Application of Dried Blood Sample on Whatman Filter Paper for Detection of *Trypanosoma evansi* from Cattle in Central Kalimantan by Internal Trascriber Spacer-1 Polymerase Chain Reaction

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ABSTRACT

*Trypanosoma evansi* is a hemoflagella parasite that infects cattle as agents of Surra. The disease causes great economic losses due to decrease in production and death. Rapid and accurate method for early detection is necessary so that the appropriate control can be implemented. In recent years, PCR has been widely applied for detection of *Trypanosoma* with higher sensitivity. *Trypanosoma*-species-specific-DNA-sequences multi copy can be used as a target parasite identification. Internal transcribed spacer regions 1 (ITS-1) is a useful marker for discriminating trypanosomes species. The aims of this study was to detect *T. evansi* in cattle from Central Kalimantan using ITS-1 PCR. A total of 44 bovine blood samples were collected using Whatman filter paper from Lamandau District of Central Kalimantan Province. PCR was carried out using ITS-1 primers on DNA template that was isolated from dried blood. The results showed that 27 out of 44 samples (61.4%) were positive for *T. evansi*. Lamandau District was suspected to be endemic area of Surra and therefore requires a special attention.

Key Words: *Trypanosoma evansi*, Internal Transcriber Spacer-1, PCR

INTRODUCTION

*Trypanosoma evansi*, a flagellated kinetoplastid haemoproteozoa, is the most prevalent pathogenic trypanosome in Asia, Africa, South, and Central America (Laha & Sasmal 2008; Desquesnes et al. 2009). It causes a major problem and known as ‘Surra’. The parasite infects a wide range of domestic and wild animals owing to its ability to be mechanically transmitted by biting flies such as *Tabanus* and *Stomoxys* (Luckins 1988; Desquesnes et al. 2009). Several cases of human trypanosomiasis by *T. evansi* have been reported recently, and therefore it is a new emerging disease in human. In Indonesia, a severe Surra outbreak occurred in Sumba in 2010-2012 causing the deaths of more than 2,000 head of livestock (Ditjenak 2012). In 2011 outbreaks of Surra in Dharmasraya districts of West Sumatra Province were also reported (Diseases Investigation Centre Bukittingi 2014). In addition, Surra cases in South and East Kalimantan were increasing from 2011-2013 but there was a few Surra case in Central Kalimantan (Disease Investigation Centre Banjarbaru 2014).

Effects of the infection in different geographical locations vary according to the strain of parasites and species of the host (Desquesnes et al. 2013a). Clinical signs are variable and non-specific (Desquesnes et al. 2013b). In Indonesia, buffalo is apparently more susceptible than bovine (Payne et al. 1991). The disease may manifest as an acute or chronic form, and in the latter case could persist for several months, possibly years. Acutely infection in cattle or buffalo usually end in death, whereas the chronic form result in unproductive animas (Partoutomo 1996). The disease in bovines is characterized by severe anaemia, nervous complications, weight loss, reduced productivity, infertility and abortion and death in some animals during the early phase of the disease (Juyal 2002). The
The present study was designed to investigate the case of Surra in cattle at the District of Lamandau of Central Kalimantan province by using ITS-1 PCR.
MATERIAL AND METHODS

Sample collection

Forty four blood samples were collected from cattle at Lamandau District of Central Kalimantan Province. Blood was collected from the jugular vein from each cattle using 5 ml disposable syringe for blood spotted on Whatman filter paper and the rest was placed in 5 ml plastic tube for other analysis.

Blood spotted on Whatman filter paper

One drop of fresh blood (5 μl) was placed into the Whatman filter paper no. 1 (Whatman Ltd, England) and was allowed to dry in the shade. Dried paper were later put separately into a tightly closed small plastics bag individually and were transported to the laboratory and then kept at refrigerator until DNA extraction was done.

DNA extraction

Extracted genomic DNA of 44 samples were conducted by using Genomic DNA Mini Kit (Geneaid). A piece of 5×5 mm dried blood in Whatman filter paper was placed into 1.5 ml eppendorf tube. The paper was incubate with 300 ml lysis buffer at 60°C for 10 minutes. After blood dissolved, paper was taken and continue to the next step DNA extraction as manufactured guidance. Genomic DNA was stored at -20 until use.

PCR amplification

PCR amplifies the ITS1 region of the rDNA gene by using forward primers ITS1F:5’CCGGAAGTTACCGATATTG’3’ and reverse primer ITS1R:5’TGCTGCTTTCAACAGAA’3’. The 480 bp PCR product was amplifed using KAPA 2G Fast PCR kit (KAPA) in a 25 μl total volume. Each reaction included 5 μl (5 unit/μl) 2 G Fast Master Mix, 0.1 (5 unit/μl) KAPA 2G Fast DNA polymerase, 0.5 μl (10 μM/dNTP) dNTP mix; 2 μl (10 mM) of each primer, 2 μl (50ng/μl) extracted DNA; 17.44 μl RNase-free water. Thermocycling profile started with initial hold for 3 min at 95°C, followed by 35 cycles of 95°C for 10 sec, 58°C for 10 sec, and 72°C for 15 sec and final extension step of 10 min at 72°C. Five microlitres of PCR products of approximately 470 bp in size was analysed by using 1.5% agarose gel electrophoresis in (Tris-acetate EDTA) buffer and stained using fluorosafe (Geneaid), before being visualized under UV transilluminator.

