# THE APPLICATION OF BIOTECHNOLOGY TO THE STUDY OF CESTODES

*John H. Cross**

**ABSTRACT**

Cestodes or tapeworms are found in vertebrate hosts worldwide. There are a great many species, but few have received much attention in biotechnologic research. Those that have been studied in any detail have been those of importance to veterinary and human medicine. The application of biotechnology to the study of taeniids has been gaining momentum in recent years. Research has been done to improve the diagnosis of larval taeniid infections, especially cysticercosis. There have been improvements in serologic testing using refined and purified antigens readily available from one species to detect antibodies of another. (*Taenia hydatigena* antigens are used to detect *Cysticercus bovis* and *Cysticercus cellulosae*). The use of Western blots of tapeworm antigens *(T. solium, T. crassiceps, Echinococcus granulosus)* has been shown to be effective in neurocysticercosis and hydatid diseases.

Studies with monoclonal antibodies have also been found to be of interest. Anti-oncospheral monoclonal antibodies have been developed to distinguish eggs of *E. granulosis* from other taeniid eggs. In another study, monoclonal antibodies from oncospheres of *T.saginata* conferred protection against oral infections with *T.saginata* eggs in calves. Other investigators reported vaccines against *Cysticercus fasciolaris* by a *T. taeniaeformis* antigen expressed in *Escherichia coli.*

Studies on DNA have been gaining momentum. DNA-based techniques have been used to detect inter- and intraspecific variations in *Echinococcus* and to characterize isolates of *E.granulosus.* DNA probes in Southern blot analysis have been used to discriminate taeniid species.

*Taenia saginata* in the Far East has become an enigma. Although the parasite is morphologically *T.saginata,* the definitive host for the parasite is not clear. Studies have shown the pig to be a possible intermediate host with larval development similar to *T.solium,* but in pig liver rather than muscle. In recent comparative studies with *T. saginata*-like worms from Taiwan and classical *T.saginata,* differences have been detected in DNA hybridization patterns.

## INTRODUCTION

Cestodes or tapeworms are found in vertebrates from all parts of the world. There are about 60,000 species of vertebrates; however, only about 4,000 species of tapeworms have been described. There appears to be, therefore, a large number of cestodes yet to be described[1].

Cestodes have received little attention in Indonesia as well as other countries in Asia. There are scattered reports of *Hymenolepis, Bertiella,* and *Raillietina* infections in humans, and sparganosis has been seen a few

* Department of Preventive Medicine, Uniformed Services University of The Health Sciences, Bethesda, Maryland USA

times. *Echinococcus* infection has been reported in dogs in Sulawesi[2], but few cases of hydatid disease in humans have been reported. Taeniasis, however, is no doubt the most important cestodiasis in Southeast Asia; human infections with *T. saginata* and *T. solium* are well documented. Furthermore, cysticercosis is a serious disease reported from pork-eating populations, such as those in Irian Jaya.

Cestode infections in fish, birds and reptiles are well documented from Indonesia as are reports of tapeworms in mammals. *T. taeniaeformis* is not uncommon in cats and its larval stage *Cysticercus fasciolaris* is often found in the liver of wild rats.

There appears to be very little interest internationally in tapeworms; except for those of importance to human and veterinary medicine. The taeniids *T.solium* and *Echinococcus granulosis* have received the most attention. This is unfortunate, as there is undoubtedly much to learn from the study of others in this diverse group of flatworms.

Fortunately the application of biotechnology in the study of taeniids has been gaining momentum in recent years. Most of these studies have been on the diagnosis of human and animal infections as well as studies on immunity and vaccines. Accomplishments have also been made in understanding the basic aspects and genetics of the parasites and this paper will review some of these recent achievements. It is not the intent to review all of the published literature, however.

## DIAGNOSIS

Advances in the diagnosis of cestode infections, especially those with larval stages in the tissues of man and animals, have been made using some new technological methods.

Computerized tomography (CT) scanning has become a valuable tool in the diagnosis of neurocysticercosis. When used properly, discrete lesions can be seen on a CT scan of the brain of persons with *Cysticercus cellulosae* infections. When magnified, scolices can even be revealed within the cysticerci[3]. Magnetic resonance imaging has also been used with success in neurocysticercosis[4].

## IMMUNODIAGNOSIS AND IMMUNOLOGICAL METHODS

The immunodiagnosis of cysticercosis has also improved. Rhoades et al.[5] and Kamanga-Sollo et al.[6] have reported good results in the serodiagnosis of porcine, human and bovine cysticercosis utilizing antigens from *T.hydatigena* metacestode cyst fluid in an enzyme-linked immunosorbent assay (ELISA).

