

Trypanosoma evansi Infection in Sumba Horses in East Sumba Regency : A Study at BBVet Denpasar

Infeksi *Trypanosoma evansi* pada Kuda Sumba di Kabupaten Sumba Timur : Sebuah Studi di BBVet Denpasar

Sarwo Edy Wibowo^{*1}, Fahmida Manin¹, Anie Insulistyowati¹, Fadel Muhammad Priyatna², Yudhi Ratna Nugraheni², Paradewa Baskara³, Aan Awaludin⁴

¹Department of Animal Health, Faculty of Animal Science, Universitas Jambi, Jambi-Indonesia

²Department of Parasitology, Faculty of Veterinary Medicine, Universitas Gadjah Mada, Sleman-Indonesia

³Department of Parasitology, Denpasar Veterinary Centre (BBVet Denpasar) Bali, Denpasar-Indonesia

⁴Department of Animal Science, Politeknik Negeri Jember, Jember-Indonesia

ABSTRACT

Background: Sumba is one of the original habitats of the Sandalwood Ponies and the presence of pony is an important element for the community. The Sumbanese herd their ponies in the savanna and rely on nature as a source of horse feed, consequently, during the dry season, the availability of the feed decreases. These conditions can potentially reduce the health status of ponies and increase morbidity or mortality from diseases caused by *Trypanosoma evansi*. **Purpose:** This study aims to determine the occurrence and intensity of *Trypanosoma evansi* infection in blood samples from horses examined at the Denpasar Bali Veterinary Centre. **Methods:** Thirty blood samples were collected from East Sumba Regency. Three milliliters of blood were taken from the jugular vein and immediately transferred into an ethylenediaminetetraacetic acid tube. Thin blood smears were subsequently prepared and examined using the Giemsa-stained blood smears method. The preparations of blood smear were examined under microscope to determine the *Trypanosoma evansi* infection and the intensity was calculated on the average number of *Trypanosoma evansi* in 100 red blood cells. **Results:** The result showed that six out of 30 horses were tested positive for *Trypanosoma evansi*. The infection intensity ranged from two to 18 parasites with an average number of 8 in the blood smear examination method. **Conclusion:** The incidence of trypanosomiasis in Sumba horses varies with each season in East Sumba Regency. The prophylactic measures that should be adopted in the particular herd of horses because this is the most significant blood protozoan parasite of equines.

ABSTRAK

Latar Belakang: Sumba merupakan salah satu habitat asli kuda sandel dan keberadaan kuda poni ini menjadi elemen penting bagi masyarakat. Masyarakat Sumba menggembalakan kuda poninya di padang savana dan mengandalkan alam sebagai sumber pakan kudanya, sehingga pada musim kemarau ketersediaan pakannya semakin berkurang. Kondisi tersebut berpotensi menurunkan status kesehatan kuda poni dan meningkatkan angka kesakitan atau kematian akibat penyakit yang disebabkan oleh *Trypanosoma evansi*. **Tujuan:** Penelitian ini bertujuan untuk mengetahui kejadian dan intensitas infeksi *Trypanosoma evansi* pada sampel darah kuda yang diperiksa di Balai Besar Kedokteran Hewan Denpasar Bali. **Metode:** Tiga puluh sampel darah diambil dari Kabupaten Sumba Timur. Tiga mililiter darah diambil dari vena jugularis dan segera dipindahkan ke dalam tabung asam etilendiaminetetraasetat. Apusan darah tipis selanjutnya dibuat dan diperiksa dengan metode apusan darah pewarnaan Giemsa. Sediaan hapusan darah diperiksa di bawah mikroskop untuk mengetahui adanya infeksi *Trypanosoma evansi* dan dihitung intensitasnya berdasarkan rata-rata jumlah *Trypanosoma evansi* dalam 100 sel darah merah. **Hasil:** Hasil penelitian menunjukkan enam dari 30 ekor kuda dinyatakan positif *Trypanosoma evansi*. Intensitas infeksi berkisar antara dua hingga 18 parasit dengan rata-rata jumlah 8 pada metode pemeriksaan hapusan darah. **Kesimpulan:** Angka kejadian trypanosomiasis pada kuda Sumba bervariasi setiap musim di Kabupaten Sumba Timur. Tindakan profilaksis yang harus diambil pada kawanan kuda tertentu karena ini adalah parasit protozoa darah kuda yang paling signifikan.

