

PHYLOGENETIC ANALYSIS OF *TRYPANOSOMA EVANSI* IN CATTLE WITH HIGH RoTat1.2 VSG GENE COPY NUMBERS: HAEMATOLOGY AND SERUM BIOCHEMISTRY FINDINGS

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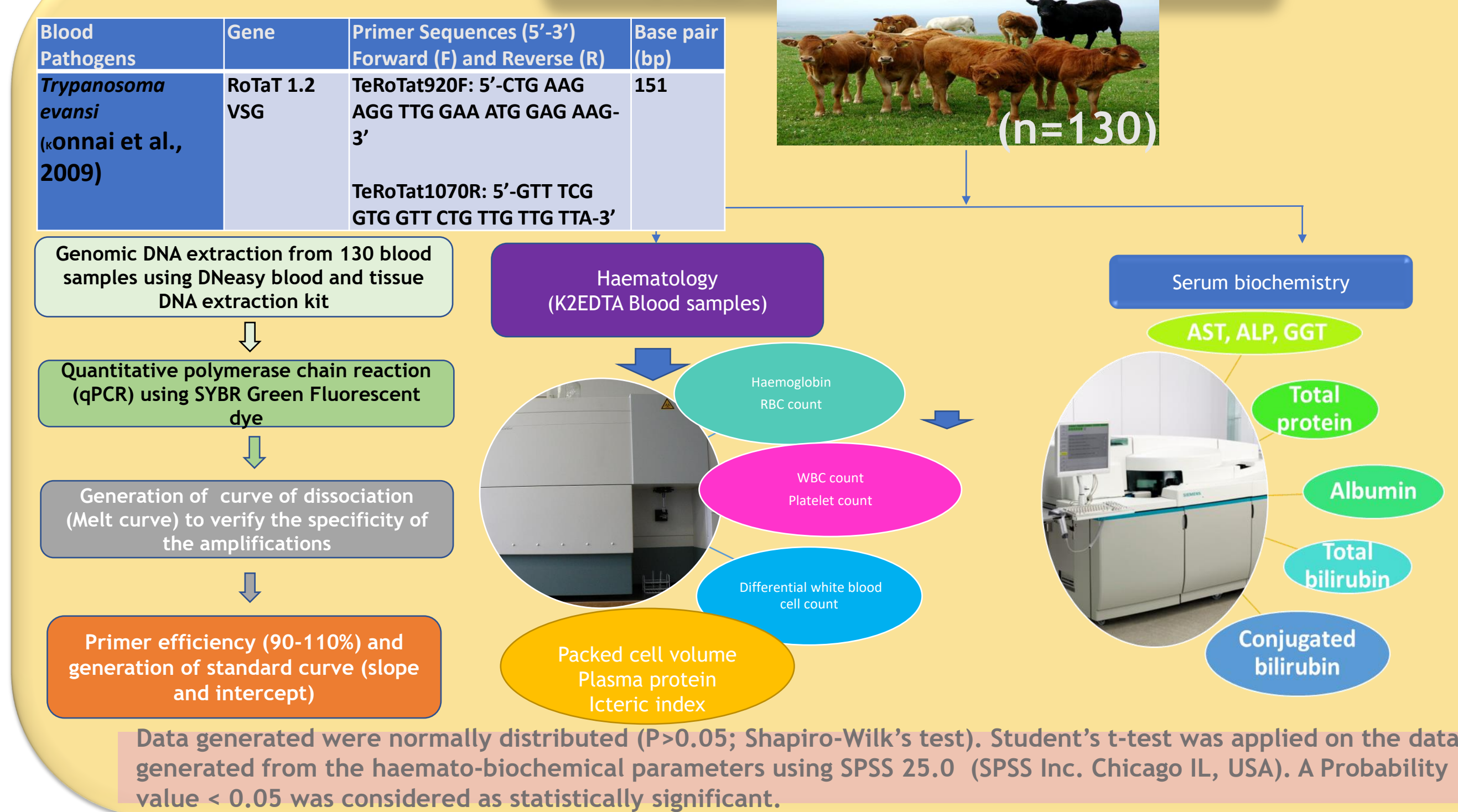
INTRODUCTION

Trypanosomes are extracellular, microscopic and haemoflagellate protozoan parasites belonging to the genus *Trypanosoma* that survive in the blood stream. *Trypanosoma evansi* causes Surra which is an economically significant disease that affects a wide range of domestic and wild animals including cattle, buffaloes, sheep, goats, pigs, horses, donkeys, camels, dogs, deer, gazelles and elephants in Asia, Africa, Central and South America, and parts of Europe. It is characterized by high morbidity and mortality (Desquesnes *et al.*, 2013). *Trypanosoma evansi* is a salivarian trypanosome transmitted mechanically from one susceptible host to another by blood sucking flies belonging to the genera *Stomoxys* and *Tabanus* (Erekat *et al.*, 2020). Vampire bats (*Desmodus rotundus*) may serve as host, reservoir or biological vectors of *T. evansi* in Latin America (Desquesnes *et al.*, 2013).

