The evaluation of GM6-based ELISA and ICT as diagnostic methods on a Mongolian farm with an outbreak of non-tsetse transmitted horse trypanosomosis

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General introduction

Kinetoplastidia

Trypanosomatida

Trypanosoma

- Stercoraria
- Salivaria

Duttoneilla

T. vivax

T. Vivax (both tsetse and non-tsetse transmitted)

Nannomonas

T. simiae

T. congolense

Trypanozoon

T. brucei

T. equiperdum

T. evansi

Tsetse transmitted

Non tsetse transmitted

Non tsetse transmitted Trypanosoma

Nagana - Causative agent: *T. vivax*,

Symptoms:

- fever
- Anemia
- discharge from eye and nose,
- paralysis

Dourine Causative agent: *T. equiperdum*

Symptoms:

- Fever
- anaemia
- local oedema of the genitalia
- lip paralysis

Surra

Symptoms:

- fever
- anemia
- emaciation

Causative agent: *T. evansi*

Current diagnosis of Non tsetse transmitted *Trypanosomosis*

Diagnostic methods		Advantages	Disadvantages
Indirect	CATT/*T. evansi*	Suitable in field	Did not cover all type of *T. evansi*
	Crude Antigen ELISA	Good for mass screening	Not suitable in field
Direct	PCR	High sensitivity	Not suitable in field
	Microscopic examination	Simple and inexpensive.	Low sensitivity

Crude antigen requires advanced laboratory technique and difficulty to standardize

In contrast of these methods recombinant antigen based ICT and ELISA are more suitable

Introduction

- The livestock sector contributes to 11.4% of the Mongolian economy (Mongolia, 2016).
- The Mongolian agricultural sector has been severely affected by various infectious diseases, and the impact of infectious diseases on the national economy is currently a pertinent issue (Altangerel et al., 2012).

- High prevalence (21 %) of trypanosomosis was reported in two western provinces of Mongolia. (Musinguzi et al., 2017, 2nd NTTAT Conference presentation)

- **Equine trypanosomosis has around 5.5% of prevalence in central part of Mongolia (Clausen et al., 2003).**
- **Equine trypanosomosis has around 15.8% by PCR of prevalence in western part of Mongolia** (Musinguzi et al., 2017, 2nd NTTAT conference presentation)

- **Therefore we need effective and easy to use diagnostic tools for equine trypanosomosis**

Introduction

- TeGM6-4r based ELISA and ICT have already shown good diagnostic value against *T. evansi* infected water buffalo, cattle, goat and sheep (Nguyen et al., 2015; Nguyen et al., 2014)

- **Comparison of this established ELISA and ICT with Crude antigen ELISA (OIE recommended) will validate these methods for the diagnosis of equine trypanosomosis**

Materials and methods

T. equiperdum → DNA extraction → Sequencing

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Compare with other *Trypanozoon* GM6

rTeGM6-4r expression → mice immunization → polyclonal antibody

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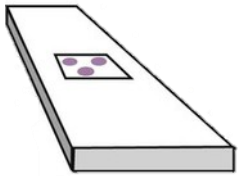
IFAT for cellular localization



Materials and methods

1. *T. evansi* Crude Antigen ELISA for reference (OIE recommended)
   - Antigen 1 µg per well and serum dilution 1:200

2. *T. evansi* rTeGM6-4r ELISA to reveal diagnostic value
   - Antigen 0.2 µg per well and serum dilution 1:200

3. *T. evansi* rTeGM6-4r ICT to reveal diagnostic value
   - 1:4 serum dilution

To determine diagnostic values:

