是□ 否□ ， 如果是，
Have you owned any dogs? Yes, No; If yes,
养过几年狗：____年；养过几只狗：（1）1 只，（2）2 只，（3）3 只，（4）＞3 只…□
No. of years dogs owned:___Yrs; No. of dogs owned: (1) 1，（2）2，（3）3，（4）＞3
家庭中照顾狗的是：父□ 母□ 爱人□ 兄弟□ 姐妹□ 孩子□ 本人□ 其他□
Who cared dogs in your family: father, mother, partner, brother sister, children, yourself, others
狗吃什么：（1）动物内脏 （2）剩饭 （3）野鼠、兔… ……□
Dog's food: Viscera House hold scraps catch wild rodents
狗接受过检查吗？是□ 否□
Have your dogs been tested/treated before: Yes No
狗粪作肥料浇地吗？是□ 否□ 狗用于放牧家畜吗？ 是□ 否□
Have you used dog faeces as fertile in the farm? Yes, No; Did dog go with livestock together? Yes, No
8．你家里有家畜吗？是□ 否□ ；如果是，绵羊□ 山羊□ 牛□ 马□ 骆驼□ 驴□ 骡□ 猪□
Do you own livestock? Yes No; if yes, sheep, goat, cattle, horse, camel, donkey, mule, pig
9．你在家屠宰牲畜吗？是□ 否□
Do you slaughter livestock at home? Yes No
10．你见过狐狸：是□ 否□ ；狼：是□ 否□；野猫：是□ 否 ；
Have you seen fox: yes no; wolf: yes no; felines: yes no
你打猎打过狐狸：是□ 否□ ；接触过狐狸皮：是□ 否□
Have you hunted fox before? Yes no; have you touched fox skin? Yes no
11．你见过的老鼠：家鼠□ 田鼠□ 旱獭□ 其他□
Have you seen rodents: *Rattus norvegicus, Microtus, Marmota*
12．饮用水：(1)自来水 (2)深井水 (3)浅井水 (4)河塘水 (5)溪水 （6）其他……□
Drinking water: (1)tap in the street (2) deep well (3) pump in the yard (4)from river (5)from scream (6)others
13．饭前洗手：是□ 否□ ；饮用未经煮沸的生水：是□ 否□ ；食用生的蔬菜：是□ 否□
Washing hands before eating: yes no; drinking un-boiled water: yes no; eating uncooked vegetables: yes no

14. 血样品采集：（1）滤纸　　（2）静脉　　（3）两者均有……□
Blood sample collection: filter-paper　　vein　　both of them

15. 腹部 B 超检查结果：（1）正常□ （2）CE□ （3）AE□ （4）可疑□ （5）单纯钙化灶□
Abdomen ultrasound: normal　　CE　　AE　　query calcified lesion
（6）其他腹部疾病:　　胆结石□　胆囊炎□　　脂肪肝□
Other abdomen abnormal:　　gall-stone　　cholecystitis　　fatty liver

如果是 CE：
If CE,
T1=单纯囊型（直径＜4cm）……□
Type 1 =univesicular (Φ<4cm)
T2=单纯囊型（直径>4cm）……□
Type 2 =univesicular (Φ>4cm)
T3=多子囊型……　　……□
Type 3 =daughter cysts
T4=内囊塌陷内囊破碎型……□
Type 4 =ruptured endocyst
T5=实变型…　　……□
Type 5 =solid cyst
T6=钙化型…　　……□
Type 6 =calcified cyst
大小（mm）：_____数量：_____
Size (mm):　　Number:
位置：　左肝□　右肝□　左右均有□　脾□
Location: left liver　　right liver, both　　spleen
肾□　　腹腔□　　肝及其他部位□
kidney, abdominal cavity, liver and other organs
症状：_____
Symptom:

如果是 AE：
If AE,
P1=病灶≤2 个肝段，无肝脏、胆道累及 …□
Single lesion≤ 2 segments, without intrahepatic vascular or biliary involvement
P2=病灶≤2 个肝段，有肝脏胆道累及…… □
Single lesion≤2 segments with the above involvement
P3=病灶占 3-5 个肝段，有肝胆道累及… □
The lesion occupied 3-5 segments
P4=病灶占 6-8 个肝段，有肝胆道累及… □
The lesion occupied 6-8 segments
其　　他型 …　　… ……□
Other types

大小（mm）：_____数量：_____
Size　　(mm):　　Number:
AE 病变有中央型坏死腔：是□　否□
Central necrosis in AE lesion:　　Yes　　No
位置：左肝□　　右肝□　　左右均有□
Location: left liver　　right liver　　both;
症状：_____
Symptom

16. 其他部位包虫病检测结果：
Other tests for other organs hydatid disease:
部位：_____
Location:
检测结果：_____
Test results:

17. 血清学检测结果：
Serology test results:
(1)快速检测：　EgCF:　+++□ ++□ +□ +/-□ –□　　EgP:　+++□ ++□ +□ +/-□ –□
Rapid DIGFA:　EgB:　　+++□ ++□ +□ +/-□ –□　　Em2:　+++□ ++□ +□ +/-□ –□
(2)酶联检测：　EgCF　　EgP
ELISA:　　EgB　　Em2

VII. Questionnaire for dog owners on hydatid disease in Xinjiang Uygur Autonomous Region, P.R,China

China: Surveillance Data for Echinococcus spp. in dogs

中国：关于有腔棘球绦虫的调查数据

Date 日期_____ Grid point 坐标点_____

全球定位系统坐标：GPS X_____

GPS Y_____

General Information 常规信息

1. Village name 村庄名称_____
2. Household name 户主名称_____
3. Are you nomadic? (please circle one) No 不是
   您是牧民吗？（请选择右边的一项） Yes-herdsman 是—放牧
   Yes-dig herbs 是—采药
   Yes-hunter 是—狩猎
4. Number of years at current location 请填写您在这个地区居住的时间为（年）_____
5. What is your occupation？请填写您在这里所从事的职业_____
6. Do you or have you ever hunted fox (explain)_____
   请叙述您以前猎捕狐狸的一些情况

Dog Information 关于狗的信息

7. Length of dog ownership(years) 请填写您养狗的时间（年）_____
8. Number of dogs currently owned 请填写您家里目前所养狗的总数_____
9. Name of dog 这只狗的名字是_____
10. General description of dog 请您对这只狗进行概括的描述_____
11. Age of dog(years) 这只狗的年龄是_____
12. Sex of dog(please circle one) Male 公
    请选择这只狗的性别 Female 母
13. Does your dog eat raw meat?(please circle one)Yes-frequently 是—经常
    请选择这只狗是否吃未经过烧煮的肉 Yes-occasionally 是——有时候
    No 不
14. Has the dog been seen eating rodents? (please circle one) Yes-frequently 是—经常
    您的狗吃其他啮齿类的动物吗？ Yes-occasionally 是—有时候
    No 不
15. Is the dog tied? (please circle one) Never 从来没有
    您拴着您的狗吗？ Yes-all of the time 是—总是
    Yes-during the day only 是—只有白天
    Yes-at night only 是—只有夜里
16. Who cares for the dog 请填写您家里经常照顾狗的人是（男性或女性）?_____
17. Do you use dog feces as fertilizer(please circle one) Yes 是的
    您用狗的粪便做肥料吗？ No 不是
    Don't have a garden 我家没有（菜、花）园
18. Are there stray dogs in the area(please circle one) Yes 有
    在附近有没有发现野狗 No 没有
19. Do you play with or pet your dog(please circle one) Yes 是的
    您和您的狗一起玩耍吗？ No 不是

Livestock Information 关于牲畜的信息

20. Do you own yaks? (please circle one) Yes 有_____
    您有牦牛吗？ No 没有
21. Do you own sheep or goats? (please circle one) Yes 有_____
    您有绵羊或者山羊吗？ No 没有

Water Source 水源

22. What is your primary water source 您家里所使用的水源是 (please circle one) （请选择）
central supply 集中提供
tap in the house 家用自来水
tap in the street 自来水（公共场所）
pump in the yard 院子里的水源
tank filled from river 供水车中的水箱
carried from river 河水
carried from stream 泉水
from a well 井水

Human Hydatid Disease 人体包虫病

23. Number of family members ultrasounded 请填写您家里有几位成员进行过超声波检查_____

24. Presence of positive cases(please circle one) 检查结果是否有人感染 Yes 有 No 没有

If yes, please fill out the following (noting how disease was confirmed and location of lesion): 如果有请指出是下列哪项（说明：疾病是由什么方式检查出来以及病变所在的位置）

Male AE confirmed 男—确诊ＡＥ　Female AE confirmed 女—确诊ＡＥ
Male CE confirmed 男—确诊ＣＥ　Female CE confirmed 女—确诊ＣＥ
Male hydatid disease suspected 男—怀疑感染包虫病　Female hydatid disease suspected 女—怀疑感染包虫病

How long ago was the most recent case diagnosed (in years) 请填写您上次检查的时间（几年以前）?

25. Was surgery performed on this case? 请填写您以前是否针对这种疾病进行了外科手术_____

Knowledge of Hydatid Disease 对于包虫病的认识

27.Correct description of hydatid disease (please circle one) 您是否能对包虫病进行正确的描述 Yes 能 No 不能

28.Correct transmission knowledge (please circle one) 您是否能把包虫病的知识传达给其他人 Yes 能 No 不能

Samples and Findings 采样以及发现

Sample collected(please circle one) 所采集的样品
Feces (ground)粪便（地面）
Feces (loop)粪便（采样仪器）
Purged sample 使用泄药采集的样品
Necropsy 尸体检验时采集的样品

Echinococcus multilocularis found (number) 发现泡状棘球绦虫的数目_____

Echinococcus granulosus found (number) 发现细粒棘球绦虫的数目_____

ELISA(please circle one) Positive 阳性 Negative 阴性

PCR(please circle one) Positive 阳性 Em/Eg Negative 阴性

Other parasites found (please specify) 其它寄生虫病例 _____

Where samples kept from this animal (please specify) 寄生虫感染的位置 _____

VIII. Publications

1. **Feng Xiao-hui,** Wen Hao, Zhang Zhaoxia, Chen Xinhua, Ma Xudong, Zhang Jinping, Qi Xinwei, Bradshaw Helen, Vuitton Dominique and Craig Philip S., Dot immunogold filtration assay (DIGFA) with multiple native antigens for rapid serodiagnosis of human cystic and alveolar echinococcosis. Acta Tropica. 2010; 113(2):114-20

2. WANG Le, **FENG Xiao-hui**, DUAN Xin-yu，WEN Hao．Direct and indirect economic burden of 999 cystic echinococcosis patients in a tertiary hospital. Chinese Journal of Epidemiology, 2010, 31(7): 835-836

3. CHU Xiang-dong, WANG Gui-zhi, **FENG Xiao-hui (Correspondence author)**, ER Xi-ding, HE Jin-hua, WEN Hao, Risk factors on human cystic echinococcosis in Hobukesar Mongolian Autonomous County in Xinjiang , Chinese Journal of Epidemiology, 2010, 31(3): 297-299

4. LIU Da-peng, **FENG Xiao-hui,** ZHANG Jing-ping, WEN Hao. Clinical appHcation of the 8-test for immunodiagnosis of human bone cystic echinococcosis, Chinese Journal of Orthopedic, 2010, 30(2): 198-202

5. WANG Gui-zhi， **FENG Xiao·hui (Correspondence author)**, CHU Xiang-dong, ERXIDING, AMINA, ZHOU Ji-xia, WANG Qiao, HE Jin-hua, WEN Hao, Epidemiologieal study on human echinococcosis in Hobukesar Mongolian Autonomous County of Xinjiang. Chinese Journal of Endemiology, 2009, 28 (2): 214-21

Books editor joined.

6. Atlas of Echinococcosis (English version), 2008,

7. Chapter 7. The Immunological Diagnosis, in Practical Echinococcosis.2007.

Posters and abstracts

8. A set of Poster for echinococcosis (with Chinese, Uygur, Kazakh, Mongolian and Tibetan languages).

9. Abstract in XXIII WORLD CONGRESS OF HYDATIDOSIS (Colonia de Sacramento – URUGUAY December 10 – 12, 2009. **Feng Xiaohui**, Wen Hao, Gongsangquzhen, Ma Xudong, Luosangqunzhen, Yang Lei, Qi Xinwei, Bartholomot Briggette, Vuitton Dominique, Craig Philip S., Human echinococcosis in Tibet Autonomous Region, P.R.China

10. Poster and abstract in XXIV WORLD CONGRESS OF HYDATIDOSIS (Urumqi – China, September 14, 2011): **FENG Xiaohui**, WEN Hao, Duan Xinyu, Qi Xinwei, ZHANG Jingpin, Craig P.S.. Epidemiological analysis on Echinococcus infections in three Mongolian communities of Xinjiang，P. R. China.

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Dot immunogold filtration assay (DIGFA) with multiple native antigens for rapid serodiagnosis of human cystic and alveolar echinococcosis

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ABSTRACT

A new 3-min rapid dot immunogold filtration assay (DIGFA) for serodiagnosis of human cystic and alveolar echinococcosis was developed using four native antigen preparations: crude and partially purified hydatid cyst fluid extracts from *Echinococcus granulosus* (EgCF and AgB), *E. granulosus* protoscolex extract (EgP) and *Echinococcus multilocularis* metacestode antigen (Em2). The overall sensitivity of DIGFA in a hospital diagnostic setting was 80.7% for human cystic echinococcosis (CE) ($n = 857$) and 92.9% for human alveolar echinococcosis (AE) ($n = 42$). Highest specificity was 93.4% with AgB extract for CE, and 90.3% with Em2 antigen for AE when CE versus AE cross-reactivity was excluded. Anti-AgB antibodies were present in 35.5% of AE cases and anti-Em2 in 7.4% of CE cases. In endemic communities in northwest China screened for echinococcosis, the sensitivity of DIGFA ranged from 71.8% to 90.7% in comparison to abdominal ultrasound; specificity for CE using AgB was 94.6% and for AE using Em2 was 97.1%. This simple eye-read rapid test can be used for both clinical diagnostic support, as well as in conjunction with ultrasound for mass screening in endemic CE and AE areas.

