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DEVELOPMENT OF TRYPANOSOMA EVANSI INFECTION DETECTION USING ELECTRONIC TONGUE AND MACHINE LEARNING COMBINATION METHOD

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ABSTRACT

Trypanosoma evansi is a parasite that can affect animal development and cause economic losses. Diagnosis of infection T. evansi generally based on anamnesis, clinical examination, and conventional methods, but these methods have low sensitivity so it is necessary to develop methods that are faster, more sensitive, and can be applied in the field**.** The research aims to develop infection detection T. evansi by using a combination of electronic tongue biosensors and machine learning. E-tongue and machine learning test results in blood samples resulted in a total variance of PCA of 75.8% and LDA of 97.96%, while in serum samples PCA was 40.52% and 97.46%. Accuracy of total LDA performance for blood samples is 100% and serum is 100%. The combination of e-tongue and machine learning can detect blood and serum samples from infected and uninfected mice T. evansi based on PCA and LDA data pattern plots.

Keywords: Trypanosoma evansi; Electronic Tongue, Machine Learning, PCA, LDA, Biosensor

Abbreviations: WBF: Wet Blood Film; TBS: Thin Blood Smear; HCT: Haematocrit Centrifugation Technique, PCR: Polymerase Chain Reaction, PCA: Principal Component Analysis, LDA: Linear Discriminant Analysis.

1. INTRODUCTION

Surra is a strategic infectious animal disease in Indonesia which causes many deaths in animals, especially horses and buffalo. Surra is caused by the blood protozoan Trypanosoma evansi (Subektiet al. 2018), and causes economic losses, affecting animal development and human health (Hassan-Kadleet al. 2019). Various methods for detecting Trypanosoma infections have been developed but generally still have weaknesses. Method Polymerase Chain Reaction (PCR) requires trained personnel, a suitable laboratory environment, and is difficult to use in most field conditions (Liet al. 2020). The inoculation method on experimental animals is less practical because it requires many experimental animals (Reidet al. 2001). The ELISA and CAT methods can cross-react with non-specific antibodies caused by other infections and the presence of specific antibodies for weeks-weeks or months after successful treatment (Benfodilet al. 2020).

Electronic tongue is an electrochemical-based biosensor consisting of one or more array sensors that are non-specific, cross-sensitive, and less selective. Electronic tongue combined with machine learning (chemometric tools) with the aim of forming multivariate statistical data patterns so that sensor signals can be analyzed (Veloso et al. 2018). Biosensor e-tongue in the medical field has been used to test biological fluids such as urine, blood, and sweat c. Based on the literature, infection T. evansi causes changes in blood composition, including a decrease in glucose, BUN, Creatinine (Darwish et al. 2019), creatinine kinase, myoglobin, give dairy dehydrogenase (Baldissera et al. 2016), creatine kinase myoglobin band (El-Deeb and Elmoslemany 2015), and blood electrolytes (Garba et al. 2016), (Abuessailla et al. 2017). Existing research shows that e-tongue and machine learning can be used to diagnose bacterial infections (Al Ramahi et al. 2019), urea level detection, creatinine, system dysfunction urinary (Gutierrez et al. 2008), diagnosis of prostate cancer patients (Pascual et al. 2016), and urea level detection, creatinine in the post fluid dialysate (Al Ramahi et al. 2019). Combination-tongue and machine learning this is a new tool and there have been no publications regarding the biosensor method for detecting infection T. evansi. This research aims to develop a combination method-tongue and machine learning as an alternative to detect infection T. evansi.

2. MATERIALS AND METHODS

2.1. Experimental Animals

Thirty mice (*Rattus norvegicus*) were purchased from the Integrated Research and Testing Laboratory (LPPT) Gadjah Mada University for experiments. Mice were placed in standard mouse cages in the diagnostic laboratory, Department of Parasitology, Faculty of Veterinary Medicine, Gadjah Mada University, Yogyakarta, Indonesia. The animals were allowed to acclimate for one week during which they were screened for hemoparasite infections. They were fed with A.D. II® (PT. Japfa Comfeed Indonesia, Tbk) and water was provided ad libitum.

2.2 Parasite

The parasite used is Trypanosoma evansi Kalimantan isolate obtained from the Banjarbaru Veterinary Center. Parasites were multiplied in mice by inoculating intraperitoneally and monitored until the development of parasitemia reached 20-30 parasites per field according to WBF.

2.3. Experimental design

Mice were grouped into five groups based on the level of parasitemia using the WBF method (positive 1 (K_2), positive 2 (K_3), positive 3 (K_4), positive 4 (K_5), and uninfected controls (K_1)). Each group contains six mice with tags a, b, c, d, e and f. Before experimental infection, the mice had blood drawn via the lateral tail vein. Mice were screened for hemoparasite infection using WBF and TBS.