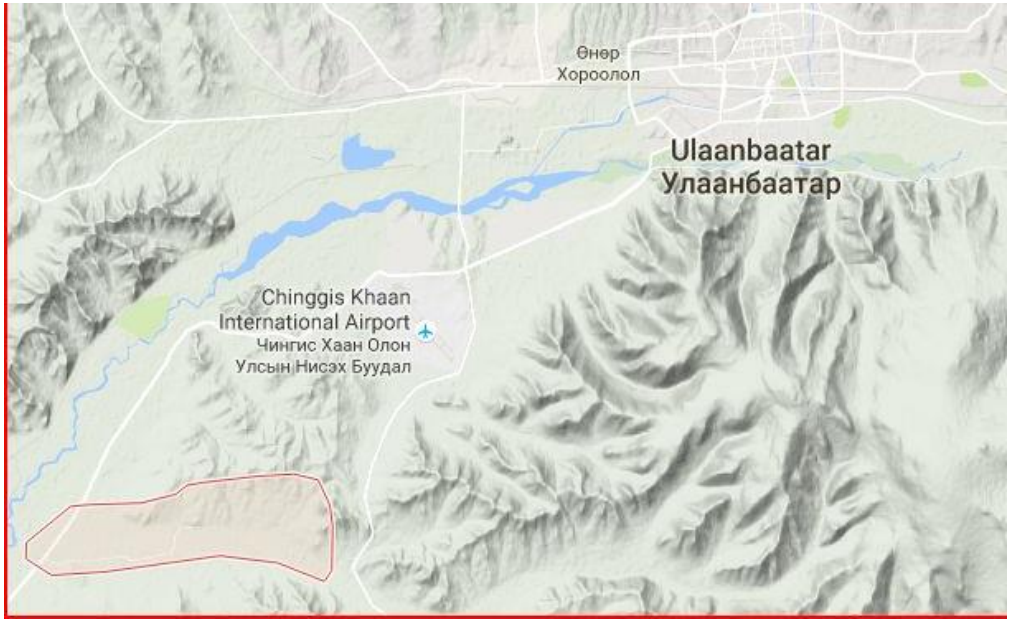
Cohen's kappa coefficient

Sensitivity and specificity

Negative and positive predicted value

Materials and Methods

Place: a remote district of Ulaanbaatar, Mongolia
Sample No: 50
Breed: Arab breed (N=5), Mixed breed (N=45)
Sex: Male (N=9), Female (N=41)
Age: 2 – 22



In addition to the serological methods ;

- PCR using whole blood DNA for all horses
- PCR using genital organ swab DNA for only three horses with clinical symptom
- Microscopic examination of genital organ swab for only tree horses with clinical symptom
  - In order to detect parasite in genital organ

The amino acid comparison of tandem repeat unit of GM6 between subgenus *Trypanozoon*.

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                 ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|...
                     5          15         25         35         45         55         65
Majority         ELAKLKASDS RSFLDPMPEG VPLSELELDK DEKFSTMEEE RRKLIAEDRE GNAARIAELE VAMNEHSH
T.equiperdum     ELAKLKASDS RSFLDPMPEG VPLSELELDK DEKFSTMEEE RRKLIAEDRE GNAARIAELE VAMNEHSH
T.evansi         ELAKLKASDS RSFLDPMPEG VPLSELELDK DEKFSTMEEE RRKLIAEDRE GNAARIAELE AAMNEHSH
T.b.brucei       ELAKLKASDS RSFLDPMPEG VPLSELELDK DEKFSTMEEE RRKLIAEDRE GNAARIAELE AAMNEHSH
T.b.ganbiense    ELAKLKASDS RSFLDPMPEG VPLSELGLDK DEKFSTMEEE RRKLIAEDRE GNAARIAELE VAMNEHSH
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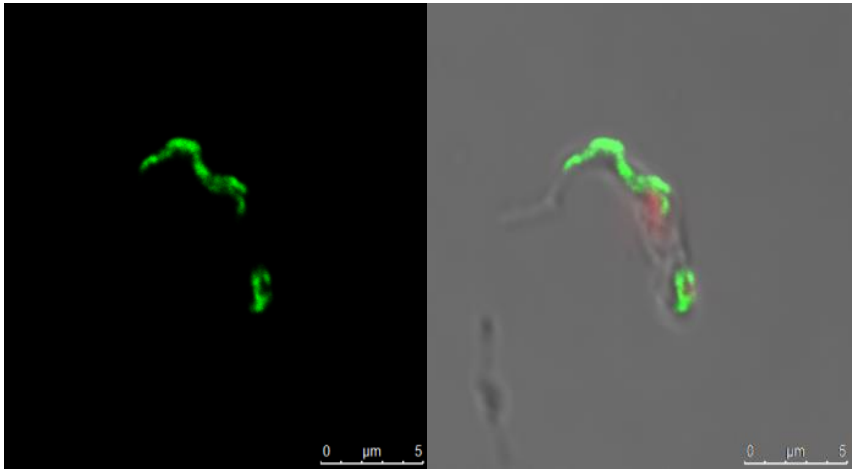
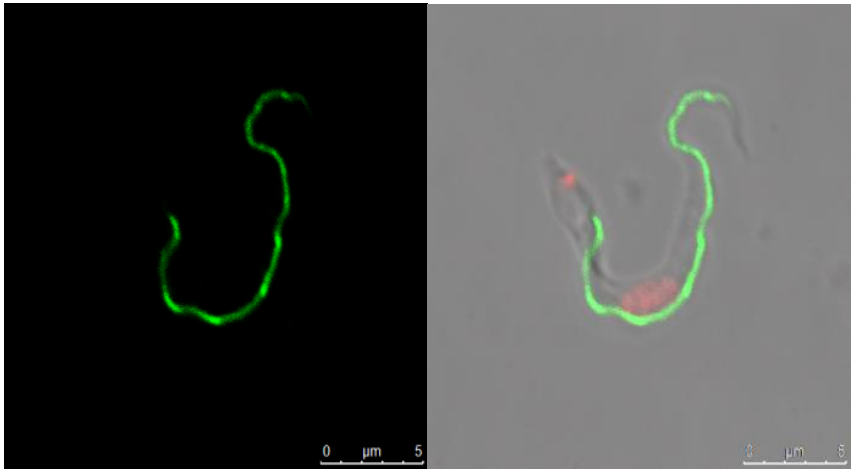
GM6 was highly conserved among *T. equiperdium*, *T. evansi* , *T. b. brucei* and *T. b. gambiense*

