

# *Trypanosoma equiperdum*: Venereal transmission and pathogenesis



Ahmed Y, Hagos A, Merga B, Goddeeris B, Duchateau L, Van Soom A, Govaere J.

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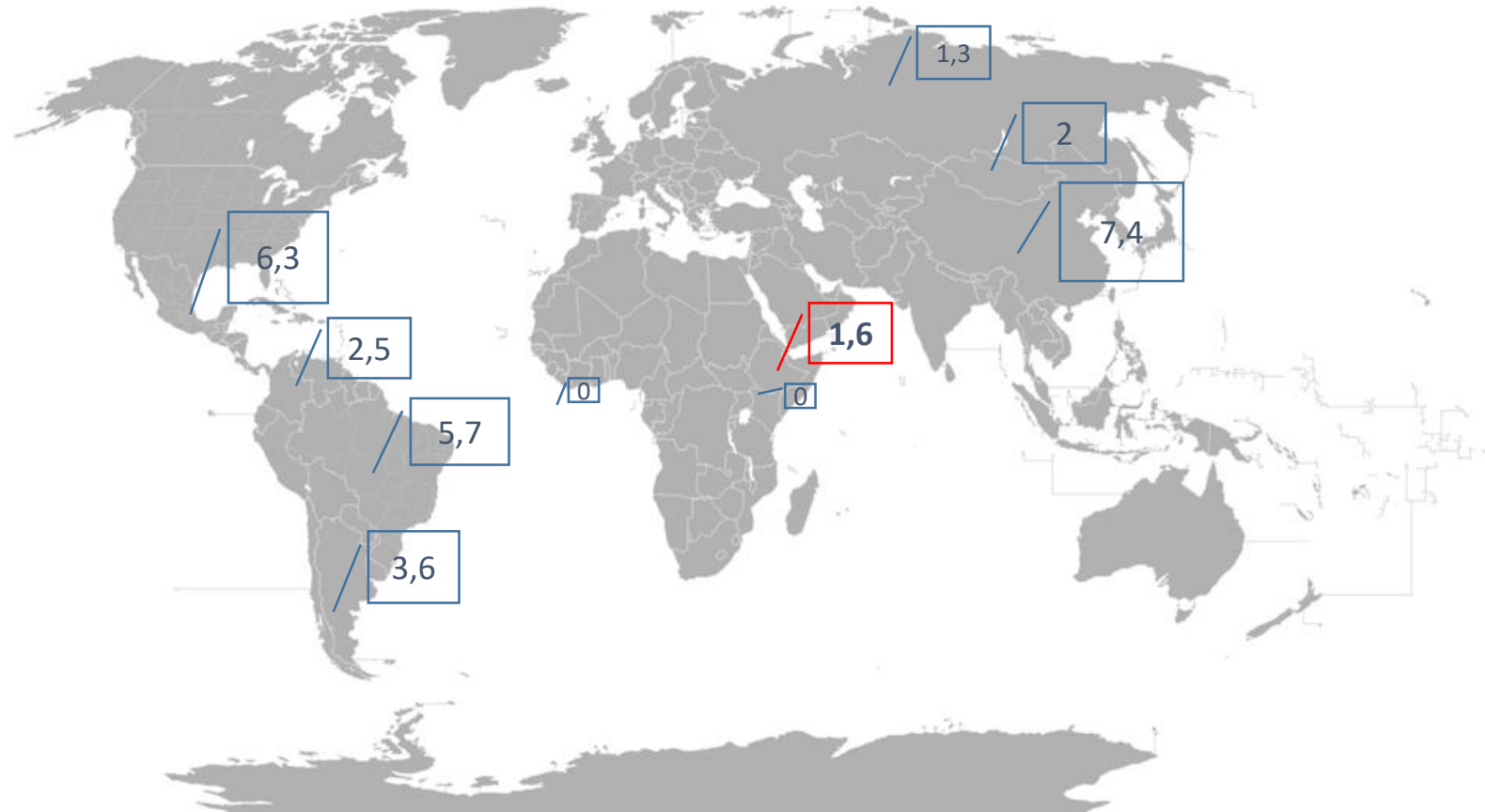
Ghent University, Faculty of Veterinary Medicine, Belgium

KUL, University of Leuven, Belgium

# Introduction

## Horses in Ethiopia ?

- 16% livestock cash value
- agricultural-, economic- and social life
- 4,5  $10^6$  horses in Africa
- 1,6  $10^6$  in Ethiopia