1. Introduction

Echinococcosis is a worldwide zoonosis caused by the larval stages of tapeworms (cestodes) belonging to the genus *Echinococcus* (family Taeniidae). *Echinococcus granulosus* and *Echinococcus multilocularis*, which cause human cystic echinococcosis (CE) and alveolar echinococcosis (AE), respectively, are highly endemic in China (Wen and Yang, 1997; Craig, 2004). Both cause serious and potentially life-threatening diseases, the latter especially with high fatality rates and poor prognosis if not diagnosed and treated in the early stages (Zhou et al., 2000; WHO/OIE, 2001; Craig et al., 2003). Mixed CE and AE cases are rare but have also been reported in China (Wen et al., 1992; Yang et al., 2006a,b). Currently, mortality for human CE may vary between 0.2% and 4.5%, and for human AE between 10% and 15% (Wen and Yang, 1997; WHO/OIE, 2001; McManus et al., 2003; Zhang et al., 2003). Early diagnosis of human echinococcosis is difficult because CE and AE cases usually have no signs or symptoms during the first few years of infection. Human echinococcosis commonly comes to the attention of clinicians because of non-specific clinical signs (e.g. upper abdominal pain, jaundice, allergic reactions), or due to incidental image findings of echinococcal cysts or lesions, or after specific mass screening surveys by ultrasound and/or serology (WHO, 1996; WHO/OIE, 2001; Zhang and McManus, 2006).

The frequent difficulty in obtaining a definitive diagnosis is one reason why immunological methods have played an important role in diagnosis of human echinococcosis (Wen et al., 1995; Rogan and Craig, 1997, 2002; WHO/OIE, 2001). Almost all traditional immunodiagnostic methods (e.g. Casoni intradermal test, complement fixation test, indirect haemagglutination test, indirect immunofluorescence antibody test, immunoelectrophoresis, and latex agglutination test), have now been replaced by the enzyme-linked immunosorbent assay (ELISA) and/or immunoblotting which are commonly performed in routine laboratory diagnosis of human echinococcosis (Rogan and Craig, 2002; Craig et al., 2003). Hydatid cyst fluid lipoprotein antigen B (AgB) from *E. granulosus*, and Em2/Em2plus, and/or Em18 antigens from *E. multilocularis*, are considered to be the most specific native or recombinant antigens for immunodiagnosis of human CE and AE, respectively (Gottstein et al., 1987; Ito, 2002; Zhang et al., 2003).

Although ELISA and immunoblotting are very useful laboratory tests for human echinococcosis, a rapid and cheap immunological

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method that can be used for initial diagnosis of clinically suspected CE or AE, and that could also be applied in community screening, would be extremely convenient. Rapid serological test formats such as dot-ELISA have been previously assessed for both human CE and AE, and although useful in conjunction with mass ultrasound screening, they were temperamental, and difficult to use and interpret (Zheng et al., 1986; Rogan et al., 1991; Eliades et al., 1998; Qiao et al., 1999; Craig et al., 2000). Dot immunogold filtration assay (DIGFA) is a rapid immunodiagnostic test similar to a 'pregnancy' test that uses colloidal gold conjugated antibody or antigen instead of enzyme or fluorescence conjugates (Faulk and Taylor, 1971; Horisberger et al., 1975; May, 1991; Chun and Chu, 1989; Dar et al., 1994; Xiao et al., 1995). Antigens are attached on a nitrocellulose membrane, and serum or whole blood applied, followed by colloidal gold conjugated anti-human antibodies to give a desired color change to indicate a positive or negative reaction.

In the current study a rapid DIGFA has been developed for human echinococcosis and assessed with four different native antigen preparations including *E. granulosus* crude hydatid cyst fluid antigen (EgCF), hydatid cyst fluid native antigen B (AgB), an *E. granulosus* protoscolex antigen extract (EgP), and an *E. multilocularis* metacestode laminated layer extract (Em2). The test was assessed in Xinjiang Medical University Hospital (Urumqi, northwestern China), which has treated over 6000 human echinococcosis cases in the last 40 years (Wen and Yang, 1997). The current study showed that the major advantages of DIGFA were rapidity, convenience, and ability to provide initial diagnosis and even differentiation of CE and AE in approximately 80% of cases either in clinical or community screening settings.

2. Materials and methods

2.1. Preparation of diagnostic antigens

Native extracts of *E. granulosus* and *E. multilocularis* were used because they can be prepared relatively easily by most laboratories. Sheep hydatid cyst fluid and protoscoleces from *E. granulosus* were collected in Xinjiang Uygur Autonomous Region (XUAR), China. Crude cystic fluid (EgCF) was partially purified by affinity chromatography using a normal human serum coupled to CNBr–Sepharose 4B to remove non-specific host reactive proteins from sheep hydatid cyst fluid (Rogan et al., 1991; Zhang et al., 2000). A crude somatic extract of *E. granulosus* protoscoleces (EgP) with >85% viability were harvested from fertile sheep hepatic hydatid cysts, prepared by ice cold homogenization and centrifugation (13,000 × *g* for 30 min at 4 °C) and partially purified by affinity chromatography as for EgCF (Zhang et al., 2000). *E. granulosus* cyst fluid antigen B (AgB) was purified from fresh sheep hydatid cyst fluid by precipitation, boiling, centrifugation and concentration by dialysis as previously described (Rogan et al., 1991; Rogan and Craig, 1997; Zhang et al., 2000, 2001). Experimental infections of gerbils (*Meriones unguiculatus*) after 3 months post-infection with *E. multilocularis* protoscoleces (in a metacestode homogenate suspension) were used to produce metacestode tissue for extraction of a laminated layer enriched antigen (Em2) by homogenization, centrifugation and affinity chromatography using rabbit anti-*E. granulosus* cyst fluid-IgG coupled CNBr–Sepharose 4B column (Gottstein et al., 1983; Zhang et al., 2001).

2.2. Serum samples and echinococcosis patients

2.2.1. Hospitalized hydatid patients

Archived serum panels used in the initial laboratory development and standardization of the DIGFA, were available from 108 post-operative hepatic CE cases, 34 post-operative hepatic AE cases, and 101 healthy controls collected from Xinjiang Medical University Hospital (XMUH) during 1998–2000. In addition 25 sera from cysticercosis (*Taenia solium*) patients were a gift from Prof. Y.H. Liu, Chongqing Medical University, PR China.

A serum panel was also available to assess hospital-based diagnosis of DIGFA and compared with standard ELISA. It consisted of 857 CE sera including 711 hepatic CE cases: among them, 516 ultrasound and/or surgery confirmed patients with less than 2 years post-surgery, 64 lung CE cases (diagnosed by X-ray or computerized tomography (CT)), 11 abdominal CE (diagnosed by ultrasound or CT), 18 multi-organ CE (diagnosed by ultrasound and CT) and 47 non-liver/lung CE cases (diagnosed by ultrasound, CT or magnetic resonance imaging (MRI)). In addition, sera from 42 liver AE cases and 1 mixed AE/CE case were assessed. In total 702 serum samples from non-hydatid disease patients were used as negative controls: non-parasite simple cystic disease 153, carcinoma 85, tuberculosis 28, solid or complicated space-occupying lesions (non-echinococcosis by imaging) 266, cirrhosis 6, abscess 13, cysticercosis 3, cholecystitis/gallstones 12, other patients treated in internal medicine (for hypertension, diabetes, and other clinical conditions) 88 and healthy individuals 5. All samples were collected and tested in XMUH during the period 1999–2006. For non-endemic controls, 35 sera from healthy people were collected from a hospital in Greater Manchester, UK, which is a non-endemic area.

2.2.2. Community screening

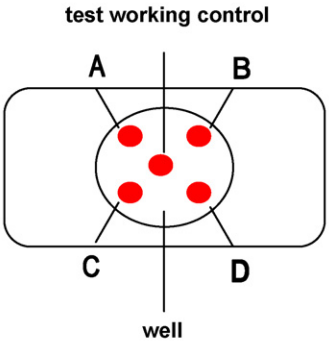
Echinococcosis endemic communities in northwest China that were screened by ultrasound and serology were: Qinghe, Hobukersaier, Wenquan, Xinyuan County and Bayanbulak Pasture in XUAR; Xiji County in Ningxia Hui Autonomous Region (AR); Ganzi County in Sichuan Province; and Dingqing County in Tibet AR. Based on ultrasound scan as the gold standard, serum samples from 160 CE and 108 AE cases, and from 2923 ultrasound normal persons were processed from these endemic communities in northwest China (Feng et al., 2002; Chen et al., 2005; Wang et al., 2001; Yang et al., 2006a,b). Ultrasound normal cases with a previous history of echinococcosis surgery were not included in the 'negative' group. All persons included gave informed consent for their serum to be collected and assessed in the study. Ethical permission was granted by the Xinjiang Medical University Hospital Ethical Committee.

2.3. DIGFA procedure

The four native antigen preparations (mostly protein antigens EgCF, EgP and AgB, and mostly carbohydrate antigen Em2) were used at a protein concentration, measured by OD value under 280 nm wavelength and then diluted to 2.2, 2.2, 0.5 and 2.2 mg/mL, respectively before optimized. Together with a quality control (diluted normal human sera), they were coated 1 μL/dot onto nitrocellulose (NCP) paper (pore size 0.45 μm, Millipore Co., Bedford, USA) fixed in a special plastic frame (5 cm × 3.5 cm) with a central well (diameter 0.7 cm) (XMUH Chinese patent: ZL 99 2 19078.9) (Fig. 1). Test serum was diluted with a 20 mM Tris–HCl (pH 8.2) based sample buffer (20 μL serum or 40 μL heparinised blood in 5 drops (about 220–250 μL) buffer) onto the NCP in the well until completely infiltrated. This was followed by 3 drops (about 130–150 μL) of 20 mM Tris–HCl (pH 8.2) washing buffer, 3 drops (about 130–150 μL) of colloidal gold conjugated anti-human IgG antibody solution and finally by Tris–HCl washing buffer. Colloidal gold was made by sodium citrate reduction of hydrogen tetrachloroaurate ($HAuCl_4$), and conjugated with goat anti-human IgG (Sigma I1886, USA; Sino-American Biotechnology Co., Luoyang, China) (Beesley, 1989; Millipore Corp., 1996; Oliver, 1999a,b; Reddy, 2006). Each reagent was added after the previous was totally absorbed (generally 1 min per step). The result could be

(i) Diagram of antigen dots:

Dot A is for EgCF, Dot B for EgP, Dot C for EgB and Dot D for Em2 antigens



(ii) Actual DIGFA result after serum test (3 minutes)

(a) serum positive CE case, (b) serum positive AE case, (c) negative control

Fig. 1. DIGFA test kit. (i) Diagram of antigen dots: Dot A is for EgCF, Dot B for EgP, Dot C for EgB and Dot D for Em2 antigens. (ii) Actual DIGFA result after serum test (3 min) (a) serum positive CE case, (b) serum positive AE case, and (c) negative control.

observed and recorded immediately after the last washing buffer had filtrated, and the whole assay usually took 3–4 min (Fig. 1). DIGFA reagents were stable when stored at 4 °C for 1 year.

The intensity of the red color as a spot from the colloidal gold conjugate indicated the degree of immune combination. A control for correct working of the test was a diluted normal human serum pool (from normal human serum pool) placed in a central well, which should always become positive if all reagents were in good working condition. The crude EgCF and EgP extracts were used primarily for anti-*Echinococcus* antibody sensitivity, while AgB was mainly specific for the confirmation of CE, and Em2 primarily for AE (Gottstein et al., 1987; Rogan and Craig, 1997; Craig et al., 2000; WHO/OIE, 2001; Zhang et al., 2003). If either one of the respective EgCF, EgP, AgB or Em2 spots appeared (a red spot), the sera were presumptively positive for echinococcosis. Color change in the AgB spot indicated high probability of CE antibodies, and the Em2 spot indicated high probability of AE antibodies. If both AgB and Em2 spots appeared, the serum was considered to be either CE or AE, respectively dependent on the strength of color change. When EgCF and/or EgP was/were positive, but both AgB and Em2 were negative, the serum was considered "doubtful". If no antigen spots were reactive, the serum was considered negative. The degree of color in general reflected the antibody activity level in a serum sample. The degree of positive color change was subjective and judged between "+" to "++++", according to the color-darkness level (Fig. 1(ii)).