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*Correspondence:

Sarwo Edy Wibowo

E-mail: sarwoedywibowo@unja.ac.id

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Kata kunci: Kabupaten Sumba Timur; Kuda Sandel; Musim; *Trypanosoma evansi*

INTRODUCTION

Horses hold significant importance within Indonesian society that is intertwined with the culture, and the economy. According to the data from [Statistics Indonesia \(2022\)](#), the horse population in Indonesia is estimated to be 394,341. East Nusa Tenggara is the province with the second largest horse population (125,302 horses) whereas Sumba Island has the largest population ([East Nusa Tenggara Statistics, 2023](#)).

On Sumba Island, the types of horses found on Sumba include the Sumba horse or Sandalwood horse, and the Timor horse, which has distinctive characteristics of 100-135 cm of height and is categorized as a pony category ([Edwards, 2023](#)). These horses have several purposes for the locals such as riding, transporting goods, herding livestock, participating in races, and engaging in traditional "pasola" ceremonies—an ancient tradition involving javelin throwing while mounted on ponies.

One of the persistent and endemic horse diseases in Sumba is trypanosomiasis (Surra), caused by *Trypanosoma evansi*. The first Surra case reported in Indonesia was in 1897 found in Java Island ([Partoutomo, 1996](#)). Livestock movement is one of the important routes of transmission of Surra disease, and blood-sucking flies (*Tabanus* sp., *Haematopota* sp., *Chrysops* sp., *Stomoxys* sp., and *Haematobia* sp.) play a crucial role in the transmission of this infectious agent ([Jones *et al.*, 1996](#)). Between 2010 and 2011, a total of 4,268 livestock in Sumba tested positive for trypanosomiasis, which caused the death of 1,760 livestock, consisting of 1,159 horses, 600 buffaloes and one cow ([Directorate of Animal Health, 2012](#)). Based on the research conducted between 2010 and 2016 in East Sumba, the impact of the Surra outbreak amounted to 25.7 billion Indonesian rupiah in economic losses ([Dewi *et al.*, 2020](#)).

Trypanosoma evansi more often infects horses, cattle, buffalo, pigs and dogs. The various serological tests have proven that *Trypanosoma evansi* is an endemic disease in Indonesia ([Payne *et al.*, 1991](#)). The overall estimated prevalence of *Trypanosoma evansi* in horses of Pakistan and Wajo Regency, South Sulawesi is notably low, standing at around 2% and 2.4%, respectively, according to studies conducted by [Nadeem *et al.*, \(2011\)](#) and [Nur \(2017\)](#). In contrast, the massive prevalence of *T. evansi* in India is reported to be 46.5% ([Bal *et al.*, 2014](#)).

The clinical manifestations of surra in animals vary; infection can be acute, subclinical, and chronic. [Desquesnes \(2004\)](#) explained that the clinical signs are influenced by the intensity of *T. evansi* infection that develops in the blood circulation. The intensity of *T. evansi* infection is affected by the immune status and the physiological conditions of the host. Mostly, the Surra is asymptomatic and detection often occurs after a chronic infection ([Directorate of Animal Health, 2014](#)). Cows and buffalo can act as reservoirs for the *T. evansi* infection. However, it is believed that infected buffaloes are more susceptible to surra than cattle since buffalo show longer and

higher parasitemia than cattle making buffalo are thought to be a potential source of infection for cattle and horses ([Mas-tra, 2011](#)). The clinical symptoms of chronic infection include fever, anorexia, dull hair, oedema in the ventral abdomen, and icterus ([Directorate of Animal Health, 2014](#)).

The livestock farming model in Sumba allows the livestock to roam freely and depend on natural resources for feed. During the dry season, the grass in the savanna dries up leading to finding water sources becoming challenging. This condition has the potential to reduce health status and increase the risk of contracting infectious agents. This study aims to investigate the occurrence and intensity of *Trypanosoma evansi* infection in blood samples from horses examined at the Denpasar Veterinary Center (BBVet Denpasar).

MATERIAL and METHOD

This study was conducted at the Denpasar Veterinary Center (BBVet Denpasar) from February 5 to April 5, 2023. The blood smear examination of Sumba horses from East Sumba was carried out at the Department of Parasitology of Denpasar Veterinary Center (BBVet Denpasar) on March 2, 2023 from 8 AM to 3.30 PM Central Indonesian Time.

Materials

The tools and materials used for sampling were a 22G multi-drawing needle, ethylenediaminetetraacetic acid (EDTA) tube, cotton wool, water, a mask, a cooler box, an ice pack, and gloves. Equipment and materials needed for sample examination were horse blood, glass object, absolute methyl alcohol, marker, alcohol (70%), Giemsa solution 10% + buffer solution (1 + 4) with a pH of 6.5, microscope with 10x ocular and 100x objective magnification, immersion oil, dye bath, fixation bath, Pasteur pipette, timer watch, and tissue. The equipment used for documentation was a digital camera.

