



National reference laboratory for
DOURINE



European Union Reference Laboratory for
EQUINE DISEASES

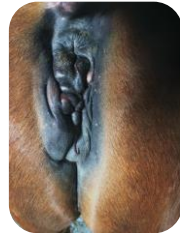


World Organisation
for Animal Health
Founded as OIE

WOAH Reference Laboratory for
DOURINE



ACTIVITY REPORT ON DOURINE AND SURRA: SCIENTIFIC NEWS



ANSES, Laboratory for Animal Health, Normandy site, FRANCE
Physiopathology and Epidemiology of Equine Diseases unit (PhEED)
Parasitology team



Charlène
Lemans



Laurent
Hébert

Content

- 1 Overview of our reference activities
- 2 Equine trypanosomosis and phylogeny
- 3 Differentiation and Quorum Sensing
- 4 WOAHA Standards
- 5 Equine trypanosomosis in the world
- 6 Conclusion

1. Overview of our reference activities

Reference activities of the parasitology team



National Reference Laboratory

- **Dourine (2006)**



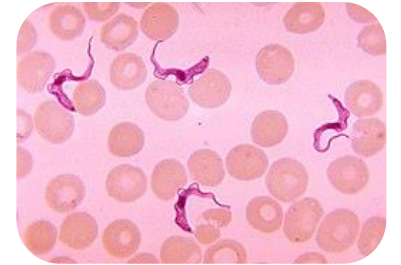
European Union Reference Laboratory for **EQUINE DISEASES** (other than African Horse Sickness)

- **Dourine (2008)**
- **Equine Surra (2021)**



Reference laboratory of the World Organisation for Animal Health (WOAH)

- **Dourine (2020)**



Equine Trypanosomoses

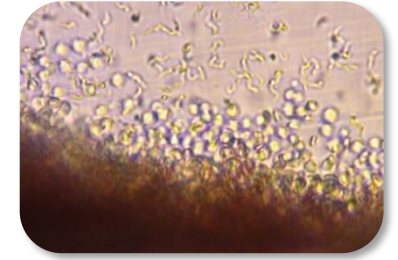


Main reference and research missions

- **Expertise, epidemiological surveillance, alert and support** to ministries.
- Develop and validate **diagnostic methods**
- **Coordinating laboratory networks:** *Interlaboratory testing, training, supply of reagents*
- **Confirmation diagnosis**
- Setting up **epidemiological studies**
- **Research:** identification, physiology, pathogenicity...

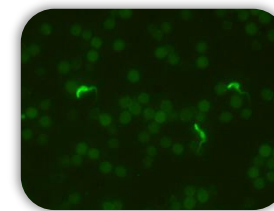
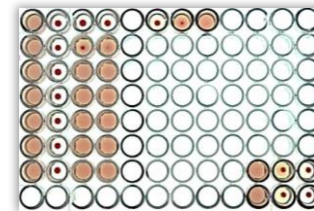
Overview of main dourine diagnostic tool

- 1 **Direct observation** of parasites
- GIEMSA, Buffy Coat, mAECT



- 2 **Molecular detection** of parasites
- PCR, genome sequencing

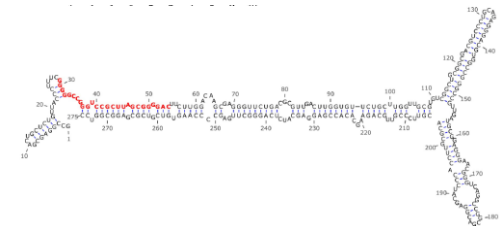
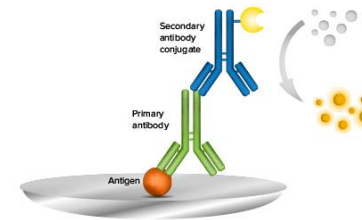
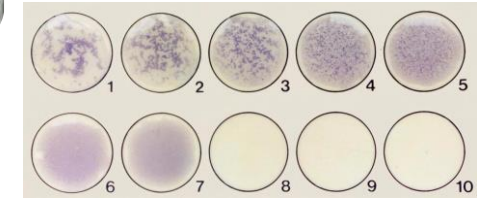
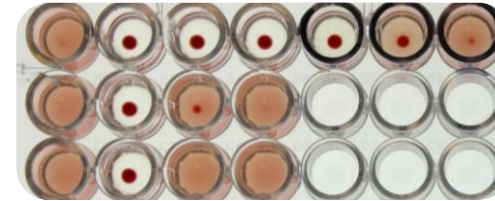
- 3 **Serological detection** of infection
- CFT, IFAT, ELISA

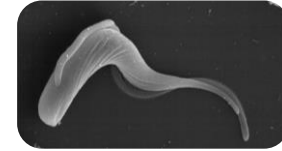


We still need sensitive and specific methods to improve the diagnosis of equine trypanosomosis

Diagnostic tools available in our unit

- Complement fixation test
- CATT/T.evansi
- ELISA dourine
- ELISA Surra
- PCR TBR
- PCR 7SL-sRNA
- Luminex GM6





Controls and reagents production

- Responsibilities and tasks of EU RL according to the Art 94 (EC) 2017/625 of the Official Journal of the European Union:

"Eu-RL shall, provide reference materials to national reference laboratories"

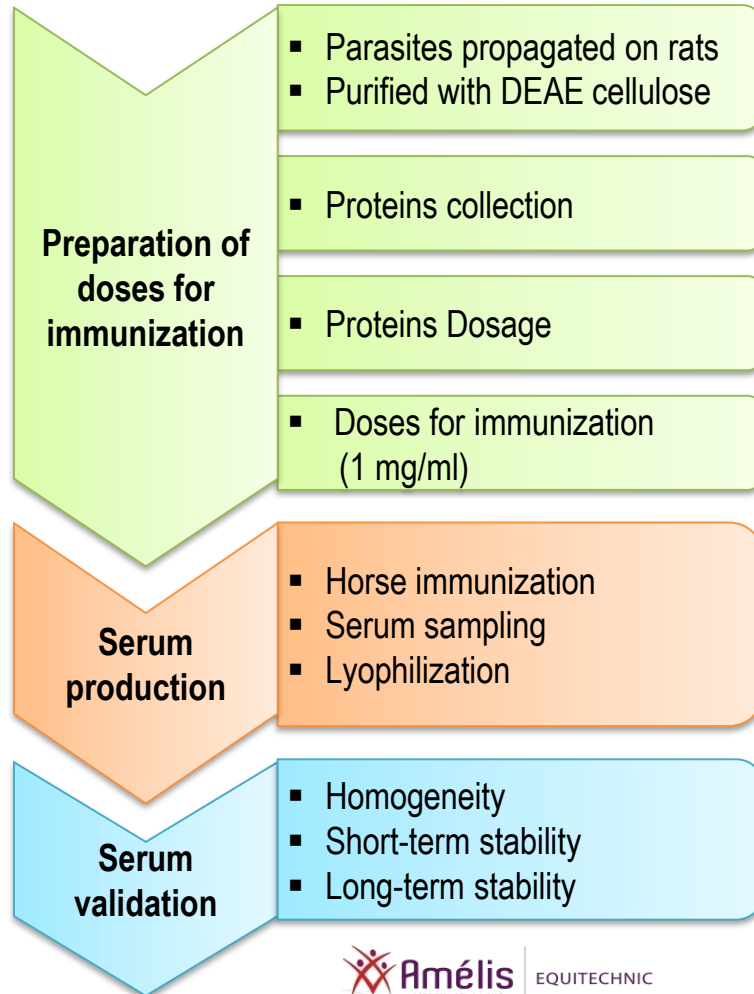
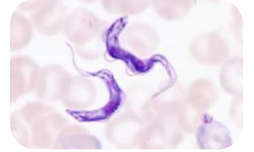
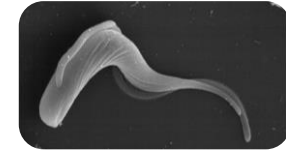
- We produce of biological material based on:

- Strain: *T. equiperdum* **OVI** (defined as reference strain by WOAAH)
- According to the **WOAH recommendations** (Terrestrial Manual chap. 3.6.3 for dourine)

- Reagent list:

Ref	Name	Free unit / year / NRL
S00654	High-titer positive serum (strain OVI)	3
S00655	Low-titer positive serum (strain OVI)	5
S00656	Dourine Antigens (strain OVI)	1
S00653	Negative serum for 8 equine diseases	1

Production of positive sera



Certificate of quality

anses French agency for food, environmental and occupational health & safety

EU-RL European Union Reference Laboratory for Equine Diseases

Dozulé Laboratory for Equine Diseases

CODE : S654

Dourine high titer positive serum
Sérum positif haut titre pour la dourine

Origin:
Serum from horse immunized with a preparation based on *Trypanosoma equiperdum* OVI strain antigens.