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<input type="checkbox"/>	<p><b>4. Genomic analyses of African Trypanozoon strains to assess evolutionary relationships and identify markers for strain identification</b></p> <p>By: Richardson, Joshua Brian; Lee, Kuang-Yao; Mireji, Paul; et al.            PLOS NEGLECTED TROPICAL DISEASES Volume: 11 Issue: 9 Article Number: e0005949 Published: SEP 2017</p> <p><a href="#">SFX</a> <a href="#">Full Text from Publisher</a> <a href="#">View Abstract</a></p>	<p>Times Cited: 0 <i>(from Web of Science Core Collection)</i></p> <p>Usage Count <a href="#">v</a></p>
<input type="checkbox"/>	<p><b>5. Genome-Wide SNP Analysis Reveals Distinct Origins of <i>Trypanosoma evansi</i> and <i>Trypanosoma equiperdum</i></b></p> <p>By: Cuypers, Bart; Van den Broeck, Frederik; Van Reet, Nick; et al.            GENOME BIOLOGY AND EVOLUTION Volume: 9 Issue: 8 Pages: 1990-1997 Published: AUG 2017</p>	<p>Times Cited: 0 <i>(from Web of Science Core Collection)</i></p> <p>Usage Count <a href="#">v</a></p>

# Introduction

- Dourine is a sexually transmitted disease of equids caused by flagellate protozoa of the species *Trypanosoma equiperdum*.
- It is known in most countries as notifiable disease (OIE 2013)
- knowledge about the prepatent infectiousness of semen is lacking
- no clear evidence based knowledge available on **how** and **when** stallions become infectious
  - ✓ **intense contact** during coitus
  - ✓ **infected semen & fluid** from prepuce

=> Objectives : to assess

- (1) the infectiousness when used in **AI**
- (2) the infectiousness of semen and prepuccial fluid during **prepatent period**
- (3) purification of semen by use of **single layer centrifugation (SLC)**
- (4) Pathogenesis and clinical features of Dourine in experimental infection
- (5) Pathology of the *T. equiperdum* infected horses (natural and experimental)

## Acclimatization

- ✓ Brought from the central highland (**No Tsetse fly**)
- ✓ Tested with CATT, parasitological methods for Trypanosome (woo test)
- ✓ Anti helminthica
- ✓ provided with quality hay and concentrates, water is ad libitum
- ✓ Ethical clearance for animal experimentation from AAU, CVMA
  - Ref. No: VM/ERC/004/07/015

## *T equiperdum* stabillate

- Doddola 943





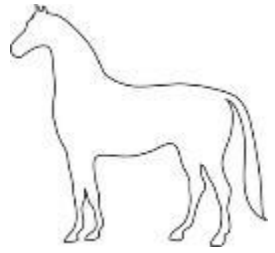
# Experimental Horses

	Name	ID
1	Catherine	M 01
2	Leen	M 02
3	Senay	M 03
4	Wube	M 04

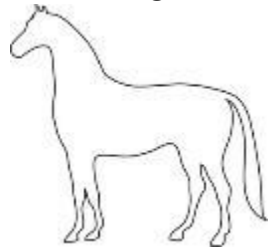
5	Nigus	S 01
6	Goddeeris	S 02
7	Hagos	S 03
8	Jan	S 04



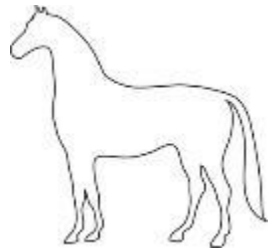




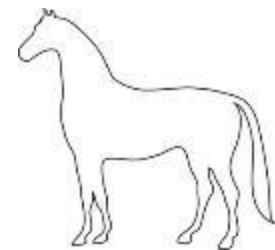
M 01



M 02



M 03



M 04

Time post AI



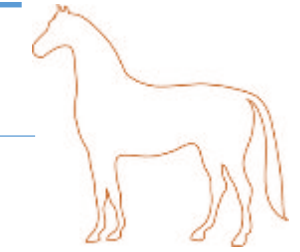
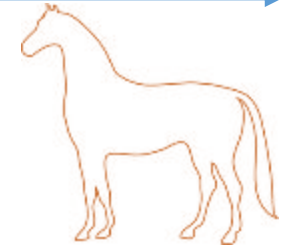
Woo  
CATT

