

Trypanocidals Effectivity against Some Isolates of *Trypanosoma evansi* Propagated in Mice

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ABSTRAK

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Surra merupakan salah satu penyakit menular pada berbagai jenis hewan yang disebabkan oleh protozoa darah. Surra disebabkan oleh *Trypanosoma evansi* (*T. evansi*) dan seringkali berakibat fatal berupa kematian yang tinggi terutama pada kuda, kerbau dan unta. Salah satu pengendalian penyakit Surra dapat dilakukan dengan pengobatan menggunakan trypanosidal yang efektif. Efektifitas trypanosidal untuk Surra harus didasarkan pada hasil uji sensitivitas. Oleh sebab itu perlu diteliti efektivitas berbagai trypanosidal terhadap beberapa isolat *T. evansi* yang berasal dari beberapa daerah kasus maupun wabah Surra di Indonesia agar diketahui kesesuaian dan efikasinya. Metode pengujian dilakukan dengan desain *pre test-post test*. Mencit DDY diinfeksi dengan beberapa isolat *T. evansi* dari beberapa daerah kasus dan diamati parasitemianya. Setelah parasitemia mencapai puncaknya, maka mencit diobati dengan masing-masing trypanosidal dengan dosis bertingkat. Selanjutnya mencit diamati perubahan parasitemianya selama kurang lebih satu bulan. Hasil penelitian menunjukkan bahwa setiap isolat memiliki kepekaan yang berbeda terhadap trypanosidal sehingga penggunaan trypanosidal cenderung spesifik lokasi. Secara umum, suramin dan *melarsomine dihydrochloride* merupakan dua trypanosidal yang paling efektif untuk beberapa isolat di Indonesia. Adapun *isometamidium chloride* tidak direkomendasikan digunakan untuk pengobatan kasus surra di Indonesia.

Kata Kunci: *Trypanosoma evansi*, Trypanosidal, Parasitemia, Surra

ABSTRACT

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Surra is one of infectious diseases in various types of animals caused by blood parasites called *Trypanosoma evansi*. It is fatally occurred, especially in horse, buffalo and camel. Surra may be controlled by effectively trypanocidals treatment based on the results of its sensitivity test. Therefore, it is necessary to study the effectiveness of various trypanocidal against some *T. evansi* isolates originating from several regions in Indonesia with surra case to determine its suitability and efficacy. The test was carried out by pre-test - post-test. Mice were infected by several *T. evansi* isolates from various infected areas. Their parasitaemia were observed. After reaching peak of parasitemia, the mice were treated by trypanocidal with different doses. Parasitaemia alteration was observed for one month. Observation results showed that all isolates had different sensitivity to the trypanocidals, so that trypanocidals application tended to specific location. Generally, suramin and melarsomine dihydrochloride were the most effective trypanocidal for some Indonesian isolates. In contrast, isometamidium chloride was not recommended to be used for surra control in Indonesia.

Key Words: *Trypanosoma evansi*, Trypanocidal, Parasitemia, Surra

INTRODUCTION

Surra is one of infectious diseases in various species of animals caused by blood parasites called *Trypanosoma evansi* (*T. evansi*). Surra is transmitted by bloodsucking flies (haematophagous flies). Horse and buffalo are sensitive to surra and often leads to high mortality. This happens in Surra outbreak on the island of Sumba, East Nusa Tenggara province in 2010 until 2012. The outbreak resulted in 1159 horses, 600 buffaloes and cows have died (Dirkeswan 2012). Surra

still occur in some parts of Indonesia, such as Kalimantan (Borneo), Banten, Lampung, Aceh, and other territories.

One methods for controlling Surra can be done using trypanocidal. Currently, there were 2 approaches, using extract of some herbs and chemically synthetic of active ingredients. Studies using extract of herbs ingredients have been carried out both *in vitro* or *in vivo*. But so far, some herbs extract did not success as anti-*T. evansi* as reported by Abdelrahman (2011), Aman (2013) and Dorneles et al (2013). On the

contrary, Nzelibe et al. (2013) tested trypanocidal activity of *Azadirachta indica* seed extract (NSE) and leaves of *Tridax procumbens* (TP) which showed a success as trypanocidal without relapse when it was combined. Those extracts were not available commercially, and economically those extractions were impossible to be applied to cow, buffalo, and horse.