Origine :
Sérum de cheval immunisé par une préparation vaccinale contenant des antigènes de la souche *Trypanosoma equiperdum* OVI.

Content:
Vial of 1.0 ml freeze dried equine serum.

Présentation :
Flacon contenant 1,0 ml de sérum sous forme lyophilisée.

Reconstitution:
Refer to the vial label to know the volume of sterile distilled water to add to restore the serum.

Reconstitution :
Se référer à l'étiquetage du flacon pour connaître le volume d'eau distillée stérile à ajouter afin de reconstituer le sérum.

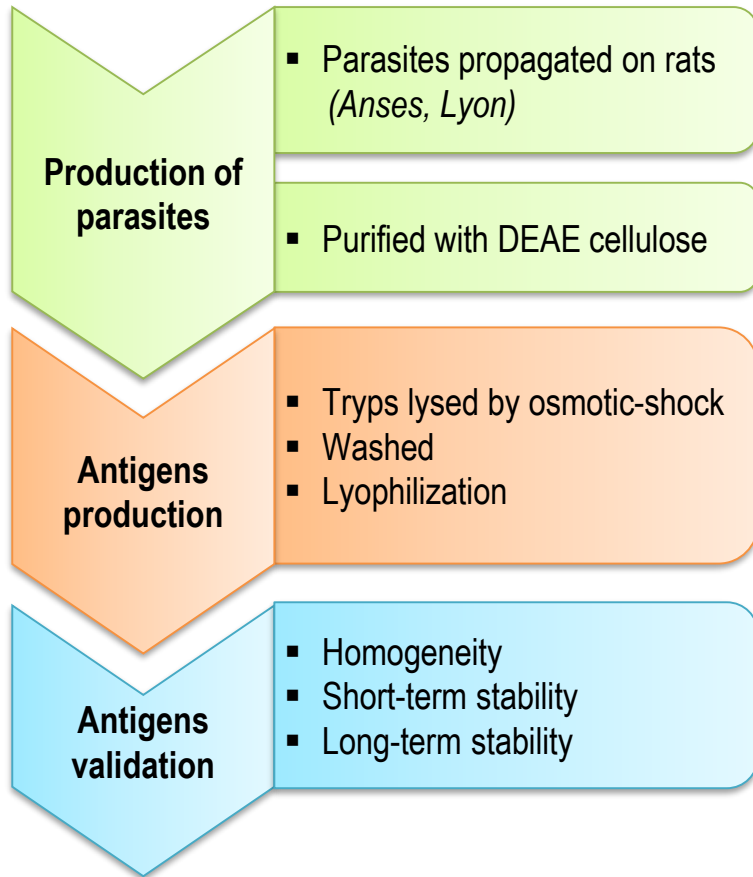
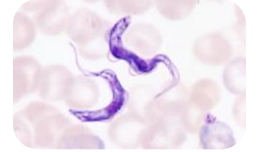
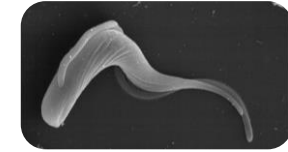
Storage conditions:
Lyophilized: Vial must be stored at 2-8°C until

Conditions de conservation :
Lyophilisé: Le flacon doit être conservé à +2-8°C avant

Depending on the adjuvant => **High** or **low** titer

Large stocks available

Production of antigens



Certificate of quality

anses

EURL
European Union Reference Laboratory for
EQUINE DISEASES

OIE
OIE Reference Laboratory for
DOURINE

Dourine antigens (OVI strain)

Reference: **S00656**

Description:

Antigens from *Trypanosoma equiperdum* OVI strain produced from rats, for the serodiagnostic of the dourine by the Complement Fixation Test (CFT) according to the OIE Terrestrial Manual, chapter 3.5.3 (2013).

The antigens do not contain antibiotic. It is important to note that no test can completely exclude the presence of infectious agents. These antigens must be considered as being potentially infectious and must be handled with all the required cautions for this kind of material.

Content:

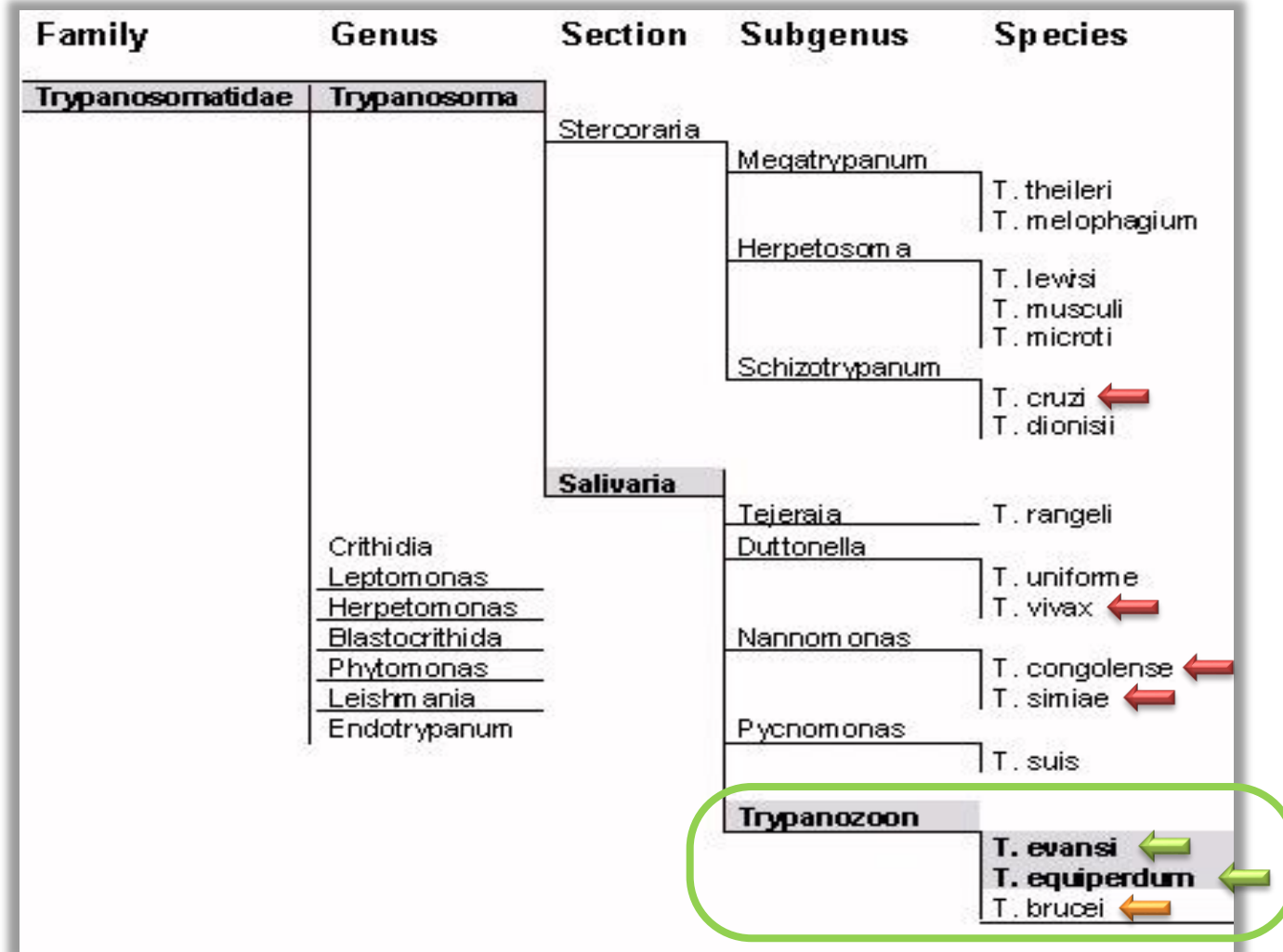
Vial of 1.0 ml freeze dried of antigens.

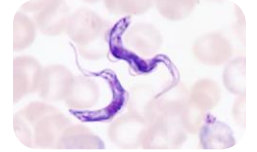
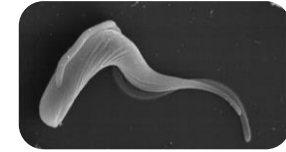
Regular production of new batches

A decorative graphic consisting of five stylized orange leaves of varying sizes and orientations, arranged in a cluster behind the main title text.






