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Parasitological and serological detection of *Trypanosoma evansi* on Bali cattle at the Pesanggaran slaughterhouse, Denpasar, Indonesia using hematological profile

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Abstract. *Apsari IAP, Suratma NA, Swacita IBN, Soma IG, Sari TK, Putra IPC, Sudipa PH. 2024. Parasitological and serological detection of* Trypanosoma evansi *on Bali cattle at the Pesanggaran slaughterhouse, Denpasar, Indonesia using hematological profile. Biodiversitas 25: 1057-1062.* Bali cattle (*Bos sondaicus* Blyth, 1842 syn. *Bos javanicus domesticus* Wilckens, 1905) are vulnerable to blood protozoan infections like *Trypanosoma evansi*. Indonesia's warm and moist environment provides ideal conditions for blood-sucking flies such as *Stomoxys calcitrans*, *Haematobia irritans*, and *Hipobosca* sp. to thrive and spread. These flies act as carriers of *T. evansi*, which can then infect Bali cattle. This study aims to detect the presence of *T. evansi* in Bali cattle. Blood samples were collected from 275 Bali cattle at the Pesanggaran slaughterhouse, Denpasar City, Bali, Indonesia. The samples were examined using the Giemsa-Staining Blood Smear (GSBS) method and hematology profile examination using Hematology Analyzer RT-7600 for Vet. Serum samples were also collected for serologic examination using the Card Agglutination Test for Trypanosomiasis (CATT) method. The hematological profile data obtained were analyzed descriptively, and the Mann-Whitney U test was used to determine the significance of hematological values between female and male cattle seropositive to *T. evansi*. The results showed that the *T. evansi* parasite was not detected in the blood smear examination. However, the serological detection with CATT revealed that 6.54% (18/275) of Bali cattle were positive for *T. evansi*. The hematological profiles of *T. evansi* seropositive cattle remained within the normal range, except for neutrophil percentage. White Blood Cell (WBC), Hemoglobin (Hb) levels, and hematocrit (PCV) values of male cattle were significantly ($P \leq 0.05$) lower than those of females. Therefore, based on serological examination, it can be concluded that the protozoa *T. evansi* is present in 6.54% of Bali cattle at the Pesanggaran slaughterhouse.

Keywords: *Bos sondaicus*, CATT, complete blood count, Pesanggaran slaughterhouse, Surra

INTRODUCTION

Trypanosomiasis is a disease that infects cattle, and it is caused by *Trypanosoma evansi*, a blood protozoan that circulates in the extracellular system (Sawitri et al. 2022). Indonesia is endemic for trypanosomiasis, which has been declared a Strategic Infectious Animal Disease (SIAD) by the Indonesian government. SIADs are animal diseases prioritized for management and control (Sawitri et al. 2018; Subekti et al. 2023). The blood-sucking flies that transmit *T. evansi* are *Tabanus* sp., *Stomoxys* sp., *Haematobia* sp., *Hippobosca* sp., and *Chrysops* spp. (Desquesnes et al. 2013). Recent research by Dwinata and Oka (2021) identified blood-sucking flies found around cowsheds on farms in Badung, Bali, namely *Stomoxys calcitrans*, *Haematobia irritans*, and *Hippobosca* sp. These blood-sucking flies indicate the possibility that *T. evansi* has spread to animal hosts in Bali.

