

Research Article

Primers application with the Tso31 gene target in the molecular identification of *Taenia solium*

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ABSTRACT

Background: taeniasis is a zoonotic disease caused by *Taenia spp.* Human taeniasis caused by *Taenia solium* can be acquired after consumption of raw insufficiently cooked infected pork meat. Pigs are intermediate host for *T.solium*. Pigs acquired this infection by eating human feces that contained *T.solium* eggs. Pigs infected with *T.solium* can be transmitted to humans. **Purposes:** identification of *T.solium* in pig is important because it is indicator of *T.solium* transmission. Microscopic examination of *T.solium* eggs is considered less effective and efficient so that many other methods are developed for *T.solium* detection such as molecular and immunology. **Method:** This method used specific primer which can detect the Tso31 gene in *T.solium*. Tso31 gene is one of the most promising antigens to differentiate *T.solium* from *T.saginata*. Pig feces samples were taken by random sampling technique from 7 pig farms in Denpasar. **Result:** from the 30 samples, we found one sample that which gave a single amplification product of 234 bp. This indicates that the pig farms in Denpasar have been infected with *T.solium*. **Conclusion:** it is necessary to do meat inspection properly in the market as well as health education about the dangers and impacts of *T.solium* infection in the community.

Keywords: taeniasis, taenis solium, tso31 gene target

INTRODUCTION

Taeniasis is a zoonotic disease caused by *Taenia spp.* There are three species which can infect humans: *Taenia saginata*, *Taenia solium*, and *Taenia asiatica*. This disease is still endemic in the developing country and has a great importance for public health (1). The human is the definitive host for these three species and harbour the adult tapeworm in the small intestine. Cattle are the intermediate host for *T saginata*, while the pigs are the intermediate host for *T.solium* and *T asiatica* (2).

Human taeniasis/cysticercosis caused by *T.solium* can be acquired after consumption of raw insufficiently cooked infected pork meat, which subsequently develops to the adult tapeworm in the human small intestine (3). Taeniasis in humans can manifest as asymptomatic or minimally symptomatic intestinal carriage or as infection with cysticerci, predominantly in the eyes, muscle, subcutaneous, and neural tissues (4).

Neurocysticercosis is an infection caused by *T.solium*. Neurocysticercosis is considered to be the most common cause of acquired epilepsy

and is thought to contribute up to 30% of epilepsy cases in the endemic areas (5,6).

T.solium has a global distribution with significant economic and public health impacts. The impacts of *T.solium* causes important economic losses, especially in the meat sector, and represent a food safety issue rather than a serious public health problem especially for the pork eating populations, as taeniasis rarely causes serious clinical signs and symptoms (2,7).

Pig is the natural animal intermediate host for *T.solium*. Pigs being coprophagic animals, usually acquired infection by direct consumption of human feces that contain *T.solium* eggs, or indirectly via contaminated water, vegetation/foods, or soil. When ingested, the eggs liberate hexacanth embryos or oncospheres in the gastrointestinal tract (3).

Animal husbandry and farming practices in Indonesia especially in Bali still remain simple and close to residential areas. Although the breeders raise pigs in pens. However, sanitation and cleanliness of the cage is not carried out properly. Balinese people's habit of eating raw pork can also be a source of *T.solium* infection.

Moreover, small-scale pig husbandry in Bali has become one of the major sources of income. Consumption of uninspected pig meat is the major source of human *T.solium* taeniasis and consequently, a major risk factor for human and pig (8). Identification of *T.solium* in pigs is important because it is an indicator of *T.solium* transmission and the risk of taeniasis/cysticercosis in the carrier or the immediate environment (9,10).

For many years, the laboratory diagnosis of taeniasis has been based on the detection of eggs by microscopic examination from fecal samples, which still remains the routine laboratory diagnosis in most laboratories. Recent studies reveal that microscopic examination alone is not reliable to differentiating

the species of *Taenia* (11). Microscopic examination also has low sensitivity and can not distinguish the morphology of *T.solium* and *T.saginata* eggs (10).