2. Equine trypanosomosis and phylogeny

Historical phylogeny of Equine trypanosomosis agents





Trypanozoon associated diseases

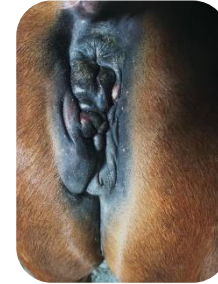
	Sub species	Disease	Transmission	Mammalian host range
Trypanozoon	<i>T. b. brucei</i>	Nagana	Vectorial  Tsetse fly	Multi-species (including equids)
	<i>T. b. evansi</i>	Surra	Mecanical    Stomoxys Tabanus Vampire bats Orally Sexually?	Multi-species (including equids)
	<i>T. b. equiperdum</i>	Dourine	Sexual  Mating, Artificial insemination Orally via milk?	Equids

While considered distinct, these diseases share many characteristics...

The clinical course in horses

1 Bloodstream / tissular stage

- Fever
- Ventral and genital edema
- Anemia and weight loss



Clinical signs vary with:

- disease stage,
- susceptibility of the host

2 Neurological stage

- Paralysis (facial, lips, hind limbs)
- Incoordination

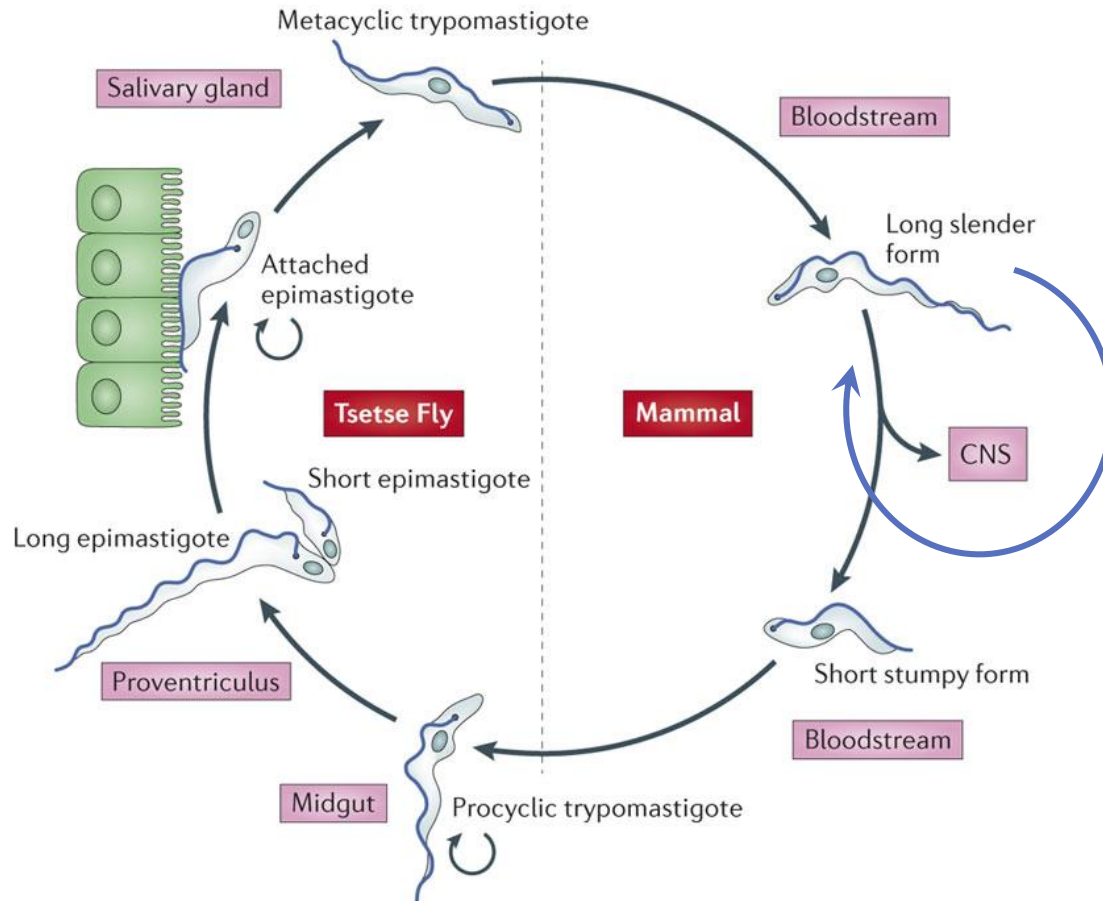


But are shared among all *Trypanozoon* infections (dourine, surra, and nagana)

Treatment success dependent on disease stage

No available vaccines against equine trypanosomosis

Trypanosoma brucei life cycle



***T. evansi* and *T. equiperdum* are monomorphic strains locked under slender form**

- Loss of transmission by the tsetse fly
- Acquisition of the mechanical transmission

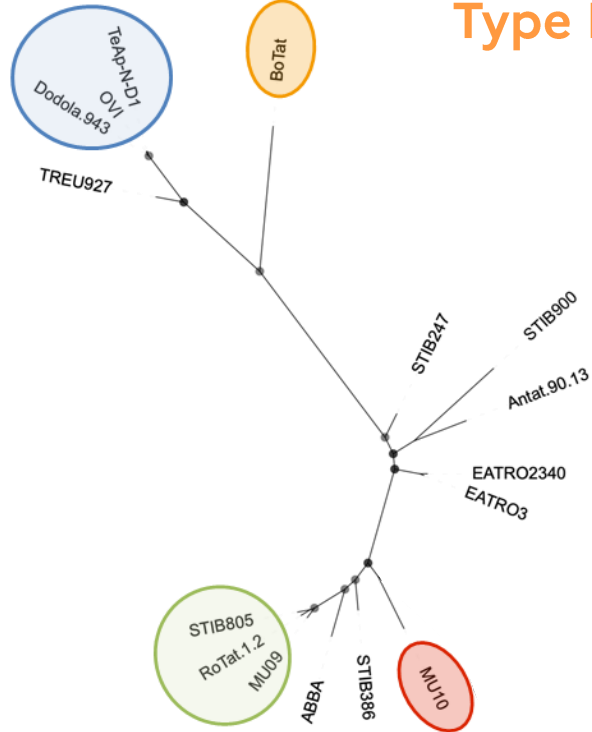
Increased capacity of dissemination

(Langousis *et al.*, 2014)

Phylogenetic relationships between Trypanozoon

T. b. equiperdum
Type A

T. b. equiperdum
Type B



T. b. evansi
Type A

T. b. evansi
Type B

- 4 clades have emerged independently
- Common point: these clades are monomorphic
- Raising the question of the link between phylogeny and pathology?

Isolation of a new strain (Suganuma et al., 2016)

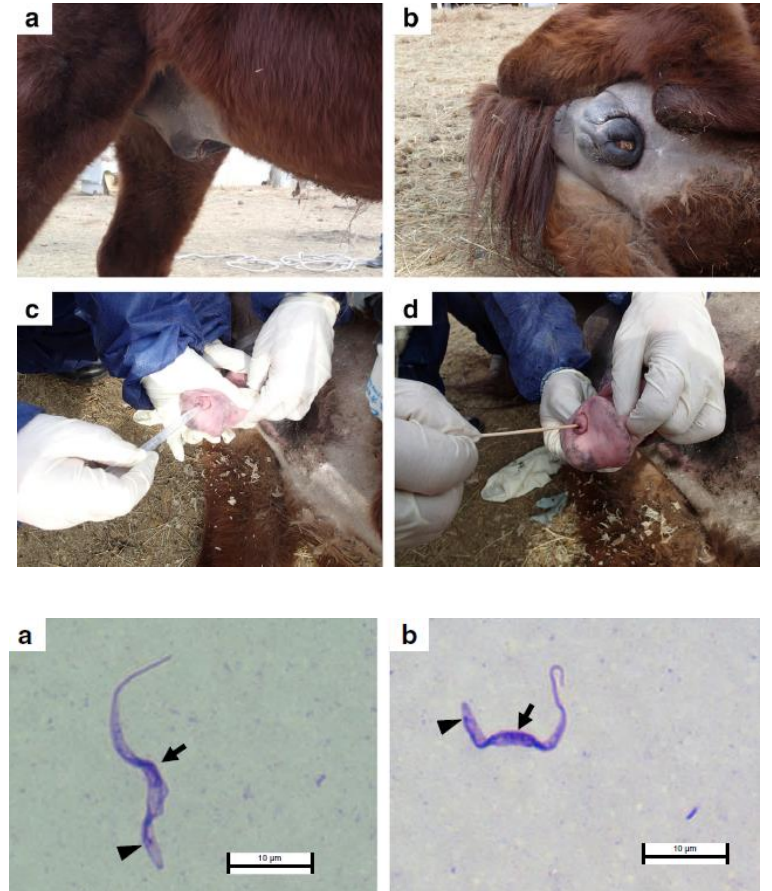
- Horse with genital edema and anemia
- Positive in serology and PCR
- Observation of trypanosomes (blood and CSF)