2.4. Experimental Infection

Monitor the development of parasitemia (20-30 parasites per field) T. evansi, 1 ml of rat blood was collected and diluted with 8 ml of physiological saline to reach a total volume of 9 ml. Using a 1 ml syringe for each rat from group K_2, K_3, K_4, and K_5 inoculated with each 0.3 ml of diluted blood estimated to contain 1 x 10^4 parasites, while group K_1 served as an uninfected control. The control group was injected with a physiological solution using the same route. The concentration of inoculated flagellates was determined using a haemocytometer (Neubauer Improved) [15]. Experimental animals were allowed to go through the course of infection.

2.5. Detection and Estimation of Parasitemia

Trypanosomes were detected by WBF, MHCT, TBS and PCR.

2.5.1. Collection of Blood Samples.

Blood was taken aseptically from each mouse via the lateral tail vein every day after infection until the parasitemia level reached positive 1 (K_2), positive 2 (K_3), Positive 3 (K_4), Positive 4 (K_5) WBF basis. Blood was also taken aseptically from each mouse intracardially, 6 ml of blood was collected. 3 ml of blood for PCR and e-tongue testing. 3 ml of blood was centrifuged to separate the serum for e-tongue and LCMS testing.

2.5.2. Wet Blood Film (WBF)

A total of 2µl of blood was dropped on the surface of a clean glass object and then covered with deck glass. Examination with a microscope with 100x magnification to detect motile trypanosomes [16]. WBF test parameters according to Ekawasti et al., (2015) [17] which have been modified as follows: positive 1: one visual field randomly shows 1-10 animals T. evansi that moves; positive 2: one field of view randomly shows 10-20 individuals T. evansi which moves. Positive 3: one field of view randomly shows more than 20 individuals T. evansi moving (parasites are not too

dense), positive 4: one visual field is randomly seen T. evansi in large numbers and dense with active movement.

2.5.3. Haematocrit Centrifugation Technique (HCT)

Blood was taken using hematocrit and blocked at one end. Centrifuge hematocrit 3000 g for 5 minutes. Trypanosomes appear to be concentrated at the junction between buffy coat and plasma using a 40x magnification microscope [16].

2.5.4. Thin Blood Smear (TBS)

One drop of blood is placed on the surface of the glass object, then the blood is wiped off using another glass object. The preparations were dried and fixed with methanol for 1 minute. Next, the preparation was dipped in Giemsa solution (one drop of Giemsa + 1 ml PBS, pH 7.2) for 25 minutes. The preparations were washed with tap water and air-dried until dry. To observe the results, the preparations were dropped with immersion oil and examined under a microscope with 100x magnification [16].

2.5.5 Polymerase Chain Reaction (PCR)

DNA Extraction. DNA extraction was carried out according to standard DNA extraction procedures from DNeasy® Blood & Tissue Kits (Qiagen, USA).DNA Amplification. The total volume of DNA amplification was 25 µl consisting of 4 µl of sample, 12.5 µl of MyTaq™ HS Red Mix (Meridian, Bioscience®), 1 µl (10 pmol primer ITS1 F), 1 µl (10 pmol primer ITS1 R), and 6.5 µl Nuclease Free Water (Thermo Scientific™). The primers used for DNA amplification were based on Njiru et al., (2005) namely Internal Transcribed Spacer 1 Forward 5'- CCG GAA GTT CAC CGA TAT TG-3' and Reverse 5' TTG CTG CGT TCT TCA ACG AA-3' (PT. Genetika Science Indonesia) [18]. PCR conditions: initial denaturation at 95°C (60 seconds); denaturation at a temperature of 95°C (40 seconds), annealing at a temperature of 56°C (40 seconds), extension at a temperature of 72°C (45 seconds) were carried out for 35 cycles and termination at a temperature of 72°C (300 seconds).Electrophoresis. Electrophoresis was performed on 2% agarose (GeneDirex®, USA), 100 volts for 45 minutes. DNA sequencing. DNA sequencing using a thermal cycler/PCR machine (Veriti 9700 ProFlex/BioRad T100), sequencing machine (Applied Biosystems 3500 Genetic Analyzer 2500) and Scoring/base caller is done with SeqA software.