Therefore, surra treatment using herbs extract still could not be expected, so that must have to rely on chemically synthetic of active ingredient which have been used for 30 to 90 years (Subekti 2014). Active ingredients that have been used as drugs or trypanocidal are *suramin*, *melarsomine dihydrochloride*, *diminazene diacetate*, *quinapyramine* and *isometamidium chloride* (Steverding 2010; Melaku & Birasa 2013). Unfortunately, some trypanosome isolates have been reported to develop resistance to some of these trypanocidals from various countries (Melaku & Birasa 2013). Therefore for an effective treatment against Surra should be based on a sensitivity test to trypanocidal (Melaku & Birasa 2013). This is because some trypanocidal may not be appropriate for certain *T.evansi* isolates. Subekti (2014) states that *T.evansi* isolates originating from different regions have different sensitivity to trypanocidal. On the other hand, trypanocidal which is currently marketed in Indonesia is *isometamidium chloride* and *diminazene diacetate*. The effectiveness of the two drugs are not known against some *T.evansi* isolates in Indonesia that is attacking livestock. Information about the differences in the sensitivity of *T.evansi* isolates of Indonesia against various types of trypanocidal also very limited. Therefore it is important to investigate the effectiveness of various trypanocidal against some *T.evansi* isolates

originating from Indonesia, especially from the region of cases or outbreaks of Surra.

MATERIAL AND METHOD

Trypanosoma evansi isolate

T.evansi isolates used in this study were from Sumba Timur, province of East Nusa Tenggara; Serang, province of Banten; Hulu Sungai Utara, province of South Kalimantan; and Pesawaran, province of Lampung. All of isolates were propagated in mice before used for infection and treatment.

Infection of experimental animals, treatment, and parasitema observation

Female DDY strain mice were acclimated and weighed for body weight (BW) grouping. Mice were divided into 5 groups with 5 mice in each group (Table 1). Each mice were infected by 10^5 trypanosoma intraperitoneally (Sones et al. 1998). Treatment was carried out when the infected mice have reached 4+ of parasitemia or equivalent to 10^8 - 10^9 trypanosoma/mL of blood.

Treatment was carried out intraperitoneally using each drugs or trypanocidal and dose applied individually according to mice body weight (Table 1) as described by FAO (Uilenberg 1998). Drug used consisted of *melarsomine dihydrochloride*, *suramin*, *diminazene diacetate* and *isometamidium chloride*. Parasitemia observation was carried out every day for one week post-treatment and continuing every two days in the subsequent observation period up to one month.

Table 1. Experimental design of some trypanocidals against different *T.evansi* isolates

Isolate	<i>Melarsomine dihydrochloride</i> (mg/kg BB)		<i>Suramin</i> (mg/kg BB)		<i>Diminazene diacetate</i> (mg/kg BB)		<i>Isometamidium chloride</i> (mg/kg BB)	
	0.25	0.75	5	10	3.5	7	0.5	1
372	5	5	5	5	5	5	5	5
373	5	5	5	5	5	5	5	5
S13	5	5	5	5	5	5	5	5
S18	5	5	5	5	5	5	5	5
A14	5	5	5	5	5	5	5	5
PLS	5	5	5	5	5	5	5	5

372 = *T.evansi* isolate from Sumba Timur, province of East Nusa Tenggara (isolated in 2012)

373 = *T.evansi* isolate from Sumba Timur, province of East Nusa Tenggara (isolated in 2012)

S13 = *T.evansi* isolate from Serang, province of Banten (isolated in 2014)

S18 = *T.evansi* isolate from Serang, province of Banten (isolated in 2014)

PLS = *T.evansi* isolate from Pesawaran, province of Lampung (isolated in 2013)

A14 = *T.evansi* isolate from Hulu Sungai Utara, province of South Kalimantan (isolated in 2014)

Parasitemia observation was carried out with 3 techniques. First by native observation using a microscope. Negative results on the observation of native then followed by observation using MHCT (*Microhaematocrit centrifugation Technique*) and BCT (*Buffy Coat Technique*) as described in the OIE (2012). Mice were declared cured if result of blood test is not found Trypanosome with native examination, MHCT or BCT until the end of the experiment. Otherwise, if the one of those observations was positive, mice were stated sick and parasitemia positive.

RESULT AND DISCUSSION

Efficacy of several trypanocidals against several *T.evansi* isolates

Test result showed that anti-trypanosome drug (trypanocidal) had different efficacy to some isolates of *T.evansi* from several regions in Indonesia. Treatment with *melarsomine dihydrochloride* in mice infected with *T.evansi* isolates 372 and 373 have been declared cured at a dose 0.25 and 0.75 mg/kg BW (Figure 1.A). Treatment with *melarsomine dihydrochloride* at a dose of 0.75 mg/kg BW, also provides a satisfactory recovery (100% cured) of the mice infected with *T.evansi* isolate S13, S18 and PLS (Figure 1A). These results are similar to previous studies in mice infected with the 371 and 375 isolates originating from Sumba Timur. In mice that have been infected with the isolate 375, *melarsomine dihydrochloride* can cure mice at a dose of 0.25 and 0.75 mg/kg BW (unpublished data). In contrast, mice that have been infected with the isolate 371, only cured by *melarsomine dihydrochloride* at a dose 0.75 mg/kg BW (unpublished data). The mice infected with the PLS isolate from Lampung only 80% is recovered at a dose of 0.25 mg/kg BW.

