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SEROLOGICAL SCREENING FOR ANTIBODIES AGAINST *TRYPANOSOMA EVANSI* IN SHEEP FROM HER'S FARM, BOGOR REGENCY, WEST JAVA PROVINCE USING ENZYME-LINKED IMMUNOSORBENT ASSAY

Skrining Serologis Antibodi terhadap *Trypanosoma evansi* pada Domba dari Peternakan Her' *Farm,* Kabupaten Bogor, Provinsi Jawa Barat Menggunakan *Enzyme-Linked Immunosorbent Assay*

Kayla Zahrah Hanafiah, Tetty Barunawati Siagian*

Veterinary Paramedics Study Program, College of Vocational Studies, IPB University, IPB Cilebende Campus, Jl. Kumbang No. 14, Bogor City, West Java, 16128, Indonesia

*Corresponding author email: tettybarunawatisiagian@apps.ipb.ac.id

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Abstract

The sheep farming subsector plays a vital role in agricultural development in Indonesia; however, its productivity can be compromised by trypanosomiasis, a disease caused by *Trypanosoma evansi* infection. This study aimed to analyze the presence of *T. evansi* antibodies in sheep at Her's Farm, an intensive farming operation located at Jl. Mandala Raya, Gang Kitri Rt. 03 Rw. 02, Cimandala, Sukaraja, Bogor Regency, using the Enzyme-Linked Immunosorbent Assay (ELISA) method. Blood samples were collected from eight sheep and quantitatively analyzed. The results are presented descriptively and supported by a literature review. The test results showed that all sheep blood samples tested negative for *T. evansi* antibodies, with an Optical Density Sample-to-Negative (OD S/N) value ≤ 2. It can be concluded that the farms studied were serologically free of trypanosomiasis. Further testing, including blood smears and molecular tests, is required to confirm the presence of *T. evansi* antigens.

Keywords: ELISA, intensive farming, serological survey, sheep, surra

Abstrak

Subsektor peternakan domba memiliki peran penting dalam pembangunan pertanian di Indonesia, namun produktivitasnya dapat terganggu oleh penyakit trypanosomiasis yang disebabkan oleh *Trypanosoma evansi*. Penelitian ini bertujuan untuk menganalisis keberadaan antibodi *T. evansi* pada domba di Peternakan *Her's Farm* dengan sistem pemeliharaan intensif yang berlokasi di Jl. Mandala Raya, Gang Kitri Rt. 03 Rw. 02, Cimandala, Sukaraja, Kabupaten Bogor, menggunakan metode *Enzyme-Linked Immunosorbent Assay* (ELISA). Sampel darah diambil dari delapan ekor domba dan dianalisis secara kuantitatif dengan hasil disajikan secara deskriptif serta didukung oleh studi literatur. Hasil pemeriksaan menunjukkan bahwa seluruh sampel darah domba memberikan hasil negatif terhadap antibodi *T. evansi* dengan nilai Optical

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Density Sample-to-Negative (OD S/N) ≤ 2. Dapat disimpulkan bahwa peternakan yang diteliti masih terbebas dari trypanosomiasis secara serologi. Pemeriksaan lebih lanjut berupa pemeriksaan apusan darah dan molekuler guna memastikan kembali keberadaan antigen *T. evansi*.

Keywords: domba, ELISA, peternakan intensif, skrining serologis, surra

INTRODUCTION

The sheep farming subsector is one of the key pillars supporting agricultural development in Indonesia; however, its productivity can be disrupted by Trypanosoma infections (Nurcahyo, 2017). This disease causes significant economic losses, although the exact magnitude is difficult to determine because of limited epidemiological data and a lack of adequate information collection in developing countries (Desquesnes *et al.*, 2013). Desquesnes *et al.* (2013) reported that Trypanosomiasis can cause substantial economic losses through reduced productivity, reproductive disorders, weight loss, and increased mortality in affected livestock. *T. evansi* is the primary cause of trypanosomiasis among various pathogenic Trypanosoma species. This is because *T. evansi* has the widest host range and geographic distribution (Warsito *et al.*, 2025). Previous studies have reported that *T. evansi* infection was found in 37.5% of sheep in Southern Algeria (Boushaki *et al.*, 2024), 18.75% in Southern Punjab, Pakistan (Tariq *et al.*, 2024), and 4% in Palestine (Ereqat *et al.*, 2020). The susceptibility of sheep to *T. evansi* infection was demonstrated in an experimental study by Rusli *et al.* (1993), who reported changes in hematological and biochemical blood parameters after inoculation of sheep with *T. evansi*.