Blood parasite infections are still a factor causing economic losses in livestock. These losses are due to death, decreased production efficiency, especially on production animal farms, and increased medical costs. Losses due to African trypanosomiasis put 50 million cattle at risk and caused the death of 3 million animals per year, as well as economic losses of 1.0-1.2 billion dollars from cattle production (Tolawak and Pal 2022). Various reports of examination results from animal health laboratories in Indonesia show that *T. evansi* infection causes losses that continue to increase yearly (Nurcahyo 2017). Indonesia's most extreme trypanosomiasis outbreak happened on Sumba Island in 2010-2012. The outbreak comes about within the passing of more than 2,000 animals. In addition, Surra was serologically detected in 16.7% (4/24) sera of farmers living in the outbreak area (Sawitri et al. 2019). The economic loss due to the epidemic in Sumba is estimated at IDR 1.4165 billion. If proper management is

not implemented, the loss could reach IDR 167.224 billion from January to June 2012 (Ekawasti et al. 2016). Investigations into trypanosomiasis were continued on Lombok Island by Ekawasti et al. (2016), an island east of Bali, where seroprevalence of *T. evansi* in cattle was 35.7% (30/114). This indicates that *T. evansi* may have spread on Lombok Island and could potentially spread to Bali Island.

In general, symptoms of Trypanosomiasis in cattle and buffalo (*Bubalus bubalis*) are chronic symptoms such as fever, anemia, edema of the legs, weight loss, decreased productivity, disruption of the estrous cycle, and loss of energy in working animals (Desquesnes et al. 2013; Wei et al. 2021). The general symptoms of trypanosomiasis are not pathognomonic enough to diagnose the disease, so laboratory methods to detect the parasite are needed to help establish the diagnosis (OIE 2021). Likewise, symptoms in cows and buffalo are subclinical, and buffalo can act as a reservoir (Rani et al. 2015; Migri et al. 2016). *Trypanosoma evansi* is a blood protozoan that parasitizes extracellularly in the blood. Blood parasite disorders in cattle can affect their hematological condition. Several previous studies found that *T. evansi* infection in cattle, buffalo, and camels (*Camelus dromedarius*) causes disturbances in the blood picture, such as lymphocytosis, followed by leukopenia during the chronic phase, a decrease in hematocrit (PCV) values, and anemia, which is reflected in low Hb values (Pandey et al. 2015; Sivajothi et al. 2015).

Diagnosing *T. evansi* in animals can be done by directly finding the parasite in a blood and lymph node. Gold standards for *T. evansi* antigen detection are the three combinations of Giemsa-Stained Blood Smears (GSBS), Hematocrit Centrifugation Technique (HCT) and Polymerase Chain Reaction (PCR) based on satellite DNA detection. As for antibody detection, the combination of Enzyme-Linked Immunosorbent Assay (ELISA) using soluble antigens from whole trypanosome lysate and card agglutination test for *Trypanosoma evansi* (CATT/*T. evansi*) based on RoTat 1.2 (OIE 2021). The CATT is a serological test to detect antibodies and is the best test for detecting *T. evansi* in buffalo in Indonesia (Reid 2002; Ekawasti et al. 2016; Sawitri and Wardhana 2024). CATT is relatively cheap, fast, and simple and can be implemented in the field for any host (Desquesnes et al. 2022). Trypanosomiasis in the Bali Province in Bali cattle (*Bos sondaicus* Blyth, 1842 syn. *Bos javanicus* d'Alton, 1823 ssp. *domesticus* Wilckens, 1905) has never been reported through serology, parasitology, and molecular testing. This study aims to detect the presence of *T. evansi* in cattle at Pesanggaran slaughterhouses directly on blood smear preparations and serologically with CATT, as well as hematological images.

MATERIALS AND METHODS

Sample

The material used was blood collected from 275 samples of cows at the Pesanggaran Slaughterhouse, Denpasar City, Bali Province, Indonesia. Blood samples were taken randomly from cattle slaughtered at the abattoir. Blood samples were collected after the animals were slaughtered and immediately collected in a sampling tube. One part of the blood was given an anticoagulant (tube with heparin), and one part was not given an anticoagulant (plain tube), from which the serum was taken later. The Faculty of Veterinary Medicine, Udayana University's animal ethics commission approved the experimental animal protocol.

