International Seminar on Zoonotic and Tropical Disease

ONE WORLD ONE HEALTH™ CHALLENGE: GLOBAL MOVEMENT ON ZOONOTIC DISEASE

Friday - Saturday, 26 - 27th June 2009
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INTERNATIONAL SEMINAR ON ZOONOTIC AND TROPICAL DISEASE

One World One Health™ Challenge:
Global Movement on Zoonotic Disease

Yogyakarta, 26-27th June 2009

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PROCEEDING
INTERNATIONAL SEMINAR ON ZOONOTIC AND TROPICAL DISEASE
One World One Health™ Challenge:
Global Movement on Zoonotic Disease

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Loop-Mediated Isothermal Amplification (LAMP) 
As an Efficient Diagnostic Tool of Toxoplasmosis

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ABSTRACT

Toxoplasma gondii infections are widely prevalent in humans and warm-blooded animals. This tissue cyst-forming coccidium belongs to the phylum Apicomplexa, a large group of mostly intracellular parasites which includes dreadful pathogens such as Plasmodium falciparum. This work investigated the possible use of loop-mediated isothermal amplification (LAMP), the most recently developed molecular diagnostic tool, in diagnosis of toxoplasmosis. A set of primers, specific to SAG1 gene, and composed of outer primers (F3, B3), inner primers (FIP, BIP) and loop primers (IF, IB) were designed from a published sequence data (GenBank Acc. no AY651825). The method appeared to be very sensitive, exhibiting a degree of sensitivity higher than PCR, either based on SAG1 gene or the repetitive 529 bp. fragment (R529). Finally, due to its ease of use, LAMP is a convenient diagnostic tool of toxoplasmosis in conditions where sophisticated equipments are hardly affordable.

Key words: diagnosis, LAMP, PCR, repetitive sequence R529, SAG1, Toxoplasma gondii

1. Introduction

Toxoplasma gondii infections are widely prevalent in humans and warm-blooded animals worldwide. It infects up to one third of the world’s human population (Tenter et al., 2000). This tissue cyst-forming coccidium is one of the most successfull zoonotic pathogens. It is an obligate, intracellular protozoan parasite that belongs to the phylum Apicomplexa, a large group of mostly intracellular parasites which comprises important pathogens such as Plasmodium species, Eimeria, Cryptosporidium, Neospora and Theileria (Kim & Weiss, 2004). T. gondii genome is haploid, except during sexual division in cats, and contains about 8 x 10^7 base pairs (Cornelissen et al., 1998). Parasite infections are often innocuous or asymptomatic in most individuals. Infection during pregnancy may lead to severe, if not fatal, infection of the fetus. Severe consequences include abortion, intracerebral calcification, mental retardation, and embryonic, fetal or neonatal death (Kravetz & Federman, 2005). In immunocompromized patients, T. gondii has emerged as an important opportunistic infectious pathogen (Zangerle et al., 1991). Encephalitis is the most clinically important manifestation of toxoplasmosis and the disease is the major cause of death among patients with AIDS.

Though virtually able to infect all warm-blooded animals and to multiply in all the nucleated cells, T. gondii sexual cycle exclusively occurs in cats. Major parasite transmission occurs by a fecal-oral cycle, carnivorism or transplacentally. Toxoplasmosis is also considered as an important water-borne disease (Dubey, 2004). The ability of T. gondii to infect a broad spectrum of cell types and hosts is most probably due to the so called "SAG1-related sequence" (SRS) proteins, an important repertoire of structurally related, yet
antigenically distinct surface proteins (Jung et al., 2004). SRS provides a redundant system of receptors for interaction. SAG1, the prototypic member of SRS, is a highly abundant surface protein, expressed on the rapidly dividing tachyzoites. T. gondii is also the most tractable model for the study of intracellular Apicomplexan parasitism as it can be cultured, easily transfected and genetically studied in vitro (Kim & Weiss, 2004).