Trypanosomiasis caused by *T. evansi*, also known as surra, is a protozoan disease that affects various animal species worldwide, including camels, goats, water buffaloes, horses, donkeys, sheep, pigs, cats, cattle, and dogs (Kim *et al.*, 2023). This wide range of host species provides *T. evansi* with significant opportunities to thrive and spread widely, including in sheep, which serve as reservoirs (Lendzele *et al.*, 2022). Parra-Giménez & Reyna-Bello (2019) reported that *T. evansi* infection in sheep is generally chronic and latent, with low levels of parasitemia, and that natural recovery can occur in some cases.

To dateTo date, information regarding the presence of *T. evansi* in sheep at the farm level remains limited. Research on the detection of the blood parasite *T. evansi* in sheep using the Enzyme-Linked Immunosorbent Assay (ELISA) method is urgently needed. The ELISA method is a widely used serological method for diagnosing blood-borne parasitic diseases because of its high sensitivity and specificity and ability to detect even small amounts of *T. evansi* antibodies or antigens (Subekti & Yuniarto, 2020). This study aims to provide a more accurate picture of the serological status of *T. evansi* in sheep, which can then serve as a basis for developing more effective and sustainable prevention and control strategies.

RESEARCH METHODS

Research Subjects

The research subjects consisted of eight thin-tailed sheep (*Ovis aries*) raised intensively at Her's Farm. The sheep included two males and six females, all approximately one year of age. All sheep included in the sample were selected from a total population of 46 based on the following inclusion criteria: body condition score (BCS) <3, obtaining approval from the owner, and showing no symptoms of trypanosomiasis based on physical examination results. The study was conducted at Her's Farm, located on Jalan Mandala Raya, Gang Kitri RT 03 RW 02, Cimandala Village, Sukaraja Subdistrict, Bogor Regency, West Java, from October 4, 2024, to February 1, 2025. Based on field observations, the sheep did not exhibit any clinical symptoms

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of trypanosomiasis. The study subjects consisted of eight thin-tailed sheep approximately one year old that were raised intensively at Her's Farm.

Equipment and Materials

This study required several pieces of equipment and materials. The equipment used included a 3-ml syringe, collection tubes, a cooler box for temporary sample storage, a pencil for labeling, a device for data recording, a 96-well Maxisorp-type ELISA microplate, a micropipette and sterile tips, a multichannel pipette, a 450 nm ELISA reader, a microplate washer, a 4°C incubator, a vortex mixer, Eppendorf tubes, beakers, a timer, and laboratory personal protective equipment (PPE). The required materials included thin-tailed sheep approximately one year old with a body condition score (BCS) of <3, sheep blood serum samples, TSA *T. evansi* antigen, PBS solution and PBS-Tween, blocking buffer with 0.5% BSA, sheep serum samples (diluted 1:800), positive and negative control sera, anti-IgG-HRP secondary antibody, TMB substrate, and 2N H_2SO_4 stopping solution. Sample collection and testing were conducted over a fairly long period, from October 4 to February 1, 2025, in this study.

Research Design

This observational study was conducted using a preliminary study approach to detect *T. evansi* antibodies in sheep using ELISA.

Research Variables

The dependent variable was defined as the *T. evansi* antibody titer based on the optical density (OD) values obtained from ELISA. The independent variables were determined based on the sample selection criteria: body condition score (BCS) <3, animal availability, and owner consent. The control variables included the TSA antigen, anti-IgG-HRP secondary antibody, ELISA procedure, incubation time and temperature, 1:800 serum dilution, and the use of positive and negative controls.

ELISA Procedure

From a total population of 46 sheep, eight were selected as research samples based on the criteria (Sugiyono, 2019). Blood samples were collected from the jugular vein using a sterile syringe, placed in collection tubes, and allowed to stand until the serum was formed. The serum was separated from the blood clot, stored under refrigerated conditions, and then sent to the Veterinary Product Quality Research and Testing Center (BRMP Veteriner) in Bogor for testing of *T. evansi* antibodies using the ELISA method. The ELISA method is one of the most widely used serological methods for detecting antibodies because of its high sensitivity and specificity (Subekti & Yuniarto (2020). The entire testing process was conducted in the laboratory in accordance with the applicable standard operating procedures (SOPs). The test results were read using an ELISA reader and reported as qualitative interpretations (positive/negative) based on the laboratory cutoff value (S/N ≤ 2). Quantitative values, such as Optical Density (OD) or the sample-to-negative (S/N) ratio for each sample, were not included in the laboratory test result report.