Methods

Blood samples were taken from 30 horses in East Sumba, East Nusa Tenggara. These horse blood samples were immediately placed into EDTA tubes. The horse blood collection technique is carried out using a suitable vacuum ([Costa and Chapman, 2017](#)). Blood samples were taken via the jugular vein. Prior to collection, the blood vessels were occluded, and the collection site was sterilized using cotton wool soaked in 75% alcohol. The blood was then drawn using a suction needle (multi-drawing needle) with a holder in the jugular vein, and then a vacuum tube was attached to the needle and the blood flowed into the tube.

The collected blood samples were examined at the Department of Parasitology of Denpasar Veterinary Center (BBVet Denpasar). The total blood samples were examined using the Giemsa-stained blood smears (GSBS), following the methods outlined by [Desquesnes \(2021\)](#). The blood smears were prepared by briefly air-drying the samples and fixing them in methyl alcohol for one minute and allowed to dry. Subsequently, the smear was stained with Giemsa solution for 25 minutes. The stain was cleaned, and the slide was washed with

tap water and then dried. Examination of blood smear was conducted at 1000x magnification with oil immersion. Observations were made to identify the parasites in the preparations. If a positive sample is found, further analysis involved counting the number of *T. evansi* parasites per 10 fields of view.

Analysis Data

The data obtained from the laboratory examination are summarized in **Tables 1** and **2** and **Figure 1**. The actual occurrence of *T. evansi* infection in horses in East Sumba Regency was determined by binomial proportion calculation based on the results of blood smear examinations.

Table 1. Results of Giemsa-Stained Blood Smears (GSBS)

| No | Sample Code | Types of Animals | Location | Sample Type | Result |
|----|-------------|------------------|------------|-------------|----------|
| 1 | 1 | Horse | East Sumba | Blood Smear | Negative |
| 2 | 2 | Horse | East Sumba | Blood Smear | Negative |
| 3 | 3 | Horse | East Sumba | Blood Smear | Negative |
| 4 | 4 | Horse | East Sumba | Blood Smear | Negative |
| 5 | 5 | Horse | East Sumba | Blood Smear | Positive |
| 6 | 6 | Horse | East Sumba | Blood Smear | Positive |
| 7 | 7 | Horse | East Sumba | Blood Smear | Negative |
| 8 | 8 | Horse | East Sumba | Blood Smear | Positive |
| 9 | 9 | Horse | East Sumba | Blood Smear | Negative |
| 10 | 10 | Horse | East Sumba | Blood Smear | Positive |
| 11 | 11 | Horse | East Sumba | Blood Smear | Negative |
| 12 | 12 | Horse | East Sumba | Blood Smear | Negative |
| 13 | 13 | Horse | East Sumba | Blood Smear | Positive |
| 14 | 14 | Horse | East Sumba | Blood Smear | Negative |
| 15 | 15 | Horse | East Sumba | Blood Smear | Negative |
| 16 | 16 | Horse | East Sumba | Blood Smear | Negative |
| 17 | 17 | Horse | East Sumba | Blood Smear | Negative |
| 18 | 18 | Horse | East Sumba | Blood Smear | Negative |
| 19 | 19 | Horse | East Sumba | Blood Smear | Negative |
| 20 | 20 | Horse | East Sumba | Blood Smear | Negative |
| 21 | 21 | Horse | East Sumba | Blood Smear | Negative |
| 22 | 22 | Horse | East Sumba | Blood Smear | Negative |
| 23 | 23 | Horse | East Sumba | Blood Smear | Negative |
| 24 | 24 | Horse | East Sumba | Blood Smear | Negative |
| 25 | 25 | Horse | East Sumba | Blood Smear | Negative |
| 26 | 26 | Horse | East Sumba | Blood Smear | Negative |
| 27 | 27 | Horse | East Sumba | Blood Smear | Negative |
| 28 | 28 | Horse | East Sumba | Blood Smear | Positive |
| 29 | 29 | Horse | East Sumba | Blood Smear | Negative |
| 30 | 30 | Horse | East Sumba | Blood Smear | Negative |

Table 2. Intensity of *T. evansi* infection in horses in East Sumba Regency

| No | Sample Code | Number of <i>T. evansi</i> /10 visual fields |
|---------|-------------|--|
| 1 | 5 | 11 |
| 2 | 6 | 8 |
| 3 | 8 | 2 |
| 4 | 10 | 3 |
| 5 | 13 | 18 |
| 6 | 28 | 6 |
| Average | | 8 |

