KEY WORDS: cysticercosis, immunodiagnosis

ABSTRACT
Cysticercosis is an important parasitic zoonosis, caused by the infection of Cysticercus cellulosae, the larval stage of Taenia solium. This disease is a serious public health problem in pigs and human beings in many developing areas worldwide. Practical, rapid, and efficient pre-mortem diagnostic methods therefore are of great significance. The cysticercus antigens and immunodiagnosis methods used for cysticercosis, including assays designed for detecting cysticercus circulating antigens and molecular approaches, are reviewed and evaluated. The questions existing in control of cysticercosis in China are also discussed.

INTRODUCTION
Cysticercus cellulosae, the larval stage of Taenia solium, is an important pathogen, which causes neurocysticercosis or even death with the encystment in the central nervous system (CNS). More importantly, recent studies suggest that neurocysticercosis is also associated with human cancer.1 People can be infected by eating uncooked or poorly cooked cysticercosis pork or by digesting eggs created by the adult tapeworm directly or regurgitating gravid proglottids from the human gut to the stomach. This disease is a serious public health problem in pigs and human beings in many developing areas or countries worldwide, and has been reported recently in some developed countries.2 Ito reported a situation in Papua, Indonesia, that appears to be one of the worst in the world.3 In Jayawijaya in 1996-1997, 46% of 18-year-olds were found to have T solium cysticercosis. In Wamena, Jayawijaya, in 2001, approximately 8% of the population carried adult tapeworms of T solium and 32% had cysticercosis.

The situation in China is serious, too. Previous investigation showed that cysticercosis was regionally prevalent, mainly due to local customs or beliefs or poor living conditions, especially in some areas of northeast, north, northwest, and southwest China. The average rate of C

Immunodiagnosis of Cysticercosis in China
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cellulosae infection was about 5.32% nationwide and the number of cysticercosis patients aggregated 3 million, suggesting that the need to control this disease is urgent and imperative.4,5

Pigs infected by C cellulosae are not only the source of infection in human beings, but they are also a huge obstacle to international trade of pork products. Statistically, direct economic damage caused by cysticercosis across China is up to 2 billion RMB per year. Therefore, it is extremely important to diagnose cysticercosis early in order to prevent spread of this disease, to decrease economic damage, and to enhance international trade.

Infection with the adult worm is usually asymptomatic in humans. Heavy metacestode infection in pigs may cause fever, muscle pains, weakness, or, if the CNS is involved, meningencephalitis or epilepsy. Initially, people stressed treatment with chemicals and used simple methods for diagnosis, such as observing facet of humans, and in pigs, touching lesions on a tongue or inspecting skeletal muscle after slaughtering, and so on. In the 1960s, immunoenzyme techniques were established and developed into various practicable, efficient methods, including enzyme–linked immunosorbent assay (ELISA) which is now universally used as a powerful tool the diagnosis of many diseases. Recently, a few improved diagnostic methods based on ELISA, including Dot-ELISA; Monoclonal antibody-ELISA; Biotin-Avidin-Dot-ELISA; and Dot-immunogold filtration assay, in which immunogold is used as a marker, have been invented. All show better performance in cysticercosis diagnosis.

It is well known that diagnostic specificity and sensitivity rely greatly on proteins selected as a marker. T solium and other helminthes, such as E granulosus, T crassiceps, T saginata, etc., are very close in evolution, sharing common proteins which increases the frequency of cross reaction in diagnosis. Cross-reaction antigens from experimental murine cysticercosis caused by T crassiceps are useful in diagnosing human cestode diseases and evaluating vaccines against porcine cysticercosis.6 Many studies demonstrate that purified glycoproteins are promising targets for diagnosis of T solium cysticercosis (both sensitivity and specificity are up to 100%).7 Although an extract enriched for T solium glycoproteins was earlier demonstrated by Restrepo,8 supply of such sufficient parasite proteins would still be a huge question and impede the application of practical and specific diagnosis of cysticercosis. Since Johnson developed the highly efficient vaccine against T ovis, with a protection rate above 90%,9 people have diverted their attention to exploiting cysticercosis-preventing vaccines and diagnosis by means of DNA recombinant techniques. Two clones, L3B1 and 9.1RTS1 differentially immunoscreened from cDNA libraries of T solium, express fusion proteins in E coli which provide good sensitivity (80%) and could differentiate cysticercosis from alveolar hydatidosis and some nematodes infections, but the cross-reactivity with E granulosus remains high.10 It is exciting that C cellulosae 18KD protein can distinguish anti-sera against porcine and human cysticercosis from ones against E granulosus, E multilocularis; indicating that recombinant 18kD provides a potential way to diagnose cysticercosis, specifically and sensitively.