OBJECTIVE

This is to determine the phylogenetic relationship of *Trypanosoma evansi* detected in Malaysian cattle and evaluate the haematobiochemical abnormalities associated with natural *T. evansi* infection in cattle with high RoTat1.2 VSG gene copy numbers

METHODOLOGY



The stage of blood protozoa infection (clinical and sub-clinical) was determined by quantitative real time PCR and presence of clinical signs. A melt curve analysis was performed to ascertain the specificity of the amplicon.

Based on qPCR results, cattle were divided into cattle with high RoTat1.2 VSG gene copy numbers and Clinically healthy cattle

RESULTS

Table 1. Haematological profile of cattle infected with *Trypanosoma evansi*.

Haematological Parameters (Mean±SE)	<i>T. evansi</i> infected cattle with high RoTat 1.2VSG gene copy numbers (n=4)	Clinically healthy cattle (n=17)
PCV (L/L) *	0.19 ± 0.01	0.43 ± 0.01
RBC count (x10 ¹² /L) *	3.02 ± 0.01	8.54 ± 0.38
Haemoglobin (g/L) *	63.00 ± 0.40	131.29 ± 3.03
MCV (fL) *	65.46 ± 1.33	48.58 ± 1.28
MCHC (g/L) *	328.25 ± 2.17	311.47 ± 1.83
Plasma proteins (g/L) *	109.25 ± 0.47	71.75 ± 1.31
Icteric index (units) *	50.00 ± 0.00	5.00 ± 0.00
Platelets (x 10 ⁹ /L) *	427.00 ± 10.97	335.88 ± 13.50
WBC count (x10 ⁹ /L) *	4.57 ± 0.13	6.92 ± 0.42
Promyelocytes (x10 ⁹ /L) *	0.00 ± 0.00	0.00 ± 0.00
Myelocytes (x10 ⁹ /L) *	0.12 ± 0.04	0.00 ± 0.00
Metamyelocytes (x10 ⁹ /L) *	0.40 ± 0.02	0.00 ± 0.00
Band Neutrophils (x10 ⁹ /L) *	0.27 ± 0.06	0.00 ± 0.00
Segmented Neutrophils (x 10 ⁹ /L) *	0.18 ± 0.02	2.68 ± 0.22
Lymphocytes (x10 ⁹ /L) *	3.97 ± 0.24	3.59 ± 0.20
Eosinophils (x10 ⁹ /L) *	0.03 ± 0.00	0.35 ± 0.10
Basophils (x10 ⁹ /L) *	0.00 ± 0.00	0.00 ± 0.00
Monocytes (x 10 ⁹ /L) *	0.10 ± 0.00	0.46 ± 0.06

*Different superscript on a parameter indicates significance difference, p<0.05. SE: Standard error

Figure 3. A single melt peak at 84.00°C for RoTat1.2 VSG of *T. evansi*.