2.4. ELISA procedure

The panels of serum samples from hospital treated echinococcosis patients were tested by both DIGFA and ELISA. The ELISA was the routine test used in the hospital (XMUH). Microtitration plates were coated overnight at 4 °C with the above four native antigen preparations at optimal concentrations (2–10 μg/mL) in 0.1 M carbonate/bicarbonate buffer (pH 9.6). The four antigens were separately coated on ELISA plates. The plates were then washed with 0.1 M PBS 0.1% Tween 20, and blocking buffer added (PBS, pH 7.2, 0.3% Tween 20, 1% bovine serum albumen (BSA), 2% gelatin) for 2 h at 37 °C; washed with 0.1 M PBS (0.1% Tween 20) and then freeze-dried (using Labconco Freeze Dry System and Stoppering Tray Dryer, Labconco Co., Kansas, USA) for storage at 4 °C until used. Human serum samples were diluted 1:100 in PBS (pH 7.2, 0.1% Tween 20, 3.75% normal sheep serum) at 100 μL/well, added in duplicate to the above four antigens coated microtitration plate and incubated for 30 min at 37 °C. Plates were washed as above then incubated with 100 μL/well of horseradish peroxidase conjugated anti-human IgG (Sigma A6029, Saint Louis, USA), diluted 1:8000 with 0.1 M PBS, 3% sheep serum and 0.05% enzyme stable reagent (Beier Co., Lianyungang, Jiangsu, China) for 20 min at 37 °C. After the final wash a substrate of 3,3′,5,5′-tetramethylbenzidine (TMB) was used and allowed to develop for 15 min, followed by a stop solution of 50 μL 0.01 M sulfuric acid. Wells were read at 450 nm with a Bio-Rad 550 plate reader (Bio-Rad Laboratories, Inc., CA, US). Positive control sera from confirmed CE or AE patients, and negative control sera from healthy individuals, were used in each microtitration plate for quality control. Sera were tested in duplicate and the positive–negative cut-off value was determined as the mean optical density of a panel of negative controls ($n = 35$) plus three standard deviations (OD cut-off for EgCF = 0.286; EgP = 0.609; AgB = 0.105; Em2 = 0.187) (Craig et al., 2000; Rogan and Craig, 1997, 2002). Sensitivity and specificity were calculated using 95% confidence intervals and significance values were also determined at the 95% probability level.

3. Results

3.1. Initial validation of multiple Echinococcus antigens (EgCF, EgP, AgB and Em2) in DIGFA

In preliminary assessment of archived serum samples the sensitivity of DIGFA for human CE ($n = 108$) was 92.6%, 90.7% and 89.8%

Table 1
Comparison of rapid DIGFA and standard ELISA applications for serodiagnosis of human echinococcosis in hospitalized CE ($n = 857$) or AE cases ($n = 42$).

Clinical diagnosis	N	DIGFA			ELISA			p
		Positive[a]	Negative	Sensitivity (%) (95% CI[b])	Positive	Negative	Sensitivity (%) (95% CI)	
CE	857	692	165	80.7 (78.1–83.3)	643	214	75.0 (73.5–76.5)	<0.01
AE	42	39	3	92.9 (88.9–96.8)	41	1	97.6 (95.2–99.9)	>0.05
AE/CE	1	1	0	100	1	0	100	
Controls[c]	702	73	629[d]	10.4[e]	72	630[d]	10.3[e]	>0.05
Total	1602	805	797		757	845		

[a] Positive means any one of the four antigen dots changing color in DIGFA, and any one of the four antigens OD value over cut-off in ELISA.
[b] 95% confidence interval, $p \pm 1.96\sqrt{p(1-p)/n}$, p means sensitivity or specificity, n means number.
[c] Controls were defined as those free from either CE or AE infection.
[d] Negative concordance was 83.8% (588/702) between DIGFA and ELISA in control group.
[e] False positive rate in control group (non-CE/AE).

Table 2
Sensitivity and specificity of AgB antigen in DIGFA and ELISA for hospitalized CE cases.

Clinical diagnosis		N	DIGFA		ELISA		p value
			Positive	Negative	Positive	Negative	
CE		857	586	271	492	365	<0.01
	AE	42	28	14	21	21	>0.05
Non-CE	Controls	702	21	681	28	674	>0.05
	Subtotal	744	49	695	49	695	>0.05
Total		1601	635	966	541	1060	

Sensitivity was 68.4% (586/857) with 95% CI 66.8–69.9% in DIGFA and 57.4 (492/857) (95% CI 54.1–60.7%) in ELISA. Specificity was 93.4% (695/744) (95% CI 91.6–95.1%) in DIGFA and ELISA. Negative concordance between DIGFA and ELISA was 94.2% (661/702) in control group.

with EgCF, EgP and AgB native antigen preparations, respectively. Of the four different native antigen preparations the highest sensitivity occurred with antigen EgCF (92.6% for CE cases and 88.2% for AE cases). For human AE ($n = 34$) sensitivity of DIGFA was 91.1% for Em2 antigen. The specificity for CE with AgB antigen in DIGFA was 88.1% with 95% confidence intervals (CI) (82.0–94.2%), and for AE with Em2 antigen was 93.6% with 95% CI (89.4–97.8%). Cross-reactivity between human CE and AE for AgB antigen was 35.3% for AE, while cross-reactivity for Em2 with human CE was 7.4%. Cross-reaction in DIGFA with serum from cysticercosis patients was observed with all four antigens (60% and 56% with EgCF and EgP; 8% and 16%, respectively for AgB and Em2). There was no statistical difference between results observed using DIGFA versus the standard ELISA using above four native antigens ($p > 0.05$).

3.2. Diagnostic evaluation of the rapid DIGFA in a hospital setting

The DIGFA was applied for immunodiagnosis of clinical echinococcosis using a panel of sera ($n = 899$, CE = 857 and AE = 42) taken from patients that were treated in XMUH over the period

Table 3
Sensitivity and specificity of Em2 antigen in DIGFA and ELISA for hospitalized AE cases.

Clinical diagnosis		N	DIGFA		ELISA		p value
			Positive	Negative	Positive	Negative	
AE patients		42	35	7	33	9	>0.05
Non-AE	CE	857	146	711	290	567	<0.01
	Controls	702	5	697	20	682	<0.01
	Subtotal	1559	151	1408	310	1249	<0.01
Total		1601	186	1415	343	1258	

Sensitivity was 83.3% (35/42) with 95% CI 72.1–94.6% in DIGFA and 78.6% (33/42) (95% CI 66.2–91.0%) in ELISA. Specificity was 90.3% (1408/1559) (95%CI 89.6–91.0%) in DIGFA and 78.0% (1249/1559) (95% CI 75.9–80.1%) in ELISA. Negative concordance between DIGFA and ELISA was 95.7% (672/702) in control group.

Table 4
Comparison of DIGFA test with abdominal ultrasound imaging in mass screening community studies in western China (data combined from Xinjiang, Ningxia, Sichuan and Tibet AR).

US result	N	EgCF positive	EgP positive	AgB positive	Em2 positive	All negative
CE	160	112	88	82	18	45
AE	108	95	83	74	84	10
Normal	2923	641	462	91	70	2175
Sensitivity	CE	70%	55%	51.3%	11.3%	71.8%[a]
For each antigen	AE	87.9%	76.9%	68.5%	77.8%	90.7%[b]
Specificity		78.1%	84.2%	96.9%	97.6%	74.4%[c]

[a] 95% CI of general sensitivity for CE was 64.9–78.8%.
[b] 95% CI of general sensitivity for AE was 85.2–96.2%.
[c] 95% CI of general specificity for both CE and AE was 72.8–76.0%.

Table 5
DIGFA test using AgB antigen for immunodiagnosis of CE in community mass screening studies (data combined from Xinjiang, Ningxia, Sichuan and Tibet AR) in comparison to abdominal ultrasound.

Ultrasound		AgB-DIGFA		Total
		Positive	Negative	
CE patients		82	78	160
	AE	74	34	108
Non-CE patients	Controls	91	2832	2923
	Subtotal	165	2866	3031
Total		247	2944	3191

Sensitivity 51.3% (82/160) and specificity 94.6% (2866/3031).

Table 6
DIGFA test using Em2 antigen for diagnosis of AE in community mass screening studies (data combined from Xinjiang, Ningxia, Sichuan and Tibet AR) in comparison to abdominal ultrasound.

Ultrasound		Em2-DIGFA		Total
		Positive	Negative	
AE patients		84	24	108
	CE	18	142	160
Non-AE	Controls	70	2853	2923
	Subtotal	88	2995	3083
Total		172	3019	3191

Sensitivity 77.8% (84/108) (95% CI 70.0–85.6%) and specificity 97.1% (2995/3083) (95% CI 96.5–97.6%).

September 1999 to April 2006. The DIGFA test had a lower sensitivity for CE (80.7%) compared with the initial laboratory based study ($p < 0.01$) for all CE patients combined including liver, lung, or other organs (Table 1). Overall specificity of DIGFA for CE was 93.4% (695/744 non-CE cases were negative); cross-reactions occurred with 28/42 AE cases (Table 2). The sensitivity of Em2-DIGFA for human AE showed no statistical difference between the laboratory based study (91.1%) and the main hospital study (90.3%) ($p > 0.05$) (Table 3). However Em2 antigen showed cross-reaction with 17% (146/857) of CE cases. The sensitivity of DIGFA for CE in different organs was: 94.4% in multi-organ CE, 83.4% for hepatic CE, 80.7% for pulmonary CE, 80% for CE in the pelvic cavity, 70% for CE in the abdominal cavity, and 56.7% for CE in other organs (including heart, kidney, brain, spine, bone, subcutaneous). Significant statistical differences were observed between DIGFA and ELISA for serodiagnosis of CE. The ELISA exhibited lower sensitivity for hospitalized human CE cases (75.0%), but higher specificity (97.6%) compared to DIGFA ($p < 0.01$). For human AE both ELISA and DIGFA had similar sensitivities (97.6% vs. 92.9%) ($p > 0.05$) but different specificities (80.1% vs. 90.3%) ($p < 0.01$) (Tables 1 and 3). Overall a false positive rate of 10.4% occurred with the DIGFA for sera from persons without echinococcosis. Negative concordance between DIGFA and ELISA in the control group was 83.8% (588/702) in total, i.e. 94.2% (661/702) for AgB and 95.7% (672/702) for Em2.

3.3. Diagnostic evaluation of the DIGFA for endemic community mass screening in northwest China

When DIGFA was used in conjunction with ultrasound in community mass screening studies, it showed good sensitivity for human AE (90.7%) but lower sensitivity for CE (71.8%). Overall specificity for CE was 78.1% and for AE was 97.6% based on ultrasound abdominal screening as the gold standard. AgB antigen had the lowest sensitivity (51.3%) in DIGFA in comparison to ultrasound confirmed asymptomatic human CE ($n = 160$), while Em2 antigen in DIGFA had a sensitivity of 77.8% for ultrasound confirmed asymptomatic AE cases ($n = 108$) (Table 4). Specificity of AgB for community detected human CE was 94.6% and Em2 for AE was 97.1% in this study (Tables 5 and 6). AgB antigen in DIGFA gave high cross-reaction 68.6% (74/108) with community ultrasound detected AE cases, while Em2 antigen cross-reacted with 11.3% (18/160) of ultrasound confirmed CE cases.

3.4. False positives and negatives

There were in total 14.6% (131/899) false negative sera among clinically defined echinococcosis patients using the DIGFA test and these were also negative using the standard ELISA. Clinical features of false negative CE cases were collapsed, degenerated, necrotic (Type CE4), or consolidated cysts, or calcified type cysts (CE5), or single, small univesicular cysts (Type CE 1), and also small single cysts deep in organ locations (WHO/OIE, 2001). A false positive rate of 10.4% (73/702) occurred in hospitalized persons in XMUH without echinococcosis. These cases included simple non-parasitic cysts, carcinoma or tuberculosis and showed no evidence of echinococcocal cysts or lesions by ultrasound or other imaging methods (X-ray or CT). Three cysticercosis cases treated in XMUH were all seronegative in DIGFA and ELISA.

4. Discussion

Gold standard laboratory tests for human echinococcosis serology are currently based on standard ELISA or immunoblot formats using *E. granulosus* hydatid cyst fluid antigen B for CE, and *E. multilocularis* metacestode antigen Em2 or antigen Em18 for AE (Gottstein et al., 1983, 1987; Zhang et al., 2000, 2001; Rogan and Craig, 2002; Ito, 2002; Craig et al., 2003; Carmena et al., 2006). Rapid diagnostic tests for human echinococcosis would provide several advantages, not least more practical application in resource-poor community settings, including them as a confirmatory tool during mass ultrasound screening surveys. Dot-ELISA rapid format has been applied in a few community based studies for human CE but has limitations since enzyme-conjugates are difficult to store and apply in field conditions (Zheng et al., 1986; Rogan et al., 1991; Qiao et al., 1999).

The current study reports the most comprehensive assessment and application of a rapid immunodiagnostic format for human CE and AE. We show that a dot immunogold filtration assay (DIGFA) exhibited the following features: (1) the test could give a reliable diagnostic result within 2–3 min using only 20 μL of serum or 40 μL heparinized blood, with no significant differences observed between serum or heparinised blood (see also Chen et al., 2005); (2) the test though qualitative was able to detect human echinococcosis in approximately 80–93% of cases and differentiate human CE and AE in about 80% of confirmed cases; (3) the DIGFA procedure is simple and no special training was required and therefore it had practical value for support of both community mass screening in conjunction with ultrasound, and for hospital-based diagnostic confirmation of echinococcosis.

Based on a panel of 1601 serum samples from advanced CE or AE patients confirmed by imaging, pathology and/or surgery in Xinjiang Medical University Hospital (XMUH), Urumqi, China, and control sera, overall DIGFA sensitivity was 80.7% (692/857) for human CE and 92.9% (39/42) for human AE. The lower sensitivity for CE in the clinical hospital setting compared with the preliminary laboratory based assessment, could be considered to be due to the inclusion of pre-operative CE cases in the hospital setting, versus mainly post-operative sera in the archived laboratory samples. Post-surgery CE sera may show higher overall antibody positivity when compared to pre-operative sera. Specificity of DIGFA for human echinococcosis overall (both CE and AE) in symptomatic hospital treated cases was 89.6% (629/702); with antigen B specificity for CE at 93.4%, and Em2 specificity for AE at 90.3%.