6

8

14

15



**Art Insemination**

*with*

*T equiperdum*-spiked  
semen

= *no* intense contact

= *no* preputial material

+

+

+

+

+

+

+

+

+

+

+

+

+

+

+

+

# 1. Infectiousness of *T. equiperdum*-spiked semen when used in an artificial insemination

- Mares inseminated with *spiked* semen -> diseased
- Mice inoculated with epididymal semen *post* CYM Tx could still become parasitaemic
- Semen in prepatent / or post CYM Tx is (can be) still infectious

## INFECTIOUSNESS OF ARTIFICIAL INSEMINATED EQUINE SEMEN IN PREPATENT PHASE OF DOURINE

Ahmed Y., Hagos A., Merga B., Alemu T., Van Soom A., Duchateau L., Goddeeris B.M., Govaere J.

COLLEGE OF VETERINARY MEDICINE AND AGRICULTURE, ADDIS ABABA UNIVERSITY, BISHOFTU, ETHIOPIA

FACULTY OF VETERINARY MEDICINE, GHEENT UNIVERSITY, BELGIUM

ETHIO-BELGIUM VLIR- ZEIN2013/PR393 TEAM PROJECT

### Introduction

Dourine, caused by *T.eq.* (*Trypanosoma equiperdum*), is known in most countries as notifiable disease (OIE 2013). Since knowledge about the prepatent infectiousness of semen is lacking, introduction of the disease is an existing threat.

### Aims

- (1) To determine the infectiousness of stallion semen in prepatent phase of Dourine.
- (2) To assess infectiousness of *T. eq.* spiked semen when used in an artificial insemination program.

### Materials and methods

Cryopreserved stablilats of the parasite (360µl) in liquid nitrogen was used for spiking at a dose of 100,000*T.eq.* /ml (Fig.1). Mares (N=4) were artificially inseminated using *T.eq.* spiked semen (Fig.2). Semen deposition was done using conventional artificial insemination techniques.

Stallions (N=4) were infected by blood transfusion collected from a mare positive for *T.eq.* (Dodola strain, isolate 943) at 6+ parasitemia level (Fig.3). Semen ejaculate collected in the prepatent period (Fig.4) (14 days after infection, (N=1)), epididymal semen with no treatment (N=1) and 4 weeks post Cymelarsan® treatment (N=2) was inoculated (intra peritoneal) in to 7, 5 and 10 mice respectively (Fig.5). Thereafter isolation of *T.eq.* in the mice was performed by wet blood smear examination from the tip of the tail.



Fig.1. Semens spiking with *T. eq.*



Fig.2. Ai with spiked semen



Fig.3. Intravenous inoculation of stallions



Fig.4. Semen collection



Fig.5. Intrapertoneal inoculation of mice

### Results

Mares inseminated with spiked semen all got clinical Dourine. Parasites were observed in wet blood smear examination and Woo test. All mares were serologically positive on card aglutination test for trypanosoma (CATT).

Mice inoculated by ejaculated semen or epididymal semen from infected, non-treated stallions all were found to be positive for *T.eq.* (7/7 and 5/5) after using conventional artificial insemination techniques. Mice inoculated with epididymal semen of cymelarsan treated, clinically healthy stallions were found parasitaemic from one stallion (5/5) but not in the second one (0/5).

### Discussion

Although the presence of *T.eq.* has been shown in seminal fluid and genital tissues (Lelli et al. 2012), disease spread after artificial insemination with infected semen has not been reported and no knowledge about infectiousness in prepatent period is reported so far.

### Conclusion

This study clearly shows the infectiousness of *T. eq.* by semen  
 (1) when used for artificial insemination in the horse and  
 (2) during prepatent phase.

Contact  
 Jan.Govaere@ugent.be  
 yasmineahmed11@gmail.com

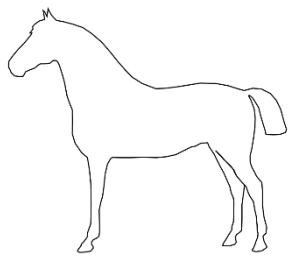
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## Introduction cont'd....

