COMPARISON OF POLYPEPTIDE PROFILE OF Trypanosoma evansi ISOLATES FROM INDONESIA AND THEIR RELATION TO BIOTYPE AND SENSITIVITY TO TRYPANOCIDAL

Ichwan Yuniarto\(^1\)*, Didik T Subekti\(^2\), Umi Cahyaningsih\(^3\), and Fadjar Satrija\(^3\)

\(^1\)Veterinary Center of Banjarbaru, Banjarbaru, Indonesia
\(^2\)Indonesian Research Center of Veterinary Science, Bogor, Indonesia
\(^3\)Faculty of Veterinary Health, Bogor Agriculture Institute, Bogor, Indonesia

*Corresponding author: 99.ichwan@gmail.com

ABSTRACT

This study aimed to determine whether the variant or biotype of Trypanosoma evansi can be seen from their polypeptide profiles using 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS PAGE) stained with Brilliant Blue Comasie. The results generally showed that the molecular weight (MW) of polypeptides from nine isolates from East Java, Central Java, Banten, South Kalimantan, Central Kalimantan, and Lampung provinces were in the range of 85.46 to 15.76 kD and each isolate has different polypeptide profile. Isolates A13 and A14 were isolated from the same place but have different polypeptide profiles. Likewise, isolates S13 and S18 also have different polypeptide profiles despite being isolated from the same place at the same time. On the other hand, isolate 372, 87, and 06 have different protein profiles but was classified in the same biotype namely biotype I. Generally, the difference in protein profile actually more related to the biological diversity of the metabolism of each Trypanosoma evansi isolate from Indonesia.

Key words: biotype, protein profile, SDS PAGE, surra, Trypanosoma evansi

INTRODUCTION

Surra is a hemaprotozoan disease caused by a blood parasite, Trypanosoma evansi. This parasite is widely spread in Southeast Asia region, Africa, and America (Davison et al., 2000; Abdel-Rady, 2008; Ravindran et al., 2008). It can infiltrate into all cattle and wild animals, such as cow, buffalo, camel, horse, sheep, goat, dog, cat, elephant, coati, capybara, and marsupials (Stephen, 1986).

In 2010-2011, surra disease caused 1760 deaths (1159 horses, 600 buffaloes, and a cow) at Sumba Island, East Nusa Tenggara Province (Ditkeswan, 2012). There were 14 Surra cases in 2012, 25 cases in 2013, and 26 cases in 2015, which were found based on blood test according to surveillance result from Veterinary Office of Banjarbaru.

This disease cannot be optimally controlled because of the high variance in Trypanosoma evansi. The variation could be seen through their difference sensibility to trypanocidal as been stated by Macaraeg et al. (2013) and Subekti et al. (2015). They both stated that isolates from different region exhibit different sensibility to trypanocidal. On the other hand, Subekti (2014) also described that isolates taken from a single habitat at the same time could exhibit different response towards the same dosage of trypanocidal.

Parasitemia pattern in Trypanosoma evansi-infected mice also indicated the presence of variance. Subekti et al. (2013) reported 3 types of parasitemia pattern among mice infected by Trypanosoma evansi from Indonesia, namely biotype 1, 2, and 3. The presence of variance is also seen from the variation in antigenic profile and characteristic of Trypanosoma evansi protein isolate (Uche et al., 1992; Singh et al., 1995; Queiroz et al., 2001; Laha and Sasmal, 2008; Aquino et al., 2010). These differences show the variance in Trypanosoma evansi species.

Research on protein profile of Trypanosoma evansi is sparse compared to Trypanosoma brucei and Trypanosoma cruzi. Subekti et al. (2017) have analyzed the diversity of polypeptide banding patterns from various Trypanosoma evansi isolates from various regions in Indonesia. The study tends to analyze the clustering of Trypanosoma evansi isolates using Hierarchical Cluster Analysis. However, its relationship with some characters such as tripanocidal sensitivity and pathogenicity (biotype) has not been described clearly. This study aimed to describe the variation or biotype of Trypanosoma evansi through profile identification of isolate protein from regions with surra case in Indonesia. The results of this study can provide basic information and will be enriching knowledge about the protein profile of Trypanosoma evansi isolates from Indonesia.
MATERIALS AND METHODS

Trypanosoma evansi Isolate

This study used the isolates from various regions with surra cases in Indonesia, which were obtained from Research Center of Veterinary Science Bogor, Veterinary Office of Lampung and Veterinary Office of Banjarbaru. Nine *T. evansi* isolates (Table 1) were multiplied through intraperitoneal inoculation in mice. The parasitemia was observed daily by cutting small portion of mice vein at the edge of its tail and smeared on a slide prior to examined under microscope.