Newer methods like immunological, and molecular methods have been used to detect *T.solium*, while a combination of two or more methods appears to provide higher sensitivity (3). Up to now the only reliable technique to distinguish *Taenia* eggs is PCR (8).

Molecular biology has been employed to detect parasites in order to enhance the identification and characterization of parasites such as *T.solium*. The goal of this study was to detect *T.solium* in pigs from fecal samples using PCR method with h specific primers which can detect the Tso31 gene in *T solium*.

METHODS

This study was used to detect taeniasis in pig farms in Denpasar, Bali. The population in this study were pig farms in Denpasar city. There are seven pig farms in Denpasar, Bali. The owner who has agreed to the informed consent is followed up by taking 30 samples of pig feces.

The samples was obtained using a random sampling technique from 7 pig farms in Denpasar. Sufficient pork feces were taken using a sterile stick. Feces samples were added with 2.5% Potassium dichromate, in a ratio of 1:2. The sample was placed in the sample container that has been provided and closed properly and stored at the room temperature. Stool samples were preserved with 2.5% potassium dichromate added with aquadest. The samples were centrifuge at 14,000 rpm for 10 minutes and the supernatant was discarded. After that, the samples were washed with aquadest three times (7). DNA from stool samples can be extracted using QIAamp-Fast DNA Stool Mini Kit (10,11).

In this study, we used nested PCR assay was used by Mayta et al to detect the *T.solium* oncosphere protein Tso31 (7). The first master mixture of PCR was performed in a total volume consisting of 25 µl, containing of 3 mM MgCl₂, 200 µM deoxynucleoside triphosphate, 0.2 µg/µl BSA, 0.8 µM primer 1, 0.125 U Taq Polymerase, and 2.0 µl of sample. PCR amplification was carried out in Biometra TAdvanced. The first PCR amplification utilized an initial denaturation step at 95°C for 3 min, followed by 25 amplification cycles, each consisting of a denaturation step at 95°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 1 min. The final extension at 72°C for 10 min. Subsequently, PCR-amplified products were electrophoresed using 2% agarose gel. The gels were stained with ethidium bromide (10 µg/ml) for 10 min. The stained gels were observed in a transilluminator and documented.

The second PCR was carried out in Biometra TAdvanced TADVANCED. The second master mixture of PCR was performed in a total volume of 25 µl, containing 2.5 mM MgCl₂, 200 µM deoxynucleoside triphosphate, 0.2 µg/µl BSA, 0.8 µM primer 1, 0.125 U Taq Polymerase, and 2.0 µl of sample. The second PCR amplification consisted of an initial denaturation step at 95°C for 3 min, followed by 25 amplification cycles, each consisting of a denaturation step at 95°C for 30 s, annealing at 60°C for 30 s, and extension at 72°C for 1 min. The final extension at 72°C for 10 min. 2 µl of PCR-amplified products were electrophoresed using 2% agarose gel. The gels were stained with ethidium bromide (10 µg/ml) for 10 min. The stained gels were observed in a transilluminator and documented.

RESULT

From 30 samples, we obtained one sample which gave a single amplification product of 234 bp (Figure 1 and 2)

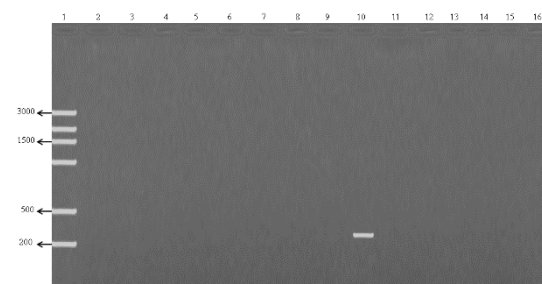


Figure 1. Tso31 nested-PCR amplification using DNA extracted from different sources. Electrophoresis was performed using 10 ul of amplification products. Lane 1 DNA ladders (solis Biodyne). Lanes 2 to 14, DNA from stool sample code A to M. Lane 16, DNA from stool sample code V. Lane 16, negative control.