Suramin effectively used for most isolates of *T.evansi* being tested. *Suramin* at a dose 5 and 10 mg/kg BW can cure 100% of mice infected with the isolates 372, 373, S13, PLS and A14. *Suramin* at a dose 10 mg/kg BW cures only 80% of mice infected with the S18 isolate (Figure 1.B). In mice that had been infected with the isolates 375 and 371, *suramin* give 75% recovery at doses of 5 and 10 mg/kg BW (unpublished data).

On the other hand, *diminazene diacetate* and *isometamidium chloride* which are widely marketed in Indonesia did not show satisfactory effectiveness compared with *melarsomine dihydrochloride* and *suramin*. *Diminazene diacetate* only effective for mice infected with the PLS isolate at doses of 3.5 and 7 mg/kg BW. In contrast, mice infected with the 372 and S13 isolates only recovered when treated with

diminazene diacetate at dose of 7 mg/kg BW (Figure 1.C). Conversely, all of mice infected by the sixth *T.evansi* isolates did not cured when treated by *isometamidium chloride*. Generally, mice still showed high parasitemia after treatment. This fact provides evidence that the six *T.evansi* isolates being tested are resistant to treatment with *isometamidium chloride* up to a dose 1 mg/kg BW.

Resistance to *isometamidium chloride* has been widely documented. Among them are reports of Macaraeg et al (2013) which states that a cure can be achieved when using *isometamidium chloride* at a dose 10 mg/kg BW in *T.evansi* isolates from the island of Luzon, and Visayas, Philippines. The dose is 10 times higher than recommended dose as used in this experiment (Subekti 2014). However, Homeida et al. (1980) reported that mice infected with *T.evansi* coming from the East Sudan at a dose of 10⁵ per mice and treated with 10 mg/kg BW of *isometamidium chloride* only partially cured (approximately 40% recovery). Two mice were cured through observations of more than 30 days, 2 mice showed a relapse (40%) and 1 mice died (20%). These results suggest that the usage *isometamidium chloride* treatment more than 1 mg/kg BW also does not guarantee a cure. Jatau et al (2010) reported that treatment with *isometamidium chloride* at a dose of 0.5 mg/kg BW failed to cure mice infected with *T.evansi*. Conversely at a dose 1 mg/kg BW given 5 days post-infection only provide temporary relief followed by relapse at all experimental animals on the eleventh day after the treatment, otherwise if treated at 8 and 11 days post-infection fails to provide relief (Jatau et al., 2010). This condition is likely due to high levels of parasitaemia so the mice die.

Generally, trypanocidal test using *isometamidium chloride* against *T.evansi* isolate from Indonesia showed the same result with Sudan isolate (Homeida et al. 1980) or Luzon and Mindanao Island isolates (Macaraeg et al. 2013) and Jatau et al. (2010). In this study did not use *isometamidium chloride* more than 1 mg/kg BW according to some consideration. First, *isometamidium chloride* is an *ethidium bromide* derivate and has long withdrawal time. Secondly, there is no guarantee of cure by providing higher doses. There is obstacle in its field extrapolation due to its expensive price. One gram/sachet of trypanocidal with active ingredient of *isometamidium chloride* in Indonesia is IDR 250,000. Treatment at a dose of 10 mg/kg BW on large animals with a body weight about 300 kg, needs about 3 grams per head. That means, an animal with a weight of 300 kg takes about 3 sachets so that the cost of drugs per head is IDR 750,000. That cost does not include other cost. It is very expensive and certainly not affordable for farmer in the most Indonesian regions.

