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Recombinant proteins for efficient African Animal Trypanosomiasis (AAT) diagnosis : new candidates tests

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I Background

1. Trypanosomes

- Unicellular and flagellate parasite from kinetoplastida order.
- Deadly parasite, transmitted by blood-sucking insects : *Glossina sp* and Tabanids and Stomoxes.
- Develop and circulates in blood and lymph vessels with high antigenic variation that makes difficult their detection.

2. African animal trypanosomiasis : Nagana

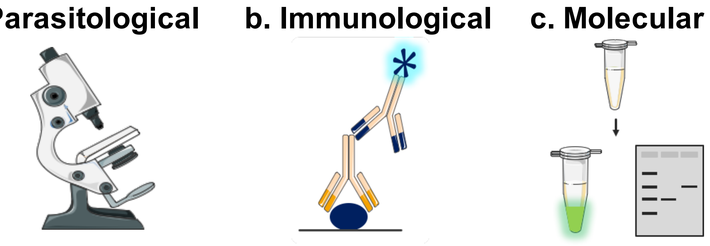
- Major neglected animal disease caused by *T. congolense, T. vivax and T. b brucei.* Main hosts : cow, sheep, goat.
- Major livestock disease associated to US $1-2 billon losses/year, with more than 50 millions animals at risk (1).
- No vaccine, inadequate diagnostics and therapeutics.

3. Current diagnostic tools and tests

- The disease control still relies on efficient diagnosis.
- Current tests are facing a problem of sensitivity, they are expensive and few targets available.

Need of new targets and new test development

a. Parasitological **b. Immunological** **c. Molecular**



Need special equipment, expensive, no specific identification, very low sensitivity (except PCR).

II Results

Objectives : Develop and standardize an easy, specific, sensitive and inexpensive diagnostic test for animal trypanosomiasis.

PhD program

Fig 1 : ParaFrap PhD south program.

Workflow

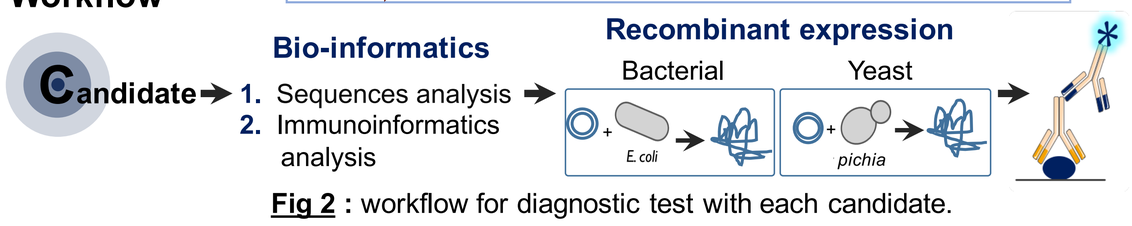


Fig 2 : workflow for diagnostic test with each candidate.

Candidates

Fig 3 : Trypanosomes specific proteins identified as potent diagnosis target.

Three candidates are in testing in the Lab. Two is presented hereafter : ***Tb*PLA-1b and *Tb*GK.**

Materials : Trypanosomes infected mouse and bovine experimental sera and bovine field sera are used in this work.

Trypanosoma brucei phospholipase antibody detecting test

***Tb*PLA** are known as virulence factor. *Tb*PLA-1b is currently study in the lab and is conserved in *Trypanoma sp.*

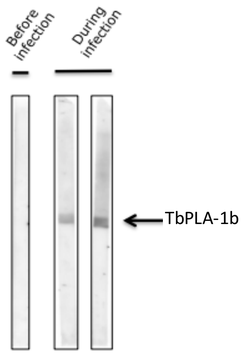


Fig 4 : *Tb*PLA-1b recognition by *T.brucei* infected mouse sera by Western blot (2).

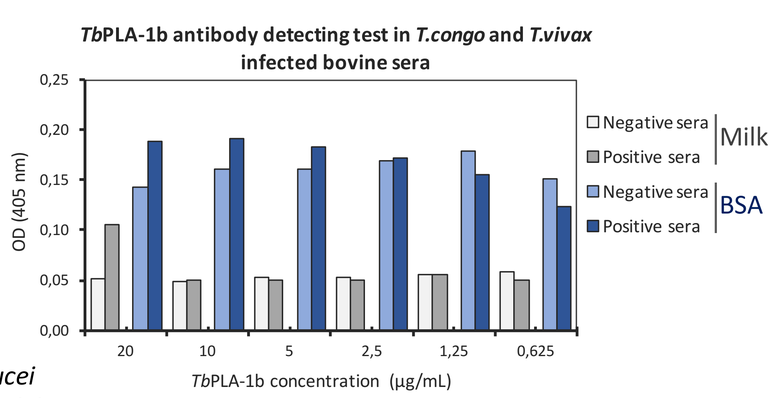


Fig 5 : Indirect ELISA test for antibody detection using different concentration of *Tb*PLA-1b. Carbonate coating, blocking (Milk 5% and BSA 0.5%).