Dourine

Urethral wash with culture medium

=> Adaptation of the parasite to *in vitro* culture

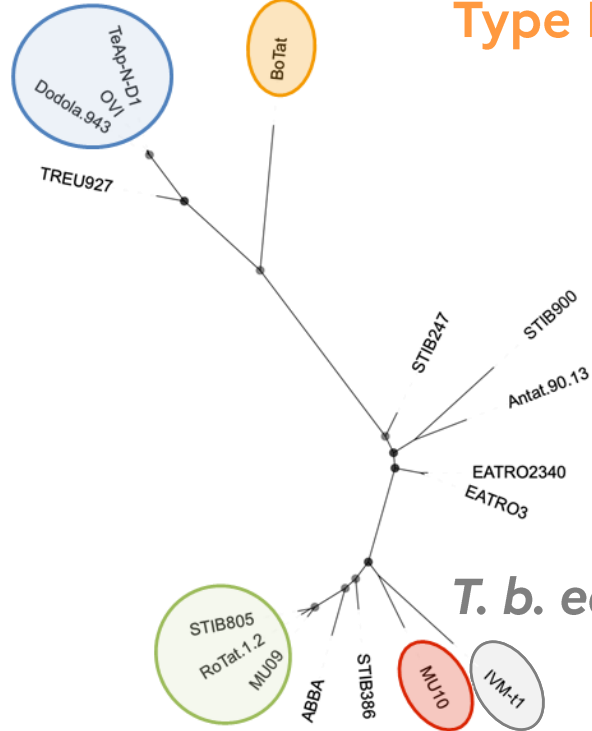
Genome sequencing (Davaasuren et al., 2019)



Phylogenetic relationships between Trypanozoon

T. b. equiperdum
Type A

T. b. equiperdum
Type B



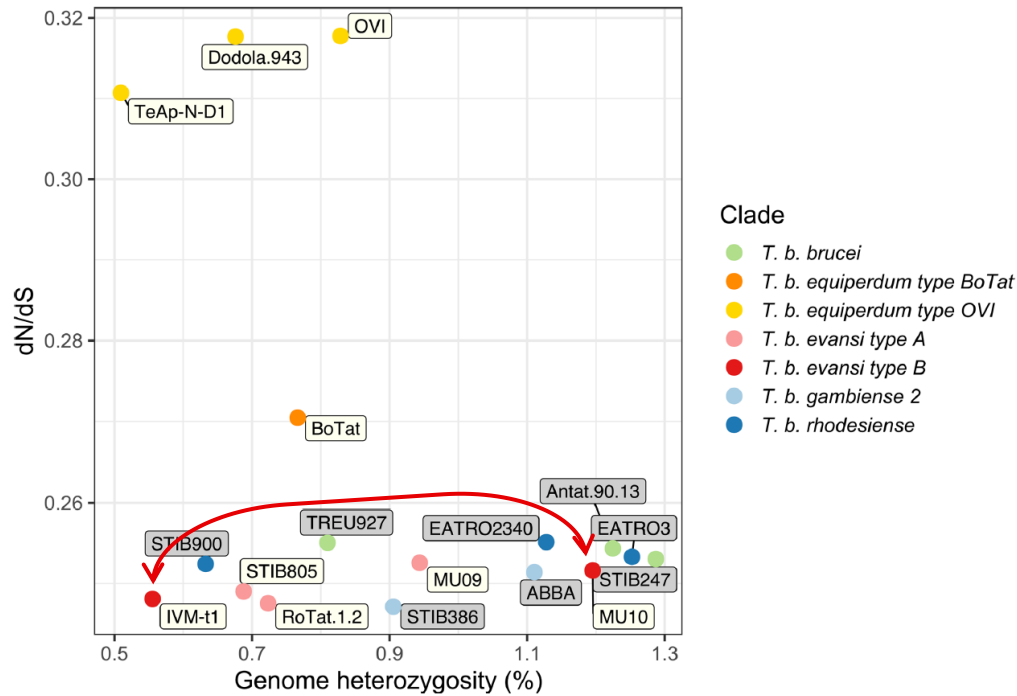
T. b. evansi
Type A

T. b. evansi
Type B

T. b. equiperdum
Type C ?

- Close to *T. b. evansi* type B despite dourine clinical signs!
- Emergence of a 5th clade?

IVM-t1 clusters with *T. b. evansi* type B



- IVM-t1 either:
 - Belongs to the *T. b. evansi* type B clade
 - Represents a 5th monomorphic lineage emergence
- dN/dS = ratio of non-synonymous to synonymous substitutions
=> measures the evolutionary pressures on coding genes
- Heterozygosity = is expected to decrease with Asexuality
- Support the hypothesis that IVMT-1 represent a 5th emergence

Conclusion

- 🍃 Clinical symptoms are not sufficient to classify a strain
 - => Phylogenomic analysis is necessary to assign a strain to a specific clade of the *Trypanozoon*
- 🍃 "*T. b. evansi*" and "*T. b. equiperdum*" do not correspond to the scientific nomenclature of "species" nor "subspecies"
 - => Review the nomenclature of clades

Collect and analyse more field samples

Is dourine a syndrome?

The incongruity between the parasites' evolutionary position and the induced pathology, mode of transmission and tissue tropism highlights **the potential for the host physiology and immune response to contribute to clinical disease** manifestation, rather than being solely parasite driven.

REVIEW

Open Access

Equine trypanosomosis: enigmas and diagnostic challenges



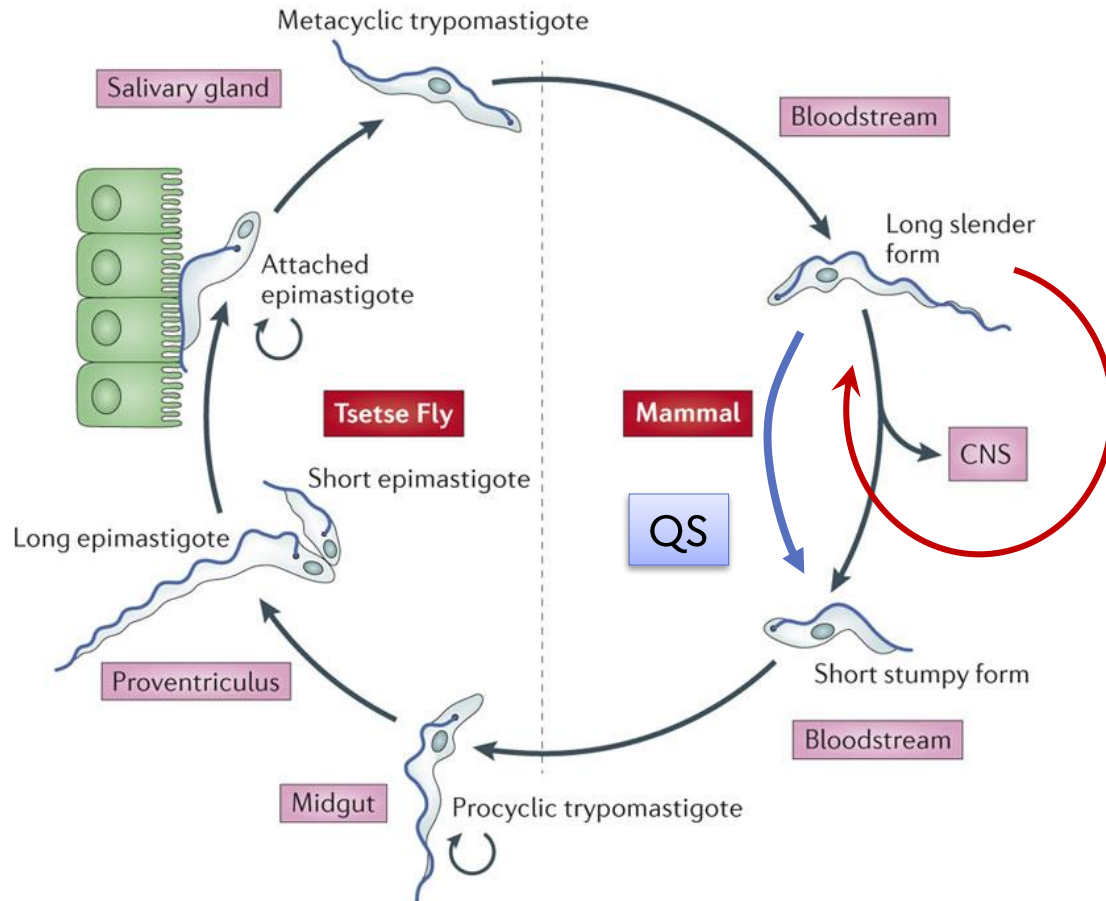
Philippe Büscher^{1*}, Mary Isabel Gonzatti², Laurent Hébert³, Noboru Inoue⁴, Ilaria Pascucci⁵, Achim Schnauffer⁶, Keisuke Suganuma⁴, Louis Touratier⁷ and Nick Van Reet¹

To address the limitations of the current diagnostics for equine trypanosomosis we recommend **not to aim for the distinction between taxa** within the subgenus *Trypanozoon*.