Soluble Trypanosoma Antigen (STrAg) Purification

The procedure used for purification of soluble trypanosoma antigen (STrAg) in *Trypanosoma evansi* was anion exchange chromatography, which was mutually developed by Research Center of Veterinary Science Bogor and Veterinary Office of Banjarbaru.

STrAg was collected from 9 isolates which were inoculated into mice with the parasitemia level reached 10<sup>3</sup>-10<sup>6</sup> trypanosome/mL blood. The mice were anestized using Ketamine (Ketalar<sup>®</sup>) at dose of 0.1 mg/kg body weight before collecting blood from its heart. The blood was then stored into 10% heparin tube and then purified with anion exchange chromatography using diethylaminoethyl anion exchange cellulose (DE52<sup>®</sup> Whatman) with phosphate-buffered saline glucose (PBSG) solution at pH 8. The suspension of DEAE cellulose with PBS glucose was inserted into 4-5 mL of polypropylene column. PBS glucose was subsequently added into polypropylene column and the filtrate was collected inside centrifuge tube and protease inhibitor (Aprotinin) was added. The tubes were centrifuged at 3500 rpm for 15 minutes. The supernatant was removed and re-suspended with PBS and Aprotinin. Freeze-thaw was conducted on the suspension until there was no complete cell form was found. Suspension was then centrifuged at 3500 rpm for 15 minutes, and then the supernatant was collected while the sediment was disposed. Supernatant were stored in the freezer at -20°C.

Protein Quantification

The protein concentration was measured using Bradford method (Bradford, 1976). Standard protein was made using bovine serum albumin (Sigma, USA) at concentrations of 0, 0.5, 0.75, 1, 1.25, and 1.50 mg/mL. 10 µL of STrAg and standard protein were collected and diluted in 190 µL of Bradford solution. After homogenization, 80 µL from standard and sample proteins (STrAg) were stored in microplates (flat bottom 96 well microplate, Nunc-Denmark) and read using enzyme-linked immunosorbent assay (ELISA) Reader (Multiskan EX Colorimeter Reader, Thermo Scientific-Finland) at 600 nm wavelength. Afterwards, the absorbance value was converted into protein level.

Protein Profile Identification

Identification of *Trypanosoma evansi* STrAg was conducted using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) technique (Laemmli, 1970). 10 µg of samples (STrAg) were mixed with buffer sample (Laemmli sample buffer + 2-mercaptoethanol, Biorad) at 1:1 ratio in microtubes. Samples were then homogenized with micropipette and then boiled at 65°C hot water for 5 minutes.

The samples and marker with molecule weight of 250-10 kD (Precision plus protein all blue standard, Biorad) were added into 12% Gel (Mini protein TGX stain-free precast gels, Biorad). Electrophoresis was then conducted at 200 volt and 300 mA for 35 minutes or until the blue color has completely dropped.

Determination of Protein Molecular Weight

The molecular weight was determined using retardation factor (Rf) from each bands with the below formula:

\[ Rf = \frac{\text{Distance of protein movement from the start point}}{\text{Gel length (distance of color movement from the start point)}} \]

The formula obtained could be in the form of linear regression, quadratic, or cubical and then used to calculate molecular weight of the samples by determining RF value of the sample (X) and the sample molecular weight (Y). The result was then inputted to the RF converter software.

Data Analysis

The results of protein profile identification using SDS PAGE methods were analyzed with descriptive statistical analysis to see the protein profile of *Trypanosoma evansi* isolates from regions with Surra cases in Indonesia.