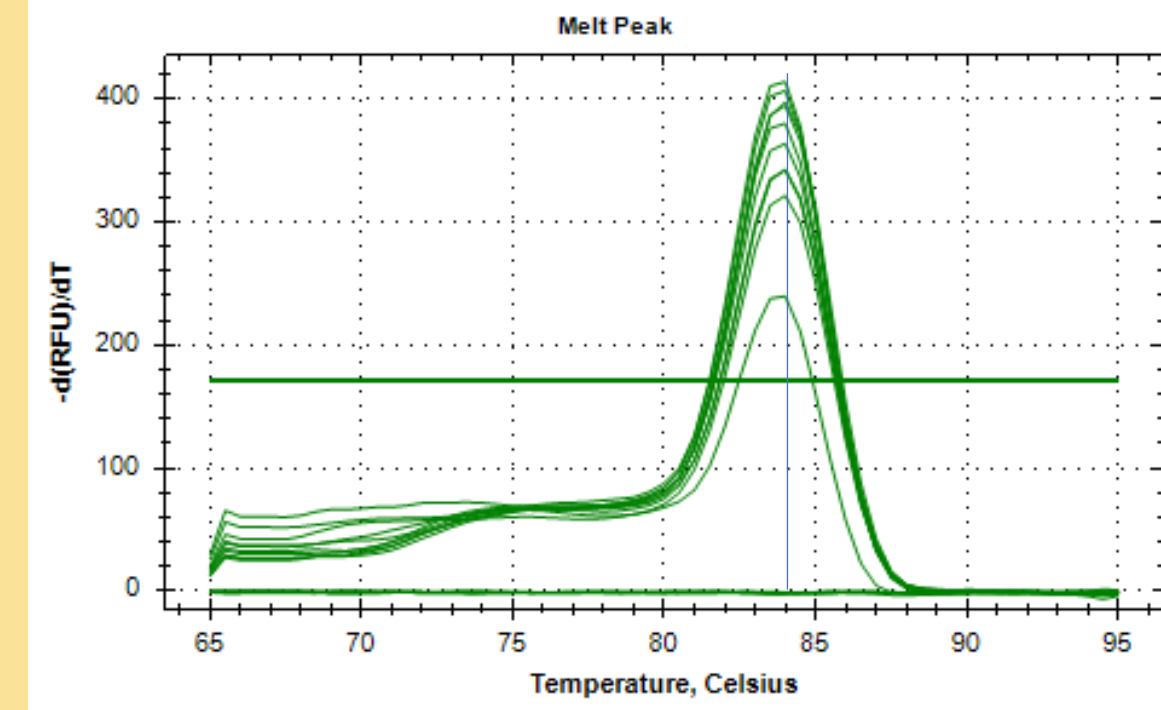


Table 2. Serum biochemistry profile of *T. evansi* infected cattle.

Serum biochemical parameters (Mean±SE)	<i>T. evansi</i> infected cattle with high RoTat1.2 VSG gene copy numbers (n=4)	Clinically healthy cattle (n=17)
Sodium (mmol/L) *	191.50 ± 3.75	139.59 ± 1.09
Potassium (mmol/L) *	6.15 ± 0.03	4.65 ± 0.09
Chloride (mmol/L) *	126.50 ± 3.18	101.71 ± 1.67
ALP (U/L) *	351.00 ± 2.89	70.31 ± 4.63
AST (U/L) *	3.50 ± 1.44	88.53 ± 3.56
γ-GT (U/L) *	57.00 ± 0.00	22.06 ± 0.80
Total proteins (g/L) *	92.40 ± 1.03	68.14 ± 1.36
Albumin (g/L) *	21.75 ± 0.38	36.75 ± 0.58
Globulin (g/L) *	70.65 ± 0.66	38.38 ± 1.16
A: G (Unit) *	0.31 ± 0.00	0.96 ± 0.06
Total bilirubin (μmol/L) *	30.30 ± 0.29	2.79 ± 0.31
Conjugated bilirubin (μmol/L) *	8.50 ± 0.12	1.84 ± 0.16
Unconjugated bilirubin (μmol/L) *	21.75 ± 0.17	0.92 ± 0.20
Inorganic phosphate (mmol/L) *	11.30 ± 0.58	2.08 ± 0.10
Creatinine (μmol/L) *	179.50 ± 2.22	128.00 ± 6.61
Urea (mmol/L) *	2.75 ± 0.40	5.54 ± 0.40

*Different superscript on a parameter indicates significance difference, p<0.05. SE: Standard error