When the rapid test was applied to community mass screening studies in western China (i.e. sites in Xinjiang, Ningxia, Sichuan, Tibet), the *Echinococcus* DIGFA showed slightly lower sensitivity (71.8% for CE and 90.7% for AE) and specificity (74.4% for echinococcosis in general, 94.6% with antigen B for CE, 97.1% with Em2 for AE) compared with the hospital-based DIGFA assessment. Recombinant antigens (e.g. rec AgB) might be used in the DIGFA test in further assessments since they are easier to standardize, but poor stability and reduced sensitivity was found to occur in initial studies (X. Feng, unpublished observations). The DIGFA test was nevertheless extremely useful in these resource-poor settings as a combined diagnostic tool in conjunction with ultrasound. Sera could be tested within 1 h of ultrasound scan and up to 200 sera tested in 1 day. Diagnosis of CE or AE was able to be confirmed in more than 80% of community detected cases using the ultrasound/DIGFA serology approach and therefore facilitated efficient clinical treatment and/or follow-up recommendations.

Reasons for lower sensitivity of DIGFA in community (vs. hospital settings) may be due to exposure without a detectable abdominal cyst lesion and especially involvement of sites not ultrasound detectable. However, "false positive" cases are actually more common in mass screenings performed in endemic areas than in non-endemic areas or in hospital settings; this has been attributed to the high proportion of asymptomatic subjects that may have spontaneously recovered after contact with the parasite (Craig et al., 2000; Yang et al., 2006a,b). Lower sensitivity may be related to false positive ultrasound images and thus misdiagnosed space-occupying lesions (e.g., neoplasia, abscesses, non-parasitic cysts the nature of which cannot be confirmed using CT scan or MRI) and/or to the presence of small cysts or lesions, or degenerate, calcified, or necrotic cysts/lesions, or more generally to undetectable levels of circulating antibodies depending on the number, size, location and condition of the cyst (Gavidia et al., 2008). The false negative rate of DIGFA for hospitalized CE cases was 19.3% (165/857) compared to 7.1% (3/42) for AE, while false positives occurred in 6.6% of CE (49/744), and in 9.7% (151/1559) of AE cases. The DIGFA test could reliably differentiate CE and AE cases from each other around 80% of the time and an Em2 positive reaction appeared in 17.1% (146/857) of CE case sera. The DIGFA results were comparable to those obtained with the standard ELISA using the above four antigens respectively (false negative for CE 25.0%, for AE 2.4%, false positive for both 10.3%). In general the standard ELISA was less sensitive ($p < 0.01$) but exhibited comparable specificity with DIGFA for human CE. The AgB preparation from *E. granulosus* hydatid cyst fluid and Em2 metacestode extract from *E. multilocularis* showed reasonable specificity (90.3–97.1%) in DIGFA for CE or AE, and were comparable to other studies using traditional ELISA formats. Determination of the carbohydrate concentration, rather than protein, may be beneficial in further optimization of Em2 use in the DIGFA because this antigen is essentially a carbohydrate (Gottstein et al., 1983, 1987; Liu and Zhao, 1993; Poretti et al., 1999; Carmena et al., 2006; Dai et al., 2001).

In conclusion, a robust 3 min eye-read dot immunogold filtration assay (DIGFA) for the rapid serodiagnosis of human cystic (CE) and alveolar (AE) echinococcosis was developed in which 4 crude or semi-purified native antigens from *E. granulosus* (EgCF, EgP, AgB) and *E. multilocularis* (Em2) were utilized simultaneously. The *E. granulosus* protoscolex (EgP) and crude cyst fluid (EgCF) extracts, provided high sensitivity for the test; while *E. granulosus* partially purified antigen B (AgB) and *E. multilocularis* antigen (Em2) ensured specificity comparable to standard ELISA. The DIGFA format was used successfully in conjunction with ultrasound for mass screenings to identify or confirm asymptomatic CE and AE cases in co-endemic communities in western China.

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·疾病控制·

999例包虫病住院患者经济负担分析

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【关键词】 囊型包虫病；疾病经济负担；人力资本法

Direct and indirect economic burden of 999 cystic echinococcosis patients in a tertiary hospital

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【Key words】 Cystic echinococcosis; Economic burden of disease; Human capital method

包虫病为人畜共患寄生虫病，在农、牧区已成为当地居民因病致贫、因病返贫的主要原因之一。为探讨造成包虫病患者经济负担的影响因素和卫生决策者提供参考依据，本研究利用2004－2008年乌鲁木齐市某三级医院包虫病住院患者资料，分析其经济负担。

1. 对象与方法：调查该医院2004－2008年包虫病患者(1022人次，其中泡型包虫病23例，囊型包虫病999例)住院治疗费用报表。由于泡型包虫病较难根治、费用较高且例数少，本研究着重分析囊型包虫病患者费用。应用Excel软件建立数据库资料，数据分析采用SPSS 13.0软件。统计学方法采用方差分析、多元逐步回归，并利用伤残调整生命年(DALY)与人力资本法结合估计间接经济负担。

2. 结果：

(1)基本情况：患者职业以农(林、牧)民最多515例(51.55%)，其次是干部208例(20.82%)。汉族599例(59.96%)，维吾尔族186例(18.62%)，哈萨克族100例(10.01%)，回族53例(5.31%)，蒙古族47例(4.70%)，其他民族14例(1.40%)。男性527例(52.75%)，女性472例(47.25%)。患者年龄3～83岁，其中以30～34岁年龄组为主173例(17.32%)，20～24岁组最少为71例(7.11%)。2004－2008年总调查病例数为999例，除2004年外，各年病例数有逐年上升趋势，依次为186、161、178、189和285例。

(2)直接经济负担分析：①住院费用：不同年龄组、性别患者平均住院费用采用均数($\bar{x}$)和中位数(M)描述。≥70岁患者人均住院费用最高为17 911.90元，其次是15～30岁组为16 126.22元(表1)。②住院费用影响因素分析：将所有调查对象的住院总费用做因子进行方差分析。结果显示年龄($F=4.004, P=0.003$)、民族($F=3.201, P=0.007$)、职业($F=2.048, P=0.047$)、治疗效果($F=44.757, P=0.000$)、住院天数($F=21.478, P=0.000$)、有无医保($F=12.811, P=0.000$)、西药费($F=127.262, P=0.000$)、手术费($F=31.582, P=0.002$)和检查费($F=41.265, P=0.001$)对住院总费用具有影响。

表1 不同年龄组、性别包虫病患者人均住院费用(元)

年龄组(岁)	人数	男性		女性		合计	
		$\bar{x}$	M	$\bar{x}$	M	$\bar{x}$	M
0～	59	9 367.21	9 596.82	9 251.31	9 184.41	9 325.96	9 541.77
15～	223	15 798.19	14 013.74	16 481.85	13 255.61	16 126.22	13 771.35
30～	420	14 478.92	14 291.32	14 569.45	13 327.40	14 521.17	13 863.08
45～	194	15 981.69	15 519.02	15 677.42	14 294.65	15 832.69	14 945.83
60～	75	14 302.73	8 683.87	15 305.90	13 518.56	14 797.62	12 381.87
70～	28	20 962.08	23 180.12	15 624.26	15 539.99	17 911.90	17 448.00
合计	999	14 817.95	13 803.85	15 082.86	13 327.40	14 943.11	13 681.90

(3)间接经济负担分析：用DALY估算[1-3]。由于目前国内尚无包虫病疾病负担相关文献。Budke等[4]将包虫病失能权重指标D按照治疗效果分为5个等级，即治愈、好转、未愈、恶化、死亡，分别赋值为0、0.2、0.239、0.809、1。本研究按照临床的划分："治愈"指经过手术治疗将包囊完整摘除且无并发症；"好转"指患者经过一段时间的药物治疗得到有效的控制，且经B超检测包囊明显缩小；"未愈"指医生建议进行手术治疗或药物治疗，而患者因为经济等因素未接受，而提前出院；"恶化"指患者接受治疗后，包囊没有缩小且引发其他并发症。由表2可知30～34岁年龄组人数最多为173例，该组患者DALY损失最多，合计达到261.41年。20～24岁年龄组人均DALY损失最多为1.88年。

用DALY与人力资本法结合估算间接经济负担。是将间接经济负担中的时间转换为货币价值，包括用工资、人均国民收入、人均国民生产总值。本文采用DALY与人力资本法结合的计算公式：间接经济负担＝人均国民生产总值(GNP)×DALY×生产力权重。由于各年龄组生产力不同，其权重亦不同。0～14岁年龄组未参加社会财富创造，其权数为0.15；15～44岁和45～59岁组分别为0.75、0.80，≥60岁又降为0.10[5]。2004－2008年5年平均GNP为15 170.6元[6]，999例患者总间接经济负担为12 032 538.63元，人均间接经济负担为12 044.58元(表3)。

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表2　各年龄组不同性别包虫病患者DALY的分布

年龄（岁）	残疾权重分类	男性			女性			DALY合计
		例数	人均DALY	总DALY	例数	人均DALY	总DALY	
0～	治愈	3	0.00	0.00	4	0.00	0.00	0.00
	好转	1	7.08	7.08	1	7.22	7.22	14.30
5～	治愈	12	0.00	0.00	6	0.00	0.00	0.00
	好转	0	0.00	0.00	1	7.49	7.49	7.49
10～	治愈	20	0.00	0.00	8	0.00	0.00	0.00
	好转	2	7.40	14.80	1	7.52	7.52	22.32
15～	治愈	20	0.00	0.00	12	0.00	0.00	0.00
	好转	0	0.00	0.00	3	7.12	21.36	21.36
	未愈	1	8.56	8.56	0	0.00	0.00	8.56
20～	治愈	32	0.00	0.00	28	0.00	0.00	0.00
	好转	4	6.91	27.62	2	7.04	14.08	41.70
	未愈	2	8.31	16.62	1	8.07	8.07	24.69
	恶化	0	0.00	0.00	1	34.27	34.27	34.27
	死亡	1	33.03	33.03	0	0.00	0.00	33.03
25～	治愈	49	0.00	0.00	49	0.00	0.00	0.00
	好转	5	6.13	30.65	7	6.24	43.68	74.32
	未愈	2	7.56	15.12	4	7.55	30.22	45.34
30～	治愈	65	0.00	0.00	64	0.00	0.00	0.00
	好转	22	5.61	123.50	12	5.80	69.56	193.06
	未愈	5	6.80	34.00	5	6.87	34.35	68.35
35～	治愈	67	0.00	0.00	47	0.00	0.00	0.00
	好转	12	4.99	59.84	9	5.10	45.89	105.72
	未愈	5	6.22	31.12	4	6.13	24.52	55.64
40～	治愈	37	0.00	0.00	43	0.00	0.00	0.00
	好转	9	4.49	40.38	6	4.55	27.31	67.69
	未愈	2	5.55	11.10	4	5.34	21.36	32.45
	恶化	0	0.00	0.00	2	18.07	36.14	36.14
45～	治愈	29	0.00	0.00	32	0.00	0.00	0.00
	好转	6	3.84	23.02	13	3.91	50.87	73.89
	未愈	3	4.59	13.76	1	4.89	4.89	18.65
50～	治愈	21	0.00	0.00	22	0.00	0.00	0.00
	好转	7	3.21	22.44	2	3.19	6.37	28.82
	未愈	2	3.89	7.79	0	0.00	0.00	7.79
55～	治愈	21	0.00	0.00	19	0.00	0.00	0.00
	好转	7	2.56	17.90	5	2.67	13.34	31.24
	未愈	3	3.18	9.53	1	12.21	12.21	21.74
60～	治愈	7	0.00	0.00	9	0.00	0.00	0.00
	好转	8	2.04	16.29	4	2.19	8.78	25.07
	未愈	2	2.35	4.70	2	2.65	5.31	10.01
65～	治愈	6	0.00	0.00	16	0.00	0.00	0.00
	好转	6	1.39	8.33	3	1.72	5.16	13.49
	未愈	7	1.74	12.17	3	2.05	6.16	18.33
	死亡	2	7.59	15.18	0	0.00	0.00	15.18
70～	治愈	5	0.00	0.00	10	0.00	0.00	0.00
	好转	2	0.86	1.72	0	0.00	0.00	1.72
	未愈	1	1.03	1.03	0	0.00	0.00	1.03
	死亡	1	3.23	3.23	0	0.00	0.00	3.23
75～	治愈	1	0.00	0.00	3	0.00	0.00	0.00
	好转	1	0.43	0.43	1	0.65	0.65	1.09
	未愈	0	0.00	0.00	2	0.84	1.68	1.68
80～	好转	1	0.05	0.05	0	0.00	0.00	0.05
合计	–	527	160.60	611.00	472	181.37	548.45	1159.45

表3　2004—2008年999例包虫病住院患者间接经济负担

年龄组（岁）	例数	DALY	生产力权重	人均间接经济负担（元）	总间接经济负担（元）	构成比（%）
0～	59	44.11	0.15	1 701.29	100 376.27	1
15～	643	842.32	0.75	14 904.94	9 583 874.84	80
45～	194	182.13	0.80	11 393.90	2 210 417.10	18
60～	103	90.88	0.10	1 338.55	137 870.41	1
合计	999	1 159.44	–	12 044.58	12 032 538.63	100

3. 讨论：本文显示住院天数、治疗效果、有无医保、年龄、不同职业的住院费用有统计学意义。其中，住院天数对住院费用影响最大，二者有正比关系。治疗效果也是住院费用的一个重要影响因素，死亡患者总费用最高（人均43 972.89元），这可能与有些患者在病情较重时才到三级医院治疗有关。本文还显示西药费、手术费和检查费对住院费用有影响，其中西药费用占最大份额，可能与患者术后多有感染或并发症，需用抗生素有关。5年该院收治囊型包虫病患者999例，共损失DALY高达1159.45（95%*CI*：991.02～1327.87）年，人均损失1.16个DALY。15～44岁组患者间接经济负担损失达9 583 874.84元，人均损失14 904.94元，均在各年龄组之首。应根据不同年龄组的特点制定相应的政策措施，以达到卫生资源更加优化配置。