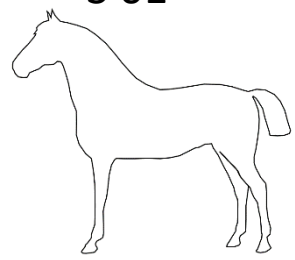
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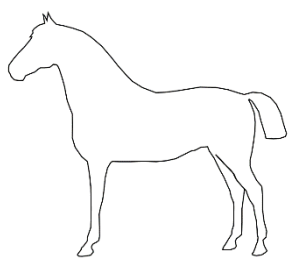
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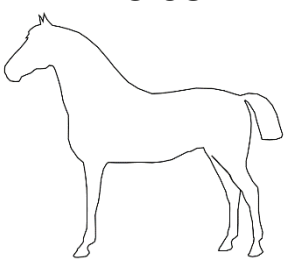
S 01



S 02



S 03



S 04

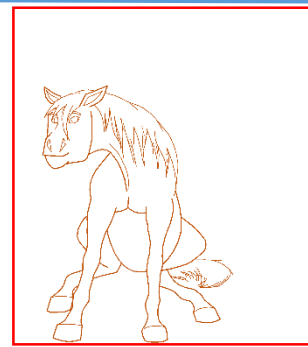
**Blood (6+)  
transfusion**

*with*

*T equiperdum*

semen

-	-
+	-
+	-
+	+



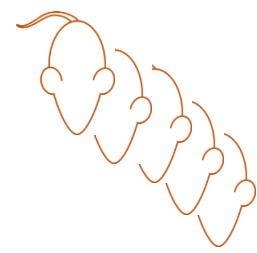
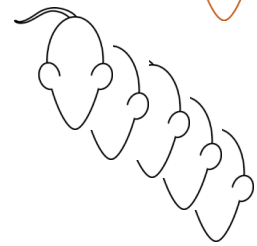
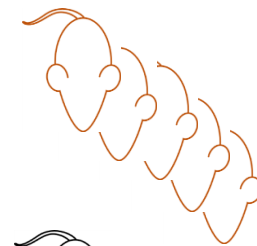
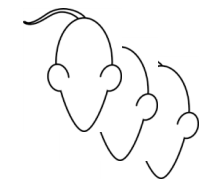
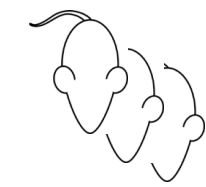
No Tx

Tx : CYMELARSAN

No Tx

Fluid  
o/preputium  
& scrotum

Epididym.  
semen



## Introduction cont'd....

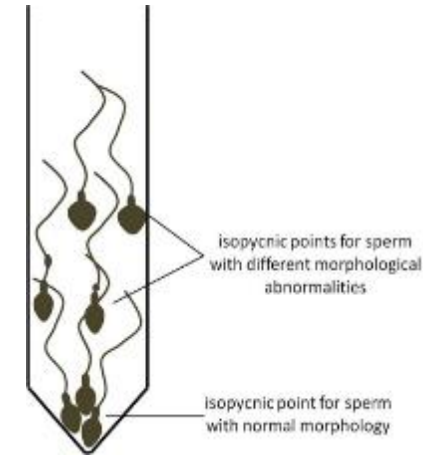
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## Hypothesis

- AI –disease transmission
- SLC – density gradient sperm selection
- successful in reducing venereal transmission of other pathogens
- **objective** - to evaluate the purification of *T. equiperdum* spiked semen by SLC



Blomqvist et al., 2011  
Morrell and Wallgren, 2011  
Morrell et al., 2013



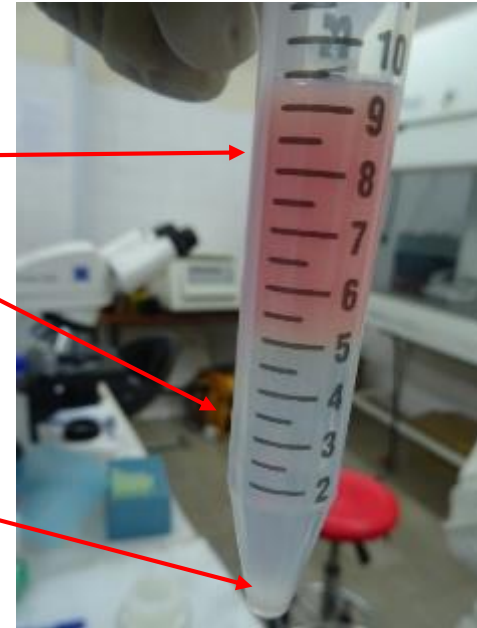
Morrell et al., 2013  
Hoogewijs et al., 2011