Blood sample collection

Blood samples were taken from 275 cows at the Pesanggaran slaughterhouse in Denpasar city, Bali, Indonesia. Blood was taken when the cow was slaughtered. One tube contained an anticoagulant (heparin), and another tube containing no anticoagulant (plain) was used to hold a blood sample. Heparin tubes were used for blood smears and hematological profile examinations (Samples and Echols 2022). Blood without anticoagulants, the serum was taken for serological examination using the CATT method (Lemans 2021).

Giemasa-Stained Blood Smear (GSBS) parasitology examination

One drop of blood from each sample (around 3-5 µL) was placed on one end of a clean glass object, and a thin blood smear was made. The preparation was air-dried to dry, then fixed with methanol for 1 minute and dried. Thin blood smear preparations were soaked in Giemsa solution (1 mL of Giemsa mixed with 9 mL of distilled water/buffer solution) for 25 minutes. The blood smear preparations were washed under running water and dried. The preparations were examined using a microscope with a magnification of 400-1000x with immersion oil (Zajac et al. 2021).

The Card Agglutination Test for Trypanosomiasis

The Card Agglutination Test for Trypanosomiasis (CATT) is a serologic test that detects antibodies in animal serum samples produced by the Institute of Tropical Medicine, Antwerp, Belgium (Songa and Hamers 1988). Bovine serum was separated from blood and taken without an anticoagulant. A 25 µL of sample animal serum (infected or suspected) was taken (previously diluted 1:4) and placed into the reaction zone on the test card. One drop (approximately 45 µL) of CATT reagent was added, and the reaction mixture was stirred using a stir bar and allowed to react on the CATT rotator for 5 minutes at 70 rpm. The reactions were compatible with positive or negative controls (Lemans 2021).

Hematological profile

Blood samples of cattle that were positive for serology (CATT) and/or parasitology (thin blood smear) were subjected to hematology tests. The hematology results were compared between male and female cattle. The hematological profile observed was the number of Red Blood Cells (RBC), total leukocytes (WBC), differential leukocytes (lymphocytes, monocytes, basophils, eosinophils, neutrophils), hematocrit value (PCV), and hemoglobin levels. The examination used the Hematology Analyzer Rayto RT-7600 for Vet (Alexey 2015).

Data analysis

Hematology data from *T. evansi* serology-positive cattle were subjected to descriptive analysis. A hematology comparison between male and female cattle positive serology for *T. evansi* was made using a Mann-Whitney U test. SPSS 22.0 software was used to analyze and present the data in tabular form.

RESULTS AND DISCUSSION

The results of the examination of 275 blood samples of Bali cattle using the thin blood test method showed that no *T. evansi* parasites were detected in the blood preparations. The blood test results were negative, meaning that the parasitological prevalence of *T. evansi* in Bali cattle at Pesanggaran abattoir in this study was 0%. This result is similar to that reported by Sulaeman et al. (2019) on beef cattle in Banyuwangi and by Ombugadu et al. (2023) report on cattle research at the Lafia abattoir in Nigeria. *Trypanosoma evansi* infection in cattle generally occurs subclinically (Chandu et al. 2021). It is often challenging to find parasites in the blood circulating in the periphery (Kim et al. 2023), as the infection tends to be chronic (Behour et al. 2019), or there is no high parasitemia (Nurcahyo 2017). Internal factors also play a role in influencing test results, namely host conditions. Host condition refers to general health both physically and immunologically (Croof et al. 2017). In this study, the cattle blood used as the research sample came from the abattoir, so the cattle were physically healthy and were not in the parasitemia phase. Cattle slaughtered at Pesanggaran abattoir have been subjected to antemortem examination by the abattoir veterinarian the day before. Only physically healthy cattle were allowed to be slaughtered at the abattoir. Surra parasitemia in cattle fluctuates (Gaffar et al. 2016). According to Charaya et al. (2022), the intermittent pattern of parasitemia in cases of *T. evansi* infection in the host is often not detected in blood tests due to its low levels.