我国目前对包虫病的研究多集中于流行病学调查分析，尚无对其经济负担研究的报道，即使是其他病种也多集中在直接经济负担的报道，从某种意义上说，与直接经济负担相比，疾病的间接经济负担虽然不是社会和家庭直接的经济支出，但却是社会劳动力有效工作时间的减少和工作能力的降低。因此，间接经济负担更能反映出疾病对社会危害程度的大小，是一种劳动力价值降低状况的体现。本研究中包虫病患者均间接经济负担损失高达12 044.58元，且随着GNP的上升呈逐年上升趋势。这与用同样方法估计的陕西省汉中市脑卒中所致农村人口间接经济负担为人均12 158.40元相近[7]。

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·现场调查·

新疆和布克赛尔蒙古自治县囊型包虫病危险因素分析

初向东 王桂芝 冯晓辉 尔西丁 贺金华 温浩

【摘要】 **目的** 分析新疆和布克赛尔蒙古自治县囊型包虫病流行相关的危险因素及探讨预防措施。**方法** 在和布克赛尔蒙古自治县采用随机抽样方法，进行人群囊型包虫病的流行病学调查，调查内容包括民族、年龄、性别、职业及生活习惯等，并对囊型包虫病相关的危险因素进行多因素logistic回归分析。**结果** 人群包虫病患病率为3.8%，血清阳性率为12.4%，其中囊型包虫病患病率为3.7%，泡型包虫病患病率为0.16%。通过多因素logistic回归分析，发现年龄和家庭屠宰牲畜与囊型包虫病患病有关，*OR*值分别是7.6(2.481～23.579)、3.2(1.297～7.809)。50～60岁年龄组患病率最高，*OR*值是<20岁年龄组的7.6倍；牧民患病率最高。**结论** 和布克赛尔蒙古自治县囊型包虫病呈持续高流行势态；年龄和有家庭屠宰牲畜行为与患囊型包虫病相关。

【关键词】 包虫病；囊型棘球蚴病；危险因素

Risk factors on human cystic echinococcosis in Hobukesar Mongolian Autonomous County in Xinjiang

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【**Abstract**】 **Objective** To study the risk factors of human cystic echinococcosis (CE) in Hobukesar Mongolian Autonomous County of Xinjiang(HMACX) and to discuss the related strategies for prevention and control. **Methods** A randomized sampling method was used to screen local residents for human CE in HMACX. CE related risk factors including ethnicity, age, sex, occupation and personal status on hygiene etc. were analyzed under multi-factor logistic regression. **Results** The prevalence rates of CE and alveolar echinococcosis (AE) were 3.7% (23/627) and 0.16% (1/627) respectively, with the seropositive rate as 12.4% (76/613). The main risk factors that significantly associated with CE were age (*OR*=7.6, 95% *CI*: 2.481-23.579) and slaughtering livestock in the households (*OR*=3.2, 95%*CI*: 1.297-7.809). Herdsmen had the highest prevalence of CE in all of the occupations in this study. **Conclusion** HMACX had been a highly endemic area for human CE, with age and family slaughtering-livestock-behavior appeared to be the main possible risk factors.

【**Key words**】 Hydatid disease; Cystic echinococcosis; Risk factors

包虫病是严重危害牧区人群健康的常见人畜共患寄生虫病，在我国西北地区广泛流行[1]，主要是细粒棘球蚴病(又称囊型包虫病，cystic echinococcosis, CE)和多房棘球蚴病(又称泡型包虫病或泡状棘球蚴病，alveolar echinococcosis, AE)两种包虫病[2]。新疆地区是包虫病高发区之一，主要以CE为主，AE为散发[3]。2001—2002年在新疆额敏县包虫病的流行病学基线调查显示，患病率为1.31%，血清阳性率为25.8%[4]。为了解和布克赛尔蒙古自治县CE流行危险因素，评价和制定预防控制措施，于2007年10月在该县进行了相关研究。

对象与方法

1. 调查地点和对象：在和布克赛尔蒙古自治县随机抽取1个牧业乡(布斯屯格牧场)和2个半农半牧乡(查干库勒乡、巴音傲包乡)进行人群CE感染的

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现场调查；应答率为98%。问卷、B超调查627人，血清学调查613人；其中男性256人，女性371人；年龄6～76岁（平均年龄28.4岁）；其中以蒙古族（166人）和哈萨克族（383人）人群为主。

2. 调查方法与内容：在实施调查前，就各种调查工具的使用方法、注意事项进行人员培训（精通蒙古族和哈萨克族语的当地疾病预防控制中心专业人员）。在各调查点逐人问卷调查，包括个人一般情况、包虫病史及可能与包虫病传播相关的危险因素等；调查对象均知情同意。并对其进行B超腹部探查，同时采集被调查者静脉血标本3 ml，采用新疆包虫病临床研究所研制的"组合抗原包虫病快速诊断试剂盒"分别检测针对抗原EgCF\EgP\EgB\Em2的特异性抗体水平，以任一抗原阳性计算血清阳性率。

3. 统计学分析：应用Excel程序录入调查问卷后，用SPSS 13.0软件分析民族、职业、年龄、家庭屠宰牲畜等生活习惯与包虫病可能的相关关系；选用logistic回归模型进行单、多因素分析，二分变量以虚拟变量形式赋值为1和2；多分变量则用指标编码设置虚拟变量，并以第一类为参照类，多因素分析采用前进法建立模型，联系强度为*OR*。

结　果

1. 基本情况：在3个乡随机调查的627人中，包虫病患病率为3.8%（24/627）；CE患病率为3.7%（23/627）；AE患病率为0.16%（1/627）；血清阳性率为12.4%（76/613）。不同职业人群间CE患病率以牧民最高为6.9%（χ^2=8.503，*P*=0.048）。见图1。发病最小年龄为6岁，最大为76岁。

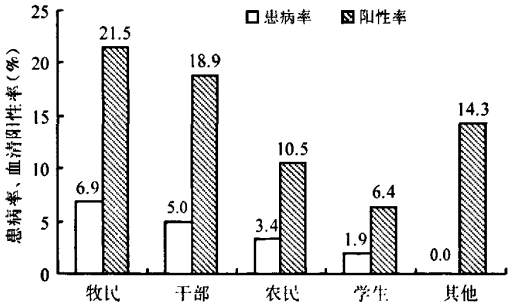

图1　和布克赛尔蒙古自治县不同职业人群CE患病率、血清阳性率分布

不同年龄之间人群CE患病率、血清阳性率中，在50～60岁年龄段的患病率为10.3%（Fisher's：χ^2=14.537，*P*=0.005）、血清阳性率21.1%最高（χ^2=20.917，*P*=0.001）。见图2。

2. 包虫病危险因素分析：对可能影响包虫感染的12个变量的赋值见表1。经过单因素logistic回归分析显示（表2），民族、职业、年龄、家庭屠宰牲畜和饮用水源5个因素与包虫感染相关。单因素分析有统计学意义的因素进行多因素logistic回归分析，剔除不显著变量，最终进入多因素模型的包括年龄和家庭屠宰牲畜2个因素，各变量的参数值见表3。

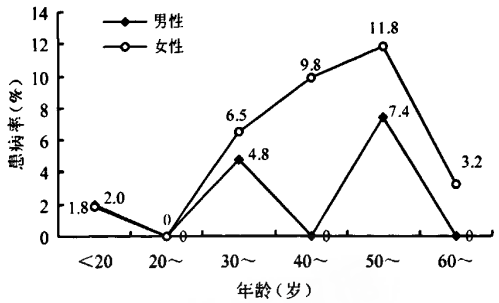

图2　和布克赛尔蒙古自治县不同年龄、性别CE的患病率

表1　分析变量及赋值

变量	赋　值
性别	1=男，2=女
年龄（岁）*	1=<20，2=20～，3=30～，4=40～，5=50～，6=60～
民族	1=汉，2=蒙，3=哈，4=其他
职业*	1=牧民，2=农民，3=学生，4=干部，5=其他
文化程度*	1=文盲，2=小学，3=中学，4=高中，5=大专以上
家庭养犬	1=是，2=否
家庭养牲畜	1=是，2=否
家庭屠宰	1=是，2=否
野犬	1=是，2=否
饮用水源*	1=自来水，2=深水井，3=浅水井，4=河塘水
饮用生水	1=是，2=否
食用生蔬菜	1=是，2=否

注：*多分类变量进行哑变量处理，以第一个为对照组

由表3可见，有2个变量进入方程，年龄和家庭屠宰为包虫病的可能危险因素。40～50岁和50～60岁的年龄组患包虫病的*OR*值分别是<20岁年龄组的4.4倍和7.6倍；在家屠宰牲畜患包虫病的*OR*值是无家庭屠宰行为的3.2倍。

讨　论

本研究显示，在和布克赛尔蒙古自治县包虫病患病率为3.8%，CE患病率为3.7%，AE患病率为0.16%，平均血清阳性率12.4%。较乌鲁木齐南郊农牧区居民CE平均患病率1.12%为高[5]。因此和布克赛尔蒙古自治县CE仍处于高发与AE散发的流行势态。

多因素回归分析证实，CE流行可能的危险因素是年龄和家庭屠宰牲畜。随着年龄的增加患CE的风险越大，这与王谦等[6]在四川藏区人群包虫病危险因素调查一致。本次调查年龄在50～60岁的患病率最高为10.3%（女性11.8%，男性7.4%），可能是包虫病的病程很长（可达数十年），存在一定的累积

表2 CE的患病率及可疑危险因素单因素logistic回归分析

因素	患病率(%)[a]	OR值(95%CI)
性别		
男	2.3(6/256)	2.001(0.778～5.146)
女	4.6(17/371)	
民族		
汉	10.5(6/57)	
蒙古	3.6(6/166)	0.319(0.098～1.032)
哈萨克	2.6(10/383)	0.228(0.079～0.654)[b]
其他	4.8(1/21)	0.425(0.048～3.757)
职业		
牧民	6.9(13/189)	
农民	3.4(2/58)	0.484(0.106～2.208)
学生	1.9(6/318)	0.260(0.097～0.697)[b]
干部	5.0(2/40)	0.713(0.154～3.289)
其他	0.0(0/22)	0
年龄(岁)		
6～	1.9(6/321)	
20～	0.0(0/44)	0
30～	6.0(4/67)	3.333(0.914～12.155)
40～	6.3(4/63)	3.559(0.975～13.000)
50～	10.3(8/78)	6.000(2.018～17.841)[b]
60～76	1.9(1/54)	0.991(0.117～8.393)
养过犬		
是	3.0(12/395)	1.589(0.689～3.660)
否	4.7(11/232)	
养家畜		
是	3.6(21/586)	1.380(0.312～6.099)
否	4.9(2/41)	
家庭屠宰牲畜		
是	2.7(13/473)	2.457(1.055～5.723)[b]
否	6.5(10/154)	
见过野犬		
是	3.9(10/259)	0.912(0.394～2.113)
否	3.5(13/368)	
饮用水源		
自来水	6.3(15/237)	
深井水	0.0(0/6)	0
浅井水	1.9(7/360)	0.293(0.118～0.731)[b]
河塘水	4.2(1/24)	0.643(0.081～5.096)
饭前洗手		
是	3.6(22/619)	3.877(0.457～32.885)
否	12.5(1/8)	
饮用生水		
是	3.2(14/439)	1.526(0.649～3.590)
否	4.8(9/188)	
食用生蔬菜		
是	3.8(20/533)	0.846(0.246～2.904)
否	3.2(3/94)	

注：[a]括号内数据分子为患病例数，分母为调查人数；[b]有统计学意义

表3 包虫病危险因素多因素logistic回归分析

因素	β	$s_{\bar{x}}$	Waldχ^2值	P值	OR值(95%CI)
年龄			13.87	0.016	
1	-17.1	5960.21	0	0.998	0.000
2	1.1	0.67	2.96	0.085	3.142(0.853～11.572)
3	1.4	0.67	4.78	0.029	4.360(1.165～16.313)
4	2.0	0.57	12.54	0.000	7.648(2.481～23.579)
5	0.2	1.10	0.02	0.880	1.180(0.138～10.126)
家庭屠宰牲畜	1.2	0.46	6.39	0.011	3.183(1.297～7.809)
常数项	-5.9	0.82	46.48	0.000	0.004

病例。女性略高于男性，女性20岁以后随着年龄的增长患病率也逐步上升；由于长期的生产、家务劳动(放牧、剪羊毛、喂犬等)，接触虫卵机会多。但男性在40～50岁时的患病率最低，原因可能是被调查人群中学生和老年人较多，年龄段分布不均，存在一定的偏倚。本次调查CE发病最小年龄为6岁，提示有新发病例，可见包虫病流行仍很活跃。因此应对血清学阳性者，特别是少年儿童定期检查，早发现、早治疗。

在和布克赛尔蒙古自治县引起CE流行的另一危险因素是家庭屠宰牲畜。由于目前当地生产方式落后，多直接从事畜牧业生产，75.4%的人在家屠宰牲畜，直接把患病动物内脏喂犬，犬是囊型棘球绦虫的主要终宿主。成年绦虫在犬体中经6～8周发育成熟，虫卵随犬粪排出且具有传染力。当地63.0%的人养犬，流浪犬及不栓养犬随处可见，犬粪中的虫卵传播到空气、土壤、水源中，造成周围植被、环境的污染，人为地促成了棘球绦虫在当地的循环链。在调查地区，人群因生产、生活之需密切接触棘球绦虫虫卵污染物品和环境、频繁受其感染，可能也是导致人体血清特异性抗体持续存留的原因。有报道显示[7]，宁夏地区包虫病危险因素主要是与民族、养犬和饮用水有关。与本次调查并不一致，其主要原因可能是当地蒙古族以牧业为主，与长期的生产生活环境和行为习惯有关。