3. Differentiation and Quorum Sensing

Trypanosoma brucei life cycle



T. b. evansi and *T. b. equiperdum* are monomorphic strains locked under slender form

Loss of Quorum Sensing capacity

(Langousis *et al.*, 2014)

Molecular mechanisms behind Quorum Sensing

Signal

- Oligopeptides

Signal reception

- GPR89

Article

Oligopeptide Signaling through *Tb*GPR89 Drives Trypanosome Quorum Sensing

Federico Rojas,¹ Eleanor Silvester,¹ Julie Young,¹ Rachel Milne,¹ Mabel Tettey,¹ Douglas R. Houston,² Malcolm D. Walkinshaw,² Irene Pérez-Pi,² Manfred Auer,² Helen Denton,³ Terry K. Smith,³ Joanne Thompson,^{1,4} and Keith R. Matthews^{1,4,*}

Cell

Signal transduction

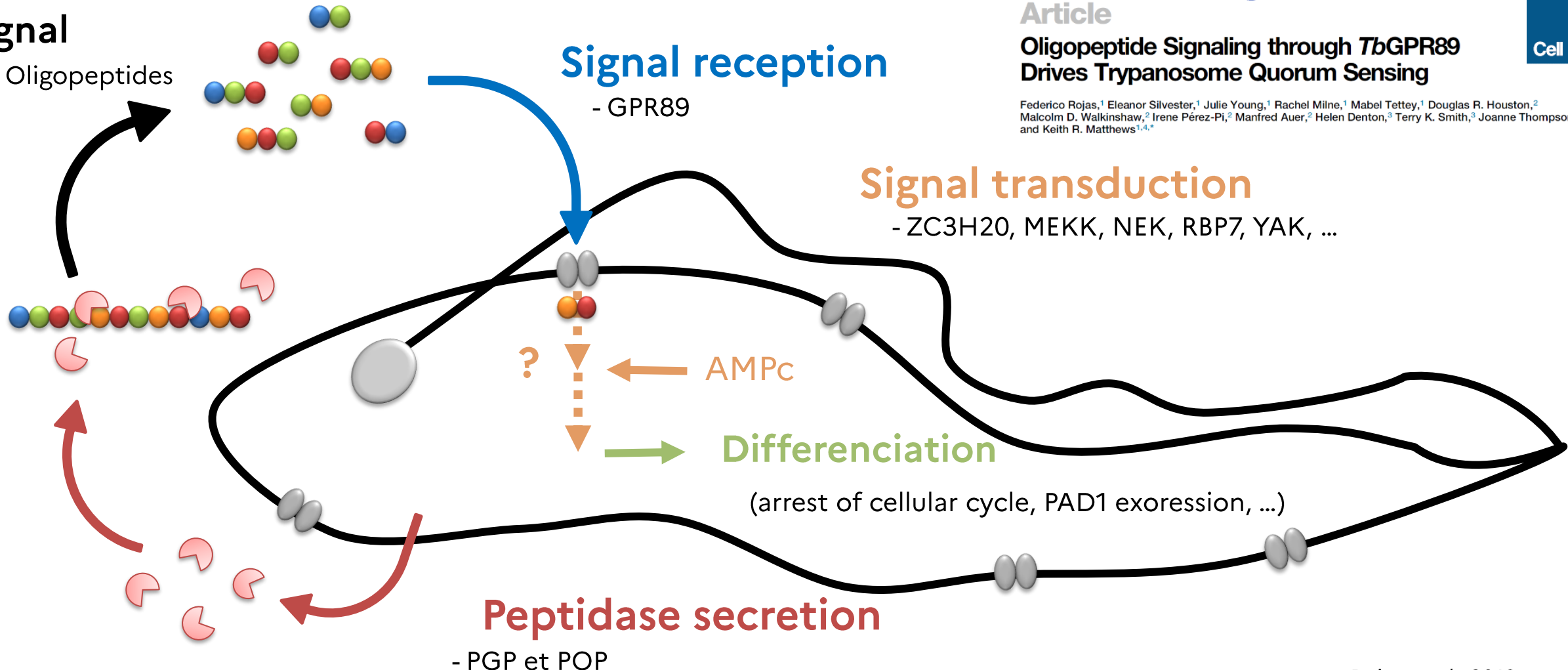
- ZC3H20, MEKK, NEK, RBP7, YAK, ...

Differentiation

(arrest of cellular cycle, PAD1 exoression, ...)

Peptidase secretion

- PGP et POP



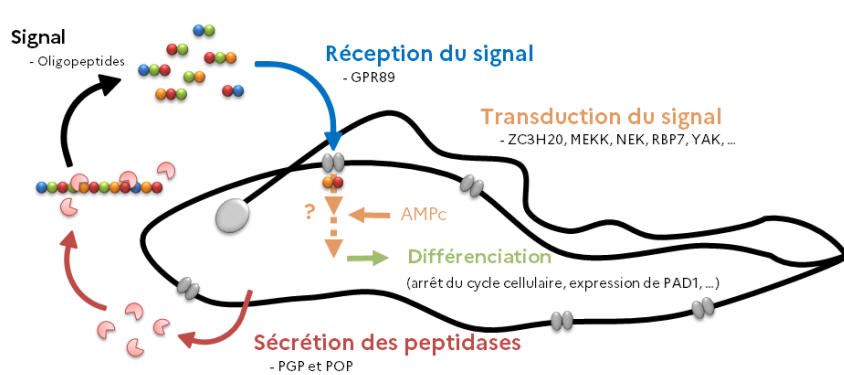
Rojas et al., 2019

Loss of differentiation capacity

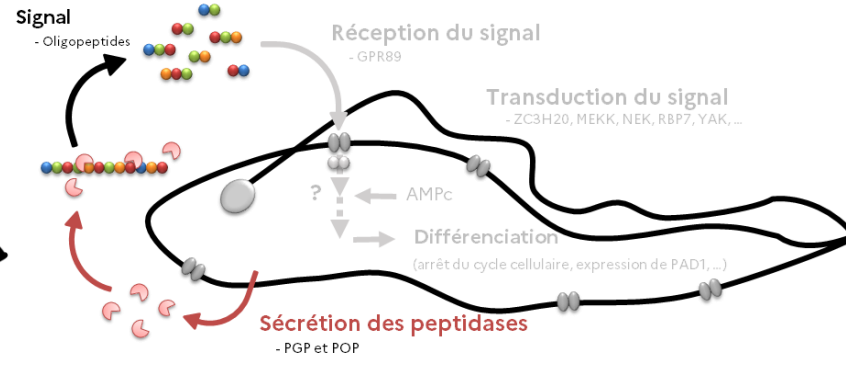
T. brucei

T. equiperdum type OVI

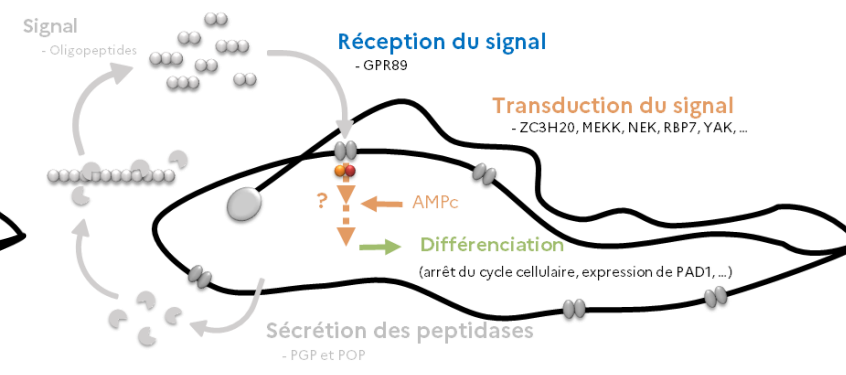
T. evansi type Rotat 1.2



Complete cycle



Lack of signal transduction ?



Lack of signal production?

Are these divergences at the origine of the different pathobiology of dourine and surra ?

Use these differences as a clade markers?