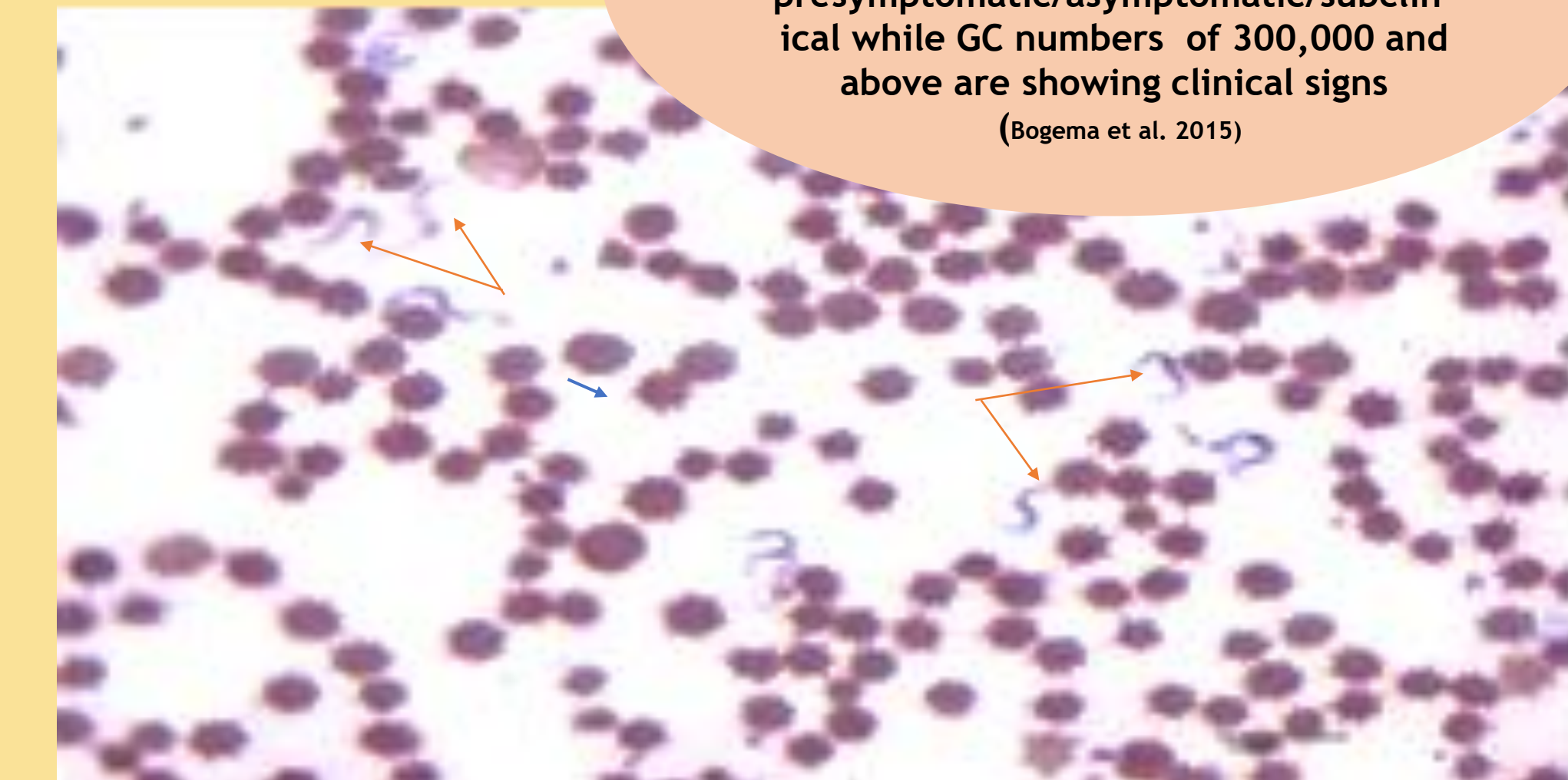


Figure 1. The detection rate of *Trypanosoma evansi* was 4/130 (3.08%;95 CI 1.20-7.64%). Double orange arrows show numerous *Trypanosoma evansi* in Giemsa stained cattle thin blood smear. Background red blood cells are created.X1000