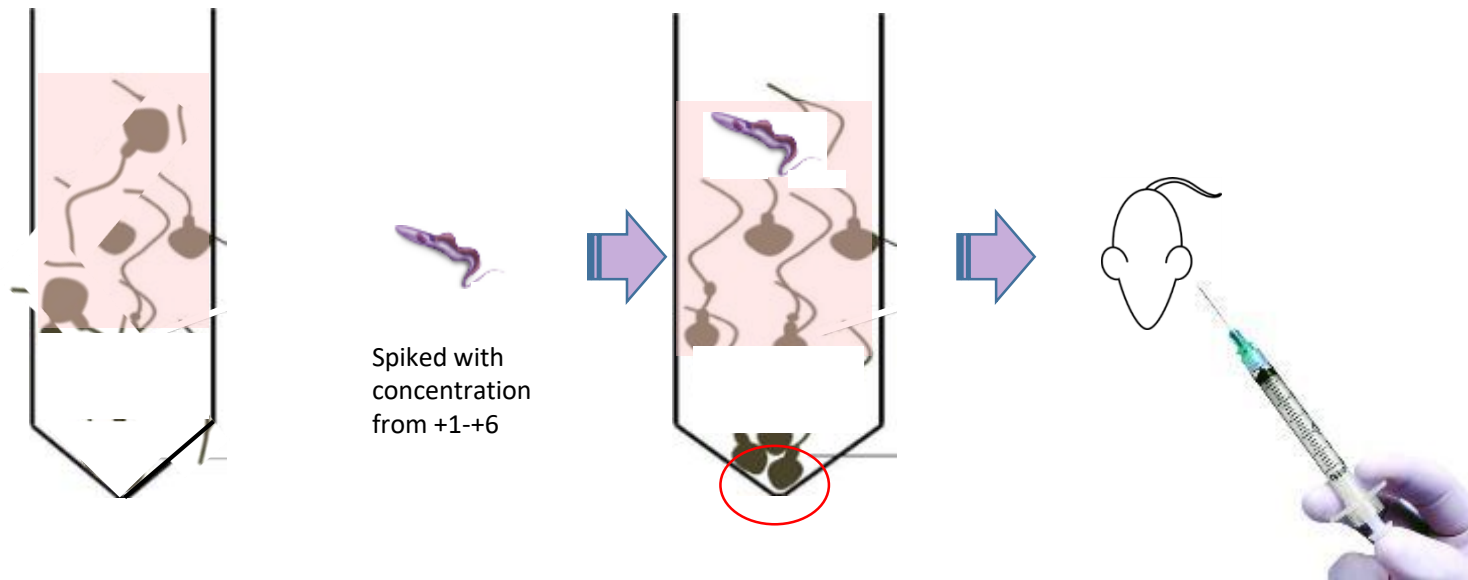
- ✓ centrifugation - 300 x g for 20 min
- ✓ aspiration of supernatant
- ✓ re dilute and centrifuge – 500 x g for 10 min
- ✓ sperm pellet