IMMUNODIAGNOSTIC METHODS FOR CYSTICERCOSIS

Throughout history, human beings have struggled with all kinds of diseases, and have obtained beneficial experiences and understandings in preventing or controlling these plagues. Since T solium and its relationship with C cellulosae
were found by Linneus in 1758 and Küchenmeis in 1855-56, respectively, researchers worldwide have dedicated themselves to cysticercosis prevention and diagnosis and treatment studies. Originally, researchers were constantly vexed at pre-mortem diagnosis for porcine cysticercosis. Serological studies revealed the possibility of reliable ante-mortem diagnosis of cysticercosis, especially through specific antibodies detection. Then immunodiagnosis became a powerful tool for control of larval \( T_{solium} \) spread. Because antigens used are mainly responsible for sensitivity and specificity of cysticercosis diagnosis, it is necessary to know what larval \( T_{solium} \) antigens are.

**Larval \( T_{solium} \) Antigen**

The larva, \( C_{cellulosae} \), is one of eukaryotic creatures with innumerable proteins, some of which are antigenic. Jiang\(^ {12} \) showed that a crude extract of cyst wall and scolex showed 22 bands on SDS-PAGE gel, but the cyst fluid only showed 16. In fact, the components of scolex, cyst wall, and cyst fluid are far more complicated.\(^ {13} \) Analyses with high performance liquid chromatography (HPLC) indicated that cysticercus cyst fluid, worm, and scolex had 12, 12, and 14 peaks, respectively. Among these, scolex and cyst fluid shared 5 common peaks, worm and scolex shared 8, and worm and cyst fluid shared 7, suggesting that different parts bear common proteins.\(^ {14} \) Some cysticercus antigens can react with anti-sera against \( T_{solium} \) adult and oncosphere, indicating that they also have common antigens.\(^ {15} \) In addition, previous studies found that proteins—which may play important roles in signal transduction and regulation of cell proliferation and differentiation during early embryogenesis, parasite invasion, avoidance of host immune surveillance, etc.—were expressed differently at various stages.\(^ {16-18} \)

Some antigens, especially ones with molecular mass above 85kD, from crude \( C_{cellulosae} \) extract serologically cross-react with other parasites. Antigen B, identified by Flisser,\(^ {19} \) showed potential use as a serodiagnosis marker. It was able to recognize 84% of the sera collected from neurocysticercosis patients. This antigen, which contains two antigenic determinants with molecular weights of 95 and 105kD, is easily separated from crude \( T_{solium} \) extracts. Using immunological methods, Olivo\(^ {20} \) showed that antigen B was also found in adult and larva of \( T_{hydatigena} \), which can lead to porcine infection. Therefore, the antigen is limited in differential diagnosis between \( T_{solium} \) and \( T_{hydatigena} \).

Except some molecules (94.0 and 85.5kD) that may give rise to false-positive reactions,\(^ {21} \) others of cyst fluid antigens are useful in differential diagnosis because, although they may be lack of high sensitivity, they show high specificity. Cyst fluid is enriched in glycoproteins (GPs), which are good candidates for diagnosis because of high sensitivity and specificity,\(^ {22} \) while cyst wall and scolex contain few identified with periodic acid-Schiff (PAS).\(^ {12,13} \) The results of immuno-peroxidase labeling (IP) and indirect fluorescent antibody (IFA) also show that the tegument of \( T_{solium} \) contains no specific antigens which are found in other Platyhelminthes.\(^ {23} \)

