

## Molecular Detection of Toxoplasmosis Using Specific Primers P30, B1, and rDNA

(PELACAKAN TOKSOPLASMOSIS SECARA MOLEKULER  
DENGAN PRIMER SPESIFIK P30, B1, DAN rDNA)

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### ABSTRACT

Study in order to develop molecular techniques using specific primers for the early diagnosis of toxoplasmosis have been conducted. Detection of *Toxoplasma gondii* genome was performed using polymerase chain reaction (PCR) technique. The primers used in this study were rDNA, P30, and B1. The PCR products were further run using gel electrophoresis (gel 1.5% – 2.0%) and the band was documented. Toxoplasma was detected at 500 bp and 600 bp using primer P30 and B1, respectively. Whereas using primer rDNA no band was observed. It was assumed that primer rDNA was not sensitive since the target amplification was 88 bp.

Keywords: toxoplasma, PCR, specific primer

### ABSTRAK

Telah dilakukan pelacakan toksoplasmosis secara molekuler dengan menggunakan primer spesifik. Tujuan dari penelitian ini adalah untuk melacak kasus toksoplasmosis dengan menggunakan beberapa primer dalam rangka diagnosis dini terhadap toksoplasmosis. Teknik PCR dilakukan dengan melakukan deteksi genom *T. gondii* yang telah diisolasi sebelumnya. Primer yang dipakai adalah rDNA, P30 dan B1. Hasil amplifikasi PCR kemudian di *running* dan dielektroforesis dengan gel 1,5%-2%, dan hasilnya didokumentasi dengan foto. Hasil penelitian menunjukkan bahwa dengan menggunakan primer P30 dan B1 dapat dideteksi toksoplasma sebesar 500 dan 600 bp, sedangkan hasil dari primer rDNA tidak tampak. Deteksi toksoplasmosis dengan rDNA kemungkinan kurang sensitif untuk reaksi PCR ini, karena target yang diamplifikasi hanya 88 bp yang akan menyulitkan pada saat elektroforesis.

Kata-kata kunci : toxoplasma, PCR, primer spesifik toksoplasma, ,

### INTRODUCTION

Toxoplasma is a zoonotic disease that may cause many problems both to human and animals. Toxoplasmosis prevalence in Indonesia is predicted to increase as the change of lifestyle occurs. Based on the data from the Animal Farming Department year 2003, the prevalence of toxoplasmosis in Kabupaten Sleman was 70% in sheep and goats. There are many cases of abortion in sheep and goat due to toxoplasmosis, therefore, toxoplasmosis has become an important zoonotic disease. About 20-100% sheep in the world are seropositive to toxoplasmosis.

Meanwhile, about 2.2 % lamb in England are seropositive (Dubey, 1994). The host of toxoplasmosis might be infected through three ways: (1) consuming not well-cooked meat which was infected by tachyzoit (acute phase), (2) swallowing any bradyzoite form (chronic phase) by consuming any food and drink that contaminated with oocyst from the infected cat faeces, and (3) transplacental infection during the pregnancy period. The clinical diagnose of toxoplasmosis in animals and human are hard to confirm since the disease is asymptomatic or subclinical in chronic infection. While in the

acute infection, the symptoms are similar to other infectious diseases. Thus, it is important to do another convincing diagnose by isolating the parasite and inoculating the suspected tissue on mice or any other testing animal (Soulsby, 1982). Skin test method using tachyzoit membrane protein of *Toxoplasma gondii* was done by Nurcahyo *et al.*, (2003). Skin test method also can be applied for the detection of toxoplasmosis in deer.

In this study, toxoplasma diagnosis was done by using PCR whereas ribosomal DNA of *T. gondii* was amplified. Some genes have been used for the detection of toxoplasmosis, such as gene B1, which was commonly done by repeating 35 times in chromosome 9. Some researchers mentioned that gene B1 is non polymorphese although it is very beneficial as the target to detect PCR (Susanto *et al.*, 2002). Gene Ribosomal DNA in term of tandem also has *single cluster* with 100 copy in chromosome 9 having repeat unit 8.2 kb which contains *small subunit* at 5.8 S RNA, *large subunit* at 5 S RNA and *untranslated region*. Hence rDNA is so sensitive for PCR detection (Tenter, 1994; Lamoril *et al.*, 1996). Therefore, rDNA is very potential for molecular diagnosis in Toxoplasmosis.