Vaccination and early diagnosis of toxoplasmosis may constitute successful measures to control the spread of the disease. Various types of vaccine have been developed and tested. They include attenuated live parasites (Freyre et al., 2007), crude or recombinant antigens (Yang et al., 2004), and more recently DNA-based (Nielsen et al., 2006) or transgene vaccines (Wang et al., 2007). Attenuated T. gondii may also be used as carrier for DNA vaccine delivery (Charest et al., 2000). An early diagnosis of the disease is also essential in order to take suitable preventive measures and the availability of efficient and sensitive diagnostic tools may prevent disastrous consequences. Various diagnostic methods, based either on antigens (serological diagnosis) (Pietkiewicz et al., 2004) or nucleic acid (molecular diagnosis) (Homan et al., 2000; Filisetti et al., 2003) have been reported.

Loop-mediated isothermal amplification (LAMP) is the most recently developed molecular detection method (Notomi et al., 2000; Parida et al., 2008). This new generation of innovative gene amplification technique is known to be a very sensitive and fast detection method. It relies on autocycling strand displacement DNA synthesis by the Bst DNA polymerase, and the reaction is carried out under isothermal conditions, usually at 63°C the optimum temperature of the polymerase. Amplification proceeds by displacing and releasing single-stranded DNA (ss-DNA). Positive response can also be visually observed due to the production of whitish precipitate of magnesium pyrophosphate. This makes the method particularly attractive for routine analyses in conditions where sophisticated equipment are hardly available. A set of LAMP primers is composed of six primers (outer primers F3, B3; inner primers FIP; BIP; loop primers IF, IB), specifically recognizing eight distinct regions of the DNA target. This makes the amplification method highly specific for pathogen detection. The inner primers FIP and BIP consist of two parts, F1 and F2 and B1 and B2, respectively, linked by four T residue. F1 and F2 in one hand and B1 and B2 on the other hand are of opposite orientation. This helps the formation of a stem-loop structure at the end of the newly synthesized strand. Primers IF and IB further improve amplification efficiency. Amplification is initiated by an inner primer (e.g., FIP), followed by strand displacement by an outer primer. The ss-DNA produced form a stem-loop structure at its 5'-end and serves as a template for the second inner primer (BIP), giving rise to DNA structure with stem-loop at each end. This non-cycling process is followed by a cycling and exponential amplification. The strand displacement by an inner primer results in the production of the original structure, gaining an additional stem-loop. The process continues giving rise to the final structure which is a stem-loop cauliflower-like DNA structure, with alternately inverted repeats of the target in the same strand.

This paper describes a LAMP technique, based on SAG1 gene, developed as a rapid, sensitive and convenient detection tool of T. gondii. Efficiency of amplification was compared to that of conventional PCR.

2. Materials and methods

2.1. Tachyzoites, organs and templates for amplification

T. gondii tachyzoites (RH strain) were propagated in vitro under standard procedures, by serial passages in human foreskin fibroblast (HFF) monolayers in Dulbecco's modified Eagle medium (DMEM, Invitrogen) supplemented with 10% fetal bovine serum at 37 °C and under 5% CO2. Tachyzoites were collected, filtered on a 3 μm polycarbonate membrane (Whatman), pelleted at 750 g for 10 min and washed twice with PBS. DNA was extracted using DNAZOL reagent (Invitrogen Life
Various organs (10-20 mg) were sampled (brain, heart, liver, spleen, kidney) from 6 week-old mice experimentally infected with tachyzoites of a virulent Indonesian isolate IS-1 (type I) (Sri Hartati et al., 2006) and killed at 4-5 days post-infection. of *T. gondii*. Supernatant of samples (10-20 mg), homogenized in TN (20 mM TRIS-HCl, 0.4 M NaCl, pH 7.4), was obtained by centrifugation at 12,000 g, for 5 min at room temperature. DNA was extracted using DNAZOL reagent as above. Cloned SAGI gene (*pGEX-SAGI*) was used as positive control and *pGEX-2T* as negative control. Different amounts of template were used.