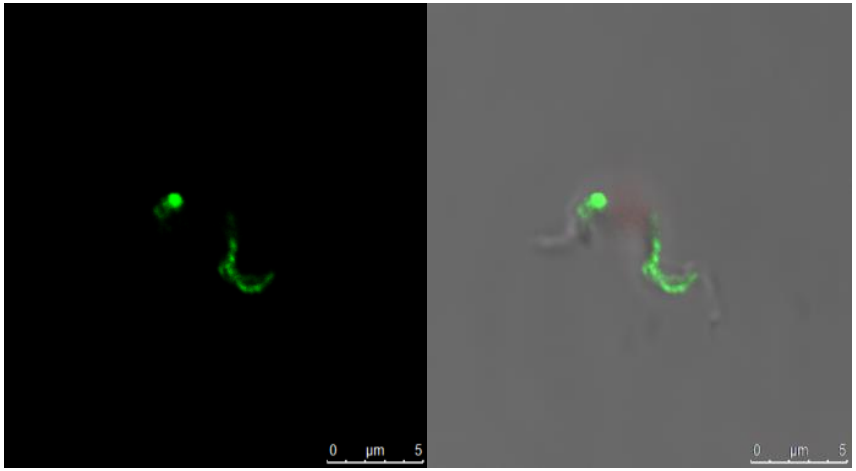
The cellular localization of *T. equiperdum* GM6 antigen



T. brucei



T. equiperdum



T. evansi

T. equiperdum GM6 was localized along the flagella.

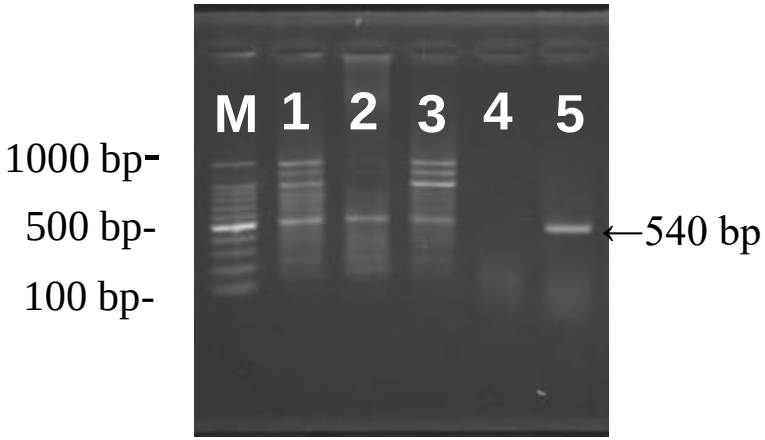
Anti *T. evansi* GM6 antibody can recognize all tree parasites

Comparison of the results among the different sero-diagnosis tests

		Crude Antigen ELISA								
		Positive	Negative	Total	Sensitivity	Specificity	Agreement	PPV	NPV	Kappa
rTeGM6 ELISA	Positive	17	6	23	74 %	85 %	80 %	81 %	79 %	0.6
	Negative	4	23	27						
	Total	21	29	50						
rTeGM6 ICT	Positive	12	2	14	57 %	92 %	78 %	86 %	72 %	0.5
	Negative	9	27	36						
	Total	21	29	50						

Microscopic examination and PCR in genital organ swab

In genital organ swab			Blood DNA
Sample	**Microscopic examination**	**PCR**	**PCR**
14	+	+	-
15	-	+	-
20	-	+	-



PCR of genital organ swabs were positive although DNA samples from whole blood were all negative by PCR.

M-DNA size marker, 1-ID14, 2-ID15, 3-ID20, 4-NC, 5-PC

Discussion

- GM6 was highly conserved among *Trypanozoon*.
- The sensitivity of rTeGM6-4r based ELISA was relatively high and it had moderate agreement
- Sensitivity of rTeGM6-4r based ICT was relatively low, although it had higher specificity and moderate agreement.
- Because *T. equiperdum* is primarily a tissue parasite, they do not commonly parasitize in blood circulation (Brun et al., 1998). PCR results (Blood: Negative, Genital organ: Positive) suggested that these horses had Dourine not Surra.

Conclusion

rTeGM6-4r based diagnostic methods showed good diagnostic value for *T. equiperdum* in horses.