### Table 1. *Trypanosoma evansi* from Surra case region in Indonesia

<table>
<thead>
<tr>
<th>No</th>
<th>Code</th>
<th>Origin</th>
<th>Animal</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>87&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Bangkalan Regency, East Java Province</td>
<td>Buffalo</td>
<td>1988</td>
</tr>
<tr>
<td>2</td>
<td>06&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Pekalongan Regency, Central Java Province</td>
<td>Buffalo</td>
<td>1996</td>
</tr>
<tr>
<td>3</td>
<td>372&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>East Sumba Regency, Province Nusa Tenggara Timur</td>
<td>Buffalo</td>
<td>2012</td>
</tr>
<tr>
<td>4</td>
<td>PLS&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>South Lampung Regency, Lampung Province</td>
<td>Buffalo</td>
<td>2013</td>
</tr>
<tr>
<td>5</td>
<td>A13&lt;sup&gt;c&lt;/sup&gt;</td>
<td>North Hulu Sungai Regency, South Kalimantan Province</td>
<td>Buffalo rawa</td>
<td>2013</td>
</tr>
<tr>
<td>6</td>
<td>A14&lt;sup&gt;c&lt;/sup&gt;</td>
<td>North Hulu Sungai Regency, South Kalimantan Province</td>
<td>Swamp buffalo</td>
<td>2014</td>
</tr>
<tr>
<td>7</td>
<td>S13&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>Serang Regency, Banten Province</td>
<td>Buffalo</td>
<td>2014</td>
</tr>
<tr>
<td>8</td>
<td>S18&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>Serang Regency, Banten Province</td>
<td>Buffalo</td>
<td>2014</td>
</tr>
<tr>
<td>9</td>
<td>SPT&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>East Kotawaringin Regency, Central Kalimantan Province</td>
<td>Buffalo</td>
<td>2014</td>
</tr>
</tbody>
</table>

Superscript showed the origin institution of the isolate = Indonesian Research Center of Veterinary Science Bogor, = Veterinary Office of Lampung, <sup>c</sup> = Veterinary Center of Banjarbaru
RESULTS AND DISCUSSION

In general, all *Trypanosoma evansi* isolates showed different protein profile. The differences were insufficient to conclude that the isolates belong to different species other than *Trypanosoma evansi*. The difference could be identified from the number of proteins (bands) identified and thickness of protein (polypeptide intensity) in each isolates. In our study, *Trypanosoma evansi* protein ranges between 85.46-15.76 kD with different number and distribution of molecular weight in each isolates. Three isolates from Kalimantan had different range of molecular weight, i.e. A13 isolate (55.83-16.09 kD) with 13 identified proteins, A14 isolate (71.92-15.76 kD) with 14 proteins, and SPT isolate (70.23-16.21 kD) with 14 proteins. There were S13 (85.46-16.04 kD) and S18 (83.14-16.34 kD) isolates from Banten, with 20 and 23 proteins respectively. The

![Image](image1.png)

**Figure 1.** Differences of protein profile and polypeptide intensity of STrAg A14, PLS, S13 and S18 which also had different respond toward trypanocidal

![Image](image2.png)

**Figure 2.** Differences of protein profile and polypeptide intensity of STrAg A13 and A14, STrAg S13 and S18 which isolated from the same region
PLS isolate (83.75-16.13 kD) from Lampung had 18 proteins and 372 isolate (84.6-16.23 kD) from East Nusa Tenggara had 17 proteins. Lastly, 2 isolates from Java, isolate 87 from East Java and isolate 06 from Central Java, had molecular weight range 74.29-16.12 kD and 59.88-16.27 kD, respectively and 12 and 11 proteins, respectively.

The 60-30 kD protein was identified from all isolates although each had different thickness of protein band. On the other hand, not all isolates had >60 kD and <30 kD protein, e.g. A13 isolate from South Kalimantan and 06 isolate from Central Java did not have >60 kD molecular weight (MW). The 18-17 kD protein was only identified in PLS isolate from Lampung and S18 from Banten. The 15 kD protein was only found in A14 isolate from South Kalimantan.

Our result was similar to Uche et al. (1992) who described the presence of protein profile difference in Trypanosoma evansi from Indonesia, Egypt, and Yemen with SDS PAGE methods. Singh et al. (1995) also reported a similar result, in which there was a difference in protein profile of cell membrane and flagella from 7 isolates from animals in various regions of India. However, Laha et al. (2008) showed that three isolates from buffalo, horse, and cattle did not show significant difference in protein profile.

The result of our protein profile difference was similar to Subekti et al. (2015), who analyzed A14, S13, S18, and PLS isolates' have different sensibility toward trypanocidal. Administration of melarsomine dehydrochloride at 0.25 mg/kg body weight on A14 isolate showed 80% relapse rate, while the relapse rate was only 25% for both S13 and S18, and 20% for PLS (Subekti et al., 2015). Relapse means the return of parasitemia in mice after treatment and deemed cured from previous observation. Subekti et al. (2015) also described that S13 isolates showed 100% cure with administration of diminazene diaceturate at 7 mg/kg body weight, while the S18 isolates did not. This indicates that A14, S13, S18, and PLS isolates have different protein profiles and different sensibility towards trypanocidal (Figure 1).