In summary, it is advisable and recommendable that different antigens be used in different diagnostic methods, and that multiple antigens, which are separated and purified from closely related species that show high serocrossreaction with \( T_{solium} \) be used in differential diagnosis according to Kumar\(^ {24} \) and Zhang.\(^ {25} \)

**IMMUNODIAGNOSTIC METHODS FOR CYSTICERCOSIS**

In 1974, Herbert found that the immune
system might participate in removal of cysticerci from the host. Later, he proved that it was possible to diagnose cysticercosis of living animals through specific antibodies. In patient’s serum IgG is a dominant immunoglobulin; moreover, IgA, IgM, and IgE are also involved because of higher levels seen in cysticercosis patients than those of uninfected persons.26–28

**Intradermal test**

Originated by Casoni in 1911, the intradermal test (ID) was designed for cysticercosis diagnosis. The method is simple, practical, and highly sensitive.29,30 However, ID shows cross-reactivity and false positives, and causes immune reactions at the inoculated region or in whole body.29 It is advantageous to be used for epidemic investigation.

**Agglutination tests**

**Latex agglutination test (LAT)** — In LAT, antigens/antibodies are combined to latex as a particle vector which visualizes the reactions between antigens and their specific antibodies through agglutination. One study showed that LAT had a positive rate up to 81.4% and a false positive of 2.7% in the diagnosis of cysticercosis in humans.31

**Charcoal agglutination test (CAT)** — Activated charcoal is used as a vector in this assay. Previous studies indicate that CAT is lacking in specificity and stability, leading to high cross-reactivity.32,33

**Haemagglutination test (HA)** — Activated red cells, mainly derived from sheep because of easy availability, are absorbed with pre-prepared specific antigens or antibodies. Using IHA for cysticercosis diagnosis, Lu34 reported that 1 of 78 pigs with infection of *C. tenuicollis* showed cross-reactivity with specificity of 98.72%, and that 163 of 178 cysticercosis pigs were detected with accuracy of 91.5%. Diagnosis of human cysticercosis with IHA has also been evaluated.35–37 HA lacks repeatability and accuracy since its efficacy varies with many factors. Application of frozen sensitized red cells and monoclonal antibodies seems to increase the specificity of HA.38,39 HA is still extensively applied to clinical diagnosis because of its high sensitivity and ease of use.

**Precipitation reactions**

**Double diffusion test (DD)** — Zhai39 detected circulating antigen of 103 cysticercosis pigs and 133 healthy pigs with DD. The results suggest that DD was time consuming and had low sensitivity.

**Counter-immunoelectrophoresis (CIE)** — CIE is a good way to diagnose cysticercosis because of high specificity, sensitivity, and repeatability, partially attributed to the concentration of the antibody and antigen. Moreover, it is time saving (<2 hours). Zhao40 used enzyme-linked antigen CIE to detect patients’ sera with specificity and false positive of 94.4% and 2.2%, respectively. The drawback is that CIE is difficult to standardize.

**Indirect fluorescent antibody test (IFAT)**

Wen41 demonstrated that the accuracy of IFAT was 95.3%, and no cross-reactivity was found with anti-sera examined against other parasite diseases. Because of special equipment requirement, the application of IFAT is greatly limited.

**Enzyme–linked immunosorbent assay (ELISA)**

At present, ELISA is universally and extensively used for the diagnosis of human and porcine cysticercosis with high specificity and sensitivity (both range from 90% to 100%), which greatly depend on selection of antigens.42–44
Several methods based on ELISA are established, including Dot-ELISA, dry blood paper-ELISA, avidin-biotin-ELISA and monoclonal antibody-ELISA (McAb-ELISA), all of which show higher specific and sensitive and more convenience than ELISA. The sensitivity and stability of Dot-ELISA will increase significantly with fixing antigens absorbed on filter with 40% ethanol and using the buffers with 10mM EDTA.