spiked semen

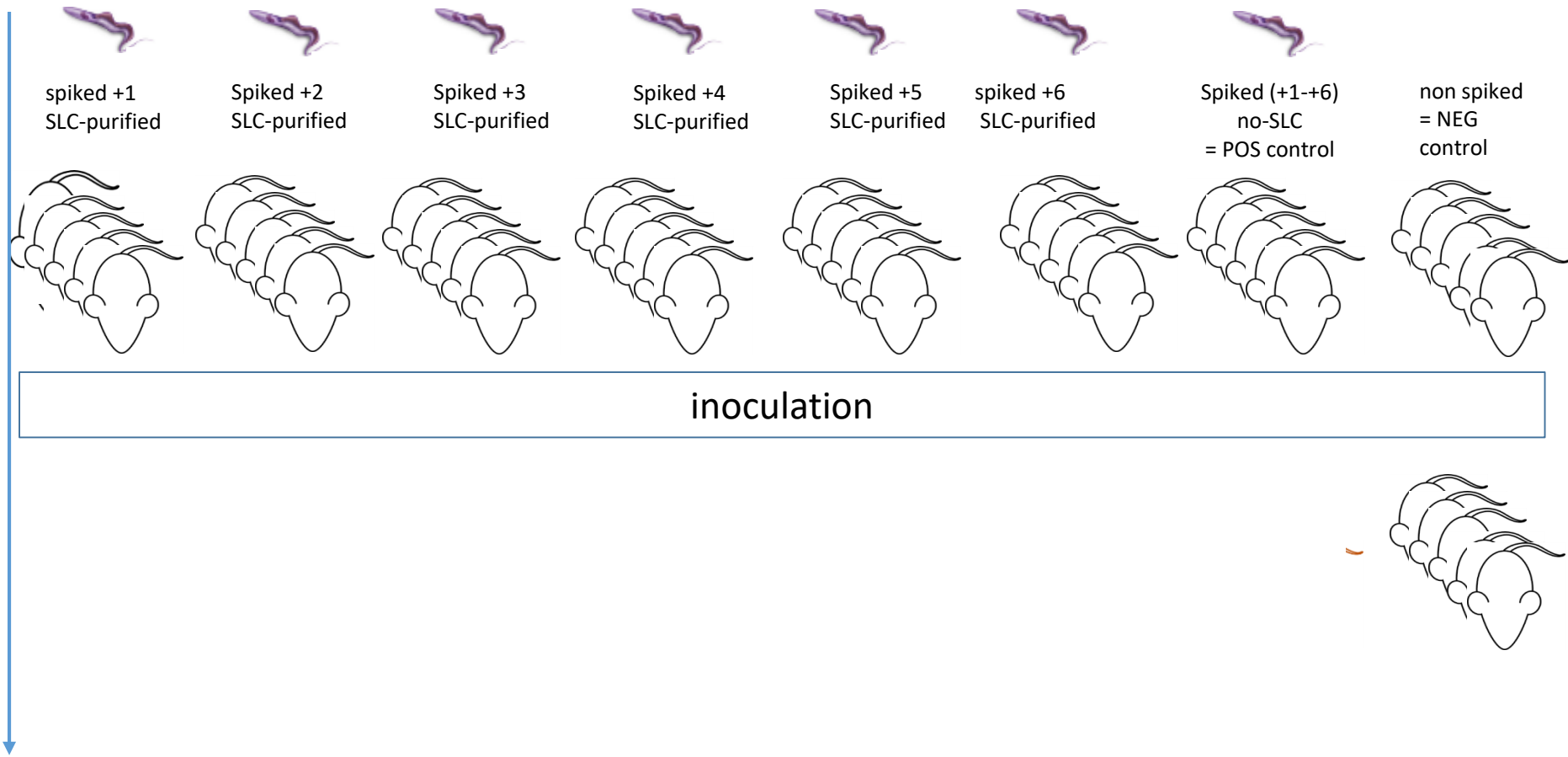
Androcoll E

sperm pellet





*Gegenart/Schutterstock*



# Discussion

- previous findings SLC of semen reduced
  - ✓ porcine circovirus
  - ✓ equine arteritis virus
  - ✓ bacteria from boar semen
- Androcoll-E™ = silane-coated silica in **buffered** salt solution
- Extender and Androcoll-E™ **pre warmed (37°C)**
- Cryostabillates – Liquid Nitrogen (**-196°C**)
- number of parasites in the semen vs blood

Blomqvist et al. 2011

Morrell et al. 2013

Morrell and Wallgren, 2011

## Conclusion

- purification of semen is possible ( $\downarrow T$  eq)
- effect of pH, temp. and osm. on Trypanosomes

### 3. The possibility to clean up parasite infected semen by use of SLC

## 2<sup>nd</sup> International Conference on Non Tsetse Transmitted Animal Trypanosomosis

### Abstract

Send back to [sdesager@itg.be](mailto:sdesager@itg.be) before 30 September 2017

Title (maximum 30 words)

**Purification of Trypanosoma equiperdum spiked equine semen by single layer centrifugation**

Authors (underline surnames)

Ahmed Yasmine Ebrahim<sup>123</sup>, Hagos Ashenafi<sup>2</sup>, Merga Bekana<sup>2</sup>, Alemu Tola<sup>2</sup>, Van Brantegem Leen<sup>3</sup>, Van Soom Ann<sup>3</sup>, Duchateau Luc<sup>3</sup>, Goddeeris Bruno M<sup>3</sup>, Govaere Jan<sup>3</sup>

