Investigation of Camel Trypanosomiasis in Egypt

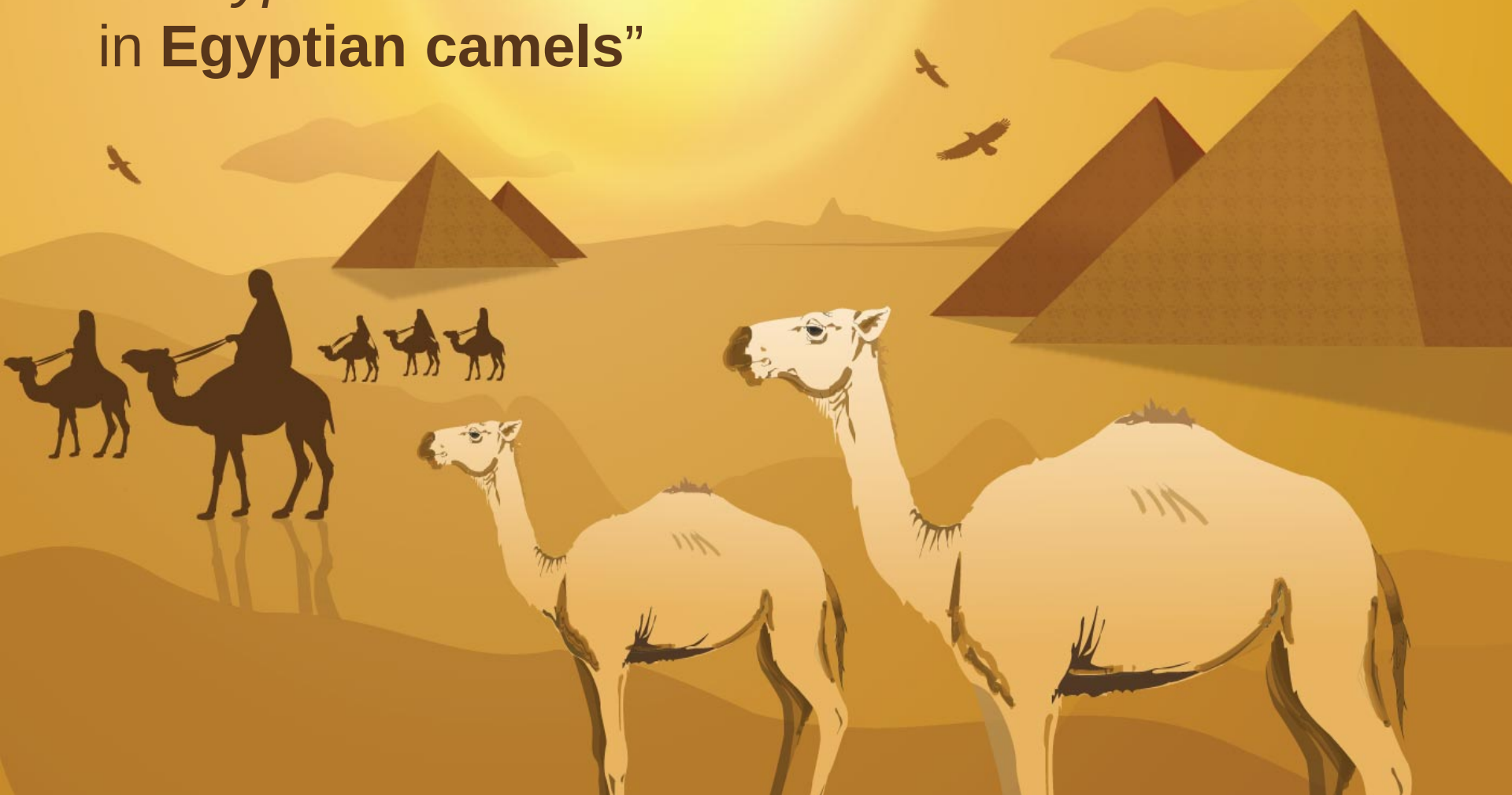
Helen Price

2nd International NTTAT Conference in Antwerp - 18 December 2017

Newton-Mosharafa Fund

The project

"Investigating **drug resistance** and **new treatments** for *Trypanosoma evansi* infection in **Egyptian camels**"



The Team

- Prof. Somaia Abou-Akkada
- Prof. Karam Imam Ashmawy
- Amira Wahid Deweir
- Sherif Kamal El Shanat