Macaraeg et al. (2013) also reported different sensibility toward trypanocidal. Luzon isolates needed diminazene at 5 mg/kg body weight to cure all mice while Visayas isolate needed 10 mg/kg body weight and

![Figure 3](image3.png)

**Figure 3.** Differences of protein profile and polypeptide intensity of STrAg 372, 87 and 06 which had same parasitemia pattern, the biotype 1

![Figure 4](image4.png)

**Figure 4.** Parasitemia pattern of biotype 1 (A), biotype 2 (B) with undulant parasitemia (inside the box), biotype 3 (C) (Subekti et al., 2013)
Mindanao isolate only needed 3 mg/kg body weight. Isolate from the same region could show difference in protein profile (Figure 2) such as STaAg A13 and A14 which came from North Hulu Sungai Regency, South Kalimantan, but were isolated at different time. A13 was isolated at 2013, while A14 at 2014. Similar condition also occurred in STaAg S13 and S18 which came from Serang Regency, Banten. Both were isolated at 2014. In Figure 3, we could see the distinction between STaAg A13 and A14, also STaAg S13 and S18. An arrow on STaAg A14 showed 71.92 kD and 19.95 kD MW protein which were identified in STAaG 14 but not in A13. The thick arrow showed a protein that is identified as major protein in STaAg A13 while it is identified as minor (thin) protein in STaAg A14. A similar condition is shown by arrow at 71.45 kD MW in STaAg 18 which is absent in S13. Arrow head also shows proteins identified as major (thick) protein in STaAg S13 that is identified as minor protein in STaAg S18, and vice versa.

Protein profile difference may not necessarily cause different biological patterns, such as in STaAg 372 from East Nusa Tenggara, STaAg 87 from East Java, and STaAg 06 from Central Java. Based on Subekti et al. (2013), isolates 372, 87, and 06 are classified as biotype 1 that are highly pathogenic to mice. Subekti et al. (2013) classified Trypanosoma evansi isolates from Indonesia into 3 groups based on parasitism pattern and its virulence speed. Biotype 1 is characterized by high parasitemia level (10²-10⁶/mL of blood) in a short time accompanied by mass death of mice in 2-4 hpi. Biotype 2 is characterized by undulant parasitemia which is biological regulation of Trypanosoma evansi in order to breed while keeping its host alive whereby they reduce population density in the bloodstream when their number is high and then increase when their number is low. Biotype 3 is characterized by high parasitism pattern that is maintained for a long duration without undulant parasitemia (Figure 4). That could be occurred because of similar specific protein in the three isolates.

The present result showed that the difference in protein profiles in the three Trypanosoma evansi isolates classified as biotype 1 is not related to their parasitism pattern and virulence. The virulency that causes the death of mice in a short period is more related to immune responses involving highly proinflammatory cytokines in the blood of mice. Sawitri et al. (2017) reported high levels of proinflammatory cytokines namely TNFα and IFNγ in mice infected with highly virulence Trypanosoma evansi isolate. Therefore the rapid death of mice is associated with high levels of TNFα and IFNγ in the blood. On the other hand, the difference in protein profile are thought to be related to differences of their response to trypanocidal. However, the difference in protein profiles is actually more related to the biological diversity of the metabolism of each Trypanosoma evansi isolate from Indonesia, even when the isolates were isolated from the same region and at the same time.

CONCLUSION

In this study, protein profiles from 9 isolates from different regions with surra cases in Indonesia were identified, namely STaAg A13 with 13 proteins and 55.83-16.09 kD MW, STaAg A14 with 14 proteins and 71.92-15.76 kD MW, STaAg SPT with 14 proteins and 70.23-16.21 kD MW, STaAg PLS with 18 proteins and 83.75-16.13 kD MW, STaAg 13 with 20 proteins and 85.46-16.04 kD MW, STaAg S18 with 23 proteins and 83.14-16.34 kD MW, isolate 372 from East Nusa Tenggara with 17 proteins and 84.6-16.23 kD MW, isolate 87 from East Java with 12 proteins and 74.29-16.12 kD MW and isolate 06 from Central Java with 11 proteins and 59.88-16.27 kD MW. The difference in protein profile actually more related to the biological diversity of the metabolism of each Trypanosoma evansi isolate from Indonesia than to be associated to biotypes or trypanosidal sensitivity.

REFERENCES