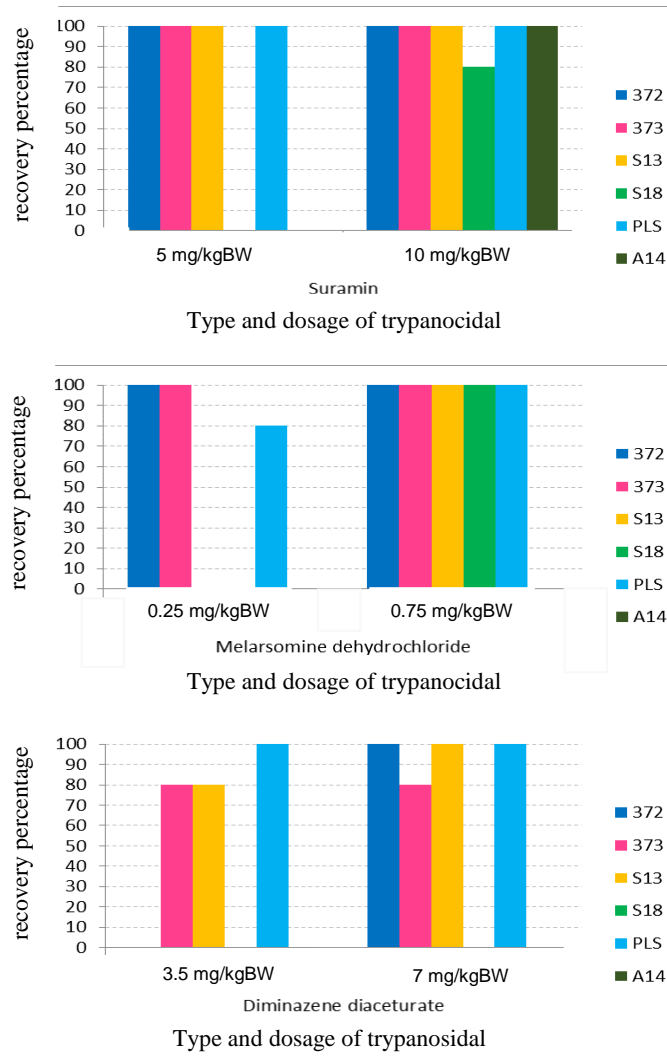


Figure 1. The recovery rate of 80% and 100% of mice infected with several isolates of *T. evansi* and treated by several trypanocidal (A) *Melarsomine dihydrochloride*; (B) *Suramin*; (C) *Diminazene diaceturate*
 372 = *T. evansi* isolate from East Sumba, East Nusa Tenggara (isolated in 2012)
 373 = *T. evansi* isolate from Sumba Timur, East Nusa Tenggara (isolated in 2012)
 S13 = *T. evansi* isolate from Serang, Banten (isolated in 2014)
 S18 = *T. evansi* isolate from Serang, Banten (isolated in 2014)
 PLS = *T. evansi* isolate from Pesawaran, Lampung (isolated in 2013)
 A14 = *T. evansi* isolate from Hulu Sungai Utara, South Kalimantan (isolated in 2014)

The phenomenon of relapses in trypanocidal sensitivity test

T. evansi categorized as sensitive to trypanocidal if the infected animal were healed 100% until the end of the experiment. Therefore, trypanocidal at the dose considered to have excellent efficacy. Sometimes can occur in some animal in the experimental group that showed a relapse. Relapse is the occurrence of parasitaemia in mice that have been treated and declared cured on previous observations. The parasitaemia will remain occur until the end of the observation during the experiment or animals die after

relapse. The animals were kept showed parasitaemia after treatment with trypanocidal, then the drug considered ineffective and isolates are not sensitive due to the their resistance to the drug. Resistance can occur at certain dose levels or at all dose levels.

The phenomenon of relapse may be linked to several possibilities. First, the drug levels in the blood are not sufficient to kill all parasites. Thus most of the parasites can not come into direct contact with the drug. This possibility is indicated by the presence of most of the animals who relapse within a short time and then disappear again until the end of the experiment. Elimination of parasites after relapse may be caused by

contact with drugs that are still high levels in the blood while the parasites who relapse are very few.

The second possibility is the existence of sub-populations or clones that are resistant. Sub-population was minor population, so that the sub-population will take time to reproduce until it appears as parasitaemia. Indication of the existence of sub-populations of *T.evansi* in one isolate was reported by Subekti et al. (2013) and De-Menezes et al. (2004). In this type, the parasite that relapses generally will not decline again until the end of the experiment. If the therapeutic dose was increased, the parasite will disappear or remain relapse. This evidence indicates that the sub-population who relapse at higher doses is Trypanosome population that has a stronger resistance to the drug.

Relapse on treatment using *Melarsomine dihydrochloride*

The experimental results demonstrate that treatment with *melarsomine dihydrochloride* shows there are some isolates *T.evansi* who relapse (Figure 2A). Isolate experiencing highest relapse was A14 from Hulu Sungai Utara, South Kalimantan. In isolate A14, 80% was relapse at dose 0.25 mg/kg BW and 50% was relapse at dose 0.75 mg/kg BW. This result indicated that higher dose was needed for isolate A14 treatment.