2.6. Electronic Tongue and Machine Learning

2.6.1. Making Working Electrodes.

The working electrode is composed of twenty-eight silver electrodes (thin layers) coated with coating with technique dipping with a combination of lipids (Tetradodecylammonium bromide (Sigma-Aldrich, Switzerland), Methyltrioctylammonium chloride (Sigma-Aldrich, Switzerland), Oleic Acid Vegetable (Merck-Germany), Gallic acid (Sigma-Aldrich, China), 1-Hexadecanol

(Sigma-Aldrich, Germany), Octadecylamine (Sigma-Aldrich, Switzerland), 1,2,4-Benzenetricarboxylic acid (Sigma-Aldrich, Switzerland)) dan Plasticizer (2-Nitrophenyl octyl ether (Sigma-Aldrich, Switzerland), Until(2-ethylhexyl) sebacate (Sigma-Aldrich, Germany), Until(1-butyl pentyl) adipate (Sigma-Aldrich, Switzerland), Dioctyl phenylphosphonate (Sigma-Aldrich, India)) dissolved in Tetrahydrofuran (Merck, Germany). Combination of 7 and 4 lipids Plasticizer on the sensor membrane-tongue (Table 1). Silver electrodes (thin layer) connected to silver wires (purity 99.9%). Each silver cable at the sensor end is connected to a copper cable to connect to the data Logger. All silver connections are wrapped with Poly (Vinyl Chloride) (PVC) Sigma-Aldrich, Switzerland) to prevent damage.

Table 1. Composition of the sensor membrane-tongue. All materials in PVC (32%).

Sensor	Lipid (3%)	*Plasticizer* (65%)
S1	Tetradodecylammonium bromide	2-Nitrophenyl octyl ether
S2		Bis(2-ethylhexyl) sebacate
S3		Bis(1-butylpentyl) adipate
S4		Dioctyl phenylphosphonate
S5	Methyltrioctylammonium chloride	2-Nitrophenyl octyl ether
S6		Bis(2-ethylhexyl) sebacate
S7		Bis(1-butylpentyl) adipate
S8		Dioctyl phenylphosphonate
S9	Oleic Acid Vegetable	2-Nitrophenyl octyl ether
S10		Bis(2-ethylhexyl) sebacate
S11		Bis(1-butylpentyl) adipate
S12		Dioctyl phenylphosphonate
S13	Gallic acid	2-Nitrophenyl octyl ether
S14		Bis(2-ethylhexyl) sebacate
S15		Bis(1-butylpentyl) adipate
S16		Dioctyl phenylphosphonate

S17	1-Hexadecanol	2-Nitrophenyl octyl ether
S18		Bis(2-ethylhexyl) sebacate
S19		Bis(1-butylpentyl) adipate
S20		Dioctyl phenylphosphonate
S21	Octadecylamine	2-Nitrophenyl octyl ether
S22		Bis(2-ethylhexyl) sebacate
S23		Bis(1-butylpentyl) adipate
S24		Dioctyl phenylphosphonate
S25	1,2,4-Benzenetricarboxylic acid	2-Nitrophenyl octyl ether
S26		Bis(2-ethylhexyl) sebacate
S27		Bis(1-butylpentyl) adipate
S28		Dioctyl phenylphosphonate

The reference electrode uses the Ag/AgCl HI70725 double junction reference electrode® (Hanna Instruments, USA).

2.7 Sample testing

EDTA blood and serum samples were placed at room temperature, then homogenized using an inversion technique by inverting the tube 8-10 times to obtain accurate results according to actual conditions (Dacie and Lewis 1991). A total of 0.1 ml of blood or serum sample is placed in Baker glass measuring 50 ml. PBS (PBS Buffer, Abclonal®) was added to reach a final volume of 10 ml. This is done to ensure that the sensor device is completely submerged in the sample solution. Potentiometric measurements were carried out with a modification of the method (Tazi et al. 2018) namely for 60 seconds and replicated 10 times for each sample to ensure the stability and repeatability of each sensor. Every time the sample is tested, the sensor is rinsed using 2 solutions, the first is a 96% ethanol solution: 0.01M HCl solution of 3:7 and the second is using distilled water to ensure it is free from the previous sample (Zhang et al. 2015). Testing carried out-tongue in 0.01 M HCl solution at the beginning and end of the test for each different sample to ensure stability-tongue.

2.8. Machine Learning

The matrix data used for analysis is the signal profile-tongue (28 lipid/polymeric membrane sensors) recorded on each sample. PCA and LDA techniques were applied to reduce 28 sensors to a small number of new variables and visualize and classify sample group data into positive (infected) and negative (healthy).