Keele University

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Imperial College London

- Prof Ed Tate
- Dr. Andy Bell

- Prof. Robert Nash

PhytoQuest
unlocking iminosugars

THE UNIVERSITY OF EDINBURGH

Dr. Achim Schnaufer

UNIVERSITY OF BIRMINGHAM

Dr. Farhat Khanim

Project Aims

- Determine disease prevalence in study regions
- Assess the extent of drug resistance in field isolates
- Provide training opportunities for staff from the Faculty of Veterinary Medicine (FVM) at the University of Alexandria (UA)
- Improve research capabilities at the FVM to enable future large scale monitoring of *T. evansi* infection in camels using molecular biology techniques
- Perform drug screen of selected libraries of drugs to identify efficient trypanocidal compounds
- Test the most promising compound in a mouse infection model (Edinburgh)

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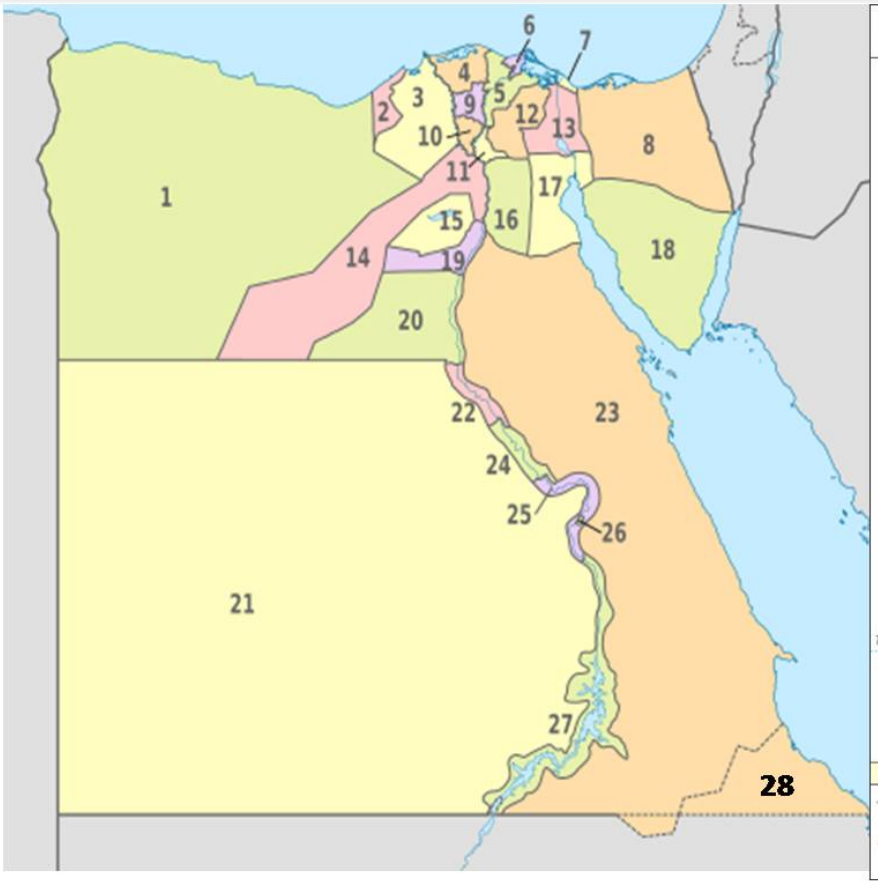
Project Aims



The intended study sites (provinces) for collection of samples.

1- Matrouh
2- Alexandria
3- Behera
27- Aswan
28- Haleib & Shalateen

Drug Screening – methodology

- Initial single dose screening (~1000 compounds) on a *T. evansi* established strain using Alamar Blue assay

  Libraries to be tested:

  (1) **pure compounds** **from plants** grown in **temperate** regions (Phytoquest)

  (2) **i**nhibitors of *T. brucei* enzyme ***N*-myristoyltransferase** (Imperial College London)

  (3) **off-patent drugs** tested at human Cmax (Birmingham)

- Determination of IC50 values for hit compounds
- Cytotoxicity analysis using a mammalian cell line (HepG2).
- Existing data for *T. brucei* will inform choice of compounds.
- The most promising candidate will be tested in a *T. evansi* mouse infection model

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Drug Screening – methodology

- Initial single dose screening:
  - **127** PhytoQuest compounds + **13** NMT inhibitors
  - Concentrations: **10 uM** and **1 uM**
  - In duplicate
  - PrestoBlue Assay
  - Positive Control – parasite culture + 0.5% DMSO
  - Negative Control – parasite culture +2uM Amfotericin B
- Definition of a **hit**:

  The compound whose **1uM concentration** would **inhibit growth of more than 50% of *T. evansi* parasites**, given that the **standard deviation** of the duplicate measurements **would not be higher than 5.**

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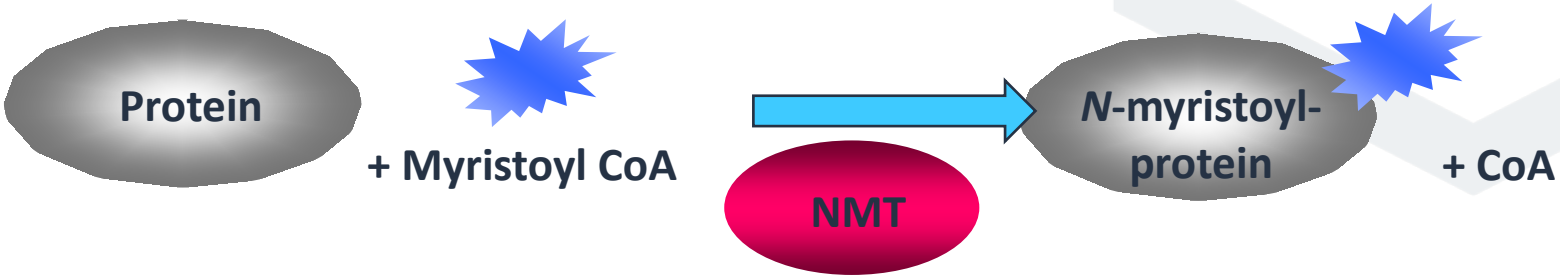
N-Myristoylation and NMT

- Attachment of fatty acid myristate to N-terminus of target protein
- Aids subcellular targeting to membranes and protein-protein interactions
- Catalysed by the enzyme myristoyl CoA: protein *N*-myristoyl transferase (NMT)



- NMT is a validated drug target in fungal pathogens
- Conservation of Myristoyl CoA binding site
- Variation of peptide binding site

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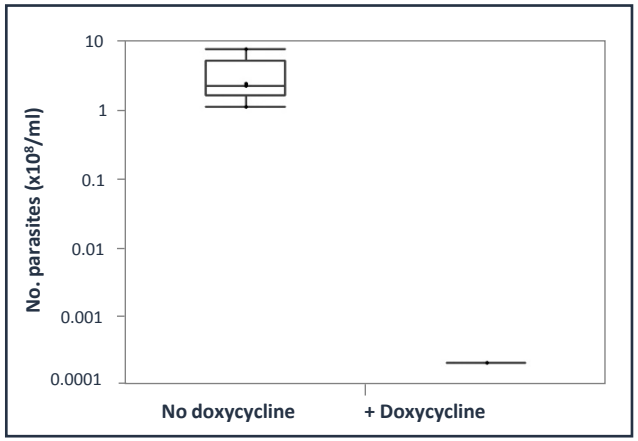
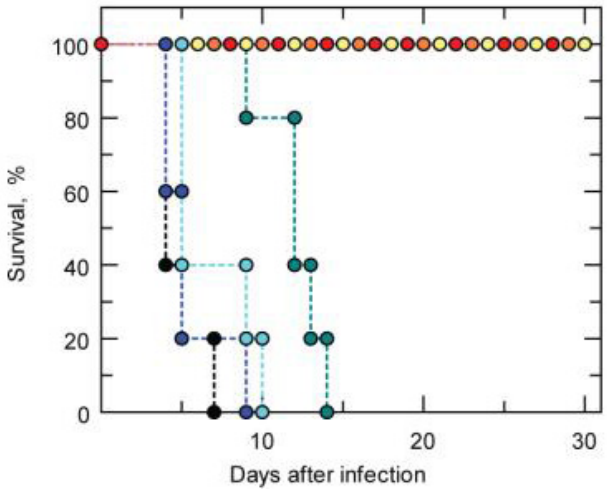
NMT inhibitors in *Trypanosoma brucei*



HTP screening (DDU, Dundee) identified a hit series with nanomolar activity against bloodstream form *T. brucei*

RNAi shows that NMT is essential in vivo: parasites unable to infect mouse model (Price *et al*, 2010)

DDD85646 cures acute HAT in vivo but not chronic CNS stage (Frearson *et al*, 2010)



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Thank you