The GSBS method can be used for detailed morphological analysis and identification of the subgenus *Trypanosoma* (OIE 2012). However, this method has low sensitivity (El-Naga and Barghash 2016; Rochmadiyanto et al. 2018) because the host must experience high parasitemia of >500,000 trypanosomes/mL of blood (OIE 2021). Compared with real-time PCR, the relative sensitivities of GSBS and CATT were 30.4 and 37.0% in *T. evansi*-infected camels, respectively (Habeeba et al. 2022). CATT has a sensitivity of 78% and a specificity of 100% in cattle (Rochmadiyanto et al. 2018). OIE (2012) has validated that CATT's specificity is 100%. Meanwhile, Reid and Copeman (2003) reported that the sensitivity and specificity of CATT in cattle in Indonesia were 83 and 96% with serum dilution of 1:4, respectively.

CATT results on the serum serology test of Bali cattle showed that 18 positives were detected out of 275 Bali cattle sera. The seroprevalence of *T. evansi* in Bali cattle was 6.54% (18/275). This seroprevalence differs from that found by Aregawi et al. (2019), which ranged from 10-29% (1693/7981) in cattle from various countries with various serological tests and meta-analyses. Seroprevalence in cattle in Mizoram, India, using the CATT test, was detected at 36.59%, while with the ELISA using recombinant variable surface glycoprotein antigen, 40.35% (Chandu et al. 2021). This difference is due to the different antigen-based detection methods used. Similarly, in fighting bulls in Southern Thailand, the detection of *T. evansi* using ELISA with crude antigen seroprevalence was 22.6%, while PCR based on TBR primers did not detect parasite DNA (Kamyingkird et al. 2020). In visibly sick cattle, the prevalence of *T. evansi* detected by ELISA was still high at 43.8% (14/32) in Eastern India (Laha and Sasmal 2009). The prevalence of buffaloes in Jabalpur, India, was 15.78%, with sex and age affected prevalence (Gangwar et al. 2019). This situation is influenced by the topography of the area and the way livestock are raised. The seroprevalence of *T. evansi* in Bali cattle (6.54%) was small compared to the prevalence in other countries, possibly because the Bali cattle in this study came from cattle that would be slaughtered at the abattoir. This means that the cattle were physically healthy. A low prevalence of *T. evansi* in healthy cattle was also reported in East Sumba, Indonesia, eight years after the surra outbreak in Sumba Island. The infection rates by CATT *T. evansi* were 9.7% (3/31), and by GSBS, 0% (0/31) (Sawitri and Wardhana 2024). Likewise, in South Sulawesi, Indonesia, only one out of 100 cattle tested positive for *T. evansi* using microscopic observation, but PCR analysis revealed a higher prevalence of 3%. No CATT *T. evansi* test was performed in this study (Setiawan et al. 2021). A comparison was conducted between CATT/*T. evansi* and Giemza stain thin blood smear tests in cattle and buffaloes in endemic areas, Central Java, Indonesia. The study's results stated that the finding of blood parasites with the blood smear method was relatively low at 9.8% compared to CATT/*T. evansi* at 46.0% (Sawitri et al. 2018).

Table 1 shows that the mean values of WBC, lymphocytes, monocytes, eosinophils, RBC, and Hb in this study are within the normal range according to Weiss and Wardrop (2010), Siswanto (2011), Adam et al. (2015), and Brooks et al. (2022), except that the neutrophils and PCV values were slightly higher than the normal range. In this research, we did not measure the absolute value of neutrophils; we only measured the percentage of neutrophils. The mean percentage of neutrophils in this study was 56.74±12.38%, which is higher than the normal bovine value of 15-45% (Weiss and Wardrop 2010). However, other studies have shown that cattle infected with *T. evansi* in Tirupati ($2.3\pm0.1\times10^3/\mu L$) (Sivajothi et al. 2014) and Basrah ($4.2\pm0.5\times10^3/\mu L$) (Alsaad et al. 2021) were still within the normal range. The normal value of absolute neutrophils in cattle is $0.8\text{-}5.0\times10^3/\mu L$ (Brooks et al. 2022). The state of increased neutrophils (neutrophilia) is thought to be an inflammation-related symptom in cattle (Bassel and Caswell 2018). In the more severe acute stages of inflammation, the bone marrow will increase neutrophil production. In chronic inflammation, neutrophilia may occur, or the neutrophil count may be normal (Sivajothi et al. 2015).