4. WOAAH Standards

WOAH standards

Proposition to gather equine trypanosomosis within a single chapter as been rejected by WOAH in 2018

	Dourine	Surra	Nagana
Terr. Manual	<p>CHAPTER 3.6.3. Updated DOURINE IN HORSES <i>(TRYPANOSOMA EQUIPERDUM INFECTION)</i></p>	<p>CHAPTER 3.1.21. SURRA IN ALL SPECIES <i>(TRYPANOSOMA EVANSI INFECTION)</i></p>	<p>CHAPTER 3.4.14. NAGANA: INFECTIONS WITH SALIVARIAN TRYPANOSOMOSES (EXCLUDING TRYPANOSOMA EVANSI AND T. EQUIPERDUM)</p>
Disease card	<p>DOURINE Updated <i>(INFECTION WITH TRYPANOSOMA EQUIPERDUM)</i> Aetiology Epidemiology Diagnosis Prevention and Control References</p>	<p><i>TRYPANOSOMA EVANSI</i> INFECTIONS (INCLUDING SURRA) Aetiology Epidemiology Diagnosis Prevention and Control References</p>	<p>TRYPANOSOMOSIS (TSETSE-TRANSMITTED)</p>
Case definit°	<p>Oie New WORLD ORGANISATION FOR ANIMAL HEALTH <i>Protecting animals, preserving our future</i> CASE DEFINITION FOR INFECTION WITH <i>TRYPANOSOMA EQUIPERDUM</i> (DOURINE)</p>	<p>Oie New WORLD ORGANISATION FOR ANIMAL HEALTH <i>Protecting animals, preserving our future</i> CASE DEFINITION FOR INFECTION WITH <i>TRYPANOSOMA EVANSI</i> (SURRA)</p>	
Terr. Code	<p>CHAPTER 12.3. DOURINE</p>		<p>CHAPTER 8.18. INFECTION WITH <i>TRYPANOSOMA BRUCEI</i>, <i>T. CONGOLENSE</i>, <i>T. SIMIAE</i> AND <i>T. VIVAX</i></p>

WOAH standards

Dourine

Terr.
Manual

CHAPTER 3.6.3. **Updated**
DOURINE IN HORSES
(*TRYPANOSOMA EQUIPERDUM* INFECTION)

Disease
card

Updated
DOURINE
(INFECTION WITH *TRYPANOSOMA EQUIPERDUM*)
[Aetiology](#) [Epidemiology](#) [Diagnosis](#) [Prevention and Control](#) [References](#)

Case
definit^o

New
 WORLD ORGANISATION FOR ANIMAL HEALTH
Protecting animals, preserving our future
CASE DEFINITION FOR INFECTION WITH *TRYPANOSOMA EQUIPERDUM* (DOURINE)

Terr.
Code

CHAPTER 12.3.
DOURINE

Possible *in vitro* production of dourine antigens

G. Schares 14:00: *In-vitro production of Trypanosoma equiperdum antigen for CFT – time consuming, expensive, but possible*

“One hypothesis asserts that the disease condition “dourine” is actually a host-specific immune response to either T. equiperdum, T. brucei or T. evansi infection”

No need to characterize the trypanosome strain to declare a dourine or a surra case

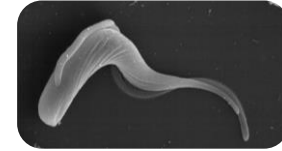
Previous WOAAH criteria for dourine diagnosis

- 1 [
 - A positive result with: CFT or PCR or IFAT

AND

- 2 [
 - i) Clinical signs compatible with dourine
 - OR
 - ii) An increase in serological CFT titer between two consecutive tests
 - OR
 - iii) Epidemiological links with a confirmed case of dourine

No need to characterize the trypanosome strain to declare a dourine



WOAH definition of a surra cases

- Observation of **trypanosomes** with *Trypanozoon* morphology **AND EITHER**

 - a. Detection of **genetic material** specific to *T. evansi*

OR

 - b. The animal is **epidemiologically linked** to a confirmed case of surra

OR

 - c. **Epidemiological context** supports infection (clinical signs, endemicity...)

- Detection of **genetic material** specific to *Trypanozoon* **AND EITHER**

 - a. The animal is **epidemiologically linked** to a confirmed case of surra

OR

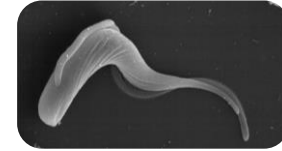
 - b. **Epidemiological context** supports infection (clinical signs, endemicity...)

- Detection of **antibodies** specific to *Trypanosoma spp.* **AND EITHER**

 - a. The animal is **epidemiologically linked** to a confirmed case of surra

OR

 - b. **Epidemiological context** supports infection (clinical signs, endemicity...)



WOAH definition of a dourine cases

- Observation of **trypanosomes** with *Trypanozoon* morphology **AND EITHER**
 - a. The animal is **epidemiologically linked** to a confirmed case of dourine

OR

 - b. **Epidemiological context** supports infection (clinical signs, endemicity...)
-
- Detection of **genetic material** specific to *Trypanozoon* **AND EITHER**
 - a. The animal is **epidemiologically linked** to a confirmed case of dourine

OR

- b. **Epidemiological context** supports infection (clinical signs, endemicity...)

- Detection of **antibodies** specific to *Trypanosoma spp.* **AND EITHER**
 - a. The animal is **epidemiologically linked** to a confirmed case of dourine

OR

- b. **Epidemiological context** supports infection (clinical signs, endemicity...)

In case of suspicion (dourine or surra)

- ▮ **Isolate the animals** (also from insect vectors if possible)
- ▮ **Collect samples and send them to a diagnostic laboratory**
 - Whole blood (blood smears), serum, edema (aspirated the fluid)
- ▮ **Proceed to an epidemiological study** (potential contact with infected animals)
- ▮ **Control the evolution of antibody titer**
 - Plan a **second** serum sampling 15 to 20 days after the first
 - Plan a **third** serum sampling 15 to 20 days after the second
- ▮ **Control the clinical evolution of the animal**

Notify to the competent authorities

Possibility to contact EU RL for assistance and technical advices



5. Equine trypanosomosis in the world

Few countries report Dourine cases

	2018	2019	2020	2021
■ Kyrgyzstan	855	1305		
■ Russia	155	185	87	50
■ Namibia	20	2		
■ Botswana	1			
■ South Africa	1			
Total	1305	1492	87	50



A probably underestimated situation

Few countries report Equine Surra cases

	2018	2019	2020	2021
Venezuela	106	77		
Pakistan			26	4
Malaysia			4	
Uruguay				11
Total	106	77	30	15



A probably underestimated situation

Surra reported cases

	2018	2019	2020	2021
Ethiopia	20	218		
Nepal	5	4	7	
Somalia	74	455	296	148
Tunisia	343	111		
Venezuela	106	79		
Saudi Arabia		4 441	111	232
Ivory Coast		1	68	
Iran			8	15
Malaysia			4	
Pakistan			304	5
Uruguay				11
Total	548	5309	798	411