***Tb*PLA-1b induces a specific antibodies during *T. brucei* infection in mouse. Very low reactivity of *T. congolense* and *T. vivax* infected cattle sera to *Tb* PLA-1b. Specific antibody present in low concentration ?**

*Tb*PLA-1b antibody detection in immunoglobulin enriched bovine sera (*T.congolense*/*T.vivax* infected)

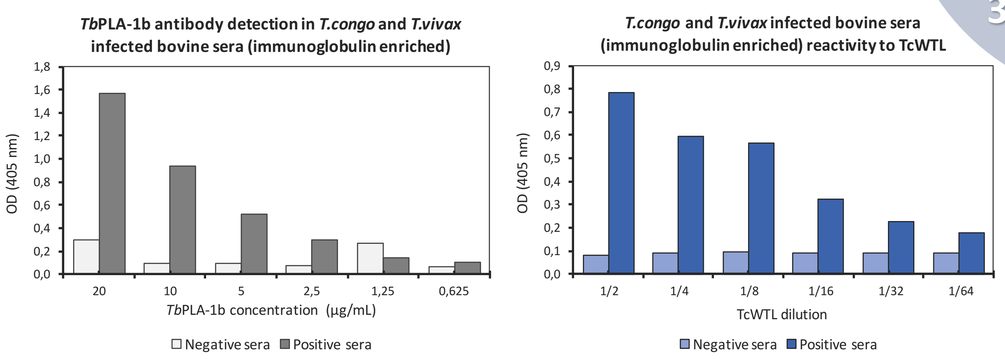


Fig 6 : *Tb*PLA-1b antibody detection by indirect ELISA in immunoglobulin enriched bovine sera with NH_2SO_4. **Left,** *Tb*PLA-1b in carbonate coating and milk blocking ; **right**, positive control with trypanosomes total extract (WTL : **W**hole **T**rypanosome **L**ysate in PBS coating and BSA blocking).

High reactivity of positive sera to TbPLA-1b, proportional to its concentration, equivalent to WTL reactivity. The result suggest a specific antibody presence but need to be confirmed. Immunoglobulin enrichment will be a limiting step for diagnosis.

Combination strategy : *Tb*PLA-1b & *Tb*GK (*T.brucei* Glycerol Kinase) ?

Trypanosoma brucei glycerol kinase a key for AAT diagnosis based on combination strategy

- glycerol-3-phosphate dependent ADP phosphorylation : ATP generation
- Very abundant and localized in the glycosome.

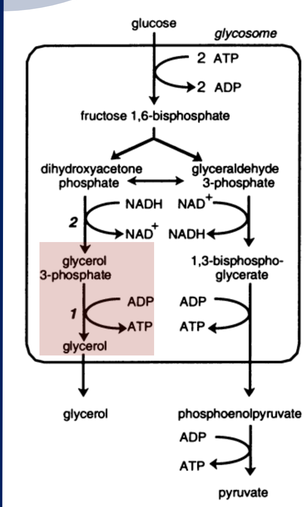


Fig 7 : *T. brucei* BSF Glucose metabolism under anaerobic condition (3).

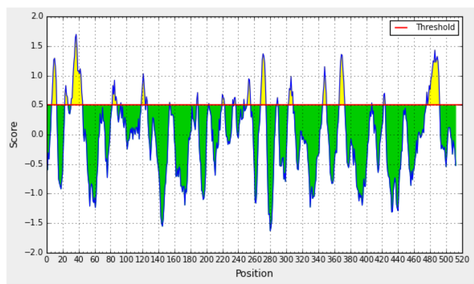


Fig 8 : *Tb*GK antibody detecting test. **(Right up)** Sequential epitopes prediction using BepiPred-2.0. **(Right down)** *Tb*GK antibody detection in *T.bg*, *T.congo* and *T.vivax* infected mouse sera. *Tb*GK specific antibody as positive control.

Specific anti-GK antibody detected in virulent *T.vivax* infected mouse sera.

Test confirmation with bovine sera (...).

Combination *Tb*GK_new candidate in the way.

III Conclusion and Perspectives

Conclusion:

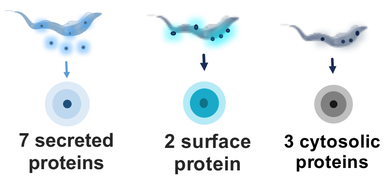
- African animal trypanosomiasis diagnostic suffers from a lack of sufficiently specific candidates for a field test. Here we tested two new candidates for AAT serological diagnostic. We demonstrated that specific anti-*Tb*PLA-1b and *Tb*GK antibodies are secreted during trypanosomes infection in mouse and bovine (enriched sera, anti-*Tb*PLA-1b).

To be continued… Confirm the specific antibody detection in trypanosomes infected bovine sera.

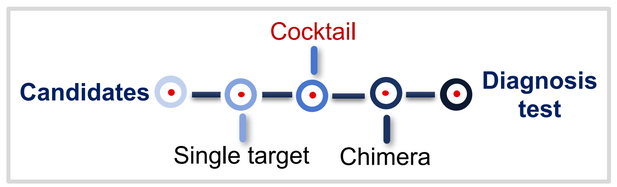
- Because of large antigenic variation of trypanosomes, the alternative way for efficient AAT diagnostic will be a recombinant proteins combination.

Perspectives

- **New candidates screening**



AAT diagnostic test development strategy using recombinant proteins



12 new candidates will be expressed and tested in single test. The most sensitives will be mixed in cocktail to develop an efficient diagnostic test. For the test standardization, proteins will be expressed in chimera.

References : **1**. Pillay et *al*, 2013; **2.** Monic et *al,* in preparation; **3** Kralova et *al,* 2000