Isolate S13 and S18 (from Serang, Banten) relapsed by 25% at dose 0.25 mg/kg BW. Similarly, isolate PLS (from Pesawaran, Lampung) relapsed by 20% at dose 0.25 mg/kg BW. *Melarsomine dihydrochloride* at a dose of 0.75 mg/kg BW effective for treatment in 83.3% of *T.evansi* isolates that have been tested, and even provide excellent efficacy to the 372 and 373 isolates from Sumba Timur, as it only requires a dose of 0.25 mg/kg BW.

Relapse in treatment using *melarsomine dihydrochloride* was also reported by Akbar et al. (1998) in Pakistan who infected *T.evansi* into camel. Result showed that *melarsomine dihydrochloride* at dose 0.25 mg/kg BW had cure rate by 66.66%. However, Kabi et al. (2009) reported that there was relapse in isolate *T.evansi* from Uganda in treatment using *melarsomine dihydrochloride* at dose 0.125 mg/kg BW. It also showed that 100 % mice died on 18th day infection. However, at dose 0.25 to 1 mg/kg BW, all of mice was cured (Kabi et al. 2009). Two isolates from Sumba Timur (isolate 372 and 373) had similar sensitivity with *T.evansi* isolate from Uganda used by Kabi et al. (2009) which was cure with *melarsomine dihydrochloride* at dose 0.25 mg/kg BW. The other fourth Indonesian isolates (isolate S13, S18, A14 and PLS) require *melarsomine dihydrochloride* with higher doses than two isolates of Uganda. This is due to recovery of 100% is only achieved when using doses ≥ 0.75 mg/kg BW.

Relapse on treatment using *Suramin*

On treatment with *suramin*, *T.evansi* isolates who relapse less than those treated using *melarsomine dihydrochloride* (Figure 2B). These results provide evidence that the sixth isolates of *T.evansi* that have been tested have better sensitivity to *suramin* compared to *melarsomine dihydrochloride*. Generally, the use of *suramin* at a dose 10 mg/kg BW was effective for the treatment of 83.33% *T.evansi* isolates tested. *Suramin* even provides excellent efficacy in 66.67% of isolates were tested, namely 372, 373, S13 and PLS as it only requires a dose of 5 mg/kg BW. Isolates experiencing relapse were isolate S18 and A14. Isolate A14 was 100% relapsed in treatment using *suramin* at dose 5 mg/kg BW but had no relapse at dose 10 mg/kg BW. In isolate S18, 20% animal treated by *suramin* relapsed at dose 5 mg/kg BW or 10 mg/kg BW. This indicates the possibility of resistance to *suramin* in these isolates.

In Indonesia, some *T.evansi* isolates were resistant to *suramin* at dose 10 mg/kg BW (Payne et al. 1994). Zhou et al. (2004) also reported *T.evansi* isolates from China that were resistant to *suramin* and did not recover at a dose 10 mg/kg BW. Gillingwater et al. (2007) also reported that the STIB 780 and 781 isolate from Kenya were resistant to *suramin*. Korir et al. (2013) said that isolate EATRO 1886 from Busoga, Uganda (*Trypanosoma brucei rhodesiense*) also resistant to *suramin* at 2.5 mg/kg BW. Such evidences showed that resistance to *suramin* was a natural phenomenon.

Relapse on treatment using *Diminazene diacetate*

Isolates who relapse after treatment using *diminazene diacetate* were more higher than *suramin* or *melarsomine dihydrochloride*. At dose 3.5 mg/kg BW, there were 5 isolates relapsed (Figure 2C). Isolate 372 and S13 relapsed by 60% and 20% respectively. Isolate A14, 373, and S18 relapsed at dose 3.5 mg/kg BW and 7 mg/kg BW. These results indicate there were sub-populations that are resistant to *diminazene diacetate* in stock population of A14, 373 and S18 isolates. Therefore, *diminazene diacetate* only slightly effective at a dose 7 mg/kg BW (33.33% isolates were recovered), while at a dose of 3.5 mg/kg BW only 16.67% of isolates were recovered.

In Indonesia, *diminazene diacetate* was given at a dose 3.5 mg/kg BW. Applications of *diminazene diacetate* with a dose 3.5 mg kg BW for Surra was wrong because the dose is too low despite frequent use in the field (Desquesnes et al. 2013). Although the clinical symptoms disappear but actually a small number of parasites in the blood are still alive and will multiply and cause a relapse in animals (Desquesnes et al. 2013; Gutiérrez et al. 2013). In the event of trypanosomosis caused by *T.b.brucei*, *T.congolense* and

T. vivax, *diminazene diaceturate* was recommended at a dose 3.5 to 7 mg/kg BW intramuscularly (Gutiérrez et al. 2013; Desquesnes et al. 2013). Conversely, in the case of surra caused by *T. evansi*, the recommended therapeutic dose was 7 mg/kg BW intramuscularly (Gutiérrez et al. 2013; Desquesnes et al. 2013). Therefore, in Indonesia, when *diminazene diaceturate* applied only once treatment will lead to relapse and the case will re-emerge. However, when given twice administration making the accumulative dose being 7 mg/kg BW, is expected to reduce the possibility of relapse even though most of the animals are likely to remain relapse because their resistance to *diminazene diaceturate*.