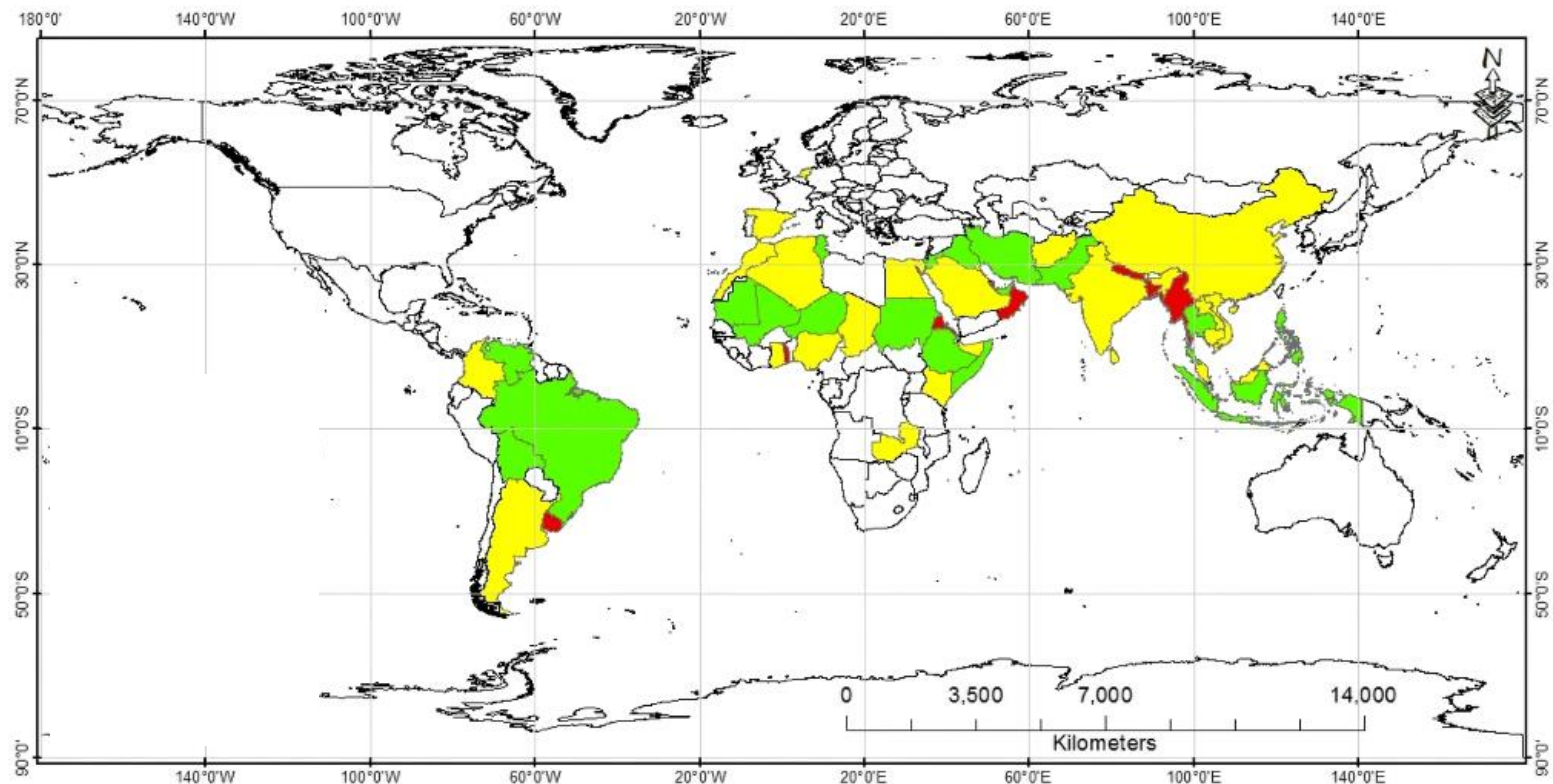


A probably underestimated situation

Surra: WOAH reporting versus literature

Based on:

- WOAH reports since 2009
- Publication from 1906 to 2017



- No publication, no WOAH reporting
- Published evidence and WOAH reporting
- Published evidence and no WOAH reporting
- No Published evidence and WOAH reporting

Equine trypanosomosis in Mongolia

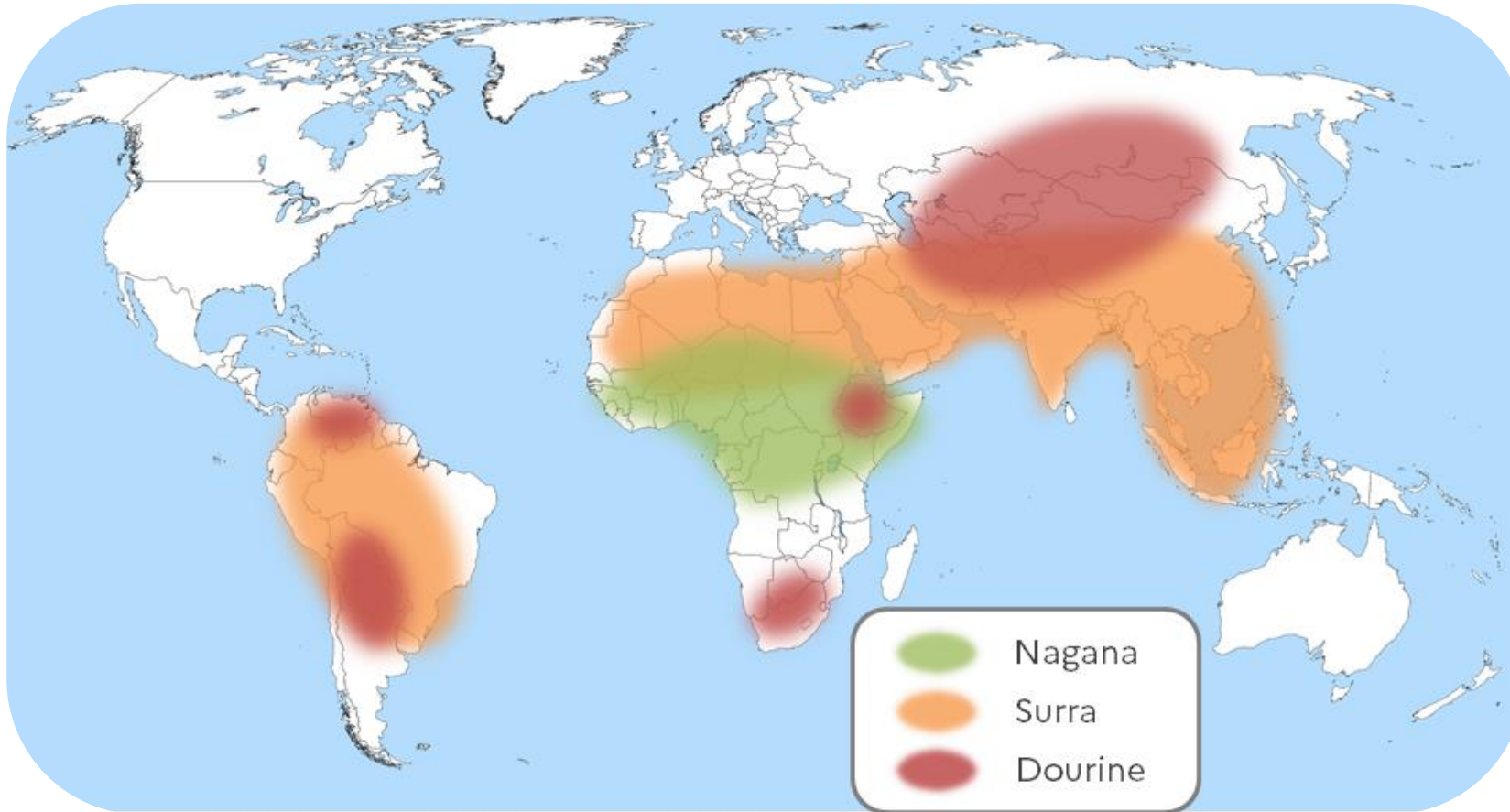
- 3,641 sera randomly sampled
- Tested by ELISA (rTeGM6-4r)
- 173 positive sera
- 2 regions with prevalence > 10%



Mizushima *et al.*, 2019

Equine trypanosomosis constitutes a real sanitary problem in Mongolia

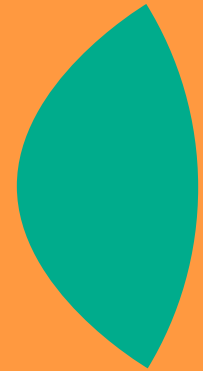
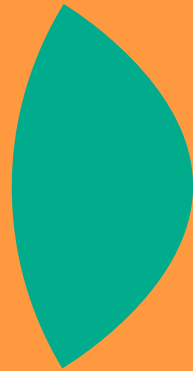
Schematic distribution of equine trypanosomosis



Stamping out policies allows some countries to be free from these diseases
(except for sporadic cases)

Emphasizes the need for control during international movement of equidae

Conclusion

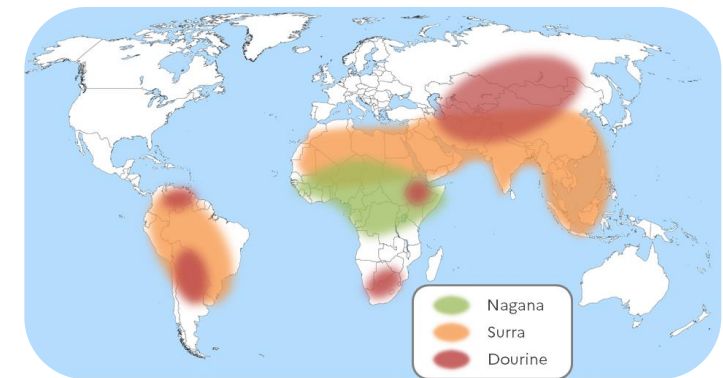


Diagnostic challenge of dourine, surra and nagana

- The relationships between *Trypanozoon* clades and pathologies remain unclear
- A parasitemia often low: limits the use of parasitological and molecular tests
- Serological tests (CFT, IFAT, CATT, ELISA) cross reactions with all *Trypanozoon*
- Is the distinction between taxa within the subgenus *Trypanozoon* advisable?

