Development of In-house DNA Extraction Method for *Trypanosoma evansi* from Dried Blood Samples on FTA Cards

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Abstract

Background: Surra, caused by *Trypanosoma evansi*, is an important parasitic disease that affects animal health and livestock production worldwide, including in Thailand. PCR-based diagnostics offer high sensitivity, capable of detecting the parasite even at low concentrations. However, field application remains limited due to the need for transporting fresh blood samples under controlled temperature conditions and the high cost of commercial extraction kits. Dried blood spots (DBS) on storage cards offer a practical alternative; nevertheless, efficient and cost-effective DNA extraction methods from this matrix remain limited. Therefore, the development of an in-house extraction method suitable for DBS is essential to enhance accessibility and support large-scale surveillance.

Objectives: This study aimed to develop and evaluate methods for extracting *T. evansi* nucleic acids from dried blood on FTA cards.

Methods: An isolate of *T. evansi* (archived at NIAH, Thailand) was serially diluted in confirmed negative bovine blood to obtain concentrations ranging from 10^1 to 10^6 parasites/mL. Each dilution was spotted onto FTA cards. DNA was extracted from three 3-mm punches of each DBS using seven methods, including a commercial kit and six modified protocols: (1) SDS–Proteinase K; (2) Proteinase K-Chelex; (3) SDS-TE (95°C); (4) SDS-TE (room temperature); (5) TE-Chelex; and (6) TED10(TE+10% DMSO)-Chelex. PCR targeting the *T. evansi*-specific repetitive sequence (TBR1/TBR2 primers; 164 bp) was performed under standard conditions. Testing with positive control samples was performed to determine the limit of detection (LOD), which all three replicate samples were consistently detected.

Results: The TE–Chelex method achieved a limit of detection of 10^3 parasites/mL, which was comparable to that of the commercial extraction kit. The TED10-Chelex method showed similar sensitivity but required more complex processing steps. The SDS–Proteinase K method and the Proteinase K–Chelex method showed lower sensitivity, with LOD values of 10^6 parasites/mL. The SDS–TE methods at both temperatures did not yield detectable amplification under the conditions tested.

Conclusion: The developed in-house TE-Chelex extraction method is a reliable and cost-effective alternative for the molecular diagnosis of *T. evansi*. It provides comparable sensitivity to a commercial kit while offering a simpler workflow and reduced reagent requirements.

Keywords: *Trypanosoma evansi*, PCR, DNA extraction, Dried blood spot, FTA card

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