Akbar et al. (1998) reported that *diminazene diaceturate* at dose 3.5 mg/kg BW cured by 66.66% of infected camels and the rest was relapse and dead. Similar with this study, mice infected with S18 isolates had relapse on 8-10th day post-infection after being treated with *diminazene diaceturate* at a dose of 3.5 mg/kg BW and died at 15-16th day post-infection. Conversely at dose 7 mg/kg BW, there was relapse without followed by death until the end of study. As well as in 373 isolate who showed relapse without followed by death until the end of the study. In contrast to the isolates A14 who relapse after treatment with a dose 3.5 mg/kg BW and followed by death, while at a dose 7 mg/kg BW, 20% relapse and die and the rest (80%) only relapse.

Mohammed (2008) has also been infecting *T. evansi* isolates from Saudi Arabia in Swiss-Webster mice and treated with *diminazene diaceturate* at a dose 3.5 mg/kg BW. The experimental results showed that 60% of mice cured while the rest were died without relapse. These results also indicate that the isolates from Saudi Arabia had a sub-population were resistant to *diminazene diaceturate*. Therefore, 40% of the infected animal does not occur parasitaemia reduction and resulting in death. Qadeer et al. (2015) reported that the goats were infected with *T. evansi* and treated with *diminazene diaceturate* at a dose 3.5 and 7 mg/kg BW have relapse (100%), while the recovery without relapse only occurred at a doses 10.5 mg/kg BW.

Kabi et al. (2009) reported that Swiss Webster mice that have been infected with *T. evansi* isolates from Uganda and treated with *diminazene diaceturate* (dose 1.75 to 14 mg/kg) were all dead on 18th day post-infection. These results correspond with the results of Zhang et al that proves the failure of *diminazene diaceturate* to kill *T. evansi* isolate from China, the Philippines and Ethiopia, both *in vitro* and *in vivo* (Kabi et al. 2009). These results indicate that the *T. evansi* isolates from Uganda used by Kabi et al. (2009) had higher resistance than Saudi Arabia isolate used by

Mohammed (2008), Pakistan isolate used by Akbar et al. (1998), and six isolates from Indonesia were used in this study.

Macaraeg et al. (2013) also have infected mice with *T. evansi* isolates of the island of Luzon, Visayas and Mindanao. Luzon isolates require *diminazene diaceturate* with a therapeutic dose of 5 mg/kg BW to cure mice (100%) and failed to cure at lower doses. Visayas isolate require *diminazene diaceturate* at a dose 10 mg/kg BW to achieve 100% recovery, while at doses of 5, 3 and 1 mg/kg BW the recovery rates were 80%, 60% and 0% respectively. *Diminazene diaceturate* at a dose 3 mg/kg was able to cure (100%) mice infected with Mindanao isolates, while at a dose 1 mg/kg BW only cured 20% experimental animal. However, in experiments conducted by Macaraeg et al. (2013) does not provide information whether there is any incidence of relapse.

The results showed that isolate *T. evansi* from different island in Philippine had different sensitivity associated with therapeutic doses are used. *T. evansi* isolates from Luzon and Mindanao were sensitive to *diminazene diaceturate* at dose 5 and 3 mg/kg BW respectively. Visayas isolate require dose above 7 mg/kg BW. The similar result was obtained in experiments using six isolates from Indonesia. Isolates 372 and S13 only recovered 100% when using *diminazene diaceturate* at a dose 7 mg/kg BW. However, four other isolates remained unrecovered at a dose of 7 mg/kg BW and even the S18 and A14 isolates showed relapses (100%).

Determination of effective Trypanocidal for Surra Disease in Indonesia

Based on these results, it was difficult to determine the most appropriate trypanocidal used for the treatment of Surra in all regions of Indonesia. This is due to the diversity of the effectiveness of some trypanocidals on different *T. evansi* isolates. Evidence from this study indicates that each isolate has a different sensitivity to the diverse trypanocidal. Those differences require interpretation which leads to a specific location for the use of trypanocidal (Table 2). *T. evansi* originating from different areas will give different responses to the type and dosage of trypanocidal being used. Example from this study, the effective trypanocidal against A14 isolate is *suramin* at a dose 10 mg/kg BW. Instead, the most effective trypanocidal against 372 and 374 isolates are *melarsomine dihydrochloride* at a dose 0.25 to 0.75 mg/kg BW, *suramin* at a dose 5 to 10 mg/kg BW and *diminazene diaceturate* at 7 mg/kg BW. Similar conditions also apply to isolates from other areas also demonstrates different sensitivities.