2.2. LAMP reaction

A set of LAMP primers of SAGI gene [localization within the mature SAGI gene and orientation in sense (S) or antisense (AS) given in brackets], consisting of outer primers F3 (S.57-74), B3 (AS.266-249), inner primers FIP (F1.SA.142-120,ttt,F2.S.76-94), BIP (B1.S.174-192,ttt,B2,AS.242-224) and loop primers IF (AS.118-96), IB (S.195-215), were “manually” designed, based on a sequence data (GenBank acc. no. AY651825). They were those previously described (Krsteva et al., in press). The amplified zone of the target is 210 bp length (between F3 and B3). Primers F3 and B3 were also used in conventional PCR.

LAMP reactions were carried out at 63°C for 60 min. The reaction mixture contained 2.0 μM each of FIP and BIP, 1.0 μM each of IF and IB, 0.2 μM each of F3 and B3, 1400 μM of dNTP mix (Promega, Madison, WI, USA), 0.6 M betaine (Sigma-Aldrich), 6 mM MgSO4, 8 U of Bst DNA polymerase (large fragment: New England Biolabs Inc.), along with 1 x of the supplied buffer and specific amounts of template, in a final volume of 25 μL. Minimal time for amplification was determined by incubation for 20, 40 and 60 min. Amplification was also tested at 65°C. The degree of sensitivity was established by performing reaction at 63°C for 60 min, using serial 10-fold dilutions of template.

Amplification products were analyzed by electrophoresis on ethidium bromide-stained 2% agarose gel. LAMP-positive results are characterized by the appearance of ladder-like pattern on agarose gel, with many bands of different sizes up to the loading well. Positive responses were also visually detected due to the whitish colouration or by adding SYBR Safe and observation under a UV lamp.

2.3. PCR reaction

PCR was carried out in a final volume of 25 μL, using the LAMP outer primers F3 and B3 with the following conditions: prior denaturation at 94°C for 3 min; 25 cycles of denaturation at 94°C for 30 sec, annealing at 60°C for 30 sec, elongation at 72°C for 30 sec; additional elongation at 72°C for 5 min. PCR was also performed under the same conditions with primers RF3 (S.207-224) and RB3 (AS.444-424), specific to the repetitive 529 bp. DNA fragment (R529), “manually” designed based on sequences from GenBank (Acc. no. AF146527 and EF195646). The amplified DNA fragment is 238 bp long. Amplification products were analyzed by electrophoresis on ethidium bromide-stained 2% agarose gel.

3. Results

3.1. Search of conditions for LAMP and application to samples

Test of LAMP was done using cloned SAGI gene (*pGEX-SAGI*) or *pGEX-2T* as template or in absence of any template. Reaction was performed at 63°C or 65°C for 20, 40 and 60 min. Amplification worked well at both temperatures and was already fully efficient at 40 min incubation. A ladder-like pattern of bands of sizes up to the loading well was observed on agarose gel. Results were also visually established. Primers were specific to SAGI gene as negative response was obtained with *pGEX-2T*. The experiment also showed that the loop primers did not induce self-amplification of primers, in absence of any template. It has been shown that this occurs with certain sequences of loop primers (Pillai et al., 2006). For standardization, LAMP reactions were henceforth carried out at 63°C, the optimum temperature of Bst polymerase, for 60 min.
Application of LAMP to organs harvested from experimentally infected mice showed that, using supernatant infected samples, one organ, i.e., heart, was positively responding. When DNA was used as template, except for brain, all the organs sampled were positively responding. The fact that the brain gave negative response, indicated that the designed SAG1-based primers were specific to T. gondii and no cross-reaction occurred with sequences of the mouse genome. The results also indicated that LAMP can be performed on extract from organ without prior DNA extraction.