综上所述，本项调查结果提示，和布克赛尔蒙古自治县CE患病率居高不下的原因是人群的年龄和家庭屠宰牲畜。因此应广泛开展卫生宣传教育，进行牧犬驱虫，加强犬的管理；并对家庭牲畜屠宰进行宣传，不要把患病的动物内脏直接喂犬，要煮熟或深埋处理。养成良好的饮食习惯，饮用开水；同时，查治患者，坚持疫情监测。

（感谢和布克赛尔蒙古自治县人民政府、卫生局和疾病预防控制中心，新疆包虫病临床研究所，新疆医科大学公共卫生学院对本项目现场调查、实验室检测及数据库建立的大力支持与帮助）

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·临床论著·

包虫八项检查在骨包虫病诊断中的应用

刘大鹏 冯晓辉 张静萍 温浩

【摘要】 **目的** 探讨包虫八项检查在骨包虫病诊断中的应用价值和骨包虫病的诊断策略。**方法** 对 1999 年 10 月至 2008 年 9 月因疑似骨包虫病而行包虫八项检查的 36 例患者进行回顾性研究。男 19 例，女 17 例；年龄 10~67 岁，平均 35.9 岁。采用金标渗滤法和酶联免疫法同时检测患者体内抗包虫囊液抗原(EgCF)、头节抗原(EgP)、囊液半纯化抗原 B(EgB)、泡球蚴抗原(Em2)四个抗原的抗体水平，简称包虫八项检查。全部病例的最终诊断均经手术、病理学检查或穿刺活组织检查证实。对包虫八项检查结果进行 ROC 分析，确定最佳诊断界点(阈值)，并计算灵敏度、特异度、Youden 指数、阳性似然比、阴性似然比、阳性预报值、阴性预报值。**结果** 经病理学检查证实，36 例患者中 11 例确诊为骨包虫病(胸椎包虫 2 例、肋骨包虫 1 例、腰椎包虫 3 例、骶骨包虫 4 例、髂骨包虫 1 例)。包虫八项检查的灵敏度为 90.91%，特异度为 92.00%，Youden 指数为 0.8291，阳性似然比为 11.3600，阴性似然比为 0.0988，阳性预报值为 83.33%，阴性预报值为 95.83%。**结论** 血清学检查是骨包虫病鉴别诊断的重要手段。包虫八项检查诊断骨包虫病有较高的准确性。

【关键词】 棘球蚴病；诊断；血清学；脊柱

【证据等级】 诊断性研究Ⅲ级

Clinical application of the 8-test for immunodiagnosis of human bone cystic echinococcosis *LIU Da-peng, FENG Xiao-hui, ZHANG Jing-ping, et al. Department of Orthopaedic Surgery, the First Teaching Hospital of Xinjiang Medical University, Urumqi 830000, China*

【**Abstract**】 **Objective** To investigate the clinical application value of the 8-test for immunodiagnosis of human bone cystic echinococcosis (BCE). **Methods** A retrospective study was made in 36 cases who were suspected to be BCE in our hospital from October 1999 to September 2008. The mean age was 35.9 years with a range of 10 to 67 years, and including 19 males, 17 females. All cases accepted the 8-test immunodiagnosis and the final diagnosis were confirmed by operation or biopsy. The dot immunogold filtration assay (DIGFA) and enzyme linked immunosorbent assay (ELISA) were used to detect the antibodies to antigens of EgCF, EgP, EgB, and Em2 in patients at one time in our research, the name abbreviated to the 8-test for immunodiagnosis of human bone cystic echinococcosis. The sensitivity, specificity, Youden index, positive likelihood ratio, negative likelihood ratio, positive predictive value and negative predictive value of the 8-test immunodiagnosis were calculated and recorded. **Results** Be confirmed by pathology, 11 of these 36 cases were diagnosed as bone hydatidosis (including 2 cases with thoracic vertebrae, 1 case with rib, 3 cases with lumbar vertebrae, 4 cases with sacrum, and 1 case with illum). The sensitivity was 90.91%, the specificity was 92.00%, the Youden index was 0.8291, the positive likelihood ratio was 11.3600, the negative likelihood ratio was 0.0988, the positive predictive value was 83.33%, and the negative predictive value was 95.83%. **Conclusion** Serological examinations can be considered as diagnosis and initial differentiation for BCE, the 8-test immunodiagnosis has high accurate rate for diagnosing human BEC. It's helpful for identification of BCE.

【**Key words**】 Echinococcosis; Diagnosis; Serology; Spine

包虫病是一种常见的严重危害人体健康的人畜共患寄生虫病，全世界均有报道，在我国许多省份都有分布。骨包虫病少见，文献报道骨包虫病约占包虫病的 0.5%~4%[1]。国内新疆报告骨包虫病例最多，新疆骨包虫病约占全身包虫病的 0.65%[2]。

骨包虫病患者病史较长，早期症状不明显，多以病变局部不适为主。脊柱包虫病多以脊髓、马尾受压，神经功能受损导致的麻痹为首发症状。四肢骨包虫病多以病理性骨折为表现，疼痛往往不明显。部分骨包虫病患者既往无肝包虫病史。血常规、红细胞沉降率及生化检查均可正常。

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骨包虫病的 X 线及 CT 表现缺乏特异性[3-6]。X 线主要表现为囊性或不规则骨质破坏。在四肢易被误诊为骨囊肿和骨巨细胞瘤。在脊柱往往表现为不规则的椎体骨质破坏，部分病例出现椎间隙狭窄、椎管内占位。X 线表现与结核、转移癌及骨肿瘤相似，不易鉴别[2]。

MR 是最有价值的影像学检查，脂肪抑制技术和磁共振水成像技术（MR-Hydrography，MRH）[3]是 MR 诊断骨包虫病常用的技术手段。MRI 可显示典型的包虫特征（外囊囊壁、多房性）[4,5]，但部分患者的 MRI 影像缺乏特异性。

由于发病率低，临床表现无特异性，影像学表现和结核、肿瘤鉴别困难，因此包虫病极易误诊[2]。血清学检查是诊断肝包虫病的重要手段之一，但对骨包虫病的诊断价值尚不明确。本文对因疑似骨包虫病而行包虫八项检查的 36 例患者进行回顾性分析，探讨包虫八项检查诊断骨包虫病的价值以及骨包虫病的诊断策略。

资 料 与 方 法

一、一般资料

1999 年 10 月至 2008 年 9 月因疑似骨包虫病而行包虫八项检查的患者 36 例，男 19 例，女 17 例；年龄 10~67 岁，平均 35.9 岁。最终诊断均经手术、病理学检查或穿刺活组织检查证实。

11 例确诊为骨包虫病：胸椎包虫 2 例、肋骨包虫 1 例、腰椎包虫 3 例、骶骨包虫 4 例、髂骨包虫 1 例；其他诊断：骨巨细胞瘤 2 例、动脉瘤样骨囊肿 2 例、软骨肉瘤 2 例、骨肉瘤 1 例、丛状神经纤维瘤 1 例、骨内腱鞘囊肿 1 例、神经鞘瘤 1 例、巨大神经根囊肿 1 例、骶骨脊索瘤 1 例、骨转移癌 2 例、纵隔囊肿 1 例、畸胎瘤 1 例、椎管内血管瘤 1 例、左膝关节内侧骨坏死 1 例、骨髓瘤 1 例、椎间盘脱出 2 例、脊髓中心性出血坏死 1 例、脊柱结核 1 例、腰大肌脓肿（结核）1 例，左胫骨骨髓炎 1 例。

二、包虫八项检查的检测方法

包虫八项免疫试剂盒由新疆维吾尔自治区包虫病临床研究所研制。其原理是同时用金标渗滤（dot immunogold filtration assay，DIGFA）和酶联免疫（enzyme linked immunosorbent assay，ELISA）两种方法检测患者体内抗包虫囊液抗原（EgCF）、头节抗原（EgP）、囊液半纯化抗原 B（EgB）、泡球蚴抗原（Em2）四个抗原的抗体水平，简称包虫八项检查。具体检测方法见参考文献[7]。

三、统计学处理

分别对金标渗滤法和酶联免疫法的检查结果进行 ROC 分析（接收者工作特征曲线分析）[8]，确定最佳诊断界点（阈值）。

计算灵敏度、特异度、Youden 指数、阳性似然比、阴性似然比、阳性预报值及阴性预报值。

结 果

一、包虫八项检查结果

包虫八项检查结果如表 1 所示。检查结果均为阴性的非骨包虫病例未列入表中。

二、ROC 分析

进行 ROC 分析时将受试者对四个抗原的检测结果分成六个等级：0 级，对四个抗原均呈阴性反应；1 级，对任意一个抗原呈阳性反应；2 级，对任意两个抗原呈阳性反应；3 级，对 EgCF 和 EgB 均呈阳性反应；4 级，对任意三个抗原呈阳性反应；5 级，对四个抗原均呈阳性反应。

将数据输入 SPSS 13.0（SPSS Inc.，美国）统计软件进行 ROC 分析，绘制 ROC 曲线，计算两种方法的曲线下面积、标准误、P 值、95%置信区间（表 2）。

由表 2 可见两种检测方法对诊断骨包虫病均有效（非参数检验，$P<0.001$）。分别根据各曲线离坐标左上角距离最短的一点确定最佳诊断界点（图 1）。

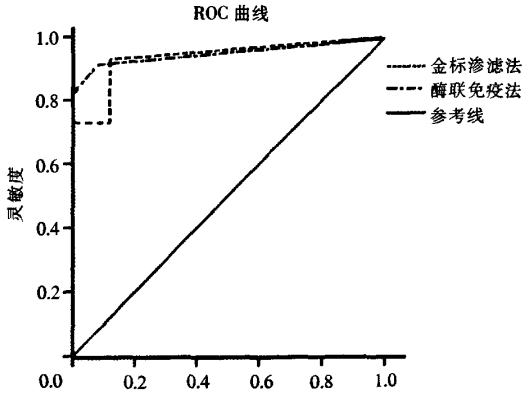

图 1 金标渗滤法和酶联免疫法的 ROC 曲线

两者的诊断界点确定为：检测结果≥3 级，则判定为阳性。即受试者血清至少对 EgCF 和 EgB 均呈阳性反应，诊断为骨包虫病。此诊断界点下两种检测方法的灵敏度和特异度如表 3 所示。

由表 3 可以看出金标渗滤法的灵敏度偏低，而特异度较高。为进一步提高诊断的灵敏度，在诊断过

表 1　本组病例的包虫八项检查结果

患者编号	最终诊断	金标渗滤法				酶联免疫法			
		EgCF(金)	EgP(金)	EgB(金)	Em2(金)	EgCF(酶)	EgP(酶)	EgB(酶)	Em2(酶)
4	L_2 椎体结核	(+)	(+)	(+)	(-)	(+)	(+)	(+)	(-)
5*	L_5~S_1 椎体包虫,椎旁包虫	(+++)	(++)	(++)	(+)	(+)	(+)	(+)	(+)
8*	骶骨包虫	(±)	(-)	(-)	(-)	(+)	(+)	(+)	(+)
9	神经鞘瘤	(-)	(-)	(-)	(-)	(-)	(+)	(-)	(-)
11	骶骨脊索瘤	(-)	(-)	(-)	(-)	(-)	(+)	(-)	(+)
15	左胫骨骨髓炎	(±)	(±)	(±)	(-)	(-)	(-)	(+)	(-)
19*	肋骨包虫	(-)	(-)	(-)	(-)	(+)	(+)	(+)	(+)
23	肋骨软骨肉瘤	(±)	(-)	(±)	(-)	(+)	(-)	(+)	(-)
25*	骶骨包虫	(+)	(+)	(±)	(+)	(+)	(+)	(+)	(+)
26*	T_2 椎体及椎管包虫	(+)	(+)	(±)	(-)	(+)	(+)	(±)	(-)
27*	T_{12} 椎体包虫	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
28*	T_5 椎体包虫	(±)	(-)	(-)	(-)	(+)	(+)	(+)	(-)
29*	骶骨包虫	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
30*	右侧髂骨包虫	(+)	(-)	(+)	(-)	(+)	(+)	(+)	(-)
31*	T_3 椎体包虫	(+)	(+)	(+)	(±)	(+)	(-)	(+)	(-)
32*	骶骨包虫	(+)	(+)	(+)	(±)	(+)	(+)	(+)	(+)

注:*为骨包虫病患者

程中对两种检测方法采用并联策略，两种检测方法中任意一种方法的检测结果≥3 级，即判定为阳性。以此诊断标准对患者进行诊断,结果如表 4 所示。

敏感度、特异度、阳性似然比、阴性似然比、阳性预报值和阴性预报值如表 5 所示。结果显示将两种检测方法并联后灵敏度提高,特异度无明显下降。

讨　论

一、血清学检查是鉴别诊断的重要手段

在影像学检查不能作出明确诊断时，血清学检查是鉴别诊断的主要手段。如本组病例 18 髂骨腺癌骨转移患者的 MRI 和骨包虫病(图 2)非常相似。病例 11 骶骨脊索瘤的 MRI 表现与病例 8 和病例 25 骶骨包虫的 MRI 表现(图 3,4)非常相似,病例 33 肋骨丛状神经纤维瘤的 CT 和 MRI 表现与病例 19 肋骨包虫(图 5,6)非常相似。依据影像学鉴别非常困难。但病例 18、病例 11 及病例 33 的包虫八项检查结果提示可以排除骨包虫病。

表 2　两种检测方法的 ROC 分析结果

检测方法	面积	标准误	*P* 值	95%置信区间	
				下界	上界
金标渗滤法	0.927	0.057	0.000006	0.816	1.039
酶联免疫法	0.942	0.057	0.000003	0.830	1.054