3. RESULTS

3.1. Wet Blood Film (WBF), Haematocrit Centrifugation Technique (HCT), Thin Blood Smear (TBS)

Parasitemia T. evansi visible through HCT methods and WBF with random results on 3 K mice$_2$ starting on day 4 post infection. Parasitemia based on the results of HCT, WBF, and TBS examinations in group K_2, K_3, K_4, and K_5 respectively occurred on days 5, 6, 7, and 8 after infection. Group K_1 negative for parasitemia on day 8 after NaCl injection. Total blood sampling K_1 8th day, K_2, K_3, K_4, and K_5 respectively occurred on days 5, 6, 7, and 8. Results of layer examination buffy coats K_2, K_3, K_4, and K_5 with a microscope can be seen T. evansi and movement, while K1 doesn't appear to exist T. evansi. TBS K examination results$_1$ not available Trypanosoma sp. while K_2, K_3, K_4, and K_5 it seems to exist Trypanosoma sp. shaped like a leaf with a sharp tip, it has a long flagella with an oval cell nucleus in the middle of the body and there is a kinetoplast at the sub terminal and visible undulant membrane (Figure 1).

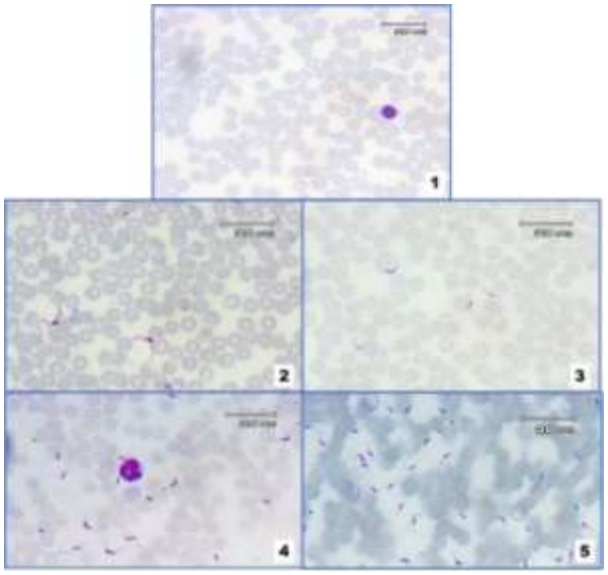

Figure 1. TBS examination results. (1): group 1; (2): Group 2; (3): group 3; (4): Group 4 and (5): Group 5. (1): not available Trypanosoma sp. (2, 3, 4 & 5): It appears to be Trypanosoma sp long slime shaped, nucleus in the center, and the presence of flagella between the red blood cells (1000x magnification).

The results of the PCR product electrophoresis were positive T. evansi on K_2, K_3, K_4, K_5 and negative on K_1 (Figure 2). Electrophoresis results from PCR testing to form DNA bands with amplicon sizes in the range of 480 bp (K_2, K_3, K_4, and K_5 (in a row (2a-5f)).

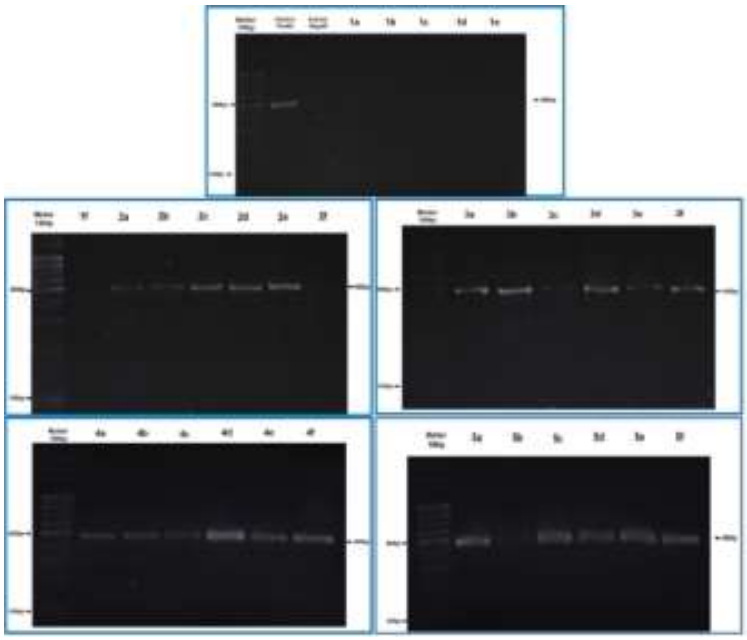

Figure 2. Electrophoresis results of ITS1 DNA amplification T. evansi using 100bp marker (left). 1a – 1f: no band visible. 2a– 5f: visible band (480bp).

Electronic tongue used is made of 28 sensors, which are divided into 2 sensor devices with codes 2-1 (sensors 1-16) and 2-2 (sensors 17-28) (Figure 3).