Table 1. Hematological values of positive serology Bali cattle for *Trypanosoma evansi*

Positive samples	WBC (10^3/µL)	Lymphocytes (%)	Monocytes (%)	Neutrophils (%)	Eosinophils (%)	Basophils (%)	RBC (10^3/µL)	Hb (g/dL)	PCV (%)
S 01	10	43	11	46	0	0	5.15	11.9	29.4
S 02	14.8	44	6	50	0	0	8.12	16	39
S 03	19.8	40	5	55	0	0	5.37	13.7	30.5
S 04	12.9	6	14	72	8	0	5.81	13.1	30.6
S 05	8.7	42	11	47	0	0	6.47	11.4	26.1
S 06	13.1	36	1	63	0	0	5.57	13	30.6
S 07	7.2	38	14	48	0	0	4.02	10	24.3
S 08	17.6	49	3	48	0	0	6.56	12.3	29
S 09	9.2	46	7	47	0	0	6.54	11.6	28.4
S 10	16	25	0	74	1	0	5.08	12.6	30.1
S 11	14	31	1	67	1	0	5.61	13.2	31
S 12	11.7	44	7	49	0	0	4.89	12.8	30
S 13	11.3	42	2	52	4	0	7.44	15.2	37.5
S 14	8.9	49	0	49	2	0	7.72	13.6	33.8
S 15	7.1	15	2	82	1	0	4.50	9.8	23.4
S 16	12.1	47	4	49	0	0	7.07	14.2	34.9
S 17	20.2	45	4	47	4	0	6.82	14.2	35.5
S 18	12	33	19	78	0	0	6.49	13.3	35
Average	12.59	37.89	6	56,74	1.17	0	6.07	12.17	31.06
Standard deviation	3.93	12.23	5.55	12.38	2.15	0	1.14	3.31	4.25
Data range	7.2-20.2	6 - 54	0 - 19	45-82	0 - 8	0	4.02-8.12	9.8-16	23.4-39
Normal	5.1-13.3[1]	45-75[1]	2 - 7[1]	15-45[1]	0-20[1]	0 - 2[1]	4.8-7.6[2]	8.2-13[2]	26.6-29.2[3]

Note: WBC: White Blood Cell, RBC: Red Blood Cell, Hb: Hemoglobin, PCV: Packed Cell Volume. [1]: Normal score according to Weiss and Wardrop (2010), [2]: Normal score according to Brooks et al. (2022), [3]: Normal score according to Siswanto (2011); Adam et al. (2015)

Table 2. Mann-Whitney U test results from hematology values between sexes of Bali cattle serology positive for *Trypanosoma evansi*

Hematology values	Male	Female	Significance
WBC (10^3/µL)	7.950	13.169	0.049 *
Lymphocytes (%)	40.0	37.188	0.725^{NS}
Monocytes (%)	12.5	5.375	0.091^{NS}
Eosinophils (%)	0.00	1.313	0.157^{NS}
Neutrophils (%)	47.50	56.125	0.157^{NS}
RBC (10^3/µL)	5.245	6.171	0.261^{NS}
Hb (g/dL)	10.70	13.156	0.049 *
PCV (%)	25.20	30.160	0.049 *

Note: NS: Not significance, ∗: Significance (P≤0.05)

This study's total leukocyte and differential leukocyte research data varied from the normal cattle leukocyte profile (Weiss and Wardrop 2010) and other publications. Several factors causing this variation in data are differences in animal species, breeds, and sex of cattle. Meanwhile, total leukocyte cell counts and leukocyte differentials vary across species (Naidenko and Alshinetskiy 2020). Differences in total leukocytes and differential leukocytes also differ in several types of cattle in Aceh (Sofyan et al. 2020).