表 3　两种检测方法的灵敏度和特异度

检测方法	灵敏度	1-特异度	特异度
金标渗滤法	0.724	0.040	0.960
酶联免疫法	0.909	0.080	0.920

表 4　包虫八项检查诊断试验结果

包虫八项检查结果	金标准(病理学检查结果)		合计
	病例	对照	
阳性	10(TP)	2(FP)	12
阴性	1(FN)	23(TN)	24
合计	11	25	36

注:TP=真阳性,FP=假阳性,TN=真阴性,FN=假阴性

表 5　将金标渗滤法和酶联免疫法并联后诊断试验的各项指标

灵敏度	特异度	Youden 指数	阳性似然比	阴性似然比	阳性预报值	阴性预报值
90.91%	92.00%	0.8291	11.3600	0.0988	83.33%	95.83%

二、包虫八项检查对骨包虫病的诊断价值

包虫病特异性抗体检测的方法包括皮内试验(IDT,Casoni 试验)、间接血凝试验(IHA)、对流免疫电泳(CIEP)、酶联免疫吸附试验(ELISA)和金标抗

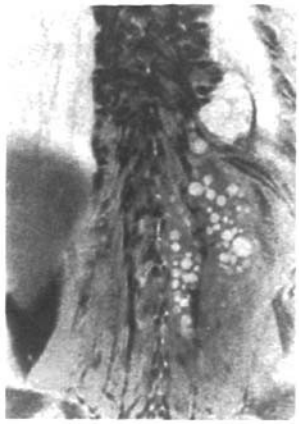

图 2 男，41 岁，脊柱冠状面 MRI 显示椎旁和腰大肌中多个高信号的圆形子囊

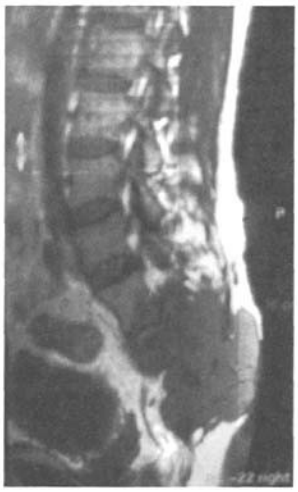

图 3 病例 25，男，43 岁，骶骨包虫矢状面 MRI 显示骶骨病灶中等信号，病灶侵入周围软组织，与肿瘤不易鉴别

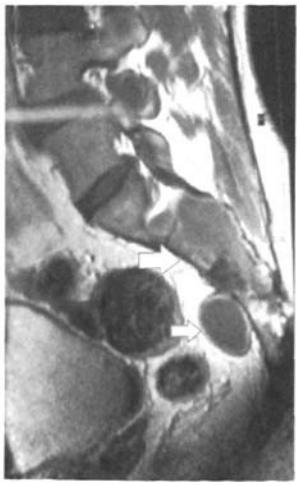

图 4 病例 8，男，44 岁，骶骨包虫矢状面 MRI 显示骶管和骶前病灶（箭头所示），中等信号，缺乏特异性

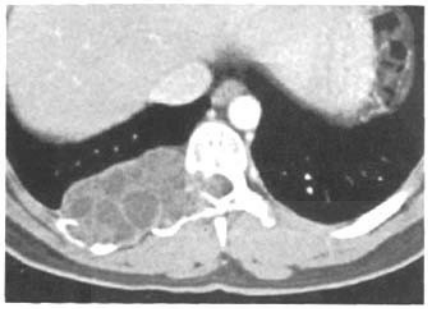

图 5 病例 19，女，20 岁，肋骨包虫 CT 显示肋骨病变膨胀性生长，破坏骨质，内有分隔

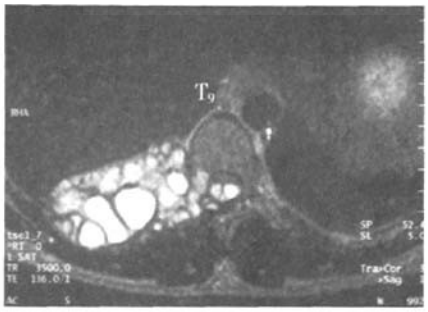

图 6 病例 19，肋骨包虫的 MRI 显示肋骨病灶膨胀性生长，内有多个囊性病灶

体等[7,9,10]。皮内试验、间接血凝试验和对流免疫电泳俗称包虫三项。使用的抗原是从包虫囊液中提取的粗抗原，非纯化的包虫特异性抗原，假阳性率高；且多采用单一抗原，无放大效应，敏感性不高，假阴性率也高。包虫八项检查可避免由于同一患者对不同抗原的反应性不同而造成的假阳性或假阴性[7]，增加了检出率。

本研究中，包虫八项检查对骨包虫病诊断的灵敏度为 90.91%，特异度为 92.00%，与付艳等[7]在大样本肝包虫病研究中的结果相近（敏感度 92.61%，特异度 91.89%）。包虫八项检查中采用的两种检测方法 ROC 曲线下面积均达到 0.9 以上，各项评价指标均显示包虫八项检查对骨包虫病的诊断有较高的价值。

机体对包虫的免疫反应与包囊的完整性、包虫的活力和包囊位置有关。包囊完整较包囊破裂患者的免疫反应轻，包囊破裂可引起过敏性休克导致患者死亡。如果包虫衰老、包囊钙化或包虫死亡，血清学检查常为阴性。一般认为肝包虫病的免疫反应比肺包虫病、脑包虫病和脾脏包虫病强。但 Lightowlers 和 Gottstein[11]认为骨包虫病往往表现出

更强烈的免疫反应，可能是由骨包虫没有纤维包膜，包虫组织和机体接触较多所致。由于本组病例较少，还无法证实这一观点。如果确实如此，将有利于血清学检查诊断骨包虫病。

三、诊断界点和诊断策略

人体对包虫病灶的免疫反应越强烈，体内的抗体水平就越高，血清学检查中呈阳性反应的抗原种类就越多，对每种抗原的抗体滴度也越高。在实际工作中，并非所有骨包虫病患者对四种抗原均呈阳性反应，同时非骨包虫病患者也会对某一种或几种抗原呈阳性反应（假阳性）。因此确定诊断界点是关键。

进行 ROC 分析按照呈阳性反应的抗原数的多少对患者的检查结果进行分级。级别的高低一方面反映了免疫反应的强弱，另一方面也反映了检查结果特异度的高低。

本研究将患者的检查结果划分为六个等级。如果进一步细分，第 2 级可以根据特异度的高低再分为六个等级（对四个抗原中任意两个呈阳性反应）。根据以往的试验结果，在细分的六种组合中，EgCF 和 EgB 两者的组合特异度最高，为了便于分析将其从第 2 级中抽出细分为第 3 级。这样可以解决在第 2 级中特异度相差太大、分级不够细的问题，也可以避免无意义的过多分级。诊断性试验的结果也证实这一诊断界点比较理想，灵敏度和特异度均达到 90%以上。

四、如何正确分析包虫八项检查结果

本组病例为一特定人群，即“在我院就诊的疑似骨包虫病的患者”，其阳性预报值和阴性预报值（分别为 83.33%和 95.83%）与针对总体人群时不同。当针对总体人群时，计算包虫八项检查诊断骨包虫病的阳性预报值和阴性预报值应知道总体人群患病率[3]。但骨包虫病的人群患病率未见文献报道，只能根据特定地区的包虫病的患病率来推算。新疆各农牧区的包虫病患病率为 1%~5%[11,12]，骨包虫病约占其中的 0.65%，推算骨包虫病患病率为 0.0065%~0.0325%，包虫八项检查诊断骨包虫病的阳性预报值为 0.073810%~0.362405%。由于骨包虫病的患病率很低，所以包虫八项检查对于总体人群的阳性预报值不高，对从总体中随机抽取的样本诊断价值不高。因此在临床工作中，用包虫八项检查诊断骨包虫病，必须结合患者的病史、临床和影像学表现，不能单纯依靠包虫八项检查结果。

对包虫八项检查结果进行具体分析时，应注意 EgCF 和 EgP 是粗抗原，其检测结果敏感度高，但易引起假阳性；EgB 是部分纯化抗原，其检测结果的敏感度略低，但特异度较高。理论上说 Em2 用亲和吸附的方法去除泡球蚴和棘球蚴中相同的抗原组分而保留下来泡球蚴特异性抗原组分，也是一种纯化抗原[13]，可根据抗体反应梯度和显色程度对泡球蚴病作出鉴别诊断。但付艳等[7]观察到部分肝细粒棘球蚴多子囊患者也对 Em2 有较强的反应，因此对此类患者要结合影像学检查等手段来明确诊断。本研究中骨包虫患者的 Em2 抗体阳性率也较高，可能与 Em2 抗原的选择性不强有关。

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·现场调查·

2007 年新疆和布克赛尔蒙古自治县棘球蚴病现况调查

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【摘要】 **目的** 了解 2007 年新疆和布克赛尔蒙古自治县人群棘球蚴病主要流行现状及其分布特征。**方法** 采用整群抽样方法，在该县抽取铁布肯乌散乡、那仁和布克牧场 2 个地区的居民，用问卷调查、血清免疫学和 B 超检查等方法进行人群棘球蚴病流行病学现况调查。**结果** 调查人群 B 超及手术史检出的棘球蚴病患病率为 9.0%(64/712)，血清学阳性率为 15.6%(111/712)，其中细粒棘球蚴病患病率为 8.7%(62/712)，多房棘球蚴病患病率为 0.3%(2/712)。不同职业、年龄、家庭屠宰牲畜和饮用水源的人群细粒棘球蚴病患病率，差异有统计学意义($P < 0.05$)，其中职业以牧民患病率[13.4%(27/201)]最高，年龄以 20 ~ < 40 岁年龄组人群患病率最高(12.8%)，但不同性别、民族及文化程度人群细粒棘球蚴病患病率和血清学阳性率差异无统计学意义($P > 0.05$)。**结论** 细粒棘球蚴病在该地区高度流行，职业、年龄及饮用水源可能是其主要的危险因素。

【关键词】 棘球蚴病； 流行病学； 数据收集

Epidemiological study on human echinococcosis in Hobukesar Mongolian Autonomous County of Xinjiang

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【**Abstract**】 **Objective** To investigate the characteristics and distribution of human echinococcosis in Hobukesar Mongolian Autonomous County (HMAC) in Xinjiang. **Methods** Using cluster sampling methods, the 2 counties (Tiebukenwusa and Narenhebuke) in HMAC were chosen as focusing areas for investigation. A survey of human echinococcosis including questionnaire, serological test and abdominal ultrasonic scan was carried out. **Results** The prevalence of human echinococcosis was 9.0%(64/712)by ultrasound and surgical history, including 8.7%(62/712) for cystic echinococcosis(CE), 0.3%(2/712) for alveolar echinococcosis(AE) and 15.6%(111/712) for total of serological positives in HMAC. CE prevalence rate of different occupations, age, family slaughtering livestock and drinking water source had significant differences($P < 0.05$). Herdsmen as the highest risk group showed a CE prevalence of the 13.4% (27/201) in comparison with other occupations. The ages between 20 to < 40 year-old were at the highest risk stage with 12.8% incidence. But CE prevalence rate of different gender, ethnic and education groups had not significant differences($P > 0.05$). **Conclusions** HMAC could be considered as a high endemic human CE region in Xinjiang. The current study reported the main risk factors may include occupations, age difference and drinking water source.

【**Key words**】 Echinococcosis; Epidemiology; Data collection

棘球蚴病(Echinococcosis)是由棘球属绦虫的幼虫寄生于人、畜体内引起的一种严重危害人群健康和畜牧业发展的人畜共患寄生虫病。我国西北地区，尤其新疆北部牧民感染细粒棘球蚴病(CE)的相对危险性较高[1-2]，1990 年新疆疾病预防控制中心在新疆 12 个地(州)，85 个县(市)，共调查了 15 289 例患 CE 的医院病例，发现和布克赛尔蒙古自治县发病率为 30.17/10 万，是 CE 的高发病区。为了解和布克赛尔蒙古自治县防治 CE 现状，2007 年 3 月作者在该县开展了人群棘球蚴病流行病学调查，结果报道如下。

1 对象与方法

1.1 调查对象：采用整群抽样方法，选择和布克赛

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尔蒙古自治县的铁布肯乌散乡(简称铁乡)和那仁和布克牧场(简称那牧场)作为调查点,在每个调查点抽取长期居住(>4 年)并对此次调查知情同意的居民,共 712 人作为调查对象,其中男 337 人,女 375 人;平均年龄 34.9 岁(范围为 7 ~ 77 岁);民族构成为:汉族 64 人、蒙古族 408 人、哈萨克族 237 人,维吾尔族 3 人;职业分布为:牧民 303 人、农民 201 人、干部 36 人、学生 160 人、其他职业 12 人。

1.2 调查内容

1.2.1 问卷调查:由经过统一培训的疾病预防控制人员(精通蒙古语、哈萨克语)对被调查者进行问卷调查,问卷内容包括人员的基本信息,与棘球蚴病相关的手术史、生产、生活行为等。

1.2.2 人群棘球蚴病调查:采用便携式 B 超(GE LOGOXP 型)对被调查者进行腹部检查。结果分为正常、CE、多房棘球蚴病(AE)、可疑、钙化、其他 6 种,存档并分析棘球蚴病的患病率。采集调查对象静脉血 3 ml,静置 1 h 后 2000 × *g* 离心 10 min,分离血清备用,用新疆棘球蚴病临床研究所研制的组合抗原棘球蚴病快速诊断试剂盒,分别针对抗原 EgCF、EgP、EgB、Em2 的特异性抗体进行检测,任意一种抗原阳性即判定调查对象为血清学阳性,用以计算血清学阳性率。