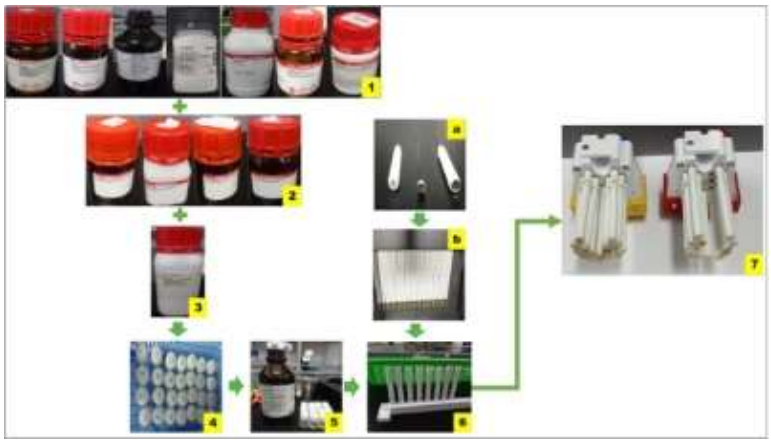

Figure 3. Manufacturing process-tongue. (1) Sensor membrane lipid composition, (2) composition Plasticizer sensor membrane, (3) PVC, (4) all sensor membrane materials in glass bottles (5) THF, (6) Sensor array, (7) Final result-tongue, (a) Fabrication of the sensor body, (b) Sensor array.

The stability of the sensor response was observed in each test. The results show a straight line which indicates that the sensor developed is quite stable (Figure 4).

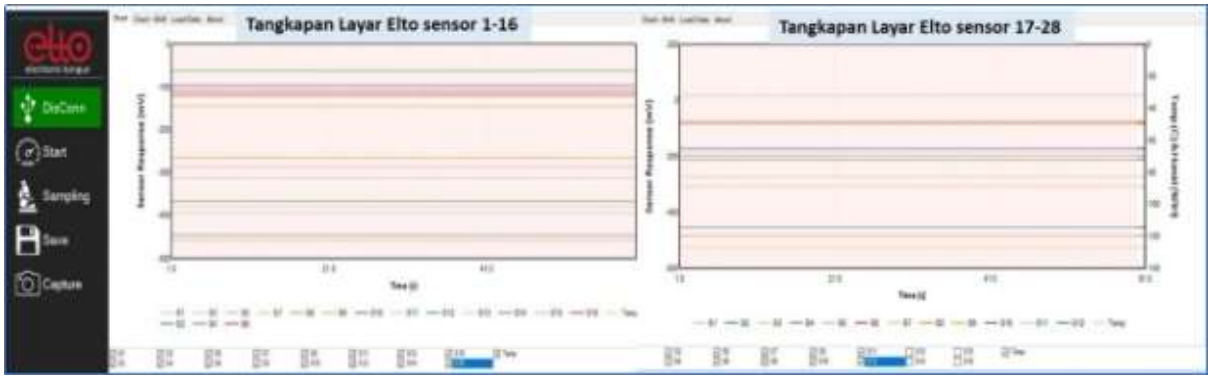

Figure 4. Screenshot of the Elto® application during testing. A straight line is visible, indicating that the tongue is stable during use in the testing process.

Blood sample testing results with-tongue saved in .csv (excel) form, then the data is processed automatically by chemometric machine learning (Software Spyder, Phyton®). The results show that the sensor response (mV) is different between samples (Figure 5).

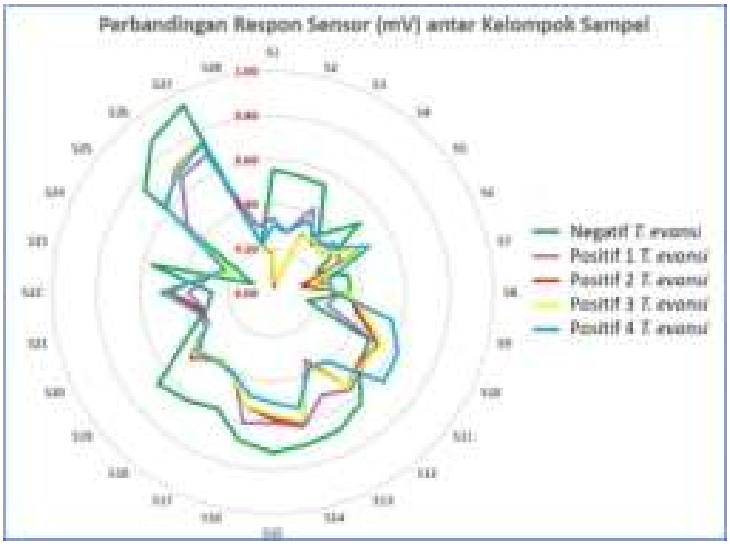

Figure 5. Radar plot illustrating the comparison of sensor responses (mV) between sample groups.