3.4 Efficiency of LAMP and comparison with conventional PCR

The sensitivity of the reaction was established using serial 10-fold dilutions of templates. Amplification efficiency was compared to that of PCR using the outer primers F3 and B3 specific to SAG1 or RF3 and RB3 specific to the repetitive sequence R529. Using pGEX-SAG as template, LAMP positive response was still observed with 1 fg of plasmid (150 molecules of target). SAG1-based PCR was still positively responding with 1 pg of plasmid though faintly. Compared to PCR, LAMP efficiency was at least 1,000-times more sensitive. Detection threshold, established using DNA extracted from isolated tachyzoites, indicated that the upper limit of detection was higher than 10 parasites but lower than 100 parasites. SAG1-based PCR was 100-times less sensitive. If the two results were combined, it appeared then that without optimization of the procedure the detection threshold of SAG1-based LAMP was between 15 and 100 molecules of target, i.e., between 15 and 100 parasites and that SAG1-based LAMP was more sensitive than SAG1-based conventional PCR.

Using total DNA extracted from infected liver as template, positive response was still obtained with DNA amount corresponding to the DNA content of ca. 50 ng of organ. It appeared so that owing to the high sensitivity of LAMP only very small amount of organ is required. 0.5 mg of organ was the minimal amount to get a positive response with SAG1-based PCR (faint band on agarose gel. When applied to the detection of T. gondii in infected organs or tissues, the sensitivity of LAMP largely exceeded that of PCR, by at least 100,000-times more. SAG1-based LAMP constitutes thus a powerful diagnostic tool suited for the diagnosis of toxoplasmosis.

Owing to its high copy number within the parasite genome, estimated to be around or even more than 300 repeat units, the repetitive 529 bp DNA fragment (R529) constitutes an ideal target in molecular detection (Homan et al., 2000). Using primers specific to R529, the sensitivity of PCR was obviously higher than SAG1-based PCR (by at least 10,000-times). Nevertheless, SAG1-based LAMP still exhibited a higher sensitivity.

We demonstrated in this experiment that the SAG1-based LAMP was found to be a very sensitive diagnostic tool of toxoplasmosis, contrarily to conventional PCR using the same target. The lower sensitivity of PCR for detecting the parasite in organs or tissues may arise from the intricate population of genes and sequences within the total DNA extracted from infected organs or tissues which comprises parasite as well as host-cell DNA. The single-copy SAG1 gene only constitutes a tiny part. It has been suggested that contrarily to conventional PCR, LAMP is not adversely affected by irrelevant DNA targets (Notomi et al., 2000). With the increasing complexity of DNA population, PCR efficiency decreases and the difference between LAMP and PCR is consequently more pronounced.

Owing to its rapidity, sensitivity and ease of use, LAMP methodology is suited for routine health controls, particularly in conditions where sophisticated and expensive equipments are not available. It is also appropriate for field application in veterinary medicine.

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Reference


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Comparison Level of Cortisol and Ratio of Neutrophil/Lymphocytes as Acute Stress Marker to Long Road Transportation of Bligon Bucks