## RESEARCH METHODS

### Toxoplasma DNA

Toxoplasma DNA isolate was taken from any tissue or cell. Tachyzoit from the infected mice was used in this study. The tachyzoit was washed twice using PBS before used. After that, proteinase K solution (1 mg/ml in NTE, pH 7,4) was prepared and leave it in the waterbath 37°C for 15 minutes. The cells were dissolved in NTE (10<sup>7</sup>sel/ml) until homogenous. The suspension is added proteinase K (final concentration 0,5%) and incubated for a night in waterbath (37°C for 24 h). After that, add phenol solution (1 : 1), for 20 minutes, 60 rpm then shake and centrifuge for 10 minute, 5000 rpm at room temperature. The top most layer of the solution was taken carefully using pippete and mixed with chloroform: isoamylalcohol (24 : 1) in 1 : 1 ratio. The mixture was centrifuged at 5000 rpm for 10 minutes. The top layer part was taken again and the steps above were repeated until there was no more interphase. After it was clean, the phase is taken

and added 1/20 vol 3 M Na acetate and 2 X volume cold ethanol (95%). The mixture was left for 10-15 minutes at 20°C until the formation of precipitation. The DNA precipitation was taken using glass stab and was dried using ethanol in vaccuum. After that, the DNA was dissolved in sterile H<sub>2</sub>O. Henceforth it is determined the DNA concentration.

### PCR Detection

The isolated DNA from the tissue/cells above was detected by PCR technique. The primer was determined based on the sequence data from the GenBank. The primers according the order 5'-3' are:

#### rDNA

Primer 1 : CAT GCC TCT TCC CCT G

Primer 2 : GGT CGC GTT GAC TTC GGT CT

#### P30

Primer 1 : TTG CCG CGC CCA CAC TGA TG

Primer 2 : CGC GAC ACA AGC TGC GAT AG

#### B1

Primer 1 : ATG TGC CAC CTC GCC TCT TGG

Primer 2 : GAA CTG TAA TGT GAT ACT GTG

The sample was prepared in a reaction using Ready to Go Mix with final volume 25 µl, composed of 2 µl DNA speciment and other materials that consist of Primer P30 R 2 µl and d H<sub>2</sub>O 19 µl. The PCR was ran through the program as follows: 95°C 5 minutes, 94°C for 2 minutes, 58°C for 1 minute, 72°C for 30 seconds, 72°C for 5 minutes, and 4°C with a cycle 30. The result of the amplification was executed eletrophoresis using gel 1,5%-2%, and the result was documented.

## RESULTS AND DISCUSSION

Advanced molecular biology is very helpful and may contribute to the development of new diagnostic devices which will be able to give better sensitivity and specificity than the conventional method. The cloning of parasite molecular and production or recombinant protein have revealed on the horizon in providing antigen material (Nurcahyo, 1998).

Figure 1

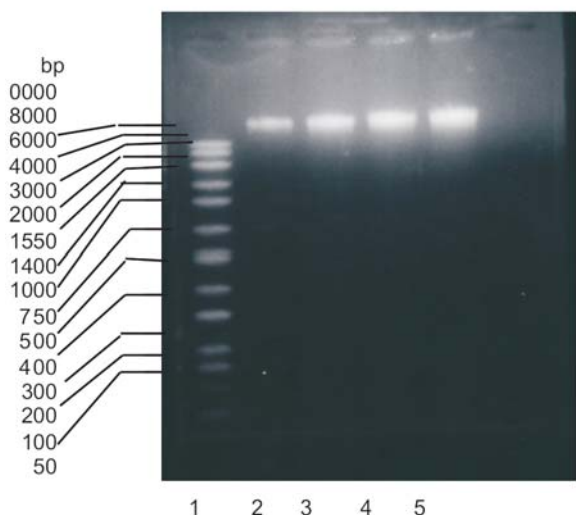


Figure 1. Result of amplification electrophoresis of isolated DNA (1: DNA Marker, 2-5 : detection of *Toxoplasma* DNA with 2,5 µl, 5 µl, 7,5 µl and 10 µl).

Figure 1 shows the isolated DNA from the cells of *Toxoplasma* tachyzoit with multilevel volume ranging from 2.5 µl, 5 µl, 7.5 µl and 10 µl DNA. There was no significant difference among the four bands which were amplified using PCR technique. Figure 2 indicates the result of amplification electrophoresis of DNA *T. gondii* using specific primer P30 (band 1-3) and primer rDNA (band 4-5). The result showed that DNA clearly detected at around 550 bp.

Figure 2.

Primer B1, P30, and ribosomal DNA are well-conserved primers. Primer B1 and ribosomal DNA are the *multiple copy* genes in genome *T. gondii* that lead the primers so ideal as the target of DNA amplification. Gene B1 has 35 times repetitive sequence with the unidentified function. In eukaryote, ribosomal DNA is often seen repeatedly up to 100 times (Jones *et al.*, 2000). Primer P30 is only expressed in tachyzoit and it codes to some membrane protein by giving less than 5% of total tachyzoit protein (Kasper *et al.*, 1985). In Figure 3, the amplification result using primer B1 shows that ribbon 1 and 2 about 600 bp of the result of primer amplification. The picture 2 line 4 and 5 are the amplification result using primer rDNA. It is not visible in Figure 2 because rDNA is likely amplified but it is not clearly detected by the primer as the size is less than 88 bp (Jones *et*