Based on the radar graph, it can be seen that each sensor produces a different potential response pattern for each taste (Figure 5). The composition and type of membrane lipids have a major influence on these potential differences. The process that occurs in the membrane when one component is removed is faster than another component based on the differences in the physical and chemical properties of the membrane and the component being separated. The results of PCA

analysis show separate plots between negative and positive samples T. evansi (positive 1, 2, 3, and 4). PCA analysis produced a total variance of 75.80% with a PC1 value of 55.58% and PC2 20.22%. Group 1 plots are quite separate from the other groups, but between positive sample groups T. evansi not clearly separated (Figure 6).

LDA analysis produced a total of 97.96% of the variance with an LD1 value of 94.46%, which means there is a first linear function that produces maximum separation from 5 groups. LD2 is 3.5%, namely the linear function reaches a maximum separation of 21.77% among all linear functions that are perpendicular to LD1. K blood sample plot$_1$ (negative T. evansi) separately from the group K plot$_2$, K$_3$, K$_4$, and K$_5$ (positive T. evansi) (Figure 6). Based on these results, analyzing chemometric LDA is suggested as an analysis chemometric which provides better separation results between groups than PCA.

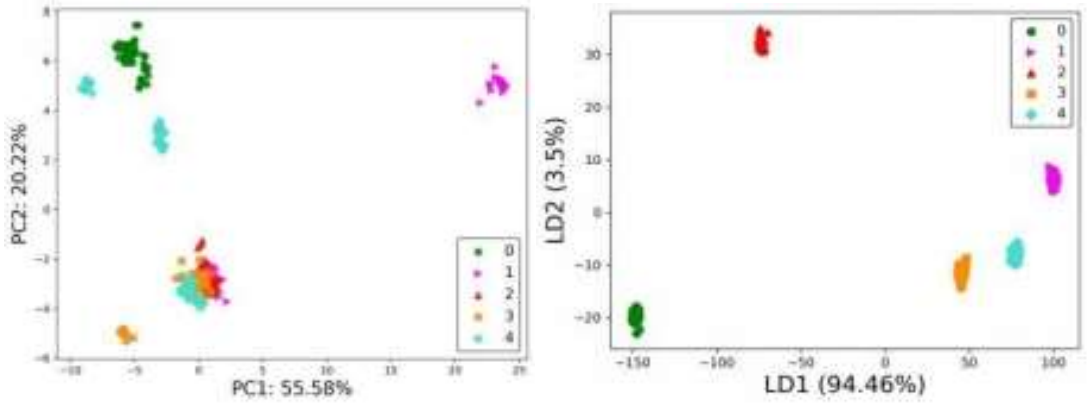

Figure 6. Analysis results of chemometrics PCA and LDA against blood samples.

LDA data is divided into data training and data test (75:25), this was done to determine the level of accuracy of testing blood samples white-tongue. Performance-tongue and chemometric LDA produces an accuracy rate of 100% (Table 2).

Table 2. Performance-tongue and chemometric LDA

	Reference				
Prediction	**Negative**	**+1 *T. evansi***	**+2 *T. evansi***	**+3 *T. evansi***	**+4 *T. evansi***
Negative	60	0	0	0	0
+1 *T. evansi*	0	60	0	0	0
+2 *T. evansi*	0	0	60	0	0
+3 *T. evansi*	0	0	0	60	0
+4 *T. evansi*	0	0	0	0	60

Serum sample test results white-tongue which has been processed with chemometric machine learning PCA and LDA show separate plots between negative and positive samples (positive 1, 2, 3, and 4) T. evansi. PCA analysis produced a total of 40.52% of the variance with a

PC1 value of 23.2% and PC2 17.32%. Plot K1 is quite separate from other groups, but among the positive sample groups T. evansi not clearly separated (Figure 7).

LDA analysis produced a total of 97.46% of the variance with an LD1 value of 94.99% and LD2 of 2.47%. Plot of blood sample group 1 (negative T. evansi) separate from plot K_2, K_3, K_4, and K_5 (positive T. evansi) (Figure 7). Based on these results, LDA is recommended as an analysis chemometric which provides better separation results between groups than PCA.