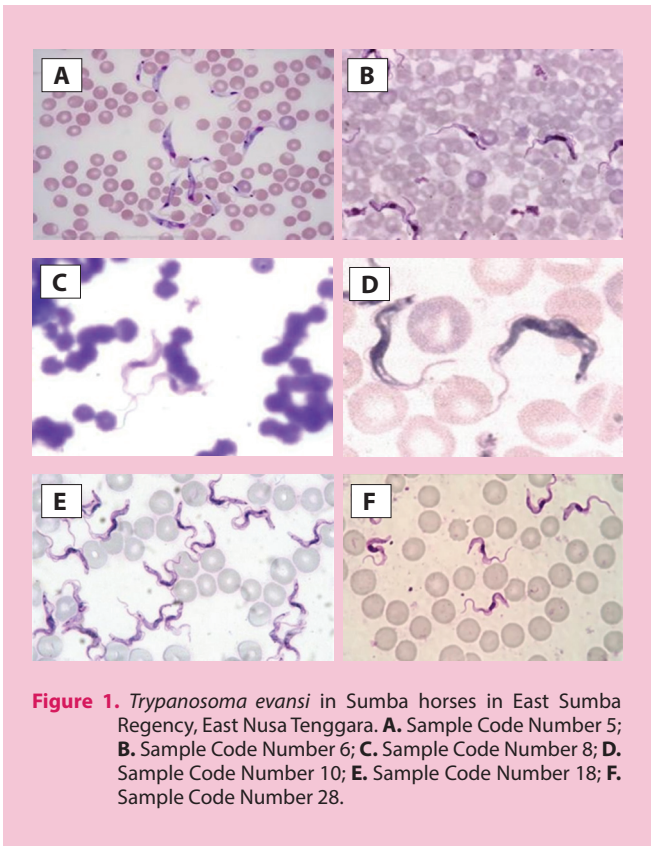


Figure 1. *Trypanosoma evansi* in Sumba horses in East Sumba Regency, East Nusa Tenggara. **A.** Sample Code Number 5; **B.** Sample Code Number 6; **C.** Sample Code Number 8; **D.** Sample Code Number 10; **E.** Sample Code Number 18; **F.** Sample Code Number 28.

RESULTS

Based on the results of the Giemsa-stained blood smears (GSBS) examination of Sumba horses at the Denpasar Veterinary Center Parasitology laboratory, six out of 30 samples tested positive for *T. evansi* (viz 05, 06, 08, 10, 13, 28). This shows that the incidence of Surra in horses in East Sumba was 20%. The complete occurrence of Surra in Sumba Horses is presented in **Table 1**. The results of the blood smear examination found that six blood samples were positively infected with *T. evansi*, in which the infection intensity ranged from two to 18 *T. evansi* parasites per 10 fields of view with an average number of eight parasites. The intensity of *T. evansi* infection in Sumba horses in East Sumba Regency at the BBVet Denpasar is presented in **Table 2**.

DISCUSSION

The occurrence of *T. evansi* in Sumba horses in East Sumba Regency was 20%, which was higher than the previous findings. [Praing *et al.*, \(2023\)](#) reported a prevalence of only 2.10% in horses in East Sumba Regency, while [Ndiha *et al.*, \(2018\)](#), reported that trypanosomiasis in horses in East Sumba was 8%. [Mursalim *et al.*, \(2017\)](#) stated that *T. evansi* infection in horses at the Kelara District Slaughterhouse, Jenepono Regency, South Sulawesi Province was 3.07 % and research by [Nur \(2017\)](#), which was carried out from June to July 2016 on horses in Wajo District, South Sulawesi, amounted to 2.4%. These rates are in contrast to the higher prevalence observed in this study. However, the prevalence reported in this study aligns more closely to the findings from

Nurcahyo *et al.*, (2019) which found that the prevalence of trypanosomiasis on Sumba Island was 13.3%. From November to December 2009, another study was conducted by Mastra (2011), in which the overall prevalence of *T. evansi* infections in horses on Sumbawa Island, West Nusa Tenggara Province reached 13.44%.

The results of this study yielded similar results to previous studies, in that trypanosomiasis still exists on Sumba Island. According to the Center for Indonesian Veterinary Analytical Studies/CIVAS, (2014) and Haryadi *et al.*, (2022), this disease is sporadic across Indonesia. Trypanosomiasis can appear anytime and is influenced by environmental factors, the animal's immune condition, and the fly population as a vector. Trypanosomiasis is found to spread throughout the world. Trypanosomiasis in horses occurs in Brazil, Ethiopia, India, Israel, Jordan, Mongolia, Nigeria, Southeast Asia, Sudan, Venezuela, and several other countries (Aregawi *et al.*, 2019, Büscher *et al.*, 2019). *T. evansi* infection is reported to be endemic in horses in Peninsular Malaysia with a prevalence of 13.9% (Elshafie *et al.*, 2013).