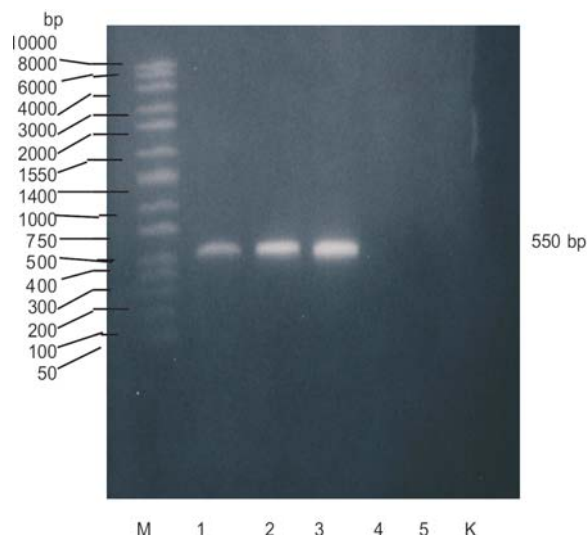


Figure 2. Result of amplification electrophoresis of DNA using specific primer gene P30 and rDNA *Toxoplasma gondii* . (M - DNA Marker, 1-3 : primer P30, 4-5 : primer rDNA, K : control PCR without any *template* addition)

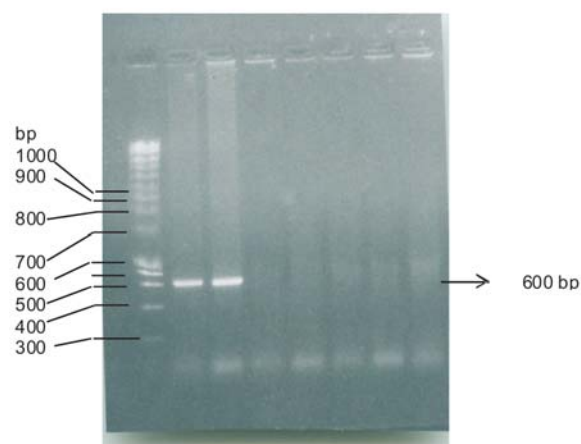


Figure 3. DNA amplification using specific primer B1 *Toxoplasma gondii* (M - DNA Marker, 1-2 : amplification using primer B1, 3-6 : negative control, K : control PCR without any *template* addition)

*al.*, 2000). Gene ribosomal DNA was less sensitive compared to DNA toxoplasma, particularly that comes from the tachyzoit stadium. Some previous studies have been carried out by optimizing PCR reaction that used primer rDNA only, but it was still not visible at 88 bp. Another possibility is the damage of primer rDNA due to some disturbances in the storing process of the

primer rDNA, such as black out during the storage.

### Figure 3

In PCR control by eliminating *Toxoplasma* template it is not shown the presence of DNA ribbon. The negative control was very important, especially to avoid any contamination that might be happen during the application and preparation of PCR. Through PCR it can be amplified and detected a specific parasite gene. By applying technical parameter that occurs in PCR, for example, a good result of PCR amplification was obtained by denaturation at 93°C for 2 minutes, *annealing* 57°C for 2 minutes and extention 72°C for 2 minutes, total cycle 30 (Nurcahyo, 1998).

The study by Grob (1994) in some human samples from the hospitals indicate that the result of DNA amplification using PCR can be amplified DNA fragment 634 bp through primer gene B1. The toxoplasmosis diagnose in animals using PCR has long been conducted in foreign countries such as a detection of abortus fetus sheep tissue or lamb born dead. Furthermore, applying PCR can distinguish other coccidia which often puzzle the diagnosis i.e. using Small Sub Unit (SSU) rRNA of *T. gondii* (Tenter, 1994). Diagnosis of toxoplasmosis was done by using gene B1 as the diagnoses target because the gene in the genome has the copy up to 35X, therefore, it was easier to amplify and detect. However, the role of gene B1 itself has not been identified clearly in toxoplasmosis. The PCR specificity will be better if it is followed by other tests, such as hybridization using internal oligonucleotide by analyzing restriction enzyme, sequencing DNA or amplifying PCR from various gene fragments (Grob, 1994).

### CONCLUSION

According to the result, it can be summed up as follows : toxoplasmosis is well detected using PCR with the specific primer P30 and B1. The electrophoresis result of P30 and B1 come up with DNA amplification 550 bp and 600 bp. Ribosomal DNA was not well-detected because the size of the fragment was small (80 bp). However, the toxoplasmosis detection using PCR can be done well depend on the DNA isolation.

### SUGGESTION

It is recommended to have sequencing DNA using primer rDNA from some samples for further detection of the gene rDNA. Moreover, it is also important to have further study using other methods such as inoculating the testing animals or making the histopatology blood smear.

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