1.3 统计方法:应用 Excel 程序进行资料输入,SPSS 13.0 软件对数据进行 χ^2 检验或 Fisher's 确切概率法分析。

2 结 果

2.1 人群棘球蚴病患病情况调查:712 例调查对象中,经 B 超及手术史检出棘球蚴病患者共计 64 人,患病率为 9.0%(64/712);而血清学检验阳性 111 人,阳性率为 15.6%(111/712),其中 CE 患者 62人,AE 患者 2 人。

2.2 不同性别、民族、职业之间人群 CE 患病率、血清学阳性率比较:不同性别人群间 CE 患病率、血清学阳性率未见明显改变(χ^2 值分别为 0.50、0.54,$P > 0.05$);本次调查因维吾尔族的样本数太少,因此只分析了汉、蒙、哈各民族间人群 CE 患病率和血清学阳性率,经统计未见明显改变(χ^2 值分别为0.77、2.75,$P > 0.05$);职业方面,因干部和其他职业的样本数较少,因此只分析了牧民、农民和学生间人群 CE 患病率和血清学阳性率,其中牧民明显高于农民和学生(χ^2 值分别为 13.24、10.02,$P < 0.05$)。见表1。

2.3 不同年龄组人群 CE 患病率、血清学阳性率比较:不同年龄组人群 CE 患病率和血清阳性率组间比较,差异有统计学意义(χ^2 值分别为 11.02、15.45,$P < 0.05$),其中 20 ~ < 40 岁和 60 ~ 岁年龄组较高,见表 2。在 20 ~ < 40 岁年龄组两性的患病率相近,而在其他年龄组男性略高于女性,见图 1。

表 1 和布克赛尔蒙古自治县人群 CE 感染情况调查

Table 1 Prevalence of human CE seropositives in HMAC

分类	分组	检查人数	B 超及手术史检出患者人数	血清学阳性人数
性别	男	337	32(9.5)	49(14.5)
	女	375	30(8.0)	62(16.5)
族别	汉	64	6(9.4)	13(20.3)
	蒙古	408	37(9.1)	66(16.2)
	哈萨克	237	17(7.2)	30(12.7)
	维吾尔	3	2(66.7)	32(13.3)
职业	牧民	201	27(13.4)	36(17.9)
	农民	303	22(7.3)	54(17.8)
	学生	160	5(3.1)	12(7.5)
	干部	36	5(13.9)[a]	3(8.3)
	其他[b]	12	3(25.0)	6(50.0)
调查人数		712	62(8.7)	111(15.6)

注:()内数字为百分数;a:5 例中 4 例是有既往病史,其中 3 例有过手术史,2 例此次检查复发;b:商人、家庭主妇因样本数少,而合并为其他,其中的 3 例患者均有既往手术史

表 2 和布克赛尔蒙古自治县不同年龄人群 CE 感染情况调查

Table 2 Prevalence of human CE in different age groups in HMAC

年龄组(岁)	检查人数	B 超及手术史检出患者人数	血清学阳性人数
0 ~	181	7(3.9)	13(7.2)
20 ~	242	31(12.8)	43(17.8)
40 ~	233	18(7.7)	48(20.6)
60 ~	56	6(10.7)	7(12.5)
合计	712	62(8.7)	111(15.6)

注:()内数字为百分数

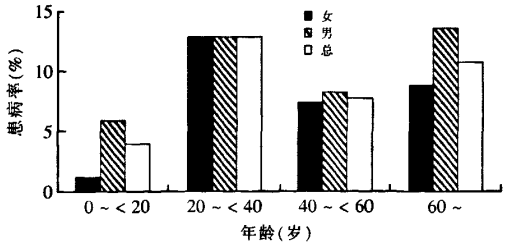

图 1 和布克赛尔蒙古自治县不同年龄、性别人群 CE 患病情况

Figure 1 Prevalence of human CE in different age-sex groups in HMAC

2.4 2 个调查点之间人群 CE 患病率、血清阳性率比较:那牧场的患病率[12.0%(30/251)]较铁乡[6.9%(32/461)]明显增高($\chi^2 = 5.13$,$P < 0.05$);血清学阳性率分别为 13.5%(34/251)、16.7%(77/461),

表 3　和布克塞尔蒙古自治县人群 CE 感染的相关因素调查结果

Table 3　Correlation analysis on infection rate of human CE in HMAC

生产生活行为	族别				职业			
	汉族人数 (n = 64)	蒙古族人数 (n = 408)	χ^2	P	农民人数 (n = 303)	牧民人数 (n = 201)	χ^2	P
养犬	50(78.1)	335(82.1)	0.58	> 0.05	229(75.6)	163(81.1)	2.13	> 0.05
饲养家畜	63(98.4)	399(97.8)	0.11	> 0.05	297(98.0)	197(98.0)	0.00	> 0.05
家庭屠宰牲畜	27(42.2)	294(72.1)	22.69	< 0.05	207(68.3)	155(77.1)	4.62	< 0.05
见过狐狸	26(40.6)	149(36.5)	0.40	> 0.05	95(31.4)	81(40.3)	4.26	< 0.05
接触过狐狸皮	4(6.3)	28(6.9)	0.03	> 0.05	18(5.9)	14(7.0)	0.21	> 0.05
见过游狗	3(4.7)	46(11.3)	2.58	> 0.05	25(5.9)	19(9.5)	0.22	> 0.05
饭前洗手	64(100.0)	401(98.3)	1.12	> 0.05	300(99.0)	197(98.0)	0.88	> 0.05
饮用生水	21(32.8)	230(56.4)	12.33	< 0.05	153(50.5)	133(66.2)	12.10	< 0.05
食用生蔬菜	20(31.3)	48(11.8)	17.03	< 0.05	49(16.2)	24(11.9)	1.75	> 0.05
饮用水源　自来水	36(56.3)	201(49.3)	1.08	> 0.05	181(59.7)	45(22.4)	68.15	< 0.05
深井水	3(4.7)	24(5.9)	0.15	> 0.05	12(4.0)	16(8.0)	3.69	> 0.05
浅井水	6(9.4)	79(19.4)	3.74	> 0.05	74(24.4)	46(22.9)	0.16	> 0.05
河塘水	0(0)	73(17.9)	13.55	< 0.05	7(2.3)	62(30.8)	83.27	< 0.05
溪水	19(29.7)	31(7.6)	28.50	< 0.05	29(9.6)	32(15.9)	4.58	< 0.05

注：()内数字为百分数

二者间未见明显改变(χ^2 = 1.23, P > 0.05)。

2.5　饮水、职业、民族等相关因素人群 CE 患病率、血清学阳性率比较：当地居民只有 40.7%(290/712)饮用自来水，而农民明显高于牧民，牧民饮用河塘水和溪水较多，且有 66.2%(133/201)的人习惯饮用生水。不同饮用水源中饮用溪水人群的患病率最高，相关因素中家庭屠宰牲畜者牧民高于农民、蒙古族高于汉族；而养犬、饲养家畜率的民族、职业组间比较，差异无统计学意义(P > 0.05)。见表 3。

3　讨　论

3.1　棘球蚴病的流行与分布：调查表明，该地区为 CE 高发区，其中那牧场的新发病率为 4.4%(11/251)，高于 Wang 等[3]调查结果(2.7%)，也明显高于 2005 年国内 12 省(区)的牧区和半农半牧区的棘球蚴病患病率(1.1%)及血清学阳性率(12.0%)[4]。当地 CE 总患病率较高，此次有 CE 手术史患者亦高达 6.2%(44/712)，但在该乡的生产、生活习惯方面，如在家宰杀牲畜率、游狗率和饮用生水率等 CE 危险因素都较低，可能与该牧场居民在前次流行病学调查中接受健康教育，改变了不良生活习惯有关。CE 新发病例的存在，说明该地区 CE 的传播链依然存在，仍然处于高流行状态。

3.2　棘球蚴病流行的相关因素：此次调查中发现，男性总患病率略高于女性，与青海省棘球蚴病流行病学分析中女性患病较高[5]的特点不同，其原因可能是该地区以牧业为主，男性多从事放牧劳动，与狗、牲畜接触较多，宰杀家畜，接触病原体机会也较多，因此易受感染。

该地区人群棘球蚴病血清学阳性率和患病率均较高，通过相关危险行为分析，可能与当地以牧业为主，造成整个生活环境污染有关，职业、年龄、家庭屠宰、饮用水源、饮用生水是当地主要危险因素，尤其职业分布，与相关文献描述一致[6-7]。当地牧民的患病率最高，家庭屠宰牲畜率明显高于农民，饮用河塘水和溪水较多，只有 22.4%(45/201)饮用自来水，且有 66.2%(133/201)的人习惯饮用生水。

本次调查结果显示人群棘球蚴病在和布克赛尔蒙古自治县的高发态势仍持续存在。建议应加强新疆牧业地区棘球蚴病防治力度，特别是在该地区引导人们改变不良日常生活习惯和注意饮食卫生知识的宣传及水源治理，以便降低棘球蚴病的感染。

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·简报·

流行性出血热的护理

李霞 刘慧敏 任奉欣

【关键词】 肾综合征出血热; 观察; 护理

流行性出血热是野鼠中流行的一种自然疫源性疾病,在我国东北地区 12 月至次年 2 月为流行高峰[1]。目前,对本病尚无特殊治疗方法,根据多年的临床经验和护理体会,流行性出血热治疗和护理的关键在于发热期进行恰当的液体疗法及免疫治疗;低血压少尿期需积极扩容,恢复有效血容量;采取综合性利尿措施,防止肺水肿,纠正酸中毒及电解质紊乱,及时发现并预防并发症的出现。

1 对象与方法

1.1 对象:以 2006 - 2008 年黑龙江省医院收治的流行性出血热患者 48 例作为观察对象,其中男 39 例,女 9 例,年龄范围为 23 ~ 52 岁。

1.2 方法

1.2.1 发热期的护理:早期卧床休息,创造舒适、安静的环境,减少噪声,减少对病人的刺激,予以高热量、高维生素、易消化饮食。随时观察体温的变化,特别是高热的患者,体温过高时应及时采取物理降温。由于此病有毛细血管中毒性损害,故不宜用酒精擦浴,尽量少用解热镇痛药,定期测量血压。患者发热后期多汗、血液浓缩,应鼓励病人多口服补液,必要时给予低分子右旋糖酐等防止休克和保护肾脏。

1.2.2 低血压期的护理:严密观察血压的变化,每 30 min 测血压、脉搏 1 次,并做好记录及时报告医生;注意补液速度,低血压早期应快速补液,必要时加粗针头或多静脉通道,但对老年体弱及心、肾功能不全者,速度应适当放慢,减少用量以防止肺水肿的发生;准确记录 24 h 尿量,尽早发现少尿倾向;低血压期患者注意保暖,禁止搬动。

1.2.3 少尿期的护理:少尿期应注意尿量每日 < 1000 ml 即为少尿倾向,< 500 ml 为少尿期,< 100 ml 为无尿期,注意观察尿量变化和尿的颜色、性质。严格记录液体出入量,以量出为入为原则。此时严格控制进液量,给予足够热量,以口服为主。输液时要注意保护血管,穿刺点由远心端向近心端循序渐进,避免针头穿破血管或液体漏出血管,以免因凝血机制障碍而加重皮肤下出血淤血;导泻的病人,必须记录大、小便的次数、量和性质,并保持床单干燥清洁;加强口腔护理,做口腔护理时动作要轻,仔细检查口腔黏膜有无溃疡、义齿、龋齿等。

1.2.4 多尿期护理:认真记录出入量,注意水及电解质平衡。本病多尿期的尿量一般每日 > 3000 ml 为依据,此时鼓励患者食用营养丰富、易消化、含钾量较高的饮食,对严重贫血者可酌情输入新鲜血液。尿量每日 > 3000 ml,补钾时应以口服为主,必要时可缓慢静脉滴入,同时注意钠、钙等电解质的补充。对尿量每日 > 5000 ml 者,可试用双氢克尿塞、去氧皮质酮、垂体后叶素、消炎痛等。由于免疫功能低下,应注意预防感染,注意病室内空气消毒,特别是加强口腔及皮肤的护理。

1.2.5 恢复期的护理:加强营养,高蛋白、高糖、多维生素饮食。注意休息,一般需 1 ~ 3 个月左右,应逐渐增加活动量,重型病例可适当延长时间。

1.2.6 并发症的护理:①观察是否有鼻出血、咯血、呕血、便血;是否有烦躁不安、面色苍白、血压下降、脉搏增快等休克的表现。根据出血部位的不同给予相应的护理,并按医嘱给予止血药。②心衰、肺水肿患者,应减慢输液或停止补液,半卧位,注意保暖,氧气吸入保持呼吸道通畅。③脑水肿发生抽搐等中枢神经系统并发症时,应镇静、止痉脱水,注意观察疗效。④高血钾病人静注葡萄糖酸钙时宜慢,输注胰岛素时应缓慢静滴,随时观察病人的生命体征,必要时可血液透析治疗。⑤进行预防流行性出血热的宣教,特别是宣传个人防护及预防接种的重要性和方法,以降低本病的发病率。向病人及家属说明,本病恢复后,肾功能恢复还需较长时间,应定期复查肾功能、血压、垂体功能,如有异常及时就医。

2 结 果

针对流行性出血热各期的特点,运用护理程序,实施整体护理,明显地提高了治疗效果,有效地预防了并发症的发生。共治愈患者 46 例,2 例危重型患者死亡。

3 讨 论

流行性出血热因各期治疗原则截然不同,且各期也有交叉重叠,并发症严重等而成为流行性出血热护理的难点,对流行性出血热的病人实施整体护理,通过护士严密观察病情,能及早发现病情变化,积极配合医生进行危重病人抢救及护理,预防并发症的发生,提高了治疗效果。

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