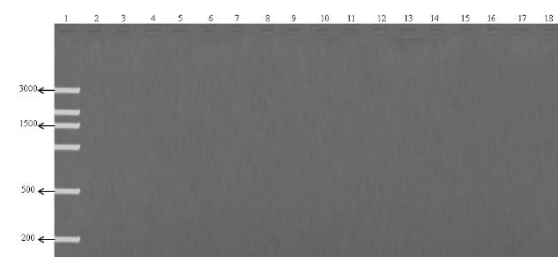


Figure 2. Tso31 nested-PCR amplification using DNA extracted from different sources. Electrophoresis was performed using 10 ul of amplification products. Lane 1 DNA ladders (solis Biodyne). Lanes 2 to 9, DNA from stool sample code N to U. Lanes 10 to 17, DNA from stool sample code W to D1. Lane 18, negative control.

DISCUSSION

Molecular biology tools have been developed to detect parasites like *T.solium*. There are some studies have been conducted to detect *T.solium* using PCR based technology. Mayta et al. performed the nested PCR method using specific primers which can detect Tso31 gene in *T.solium*. Nested PCR can specifically amplify the DNA of *T.solium* without cross-reacting with other parasites in the human intestine, such as *T.saginata*. Karamon et al described that nested PCR performed by Mayta et al.,(12) was the most useful method with requiring the least corrections during optimization (13).

The specific primer that was used can detect the Tso31 gene on *T.solium*. This method was the best method since it does not produce cross reaction with other parasites, including parasites from *Taenidae* family, which may be detected in pigs (7). Tso31 is a 31.3 kDa excretory/secretory oncosphere antigen present in *T.solium*. Tso31 gene is one of the most promising antigens to differentiate *T.solium* from *T.saginata*. Since this gene is presented in *T.solium* but not in *T.saginata*, it was recommended for differential diagnosis (14).

Verastegui et al. showed that oncosphere antigen of *T.solium* with molecular masses of 31.3 kDa is unique. This oncosphere antigen did not give reaction with other cestode infections such as *T.saginata*. Moreover, sera from pigs with antibodies to this oncosphere antigen did not cross react with immature *T.solium*, extracts from *T.solium* metacestodes, and *T.saginata* whole oncosphere (15).

Molecular methods provide a sensitive tool for identification of *Taenia spp.* and very useful for the accurate identification of cestode samples. Yamasaki et al. showed that molecular method like PCR can detect taeniid DNA in egg-free fecal samples where taeniid eggs are not present. They also suggested that PCR technique they had done seems to be dependent on the volume of fecal samples and the conditions of sample storage (15).

PCR method using specific primers to detect DNA in *Taenia spp.* has great potential in detecting *T.solium*. In addition, the PCR method is also relatively fast and sensitive for parasite identification (16,17). The molecular method is one of the methods developed to overcome the difficulty of identifying *Taenia* through microscopic examination. The difficulty of distinguishing species of *Taenia* by microscopic examination can lead to misidentification and improper treatment (18).

Proper diagnosis of the *Taenia* infection can give a proper treatment. Molecular method can differentiate between strains and provides an easy method for identification of *Taenia* (19). Proper and accurate identification of *Taenia* is required for monitoring disease prevention and vector control strategies.

Prevention of *T.solium* in pigs can be done by implementing hygienic pig farms with attention to environmental sanitation (20)(21). The pig breeders can provide cystidal drugs as anti-worm treatment (22). Precautions, control, and eradication of *T.solium* infection are very important. Humans can become infected with *T.solium* if they eat pork infected *T.solium*. Meat inspection in the market can be done to prevent this infection. This is so that pork circulating in the community is free from pathogens, including *T.solium* (23). Local government can give health education to community about the dangers of this infection (24). Information on how to properly cook pork needs to be socialized to the whole community (24,25).

CONCLUSION

We found one sample of pig stool was positive for *T.solium*. This sample comes from a pig farm which has 200 pigs. This pig farm has less stable sanitation than other farms. This indicated that pigs in Denpasar have been exposed to *T.solium*. Therefore, local government must take an action to prevent and eradicate of *T.solium* from being transmitted to humans.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare. All co-authors have seen and agree with the contents of the manuscript. We certify that the submission is original work and is not under review at any other publication.

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