Data Analysis

Data obtained from *T. evansi* antibody testing in sheep blood using the ELISA method were quantitatively analyzed using a Sample-to-Negative Ratio (OD S/N) value of ≤ 2. The results of the analysis are presented in the form of a descriptive analysis to illustrate the conditions observed in the samples. The discussion of the results is supported by relevant literature studies as the basis for the scientific interpretation.

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RESULTS AND DISCUSSION

Results

The detection of *T. evansi* antibodies in sheep was performed by collecting blood samples as the initial step in the serological testing process. ELISA testing was conducted by a reference laboratory, the and results were reported as qualitative interpretations (positive/negative) based on the laboratory's cut-off value (S/N ≤ 2). Quantitative values, such as optical density (OD) or the sample-to-negative (S/N) ratio for each sample, were not included in the laboratory test report. Based on the serological test results, all sheep blood samples were negative for *T. evansi* antibodies. These negative results indicate that no serological reaction was detected, suggesting the absence of specific antibodies against *T. evansi* in the blood samples tested. This indicates that no antigen-antibody binding occurred that reached the reactivity threshold established in the testing procedure (Subekti and Yuniarto, 2018). The negative statuses of all samples are presented in Table 1.

Discussion

During the sample collection and testing period, no serological evidence of exposure to *T. evansi* was observed in the sheep. Negative ELISA results indicated that no detectable antibodies against *T. evansi* were present in the examined sheep at the time of sampling. However, negative serological results do not completely exclude recent infection or exposure, particularly during the early phase before detectable antibody production. ELISA is primarily used to detect the presence of antibodies, not to directly detect the presence of parasites in the blood (Subekti & Yuniarto (2020). Negative ELISA results do not entirely rule out the possibility of infection in the early stages, when antibodies have not yet been optimally produced. Subekti & Yuniarto (2020) explained that antibodies against *T. evansi* can persist for a considerable time in the bloodstream. These negative results indicate that the examined sheep most likely had no history of prior infection, as the ELISA method is highly relevant for interpreting serological test results.

The ELISA tests conducted on sheep blood samples showed that no *T. evansi* antibodies were detected. This finding may be associated with the relatively good husbandry practices observed at the study farm, including adequate pen sanitation and maintenance of a clean rearing environment. Good feed management also plays a role in maintaining the physiological condition and immune resistance of sheep (Bobeck, 2020). Control of mechanical vectors, particularly blood-sucking flies, which are vectors for *T. evansi* transmission, is also believed to be a key factor in reducing the incidence of trypanosomiasis in livestock, especially in sheep (Gebeyehu & Robi, 2024). Although all samples tested negative, these findings provide important information regarding the serological status of *T. evansi* in the sheep studied and underscore the importance of husbandry management and vector control as preventive measures against trypanosomiasis. Overall, these optimal husbandry conditions can reduce the risk of Trypanosoma transmission in sheep. The intensive rearing system at the study farm may have reduced opportunities for contact between sheep and mechanical vectors, which could contribute to the absence of detectable antibodies.

Local epidemiology is an important consideration for interpreting negative results from *T. evansi* antibody detection tests using ELISA. Information on the epidemiology of trypanosomiasis in the study area has not been reported previously. Warsito *et al.* (2025) explained that the The absence of detectable antibodies may indicate a low level of exposure among the sampled sheep; however, it does not exclude the possibility of active infection or recent exposure. This condition is believed to be related to the absence of reported trypanosomiasis outbreaks or similar clinical cases in recent years, resulting in a relatively low

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likelihood of natural exposure among the sheep population (Firdausy *et al.*, 2025). Livestock movement and traffic within the study area also play a role in shaping local epidemiological patterns (Kengradomkij *et al.*, 2025). The implementation of a relatively closed husbandry system, minimal introduction of livestock from outside the region, and the existence of quarantine or health inspections prior to the introduction of new livestock are believed to reduce the risk of disease agent introduction, thereby limiting the likelihood of *T. evansi* introduction from endemic areas into the local livestock population (Pandjukang *et al.*, 2024).