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ABSTRACT

Transportation of animal is generally recognized as a stressful event and related to immune system. The purpose of this study is to compare the pattern of cortisol secretion during transportation and ratio Neutrophil/Lymphocyte (N/L) as a stress marker. Six adult bligon buck ranging in body weight 26-30 Kg were used in this study. Two weeks prior to the experiment, the animals had been given anthelminitic Albendazole to eliminate egg worm. All of animals were fed standard ration in their pen at 10% from their body weight per head daily and commercial concentrate also was given everyday. All of the animals were transported around village for 16 hours starting from 18.00 pm until 10.00 am in open small truck (3 x 2 m); eye contact with each others was provided. Blood samples were withdrawn from jugular vein using vacutainer tubes containing heparin, transferred into 1.5 mL glass tubes in aliquot, and centrifuged at 500 xg for 15 minutes. Plasma was collected and stored at -20°C. The blood samples were collected every 4 hours from ~8 hours before until 8 hours after transportation at 10.00 am. Separate whole blood samples were used to measure number of Neutrophil and Lymphocyte. Plasma was harvested and stored at -20°C until cortisol concentrations were measured using ELISA. The result showed that transportation of Bligon bucks for 16 hours affects both level of cortisol (P<0.05) and the ratio of N/L (P<0.05). The pattern of cortisol and ratio of N/L is very similar. Furthermore, the peak of cortisol was obtained at 4 hours of transportation whereas ration N/L was reached later namely 8 hours after transportation. It is concluded that ratio of N/L can be used as stress marker for Bligon goat even less sensitive than the use of level of cortisol.

Keywords: Cortisol, ratio Neutrophil/Lymphocyte, stress, transportation, Bligon

Introduction

Routine transportation of goats between farm and other cities moreover export to other countries for both slaughtering and breeder has been running almost everyday. The distance of transportation is vary start from 2 until 16 hours. Transported animals may be exposed to a variety of physical and psychology stimuli including crowding, noise, handling, isolation and extreme temperatures (Al-Kindi et al. 2005). Usually transportation of animal is generally recognized as a stressful event and related to immune system. Even tremendous study on the effects of handling and transportation of cattle, pigs and poultry, little work has been carried out to assess the effect of transported local goat on cortisol as indicator of stress.

Up to now, measurement level of cortisol is the best way to determine both acute and
chronic stress. Unfortunately this process is very expensive and not practical, it would be looked for another methods using ratio of neutrophil/lymphocyte (N/L) which is much cheaper and practical. The aim of this study are to determine 1) level of cortisol during long transportation (16 hours) in Bligon goat; 2) ratio of neutrophil/lymphocyte; and 3) to compare pattern of cortisol secretion and ratio of N/L. This physiological parameters have been proposed as sensitive indices of physiological stress response in animals that encountered long-term welfare problems such as handling and transportation.

Materials and Methods

Animals: Six adult bligon bucks ranging in body weight 26-30 Kg were used in this study. Two weeks prior to the experiment, the animals had been given anthelmintic Albendazole to eliminate egg worm. All of animals were fed standard ransom in their pen at 10% from their body weight per head daily and commercial concentrate also given everyday. Fresh water was provided as ad libitum.

Treatment: All of animals were transported around village during 16 hours starting from 18.00 pm until 10.00 am in open small truck (3 x 2 m); eye contact each others would be provided. Blood samples were withdrawn from jugular vein using vacutainer tubes containing heparin was transferred into 1.5 mL glass, then centrifuged at 500 g for 15 minutes. Plasma was collected to be stored at −20°C until cortisol concentrations were measured using commercial ELISA (Enzyme Linked Immunosorbent Assay) kit product DRG, Germany. The blood was collected every 4 hours from – 8 hours before transportation (at 10.00 am, 14.00 pm and 18.00 pm) until the time arriving after transportation at 10.00 am.

ELISA for plasma hormone concentration: The cortisol kit is a solid phase enzyme–linked immunosorbent assay based on the principle of competitive binding. The microtiter wells are coated with monoclonal antibody directed towards an antigenic site on the cortisol molecules. Endogenous hormone of sample competes with cortisol-horseradish peroxidase conjugate for binding to the coated antibody. After incubation, the unbound conjugate was washed off. The amount of bound peroxidase conjugate is inversely proportional to the concentration of hormone in sample. After addition of the substrate and stop solution, the intensity of color would be inversely to concentration of hormone. Concentrations of cortisol were determined in duplicate samples by EIA.

Statistical analysis: The differences in level of cortisol and ratio N/L were contrasted using ANOVA and continued by Tukey HSD test.