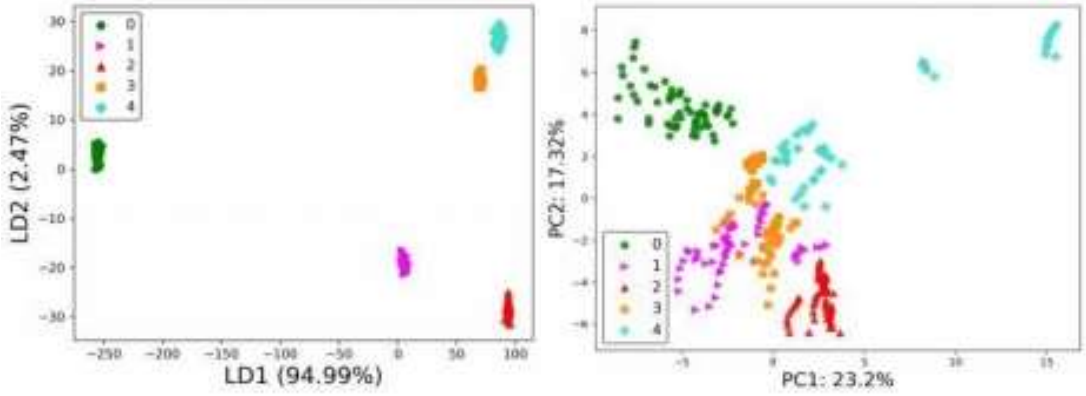

Figure 7. Analysis results of chemometrics PCA and LDA against serum samples.

Table 3. Performance-tongue and chemometric LDA of serum samples.

	Reference				
Prediction	**Negative**	**+1 *T. evansi***	**+2 *T. evansi***	**+3 *T. evansi***	**+4 *T. evansi***
Negative	60	0	0	0	0
+1 *T. evansi*	0	60	0	0	0
+2 *T. evansi*	0	0	60	0	0
+3 *T. evansi*	0	0	0	60	0
+4 *T. evansi*	0	0	0	0	60

4. DISCUSSION

The HCT method is able to detect parasitemia on the 4th day after infection because it has a detection limit of 10^2-10^3 parasites/ml, more sensitive than TBS, namely 10^5 -10^6parasit/ml (Desquesnes et al. 2013). Morphological characteristics Trypanosoma sp. as described by Desquesnes et al., (2013), when observing morphology Trypanosoma sp. in blood smear preparations with Giemsa staining, they are long slime-shaped, the nucleus is located centrally, the kinetoplast is subterminal and there are flagella that extend from anterior to posterior. The TBS method is a method that is quite difficult to use to differentiate species Trypanosoma sp. based on morphology.

This is in accordance with Njiru's researcher al., (2005) which states the PCR product T. evansi and T. brucei 480 bp. The varying lengths of ITS segments between species and interspecies

make the ITS region a very useful molecular marker for identifying mixed Trypanosoma species infections. (Desquesnes & Dávila, 2002). BLAST sequencing analysis of samples was carried out online on the website http://www.ncbi.nlm.nih.gov, shows that the results are negative in sample K_1 (1a-1f) and positive on K_2, K_3, K_4, and K_5 (in a row (2a-5f)) (Figure 2). The PCR technique can detect 1 trypanosoma/ml of blood or 1 pg of DNA Trypanosoma in host DNA (Njiru et al., 2005).

The selected lipid polymer membranes produce different interactions with the sample via electrostatic or hydrophobic interactions, due to the different composition of the polymer membranes. The lipids used produce hydrophobic and hydrophilic interactions with target molecules. Plasticizer serves to increase the absorption of solutes by softening the PVC membrane, and contributes to the interaction mechanism through polar and nonpolar components (Arca et al. 2019).

LDA data is divided into data training and data test (75:25), to determine the level of accuracy of testing serum samples white-tongue. Performance-tongue and chemometric LDA on serum samples produced an accuracy rate of 100% (Table 3).Chemometric PCA is characteristic unsupervised, able to simplify the complexity of high-dimensional data while maintaining patterns or changing data into simpler dimensions (Leveret al. 2017), whereas Chemometric LDA is of a nature supervised, classifying data distribution by minimizing the distance within the same data class and maximizing the distance between different data classes so that the data will be more grouped (Karakayaet al. 2020).

The active components used in 28 sensors have the ability to interact with a group of compounds. The active components are grouped into (1) tetradodecylammonium bromide; (2) methyltrioctylammonium chloride; (3) oleic acid; dan (4) gallic acid (5) 1-Hexadecanol (6) octadecylamine; (7) 1,2,4-Benzenetricarboxylic acid. Tetradecyl Ammonium Bromide is a quaternary ammonium salt that is often used to develop anion selective electrodes. Tetradecyl Ammonium bromide has high lipophilicity (El-Ragehy et al. 2018). Tetradecyl Ammonium bromide responds to sweet compounds such as glucose and sucrose (Kobayashi et al. 2010). Methyltrioctylammonium chloride as an organic cationic molecule it will respond to chloride ions or anions. Compound Oleic acid responds to basic compounds (Tazi et al. 2017). Gallic acid responds to sweet compounds such as glucose and sucrose (Kobayashi et al. 2010). 1-Hexadecanol responds to salty compounds such as NaCl [30].Octadecyl amine as a base it will respond to acid components or with cations (ion-dipole interactions) (Taziet al. 2017).