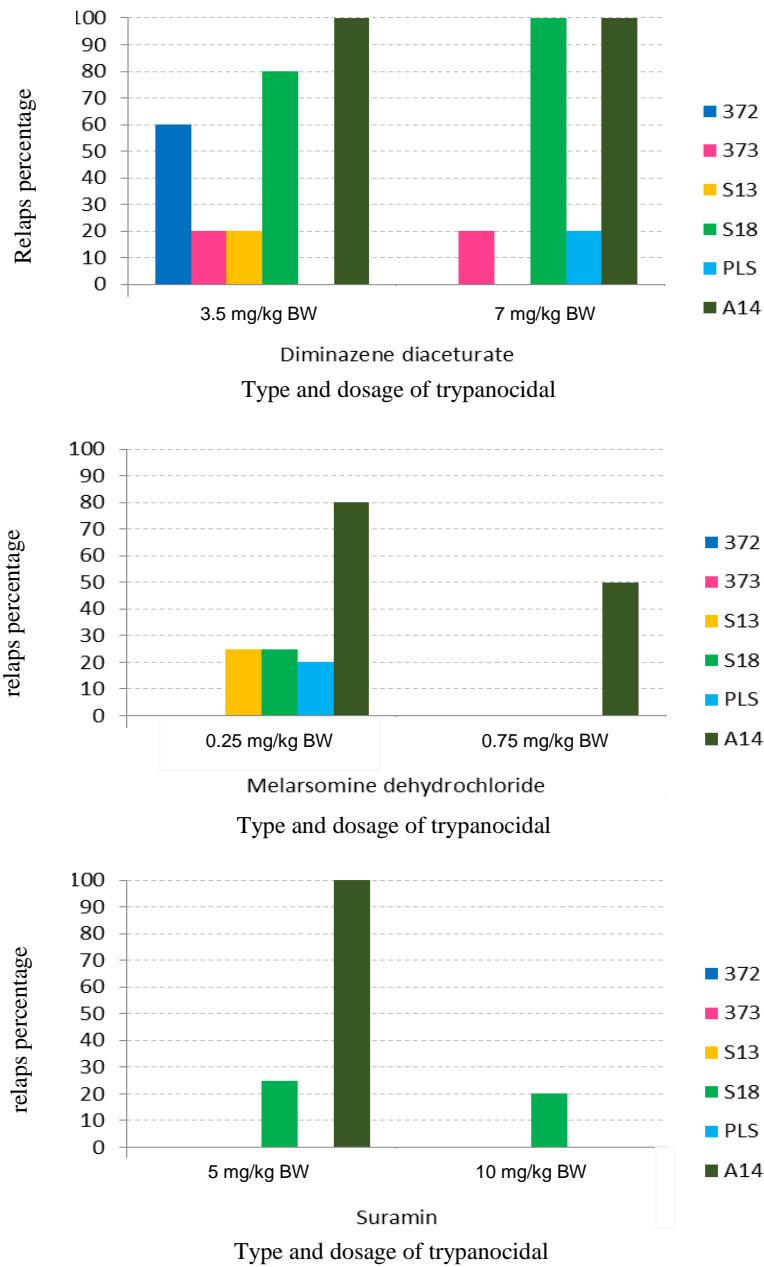


Figure 2. Percentage of relapse in treated mice by several trypanocidal. (A) *Melarsomine dihydrochloride*; (B) *Suramin*; (C) *Diminazene diaceturate*
 372 = *T.evansi* isolate from Sumba Timur, East Nusa Tenggara (isolated in 2012)
 373 = *T.evansi* isolate from Sumba Timur, East Nusa Tenggara (isolated in 2012)
 S13 = *T.evansi* isolate from Serang, Banten (isolated in 2014)
 S18 = *T.evansi* isolate from Serang, Banten (isolated in 2014)
 PLS = *T.evansi* isolate from Pesawaran, Lampung (isolated in 2013)
 A14 = *T.evansi* isolate from Hulu Sungai Utara, South Kalimantan (isolated in 2014)

Table 2. Summary of the most suitable selected trypanocidal against *T. evansi* isolate from infected area in Indonesia