Results and Discussions

Plasma cortisol levels of bucks are given in Table 1 and Figure 1, until reached the peak at 161.9±78.9 ng/mL. Cortisol exhibited significant fluctuation over time subject to pulsatile regulation by the episodic release of ACTH from the anterior pituitary and CRF (Corticotropin Releasing Factor) from hypothalamus (Liota et al. 1990; Litwack et al. 2003). Statistically, there was significant differences which found before and after transportation (P<0.05). Stress has been demonstrated to increase the number of CD4+ lymphocytes while decreasing CD8+ lymphocytes (Degabriele and Fell, 2001).

Table 1. Averages±SD level of cortisol before and after transportation. Symbol O indicate the timing of transportation.

<table>
<thead>
<tr>
<th>Period of Transportation</th>
<th>8</th>
<th>12</th>
<th>16</th>
<th>20</th>
<th>24</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortisol (ng/mL)</td>
<td>70.7±43.69</td>
<td>66.23±33.8</td>
<td>95.96±2.9</td>
<td>161.9±78.9</td>
<td>123.7±7.65</td>
</tr>
</tbody>
</table>
Based on hormone profile, cortisol is fluctuated (figure 1) by time to time, start from the lowest at 4 hours before transportation namely 66.23±33.8 ng/mL. Changes in gene expression due to glucocorticoids and catecholamines disregulate the immune system (Padgett and Glaser, 2003).

Table 2. Average±SD of ratio of Neutrophil/Lymphocyt before and after transportation. Symbol 0 indicate the timing of transportation.

<table>
<thead>
<tr>
<th>Period of Transportation</th>
<th>-8</th>
<th>-4</th>
<th>0</th>
<th>4</th>
<th>8</th>
<th>12</th>
<th>16</th>
<th>20</th>
<th>24</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ratio of N/L</td>
<td>0.87±0.40</td>
<td>0.85±0.30</td>
<td>0.74±0.51</td>
<td>1.43±1.13</td>
<td>1.67±0.57</td>
<td>1.43±0.92</td>
<td>1.12±0.33</td>
<td>1.4±0.68</td>
<td>0.79±0.38</td>
</tr>
</tbody>
</table>

In this study, number of lymphocyte (data not shown) was getting decrease and the lowest number has been occurred at the peak of ratio N/L namely 8 hours after transportation, probably that CD8+ was dominated than CD4+ lymphocytes. In acute stress of boars due to transportation, ACTH rose quickly in response to a 5-min nose snare stressor reaching a peak at 5 min after the application of stress, while the peak of cortisol levels was found 15 min later (Weaver et al., 2000).

The initial period of transport is the most stressful time for animals (Knowles et al., 1995; Broom et al., 1996). On the other hand, transportation of Bligon bucks for 16 hours were decreased level of tetraiodothyronine (T4). Decreasing of T4 levels indicated conversion of T4 to T3 to form active hormone (Astuti et al, 2009 in press). Many factors contribute to stress during transport and each species has a different stress response to transport (Manteca and de la Torre, 1996).

In this experiment, level of cortisol starting to rise at timing of transportation (0 hour) and reaching a peak at 4 hours after transportation (P< 0.05). It has been demonstrated that excessively high stocking density during transport, handling, leads to increased injury and stress (Tarrant, 1990; Cockram et al., 1996). Even many factors contribute to stress transportation, it would be predicted that handling is the most contributor to this experiment because of no high stocking density (is only 6 bucks in trucks), no heat stress (transportation was occurred evening to night) while increasing of cortisol level and ratio N/L has been done when the time of transportation and the time of arriving.