**Methods Designed for Detecting Circulating Antigens of Cysticercosis**

Further serological studies have shown that it was difficult to determine cysticercosis pigs or patients only from results of specific antibody detection because antibodies might continue to be present 1.5 to 2 years after cure. The specific antibodies can’t be detected until 1 week post challenge with *T. solium* eggs and reach the peak 6 to 7 weeks later, whereas circulating antigens exist very early and will disappear as soon as the parasite is killed. Therefore, we can detect infected animals at the early stage of infection and evaluate treatment effects from the level of larval circulating antigens directly and accurately. Methods are used for detecting circulating antigens as follows

**ELISA**—McAb-ELISA, dot-ELISA, and McAb-dot-ELISA are used for detecting larval *T. solium* circulating antigens which are present in cerebrospinal fluid (CSF) and serum, with a positive rate above 91%. The detection frequency of circulating antigens is increased through heating CSF or serum, possibly because the structure of heat-susceptible antibody is damaged but that of the circulating antigens in the antibody-circulating antigen complex is not. This leads to the release of more free circulating antigens that can combine with more antibodies.

**Immunogold techniques**—Immunogold techniques were originally established by Faulk and Taytor in 1971. They have been developed into various diagnostic methods for cysticercosis, comprising dot-immunogold staining (dot-IGS), immuno-gold-silver staining (IGSS) established by Holgata in 1983, dot-immunogold-filtration assay (DIGFA) and so on. They all are highly sensitive and timesaving, and may be advantageous in on-site diagnosis. For instance, the detectable circulating antigen is minimized up to 0.5 µg/L in DIGFA. Simultaneously, some of these assays can be used in detecting antibodies against cysticercosis. Liu reported that the average titer of the sera detected by dot-IGSS with *T. solium* cyst fluid antigen was 1:27,470, which was significantly higher than that detected by dot-ELISA.

**Enzyme-linked immunotransfer blotting (EITB)**—In EITB, *T. solium* circulating antigens in CSF or serum is separated and transferred onto NC filter. It is one of the best ways to diagnose cysticercosis, which is very useful in epidemic investigation through detecting neurocysticercosis patient saliva.

**Molecular Approaches**

With the advent of recombinant techniques, it is possible to screen, clone, and manufacture proteins with high specificity and sensitivity as diagnosis markers. The cocktail of three fusion proteins from *T. solium* larva, designed as cC1, cC2 and cP1, shows good performance with 100% positive and 8% false positive and no crossreaction with sera against other parasite infections, suggesting that the multiple recombinant antigens are advantageous in differential diagnosis. Synthetic peptides based on immunodominant components of the diagnostic antigen are also good candidates for differential diagnosis with
100% specificity and 87 to 93.7% sensitivity.\textsuperscript{62} It is suggested that several methods or diagnostic molecules with individual high specificity or sensitivity are combined in diagnosis of human and pork cysticercosis.

**QUESTIONS**

In recent years, the rate of cysticercosis infection across China has decreased due to improved living conditions and scientific education. It is unfortunate that there are no systematic, nationwide, epidemiologic investigations on which to base our pork industry management and trade policies and prevention strategies. Presently, meat inspection is a major diagnostic method which may be useful when animals are heavily infected, but not when animals carry a few viable cysticerci. Walther\textsuperscript{63} reported that 78\% of carcasses infected with >20 cysts were detected, whereas only 31\% of those with fewer cysts were detected. Efficacy of meat inspection will differ from the number and location of incisions, easily leading to missing \textit{T solium} metacestode infections. Attentions should be also paid to that the diagnosis methods, such as ELISA, EITB, tongue inspection, may all show lower sensitivity in rural pigs infected naturally with few cysticerci than such ones experimentally infected with \textit{T solium}.

The lack of good standards is a serious concern. In 2002, the diagnostic standard of cysticercosis cellulosae (GB/T 18644-2002) was published by General Administration of Quality Supervision, Inspection and Quarantine of P.R.China (AQSIQ). ELISA with cisticercus antigens purified by chromatography is used in the standard. Because the used antigens will vary with all kinds of factors, including lab’s conditions, operators, and material-disposing methods, this diagnostic standard remains skeptical and limited.

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