Differences in blood and serum sample plots using PCA, LDA and radar plots can be directly related to differences in the composition of blood/serum from negative mice. T. evansi (K_1) and positive T. evansi (K_2, K_3, K_4, and K_5). This is possibly due to parasites T. evansi in group K_2, K_3, K_4, and K_5 and the absence of parasites T. evansi in group K_1 thus causing the sensor to produce different potential values. Based on Lejon et al., (2019), the surface of Trypanosomes is less negatively charged compared to erythrocytes, leukocytes and platelets. The main component that causes the surface charge of Trypanosoma to be negative is sialic acid which binds to

glycoproteins, glycolipids and phosphate groups (Souto-Padron 2002). Apart from the existence of cells T. evansi, plot differences may also be due to T. evansi which can cause changes in blood composition include blood glucose levels, blood ion/electrolyte concentrations (calcium, phosphorus, bicarbonate), number of erythrocytes, leukocytes, platelets, blood lipids. This is in accordance with research (Bougrini et al. 2014), which states that groups of compounds that can contribute to sample discrimination bye-tongue namely minerals such as calcium, sugars such as fructose and glucose, proteins and lipids.

Decreased blood glucose levels due to infection T. evansi observed in several studies (Misra et al. 2016; Parashar et al. 2018; Reck et al. 2020). Host glucose is the primary energy source T. evansi via glycolysis (Rivero et al. 2016). In mild infections T. evansi, most of the host's glucose is used to produce energy and results in the release of pyruvate. In severe infections T. evansi, fructose, mannose, and glycerol provide additional energy sources besides glucose, and result in the release of succinate, alanine, and acetate (Moreno et al. 2015). Decreased number of erythrocytes due to infection T. evansi (Misra et al. 2016; Elshafie et al. 2018; Hussain et al. 2018), caused by erythrocyte "injury" due to whipping of trypanosoma flagella, pyrexia undulant platelet aggregation, toxins and metabolites trypanosome, lipid peroxidation and malnutrition [41]. The decrease in platelet count is thought to be due to a cytotoxic reaction due to the formation of immune complexes which ultimately causes disruption in the blood clotting mechanism. The decrease in platelet count is related to the number trypanosomes per unit of blood [42]. (Increased leukocyte count in infection T. evansi [36], [40], due to an increase in the first immunological response followed by an immunosuppressive effect trypanosome because it is influenced by changes in the variable surface glycoprotein of the infecting Trypanosoma [43]. Changes in blood ion levels, namely a decrease in calcium, Bicarbonate (HCO^{3-}) [11], phosphor [12]. Calcium is very important for the life of trypanosomes. Trypanosomes are adapted to store calcium, so the host's blood calcium decreases. Declinephosphor maybe it's because of hypoxia which is due to lower phosphorus intake [44] or due to renal excretion [12]. Decreased bicarbonate caused by renal excretion [11]. Hyperlipidemia and hypercholesterolemia were observed in positive animals T. evansi, the decrease in lipids in the blood is thought to be due to the utilization of lipid molecules by trypanosomes. Trypanosomes need cholesterol, phospholipids and total lipids for biosynthesis membranes and sustain growth [45].

Based on the results of the analysis, the combination. Tongue and machine learning is able to produce different data between positive and negative samples T. evansi, possibly due to the existence of T. evansi and/or blood/serum changes caused by T. evansi. This research shows that the combination-tongue and machine learning has the potential to be used in diagnosing infections T. evansi.

5. CONCLUSION

Electronic tongue which is made has good sensor stability. Based on the blood/serum sample data pattern plot, it shows that the combination-tongue and machine learning can detect infected and

uninfected blood/serum samples T. evansi. The results of the PCA and LDA plot scores for blood samples were 75.80% and 97.96% of the total variance, respectively. The results of the PCA and LDA plot scores for blood serum samples were 40.52% and 97.46% of the total variance. The best plot score was the LDA of blood samples which reached 96.968%. The LDA method has better pattern recognition results compared to the PCA method. This method is also able to provide better information. The performance accuracy of LDA data on blood and serum samples is 100%. Using LDA, all blood/serum samples can be differentiated into uninfected and infected samples T. evansi, as well as differentiation based on the level of parasitemia to become infected T. evansi positive 1, 2, 3 and 4.

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