Cattle infected with *T. evansi* will experience a decrease in hemoglobin, but in this study, the hemoglobin obtained was within a normal range. This may be because of examination using serologic methods with CATT/*T. evansi* cannot always distinguish whether the infection is occurring or has occurred (Ekawasti et al. 2016). Therefore, there is a possibility that the cattle were infected but are currently no longer or mildly infected. According to Kendran and Pemayun (2020), an increase in hemoglobin levels has no effect and is even said to be better and healthier. Increased hemoglobin levels can occur due to the release of catecholamines, resulting in increased blood pressure and spleen contraction.

The PCV value in Bali cattle with positive serology of *Trypanosoma evansi* in this study has an average of 31.06%, not much different from the research conducted by Perayadhista et al. (2022) of 30.8%, and Merdana et al. (2020) of 30.5% which used the same hematology analyzer method. The PCV value in this study is higher than cattle infected with *T. evansi* in Gujarat, with an average of 24.02% (Pandya et al. 2018). Based on the average PCV value of normal Bali cattle, which is 29.2% (Siswanto 2011), it can be said that the PCV value is at the normal level in this research. The state of high PCV value goes hand in hand with the high number of RBCs. In addition, an increase in PCV can also be caused by epinephrine and polycythemia (Kendran and Pemayun 2020). Conversely, a low PCV may indicate the animal is anemic (Bunga et al. 2019). The anemia may be caused by mechanical injury to the erythrocytes due to *T. evansi* attachment to the erythrocytes, causing changes in their morphology and surface oligosaccharide profile (Rossi et al. 2017).

Statistically (Table 2), male Bali cattle seropositive for *T. evansi* had lower WBC, Hb and PCV values compared to females (P≤0.05). Brooks et al. (2022) stated that adult male and female cattle in healthy conditions have no different WBC, Hb and PCV values. The difference in

results is due to the number of blood samples of seropositive males (n=2) being less than that of females (n=16). Therefore, data non-uniformity affects the statistical test. Although male Bali cattle have lower WBC, Hb and PCV values, they are still within the range of standard values (Weiss and Wardrop 2010; Adam et al. 2015; Brooks et al. 2022). Rossi et al. (2018) stated that Hb and PCV values decreased gradually from day 1 to 17 during the parasitemia phase of *T. evansi* in experimentally infected cattle. On day 10, PCV decreased to 60% and Hb 64% below pre-infection values. Sivajothi et al. (2014) also reported that WBC, PCV, Hb, and RBC values in cattle infected with *T. evansi* decreased significantly compared to controls. In the report of Sivajothi et al. (2014), the cattle used in the study had clearly observed clinical symptoms such as fever, anorexia eye discharge, dullness, and enlarged lymph nodes. However, the Bali cattle in this study did not show any symptoms based on antemortem examination before the cattle were declared fit for slaughter.

Our research showed that the seroprevalence of *T. evansi* infection in Bali cattle at the Pesanggaran slaughterhouse is 6.54% (18/275). Hematological profiles such as WBC, RBC, Hb levels, PCV, and leukocyte differential in *T. evansi* seropositive cattle are within the normal range, except for neutrophil. The presence of *T. evansi* in cattle in Bali needs to be monitored to prevent potential zoonosis in slaughtermen, veterinarians, farmers and the surrounding community involved in the Pesanggaran abattoir. In addition, surveillance and molecular diagnosis need to be conducted to trace the source of *T. evansi* found in the Pesanggaran abattoir.

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