Various factors contribute to variations in the occurrence of trypanosomiasis. Apart from differences in the diagnostic methods used and the location of the sampling site, the presence of the fly vector plays a crucial role. Environmental conditions that are suitable for vector development, such as humidity and temperature that are ideal for fly growth, season, density, and how animals are kept, are the determining factors in increasing trypanosomiasis cases (Sumbria *et al.*, 2017; Okello *et al.*, 2022; Algehani *et al.*, 2023).

Horses in East Sumba Regency are still managed traditionally, such as grazing in pastures during the day alongside other livestock such as buffalo and cows, then put in pens at night. Meanwhile, some horses may remain in pastures for extended periods. Horses bred together with buffalo and cows are one of the major factors responsible for causing *T. evansi* infection in horses, where buffalo and cows can act as reservoirs of *T. evansi* infection. Sampling conducted during the rainy season also influenced the occurrence in this study. Pastures usually produce abundant forage in the rainy season, and in the dry season, they experience drought. In the dry season, there is limited feed for livestock resulting in insufficient nutrition where lack of nutrition is one of the factors that influence *T. evansi* infection (Reid, 2002).

East Sumba Regency is located along the hilly north coast which contributes to a longer dry season compared to the rainy season. With such geographical conditions, it is a place that has less potential for the development of vectors that spread *T. evansi* (*Tabanus*, *Stomoxys*, *Hematopota*/Lyperosia) (Dewi *et al.*, 2018). Research conducted by Desquesnes *et al.*, (2013) showed that epidemiological status and geographical conditions can influence the incidence of Surra disease in a region. Therefore, taking samples during the rainy season

may contribute to the occurrence of *T. evansi* infection in this study. During the rainy season, there is an increase in the number of vectors compared to the dry season. In East Sumba Regency, the rainy season is short and lasts for three to months (January to March or December to April), while in West Sumba Regency, it lasts for four to five months (December/January to April) (Nurcahyo *et al.*, 2019).

According to Herczeg *et al.*, (2015), rainfall, air humidity, and sunlight influence the number of vectors, especially *Tabanus* sp, in the nature. Vectors require a surrounding air temperature of at least 18°C to fly. The optimal temperature for *Tabanus* sp to fly is at least 31°C to 35°C. The average temperature in East Sumba Regency is generally 22.5°C to 31.7°C (East Sumba Regency Statistics, 2022). The types of flies found in Eastern Sumba are blood-sucking flies (*Hematophagus* flies, *Stomoxys calcitrans*, *Hippobosca* sp. and *Haematobia irritans exigua*) (Sawitri *et al.*, 2019), while Oematan *et al.*, (2016) found *Stomoxys calcitrans* (45.28 %), *Haematobia irritans* (24.53%), *Tabanus* sp (13.21%) and *Hippobosca equina* (16.98%).

The intensity of *T. evansi* infection in this study was found to be between two to 18 parasites per 10 fields of view with an average number of eight parasites. This is lower than the finding of Ndiha *et al.*, (2018) which found between 13 to 71 parasites per 10 fields of view with an average number of 34.5 ± 22.7 . The variation of infection may be linked to the condition of the sampled horses where some horses only show general clinical signs of weakness, lethargy, weight loss and some show clinical signs in the form of abdominal oedema. These findings are consistent with the study conducted by Desquesnes (2004), which suggested that the clinical signs that appear are influenced by the intensity of *T. evansi* infection that develops in the blood circulation.

CONCLUSION

The occurrence of *T. evansi* infection in Sumba horses in East Sumba Regency was 20%. The intensity of *T. evansi* infection in positive samples was found to be between two and 18 parasites per 10 fields of view with an average number of eight parasites.

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CONFLICT of INTEREST

The authors have no conflicts of interest to declare.

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ETHICAL APPROVAL

This study did not require ethical approval.

AUTHORS' CONTRIBUTIONS

Conceptualizing and designing the study: SEW, FMP, FM, AI, YRN, PB. Collecting data: SEW, FMP. Performing data analysis and interpretation: SEW, FMP, YRN, AW. Drafting the manuscript: SEW, YRN, FMP, AW. Carrying out revisions: SEW, YRN.

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