Perspectives

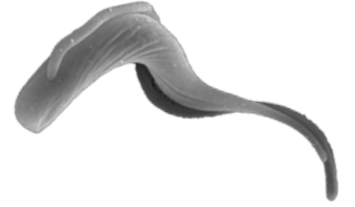
- Equine trypanosomosis remain a threat for equids
- Continue collecting samples in endemic zones
- Continue to develop diagnostic tools: REASSURED criteria



REASSURED diagnostics (Land et al., 2018)

	Criteria	Description
R	Real-time connectivity	Tests are connected and/or a reader or mobile phone is used to power the reaction and/or read test results to provide required data to decision-makers
E	Ease of specimen collection	Tests should be designed for use with non-invasive specimens
A	Affordable	Tests are affordable to end-users and the health system
S	Sensitive	Avoid false negatives
S	Specific	Avoid false positives
U	User-friendly	Procedure of testing is simple — can be performed in a few steps, requiring minimum training
R	Rapid and robust	Results within 15 min to 2 hours (without requiring additional transport and storage conditions such as refrigeration)
E	Equipment free or simple	Ideally the test does not require any special equipment or can be operated in very simple devices that use solar or battery power
	Environmentally friendly	Completed tests are easy to dispose and manufactured from recyclable materials
D	Deliverable to end-users	Accessible to those who need the tests the most

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- Liam Morrison



- Cirad - UMR INTERTRYP

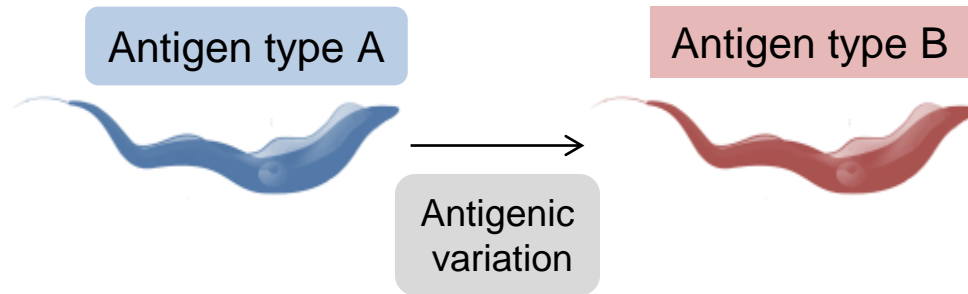
- David Berthier
- Sophie Thévenon



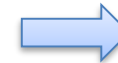
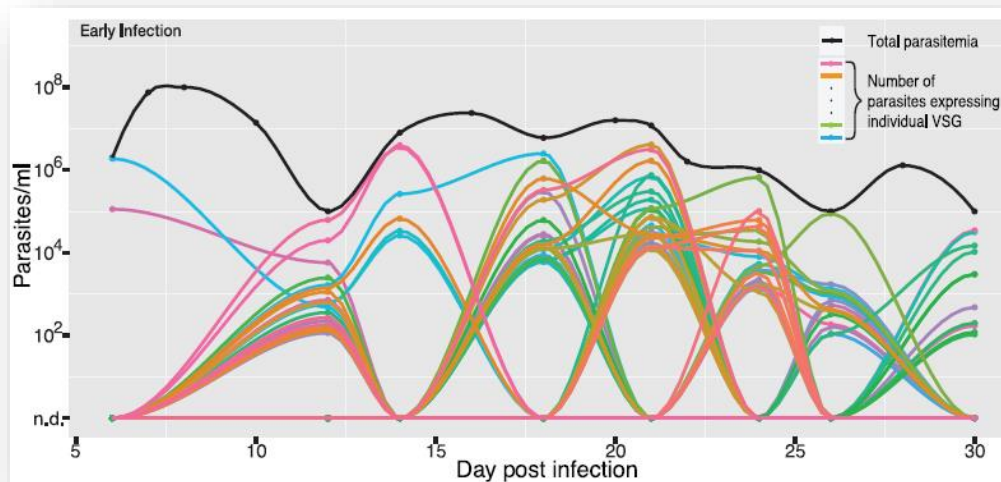
Thank you for
your attention



Antigenic variation: Variable Surface Glycoproteins (VSG) coat

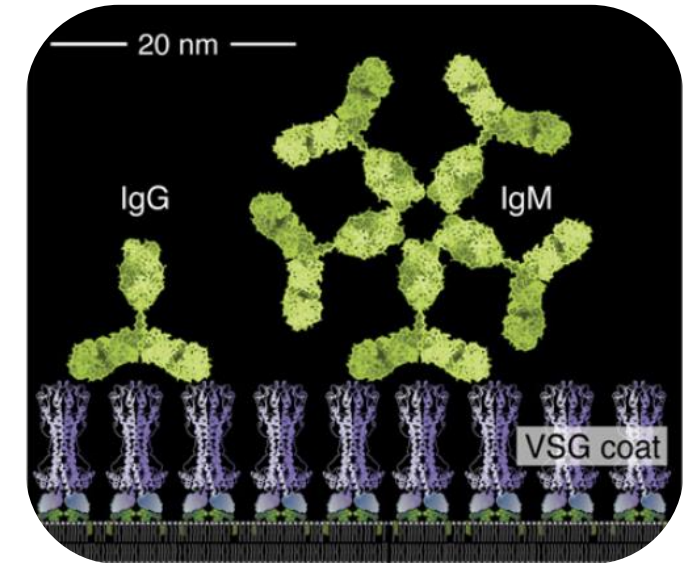


Antigenic variation in *Trypanosoma brucei*



Dynamic mechanism of immune evasion

(Mugnier et al., 2015)



(Engstler et al 2007)

WOAH standards

Dourine

Method	Purpose					
	Population freedom from infection	Individual animal freedom from infection prior to movement	Contribute to eradication policies	Confirmation of clinical cases	Prevalence of infection – surveillance	Immune status in individual animals or populations post-vaccination
Detection of the agent¹						
Microscopic observation	-	+	+	+++	-	-
PCR/ real-time PCR	-	+	+	+++	+	-
Detection of immune response						
CFT	++	+++	+++	+++	+++	-
IFAT	++	+	++	+	++	-
ELISA	+++	+	+++	+	+++	-
ICT	+	+	+	+	+	-

Surra

Method	Purpose					
	Population freedom from infection	Individual animal freedom from infection prior to movement	Contribute to eradication policies	Confirmation of clinical cases	Prevalence of infection – surveillance	Immune status in individual animals or populations post-vaccination
Detection of the agent^(a)						
Thin GSBS (or lymph or oedema fluid)	-	+	+	+++	++	-
DNA detection/PCR	+++	+++	+++	+++	+++	-
Wet blood film	-	-	-	++	-	-
TGSBF	-	-	-	++	++	-
HCT (Woo)	+++	+++	+++	+++	+++	-
BCT (Murray)	+	+	++	++	++	-
AECT	-	+	++	++	-	-
Detection of immune response						
CATT/ <i>T. evansi</i>	++	++	+++	+++	++	-
IFAT <i>T. evansi</i>	++	+++	+++	+++	++	-
ELISA <i>T. evansi</i>	+++	+++	+++	+++	+++	-
TL RoTat1.2 test	-	-	++	-	+	-

Key: +++ = recommended for this purpose; ++ recommended but has limitations; + = suitable in very limited circumstances; - = not appropriate for this purpose.

GSBS = Giemsa-stained blood smear; PCR = polymerase chain reaction; TGSBF = thick Giemsa-stained blood film; HCT = haematocrit centrifuge technique; BCT = buffy coat technique; AECT = anion exchange chromatography technique; CATT = card agglutination test; IFAT = indirect-fluorescent antibody test; ELISA = enzyme-linked immunosorbent assay; TL = trypanolysis test.

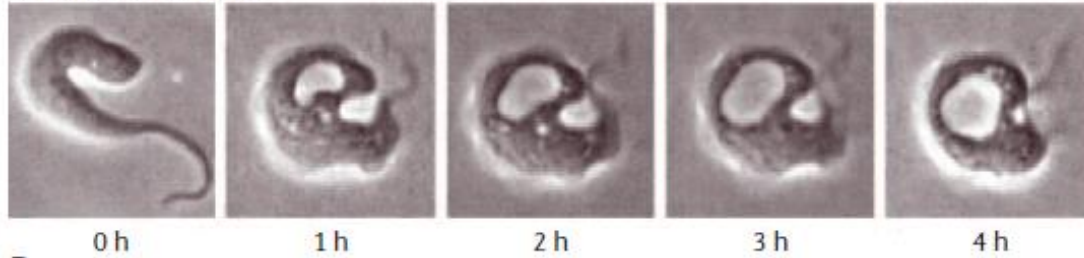
^(a)A combination of agent identification methods applied on the same clinical specimen is recommended (See Section B.3.2).

Trypanosome Lytic Factor (TLF) of human serum

APOL1 (Apolipoprotein L-1) lyse les trypanosomes

Les VE permettent le transfert de SRA

Normal human serum



La protéine SRA permet aux trypanosomes de résister à APOL1