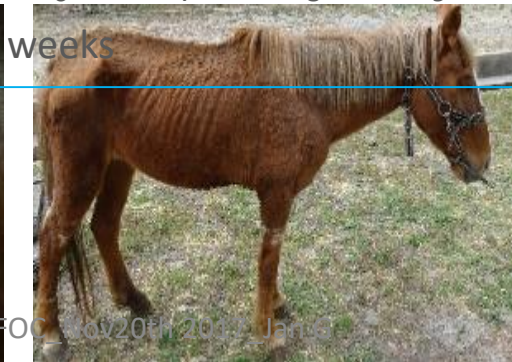
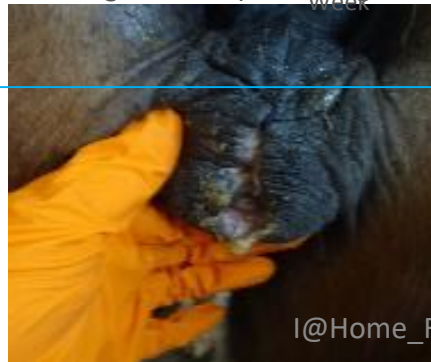
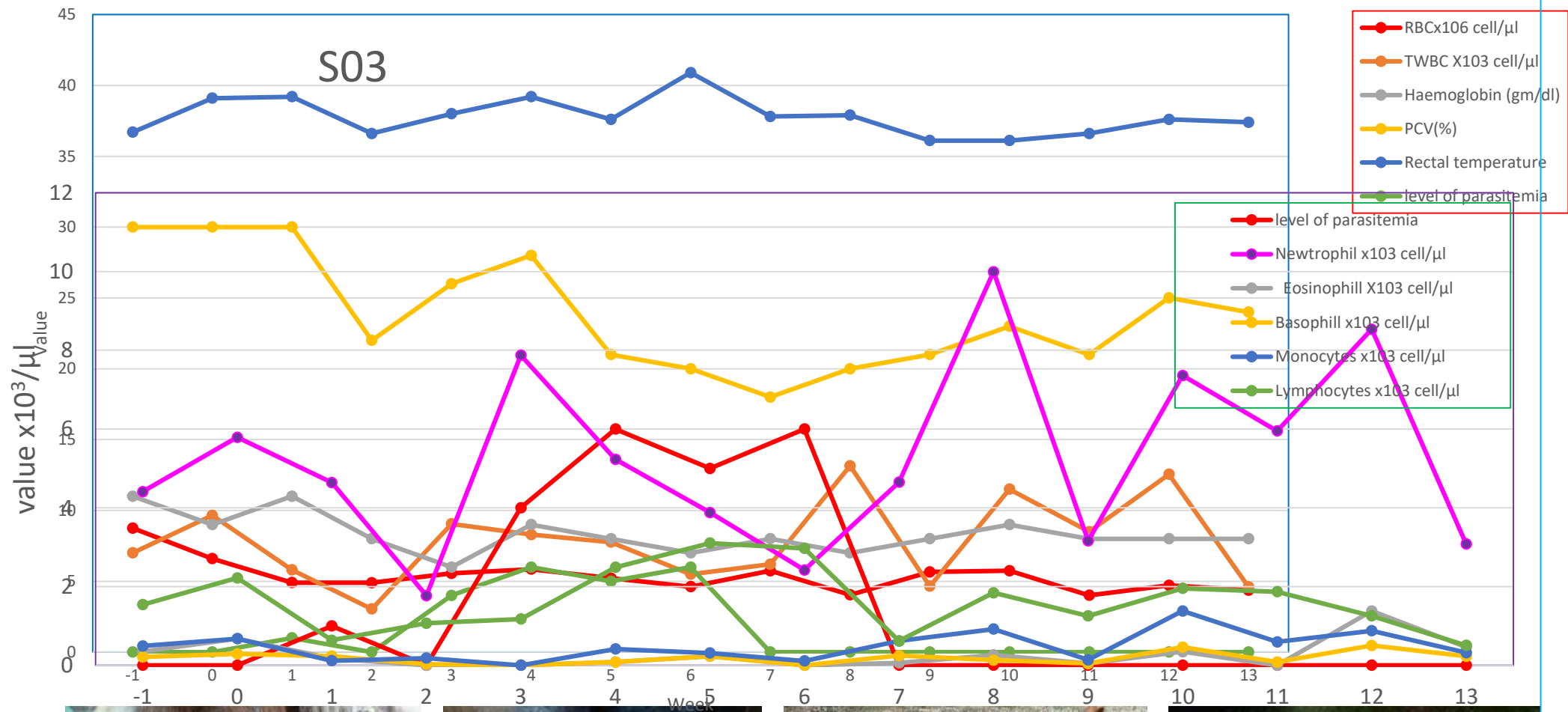