RESULTS AND DISCUSSION

PCR amplification was carried out on targeting 470 bp ITS-1 fragmen of 44 cattle blood collected from Lamandau District of Central Kalimantan Province (Figure 1). The result indicated that 33 of 44 (61.4%) samples were positive for ITS-1. These result showed high case Surra in that district.

In the survey area we didn’t find any clinical sign of Surra and no case of animal death. The absence of pathognomonic clinical signs, with the evasive nature of T. evansi, especially in chronic infections, make detection of surra in cattle very challenging (Nantulya 1990; Ngaira et al. 2003). The disease pathology varies with the host, chronic and latent disease occure in ruminants (Kundu et al. 2013).
According to the farmers, the cattle have been raising in the area implementing the integration cow-palm oil program. Surra has not been reported in Lamandau District. Nevertheless this district is very closed to West Kotawaringin District which is endemic Surra area. Distribution of Surra disease is influenced by local topography and livestock management (Mastra 2011). Surra has multiple and complex means of transmission, which vary in terms of relative significance depending on the hosts and the geographical area. Indeed, *T. evansi* is transmitted in several ways, via biting insects, sucking insects, and vampire bats, transmission can also be vertical, horizontal, iatrogenic, and per-oral with various epidemiological significances, depending on the season, the location, and host species (Desquesnes et al. 2013a).

In this area, livestock are kept in a certain pasture near oil palm plantations. There is no contact between cattle in this location with other cattle from different locations. Suspected cattle have been infected from their place of origin. According to the farmer’s information cattle derived from Kotawaringin Barat (Central Kalimantan) and Malang (East Java), so far West Kotawaringin known as Surra endemic (DIC Banjarbaru, unpublised). The existence of hematofagus flies like *Tabanus* and *Stomoxys* sp in the area might to spread Surra. As many cases remain undetected and untreated even after the completion of parasitological investigations. This situation is disappointing for the infected individual as well as the other animals in the herd. These individuals might remain being the reservoir of the infection and contribute to further distribution of the disease in the animal population.

However, because the cattle were on good condition, their immunity did not show any clinical symptoms. According Desquesnes et al. (2013) Trypanosomosis due to *T. evansi* has long been considered of the cattle, they as a mild, chronic, or asymptomatic disease in Bovinae (*Bos, Bubalus, Syncerus*, and *Poephagus*). The immune response to *T. evansi* infection in buffaloes and cows are relatively better than in horses so that ruminants are more resistant to Surra disease. The chronic incidence of the disease showing no clinical symptoms but later the animals become useless (Partoutomo 1996). While the sub-clinical...
Surra is generally not easy to diagnose based on clinical examination and conventional laboratory tests, as a result the incidence of the disease tends to undetected and neglected without any receiving proper treatment (Mastra 2011).

The results of this study indicated that Surra at Lamandau District need serious attention. Although this district is not an endemic area but this area bordered by the district of West Kotawaringin which Surra is endemic. So that livestock transportation between the two district can caused the introduction of Surra in Lamandau district. According to Partoutomo (1996) if the cattle are not on good conditions and the hematophagus flies population are abundant, it would be potential Surra transmission showing clinical symptoms and causing epidemics. Surra control in this region is urgently needed to prevent the spread to another farm site. Naturally endemic areas of *T. evansi* characterized by the outbreak with high mortality due to stable enzootic *T. evansi* in some areas in Indonesia (Payne et al. 1991). However, to make sure where *T. evansi* isolate in Lamandau district come from need further analyzed by using microsatellite marker. According to Sawitri (2016) the origin of isolates that causing Surra outbreak can be traced by using molecular analysis with microsatellite markers. Sawitri (2016) has succesfully identify and trace *T. evansi* isolates that cause outbreak at Pandeglang in 2014 with eight microsatellite loci markers.

Surveillance for *T. evansi* in Indonesia sometimes face some problem due to difficulty in collecting suitable samples from potential host species in remote areas. There is limited access to many of these areas and no permanent facilities for storing samples that require refrigeration, which means that sample collection is an expensive operation and is only done infrequently by trained operators. By using a spotted blood on filter paper method, this study provides several advantages. Firstly, the method is practically applied in the field where refrigeration facilities are limited. Secondly, the samples can be stored at room temperature, so that the transportation of samples will be easier, especially for samples collected from remote areas which have long distance from laboratory. Thirdly, the method is easier handling and can be sent by courier to another laboratory. According to Chompoochan et al. (2007) that the blood spotted in filter paper method of preservation is very easy to implement and suitable for use in epidemiological surveys for trypanosomiasis control programs in endemic areas.

**CONCLUSION**

Surra livestock has occured in the Lamandau District, Central Kalimantan Province. Mass treatment is needed to treat, prevent, anticipate and control of potential outbreak. PCR positive status should be treated using antitrypanosoma drugs with recommended dose and negative livestock could be given a prophylactic dose. Surra examination need to be recommended as one of the delivery requirements of livestock from endemic or outbreaks areas to nonendemic area.

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