Isolate	Effective drug as Trypanocidal		
	The Drugs of Choice	The 2nd Choice	Not Recommended
372	<i>Melarsomine dihydrochloride, Suramin</i>	<i>Diminazene diacetate</i> 7 mg/kg BW (100%)	<i>Isometamidium chloride</i>
373	<i>Melarsomine dihydrochloride, Suramin</i>	<i>Diminazene diacetate</i> (80%)	<i>Isometamidium chloride</i>
S13	-	<i>Melarsomine dihydrochloride, Suramin, Diminazene diacetate</i>	<i>Isometamidium chloride</i>
S18	-	<i>Melarsomine dihydrochloride, Suramin</i>	<i>Diminazene diacetate, Isometamidium chloride</i>
A14	-	<i>Suramin</i> 10 mg/kg BW	<i>Suramin</i> 5 mg/kg BW, <i>Melarsomine dihydrochloride, Diminazene diacetate, Isometamidium chloride</i>
PLS	<i>Suramin</i>	<i>Melarsomine dihydrochloride</i> (80-100%), <i>Diminazene diacetate</i> (80-100%)	<i>Isometamidium chloride</i>

Table 3. Summary of trypanocidal efficacy criteria against *T. evansi* isolate from infected area in Indonesia

Trypanocidal	Dose	372	373	S13	S18	A14	PLS
<i>Melarsomine dihydrochloride</i>	0.25 mg/kg BW	Effective	Effective	Ineffective	Effective	Ineffective	Effective
	0.75 mg/kg BW	Effective	Effective	Effective	Effective	Ineffective	Effective
<i>Suramin</i>	5 mg/kg BW	Effective	Effective	Effective	Effective	Ineffective	Effective
	10 mg/kg BW	Effective	Effective	Effective	Effective	Effective	Effective
<i>Diminazene diacetate</i>	3.5 mg/kg BW	Ineffective	Effective	Effective	Ineffective	NA	Effective
	7 mg/kg BW	Effective	Effective	Effective	Ineffective	Ineffective	Effective
<i>Isometamidium chloride</i>	0.5 mg/kg BW	Ineffective	Ineffective	Ineffective	Ineffective	Ineffective	Ineffective
	1 mg/kg BW	Ineffective	Ineffective	Ineffective	Ineffective	Ineffective	Ineffective

NA = not analyzed, it was not analyzed due to the number of live animal was less than 3 heads

The results of this study indicate that the use of trypanocidal will be divided into three conditions. The first condition is a drug that was not be recommended due to ineffective for infected experimental animals. The second condition is a drug which was only effective in large doses and in effective in small doses. This kind of trypanocidal may still be used regularly followed by an increase in dose and must be accompanied by supervision of the possibility of intoxication. The last condition is drug which was effective used at small doses. Trypanocidal in this category was recommended as the drug of choice for treatment that can be applied effectively.

Nevertheless, drug categories above are only suitable for the treatment of cases of Surra on individual animals and specific locations at the field level. Conversely, when directed as a reference for national policies for the procurement of drugs with the aim of subsidizing procurement of drugs for for a variety of cases in various regions in Indonesia would lead to difficulties in setting priorities for procurement. Therefore, it needs to set priorities by considering the cumulative effectiveness based on the results of the drug test.

Eisler et al. (2001) suggest that a trypanocidal declared effective if the experiment proved $\geq 80\%$ of experimental animals have been recovered. Referring to this provision, it can be arranged a general idea of the effectiveness of some trypanocidal against *T.evansi* isolates from Indonesia that have been tested. Generally, if using criteria of Eisler et al. (2001), *suramin* was effective on all isolates, except on A14 isolate which needed 10 mg/kg BW (Table 3). *Melarsomine dihydrochloride* at dose 0.75 mg/kg BW was effective on 5 isolates (83.33%), except on A14 isolate. *Melarsomine dihydrochloride* at a dose 0.25 mg/kg BW was only effective on 4 isolates (66.7%), except for S13 and A14. *Diminazene diacetate* declared effective on 4 of 6 isolates (66.67%) were tested when used at dose 7 mg/kg BW and only effective in 3 of 5 isolates (60%) when used at dose 3.5 mg/kg BW. *Isometamidium chloride* was known as a drug that is not effective in all isolates from Indonesia so it is not recommended for use. Therefore, based on these results was known that in general, the order of priority of procurement trypanocidal is *suramin* and *melarsomine dihydrochloride* followed by *diminazene diacetate* as the last option.

CONCLUSION

Every isolates have different sensitivities to trypanocidal, hence the use of trypanocidal tend to be specific locations. In general, *suramin* and *melarsomine dihydrochloride* is most effective for some *T.evansi* isolates from Indonesia. The *isometamidium chloride*

was not recommended to use for the treatment of Surra in Indonesia.

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