Monoclonal antibodies (MAB) have been developed to antigens from several taeniids and also have value in immunodiagnosis. MAB usually recognize one antigen unique to the parasite so cross-reactivity is decreased. This, on the other hand, reduces sensitivity due to the single epitope specificity. False reactions can be reduced, however, by using a major antigen restricted to the parasite in preparing the MAB or by use of a panel of MAB which react with different epitopes[7]. Along other lines, Shen et al.[8] used a biotin-avidin system to determine circulating immune complexes and evaluating antibody response in patients with hydatidoses. They compared the avidin-biotin-peroxidase complex ELISA with the standard ELISA using hydatid cyst fluid from sheep and found the sensitivity and the specificity of both tests to be comparable.

Larralde et al.[9] reported the value of imunoplotting in analyzing Western blot reactions between vesicular fluids from metacestodes of *T.solium*, *E.granulosus*, and *T.crassiceps*, and sera from humans with neurocysticercosis. This involves plotting the frequency with which each antigen band reacts with a set of immune sera against the frequency of the same band when reacted with another set of immune sera. Immunoplotting readily sorted out those antigens useful for discriminative immunodiagnosis from the multitude of bands in the sera of sick and healthy people.

Craig and his co-workers published an interesting paper in 1986[10] in which they reported the development of a specific anti *Echinococcus* MAB which binds in an indirect immunofluorescent test (IFAT) to egg-derived oncospheres of *E.granulosus*, but not to those of other taeniid species such as *T.hydatigena*, *T.saginata*, *T.pisiformis*, *T.multiceps* or *T.taeniaeformis*. The MAB has been designated 4E5 and the 4E5-IFAT was used to identify putative *Echinococcus* eggs recovered from the environement in sites in Kenya[11].

Korean workers have carried out a series of studies on cystic fluid of *T.solium*. In initial studies they showed that affinity purified antigen from cystic fluid had a high specificity, but a lower sensitivity as a diagnostic antigen in cysticercosis, probably because it detected a single or a limited number of antibodies among the many produced in patients with neurocysticercosis[12]. They later analyzed antigen specificity using MAB and polyclonal antibodies to *C.cellulosae* by Western blot and found cystic fluid to react with low molecular weight proteins (15, 10 and 7 Kd)[13]. Biochemical properties of a purified protein of the cystic fluid showed subunits of the 7Kd protein to be linked to the others. The protein was relatively stable and had similar biochemical characters with an antigen in hydatid cyst fluid[14].

## ISOENZYMES

In studies elsewhere, isoenzyme analysis using isoelectrofocusing in agarose was used to study *Taenia* cestodes. Gels were stained with 17 different enzymes, but only three were used to construct isoenzyme profiles. Adult *Taenia* were collected from carnivores in Kenya *(T.saginata, T.hydatigena, T.regis, T.serialis, Taenia sp.)* The samples fell into 25 zymodemes and no zymodeme contained more than one species of *Taenia*, indicating that isoenzyme analysis can reliably be used for the identification of the genus[15].

## MOLECULAR BIOLOGY

Studies on the molecular characterization of tapeworms have been increasing. DNA has been isolated and characterized from several taeniids and cloning and specific mapping done. There is evidence that DNA sequences of mitochondrial genomes may be a sensitive indicator of genetic relativeness or divergence within taxonomic groups.

Yap and associates[16] isolated mitochondrial DNA from *T.hydatigena*, *T.crassicep* and *E.granulosus*. The mitochondrial genome of *T.hydatigena* was cloned into the bacterium *Escherichia coli* and a restriction map of the recombinant molecule was constructed. Using the cloned mitochondrial genome as a probe, they were able to distinguish *T.hydatigena*, *T.crassiceps*, and *E.granulosus*. In later studies, these authors (Yap et al.)[17] developed a non-radioactive probe to differentiate taeniid cestodes.

Purified total genomes DNA labeled with photobiotin was used as a probe for the identification and differentiation of strains of *E.granulosus* and to distinguish eggs from two different species, *T.hydatigena* and *T.taeniaeformis*. DNA-based techniques have been used to detect inter- and intraspecific variations in *E.granulosus* and to succesfully characterize various isolates of *E.granulosus* (Rishi and McMannus,)[18]. These authors also showed that these techniques can provide a useful approach for the assessment of inter- and intra-specific variations in a variety of other taeniid cestodes[19]. Flisser et al.[20] constructed cloned DNA probes which can distinguish *T.solium* from *T.saginata*.