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S03

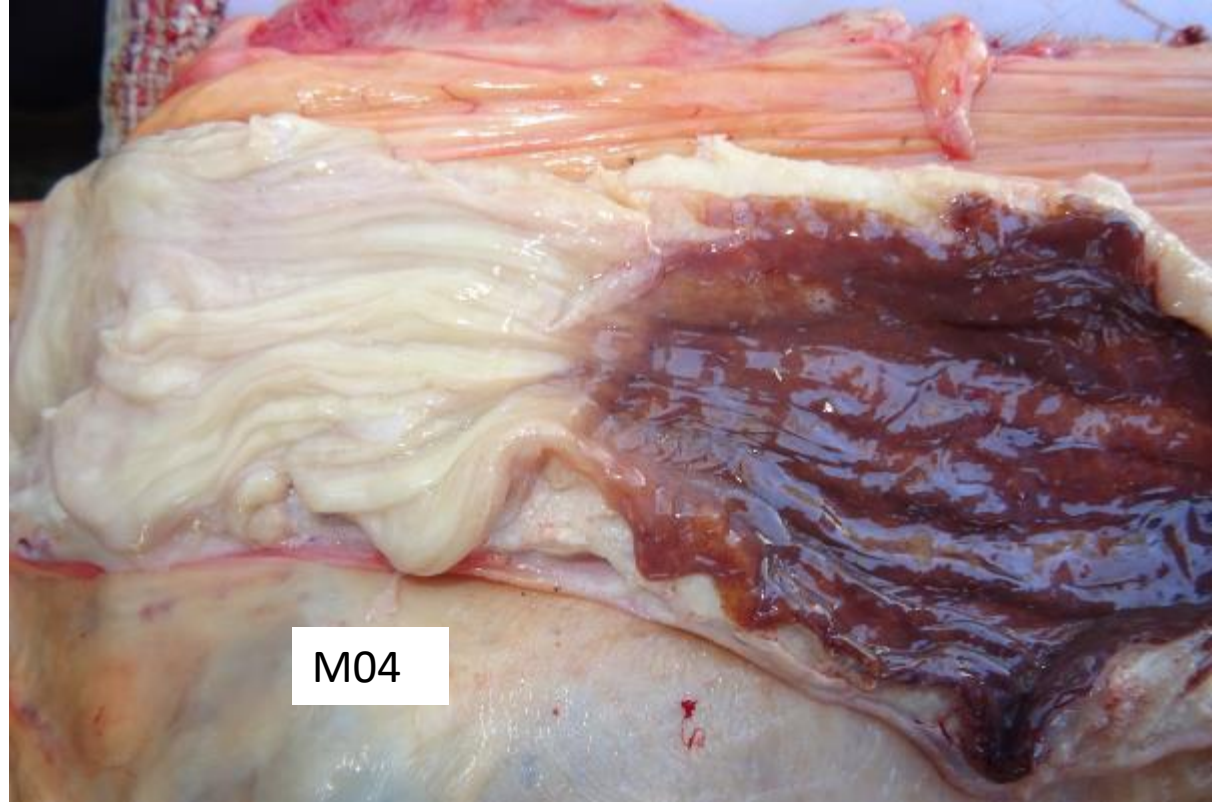


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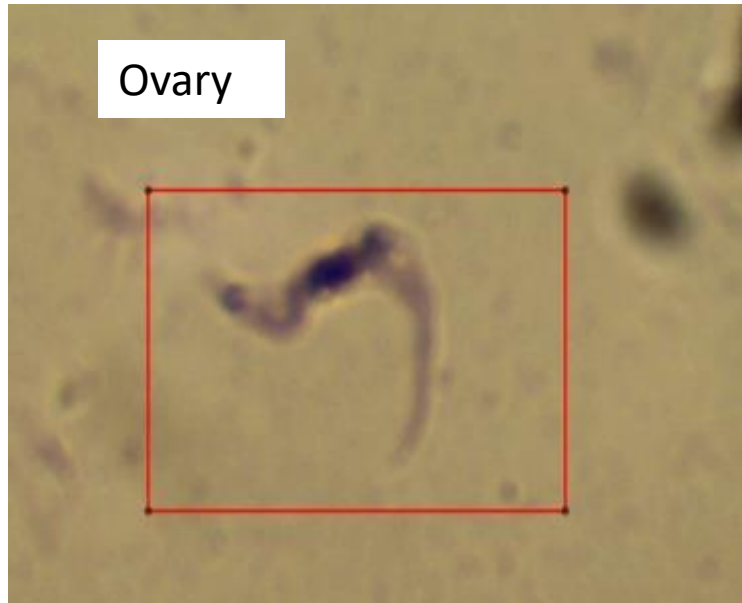




M03



M04



















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