The negative ELISA results for *T. evansi* antibodies in sheep in this study may have been influenced by various factors. The timing of blood sampling plays a crucial role in determining antibody detection results, as seroconversion or antibody formation does not occur immediately after exposure to parasites (Parra-Giménez & Reyna-Bello (2019). Specific antibodies are often not formed in sufficient quantities during the early stages of infection. These specific antibodies can be detected using ELISA; therefore, test results may show negative values even when exposure or mild infection has occurred Subekti & Yuniarto (2020).

Interpretation of ELISA results must consider the animal's immunological condition and phase at the time of blood sampling, particularly if the animal has recently been exposed to an infectious agent or is still in the early phase before antibody production reaches optimal levels Subekti & Yuniarto (2020). The timing and magnitude of the antibody response may influence the interpretation of serological results, as parasite-specific antibody responses develop dynamically during *T. evansi* infection (Onah *et al.*, 1999; Parra-Giménez & Reyna-Bello, 2019). *T. evansi* may modulate host immune responses through mechanisms involved in immune evasion (Wei *et al.*, 2021). Consequently, the levels of antibodies produced may fall below the detection limit, resulting in negative ELISA results. This underscores that the timing of sample collection reflects the dynamics of the immune response and significantly influences the accuracy of serological diagnoses. In addition to the timing of sample collection and the phase of infection, Bobeck (2020) explained that differences in immune responses among individual sheep, influenced by age, nutritional status, and physiological condition, can also affect antibody production levels; thus, these factors may influence test results. Sheep with suboptimal immune systems tend to produce low antibody titers (Bobeck, 2020). Technical factors, such as the sensitivity and specificity of the ELISA method, particularly those related to the type of antigen used, also determine the success of antibody detection. This is because antigenic variation in *T. evansi* can prevent optimal antibody binding (Jones & McKinnell).

The ELISA method has high sensitivity and specificity for detecting *T. evansi* antibodies, making it suitable for screening large numbers of samples (Subekti & Yuniarto, 2020). In contrast, blood smears have low sensitivity in cases of low parasitemia or chronic infection; the CATT test cannot distinguish between active infection and prior exposure to *T. evansi*; and although PCR is the most sensitive method for detecting *T. evansi* DNA, it requires molecular laboratory facilities and is more expensive (Kim *et al.*, 2023). Therefore, ELISA is considered suitable for determining the serological status of sheep, although combining it with parasitological and molecular methods would provide more comprehensive diagnostic results.

This study had several limitations that should be considered when interpreting the results. First, testing was conducted using only the ELISA method; therefore, detection was limited to antibodies against *T. evansi* and was not accompanied by antigen testing or DNA detection using molecular methods, such as PCR. Second, the sample size was relatively small (eight sheep); therefore, the study results cannot reflect the condition of the sheep population at large. Third, the study was conducted at only one farm; therefore, variations in management practices, environmental factors, and vector exposure levels at other locations cannot be compared. Furthermore, this study is a preliminary study aimed at providing an initial overview of the

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serological status of *T. evansi* in sheep at the study sites. Therefore, follow-up studies with a larger sample size, broader farm coverage, and a combination of diagnostic methods, such as blood smear, CATT, ELISA, and PCR, are needed to obtain a more comprehensive epidemiological picture.

CONCLUSIONS AND SUGGESTIONS

Conclusions

Based on the results of serological testing using the ELISA method, all blood samples from the eight sheep at Her's Farm tested negative for *Trypanosoma evansi* antibodies. These findings indicate that no serological evidence of exposure to *T. evansi* was observed in the tested samples.

Suggestions

Future studies should use a larger sample size, involve more farm locations, and combine serological methods with molecular methods, such as polymerase chain reaction (PCR) or direct parasite detection methods, to improve diagnostic accuracy and obtain a more comprehensive epidemiological picture of *T. evansi*.

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Table

Table 1. ELISA test results for *T. evansi* antibodies in sheep from Her's Farm with OD S/N values ≤ 2

No.	Sample Code	Test Result
1.	D-01	Negative
2.	D-03	Negative
3.	D-04	Negative
4.	D-05	Negative
5.	D-06	Negative
6.	D-07	Negative
7.	D-08	Negative
8.	D-09	Negative

Note: Sample code prefix 'D' denotes sheep.