The pattern of cortisol and ratio of N/L is very similar. Furthermore, the peak of cortisol was got at 4 hours of transportation whereas ration N/L was reached later namely 8 hours after transportation. It would be concluded that ratio of N/L can be used as stress thinker for Bligon goat even less sensitive than level of cortisol.
Reference


Dot-Blot Hybridization, Based on Subunit Env-Tm Gene, as A Diagnostic Tool of Jembrana Disease Virus (JDV)

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ABSTRACT

Jembrana disease, an acute disease of Bali cattle (Bos javanicus), is caused by Jembrana disease virus (JDV), a bovine lentivirus. The availability of early, sensitive and convenient detection methods will prevent disease outbreaks, economic losses and allow routine controls of cattle health status. We considered that dot-blot hybridization is the most suitable diagnostic tool in veterinary medicine. The possible use of subunit env-tm gene as hybridization probe was tested. This gene has been cloned in pGEX-2T and a probe, 252 bp long, was prepared by PCR and DIG-labeled. From sequence comparison, it appeared that owing to its high degree of conservation among the Indonesian JDV isolates, the probe is suitable for the detection of all the isolates so far studied. Dot-blot hybridization was carried out on total RNA extracted from sampled spleen and blood of JDV-infected and non-infected Bali cattle. Results showed that the method allowed the detection of JDV in infected animals originating from different areas. Besides it allowed the detection of the virus in total RNA as low as 10 pg. Env-tm-based dot-blot hybridization constitutes therefore an efficient and specific diagnostic tool of Jembrana disease. Due to its ease of use, it is an ideal method in conditions where sophisticated equipments are not available.

Key words: Jembrana disease virus, probe env-tm gene, Dot-blot hybridization
Primary Structure of Mature SAG1 Gene of an Indonesian
Toxoplasma gondii Isolate and Comparison
with Other Strains or Isolates

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ABSTRACT

Toxoplasma gondii is a persistent protozoan parasite able to infect any warm-blooded vertebrates. SAG1 (p30) is the prototype member of a superfamily of surface antigens called S.R.S. (SAG1-related sequence). It constitutes the most abundant and predominant antigen. We investigated on the primary structure of mature SAG1 gene and the phylogenetic relationship with other strains or isolates of T. gondii. Sequence comparison indicated a high degree of conservation of SAG1 gene through evolution, despite parasite spreading world-wide. SAG1 nucleotide sequences may be divided into two groups, independently of the isolate/strain geographic origin. Variations were mainly localized at the C-terminal half or domain 2 and some clustered in restricted areas. Sequence comparison allowed us to define the Indonesian isolate as a genuine virulent RH strain. A phylogenetic tree was constructed based on SAG1. The relationship between sequence variations and their localization within the tridimensional structure of SAG1 protein as well as their implication in function will be discussed.

Key words: Phylogeny, SAG1, Sequence variations, Strain determination, Toxoplasma gondii.
Effect of Nicotine on The Motility and Viability of *Rattus norvegicus*’ Spermatozoa

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ABSTRACT

A research has been done to determine the effects of nicotine on the motility and viability of *Rattus Norvegicus*’ Spermatozoa. Twenty adult male rats were divided into 4 groups, with 5 rats in each group. Group I was given 1 ml of aquadest (oral administration) as a control. Group II, III, and IV were treatment groups, given with 0.2 mg/kg, 0.3 mg/kg, and 0.4 mg/kg of nicotine per day. All groups were treated for 21 days. Collection of semen was done on day 22, continued with analysis of spermatozoa’s motility and viability. The result showed that the average of motility’s score of spermatozoa that was produced by right testis on Group I to IV were 3, 1.6, 1.8 and 1.4 respectively. The average of motility’s score of spermatozoa that was produced by left testis on Group I to IV were 3, 1.6, 1.8 and 1.8 respectively. Spermatozoa’s viability of Group I to IV were 191, 170.4, 160.2, and 105 respectively. Statistical analysis was done upon those two parameters, giving significant differences between control and treatment groups. The main conclusion of the research was that treatment of 0.2 mg/kg or more of nicotine by oral administration decreased the rats’ spermatozoa’s motility and viability.

*Key words:* nicotine, spermatozoa, motility, viability