More recently, Hemmings and McManus[21] isolated *E.multilocularis* antigen gene clones with a potential value in immunodiagnosis. Differential screening of the cDNA library with pools of *E.multilocularis* and *E.granulosus* human infective sera revealed 13 potentially immunodiagnostic clones. The clones were constructed from *E.multilocularis* protoscolex mRNA.

At the 12th International Congress of Tropical Medicine and Malaria held in Amsterdam, September 1988, two interesting papers were presented on the use of DNA technology to provide antigens for serodiagnosis. It is often difficult to obtain sufficient antigens from tapeworms to carry out serologic tests. Gottstein et al.[22] prepared polypeptides by recombinant DNA methods using *E. multilocularis*. They used *E.coli* to produce the antigen. In another paper, Lightowlers and Richard[23] reported their studies of expressing *E.granulosus* antigen in *E.coli*. The antigen was used to detect antigens in patients with antibodies to *E.granulosus* as well as other cestodiases.

## VACCINES

The development of vaccines using new biotechnological methods is moving forward. Harrison and Parkhouse[24] developed an anti-*T.saginata* monoclonal antibody that was used to affinity purify an antigen that gave protection in vaccinated cattle. In a more recent development, Johnson et al.[25] reported the expression in *E.coli* of a complementary DNA encoding *T.ovis* antigens as fusion proteins with the *Schistosoma japonicum* glutathione S- transferase. Vaccination of sheep with these fusion proteins gave significant, although not complete, immunity against challenge infection with *T.ovis* eggs.

## ASIAN TAENIA SAGINATA

*T. saginata* is some-what of an enigma in some parts of Asia[26]. Many people have *T. saginata* infections in Taiwan, especially among the native aborigine population and in parts of Indonesia and the Philippines. In most of these areas people often eat pork raw or partially cooked, but rarely eat beef. Dr. P.C. Fan of the National Yang Ming Medical College in Taipei has been looking into this mystery and has come to the conclusion that people obtain *T.saginata* in Taiwan from eating pig livers. In surveys conducted in Taiwan, cysticerci have been found in wild boars and domestic pig livers. In experimental studies with a variety of animals, cysticerci have been found to develop in the livers of pigs, calves and goats. Interestingly, the cysticerci were found with hooklets on the rostellum in early infections[27,28]. Taiwan *Taenia* was also introduced into pigs in the United States and the ELISA serologic response followed using cyst fluid from *T.hydatigena*. Antibody appeared at 3 weeks and continued until the

experiment was terminated at 32 weeks. Cysticerci were found only in the livers of U.S. pigs[29]. In other studies with collaborators in Korea and Indonesia (Fan et al.[30,31]) cysticerci of what is called *T.saginata* were found in livers of the experimental animals and not in musculatures.

Since there is a question on the taxonomic status of the so- called Taiwan *Taenia*, a study was conducted by Zarlenga et al.[32] (submitted) on the DNA characterization of a putative new species of *Taenia* using cloned ribosomal DNA fragments. The DNA was compared to *T.saginata* from Africa and Europe and nine other cestodes by restriction fragment length polymorphism and Southern blot analysis using $^{32}$P-labeled total cestode RNA and cloned ribosomal RNA gene fragments. Hybridization patterns of Taiwan *Taenia* DNA showed variations from that of *T.saginata* and *T.solium* as well as other cestode DNA examined. The results support biologic data indicating that Taiwan *Taenia* and *T.saginata* are very similar but distinct cestodes. Further studies are being planned to determine whether the Indonesian, Korean, Philippine and other Asian *T.saginata* are genetically similar to the Taiwan *Taenia* compared to the classical bovine-transmitted *T.saginata*.

## COMMENT

There is a great deal of new technology available today to study parasites. Parasitologists from Indonesia as well as elsewhere in Southeast Asia should take advantage of these new methods to study cestode parasites indigenous to the area. I would hasten to add that there are more immediate problems with parasites and these should be addressed first, but in the future, application of biotechnology may be of value in obtaining a better understanding of parasites and means found to control and to even eliminate them.

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## QUESTIONS AND ANSWERS :

1. Question: In regard with the protective effect, what kind of biotech procedures/products has been worked up in cestodes ?

   Answer : Initial studies indicate that vaccines are coming along using DNA technology.

2. Question: What biotechnological method would be appropriate to differentiate the egg of Taenia saginata and T.solium ?
   MAB/IFA : How is the test being done ?

   Answer : Craig 1956 published the methods (see MS)
   MAB produced in Balb C mice - collect eggs - do IFA

3. Question: Can viruses (e.g. vaccinia) be used for the production of Taenia antigens instead of using E.coli ?

   Answer : May be - but I am not sure, the system has been used in other systems.