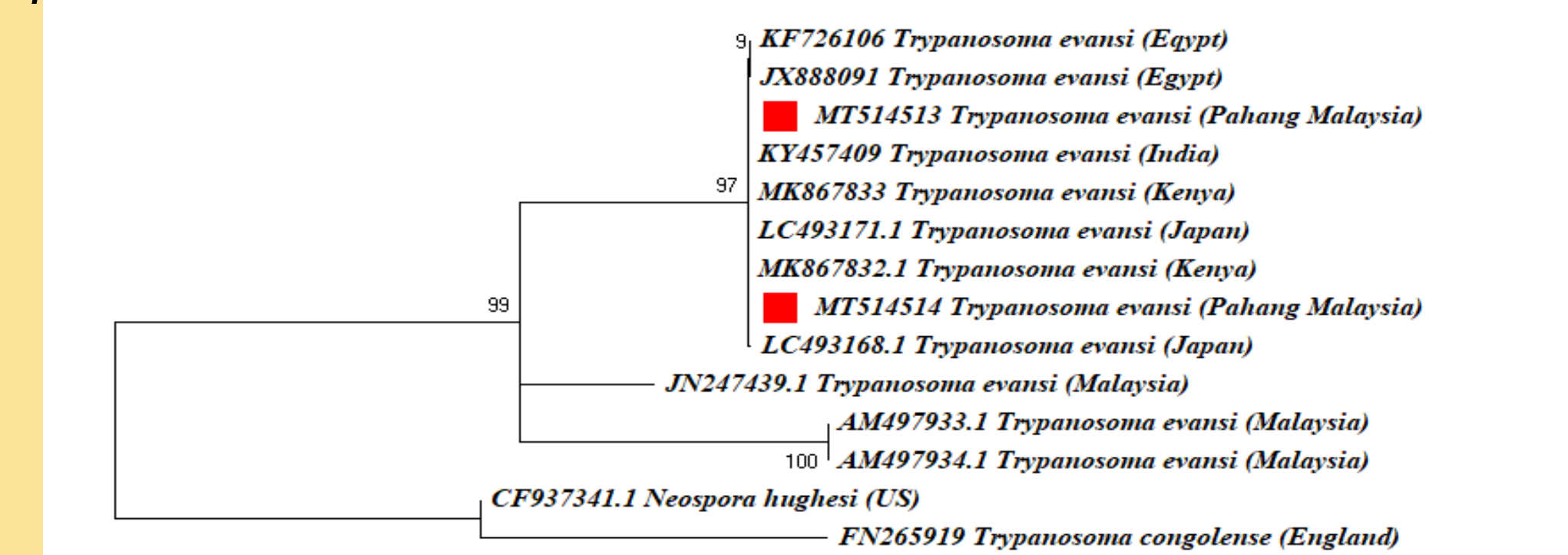


Figure 2. Phylogenetic tree showing taxonomic relationships of *Trypanosoma evansi* sequences detected from sampled cattle.

DISCUSSION

- The cattle exhibited clinical signs such as anaemia, cachexia, decrease in lactation, lethargy, progressive weight loss, nasal and ocular discharge, stiffness of the limbs, recumbency and lymphadenopathy, and had RoTat1.2 VSG gene copy numbers between 40,396,41.43 - 65,077,98.94 GC/μL. Similarity analysis using nucleotide BLAST (BLASTn) showed that the amplicon sequences of *T. evansi* from this study (MT514513.1-MT514514.1) demonstrated 100% molecular similarity with strains from Kenya (MK867832; MK867833), India (KY457409.1) and Egypt (KF726106; JX888091).
- No significant similarity was found between our sequences and previously reported local Malaysian *T. evansi* strains (JN247439.1; AM497933.1; AM497934.1).
- Phylogenetic analysis revealed that the two *Trypanosoma evansi* RoTat1.2 VSG sequences from this study (MT514513.1 and MT514514.1) grouped together with reference strains from Egypt (KF726106.1; JX888091.1), India (KY457409), Kenya (MK867832.1-MK867833.1) and Japan (LC493168.1; LC493171.1).
- Surprisingly, the three Malaysian reference *T. evansi* strains (JN247439.1; AM497933.1-AM497934.1) formed a separate branch from the *T. evansi* nucleotide sequences obtained from this study
- The haematological profile of *T. evansi* infected cattle with high RoTat1.2 VSG include low PCV, RBC count and haemoglobin concentration with erythrocytes that are larger than normal (macrocytic anaemia). Other abnormalities include high plasma proteins and icteric index, low leukocyte count with high myelocyte, metamyelocyte and band neutrophil counts, and low mature neutrophil and monocyte counts.
- Serum biochemistry findings include hyperkalaemia, hypernatraemia, hyperchloridaemia, hyperproteinaemia, low serum aspartate aminotransferase activity, high serum activities of alkaline phosphatase and gamma glutamyl transferase, hypoalbuminemia, hperglobulinemia, low albumin to globulin ratio and hyperbilirubinemia due to high level of unconjugated bilirubin. Serum inorganic phosphote and creatinine levels were high with a low serum urea level.

CONCLUSION

Natural *T. evansi* infection in cattle with high RoTat1.2 VSG gene copy numbers is associated with macrocytic anaemia, neutropenia, degenerative left shift, liver and kidney damage, jaundice and alterations in electrolyte levels. *T. evansi* nucleotide sequences from this study had 100% molecular similarity with *T. evansi* strains from Kenya, India and Egypt.

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