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French agency for food, environmental and occupational health safety

EU Reference Laboratory
for equine diseases

“Trypeq” project on dourine treatment

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fondation hippolia
RECHERCHE ET INNOVATION ÉQUINE

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Evolution of dourine infection

✔ **An infection course poorly documented due to the limited case number**

✔ **Divided in two main phases** (by analogy with Human African Trypanosomiasis):

- Invasion of tissues and blood:
  - Local œdema (genital, ventral or dermal)
  - Weight loss
  - Moderate fever
  - Anaemia
  - Increased number of monocytes

Early phase

- Invasion of central nervous system:
  - Facial and lip paralysis
  - Incoordination

⇒ Lead to inability to rise and death

Late phase

Treatment of dourine

- **OIE Terrestrial Manual 2013**

  "The only effective control is through the slaughter of infected animals"

- **Slaughtering of infected animals is impractical for endemic countries**

- **Different trypanocidal drugs are available**

  - Frequent relapses are reported following trypanocidal treatment

- **Utilisation of Cymelarsan® for surra treatment**

  - For camels Cymelarsan® is effective (curative) against *T. evansi*

Is Cymelarsan® applicable for dourine treatment?

Report of Cymelarsan® efficacy against *T. equiperdum*

Hagos et al., 2010

- 6 horses infected by *T. equiperdum* Dodola
- 20 days post infection:
  - 2 control horses
  - 2 horses received one dose of 0.25 mg/kg of Cymelarsan®
  - 2 horses received one dose of 0.5 mg/kg of Cymelarsan®

→ No *T. equiperdum* relapse in blood 320 days post treatment

Questions remaining

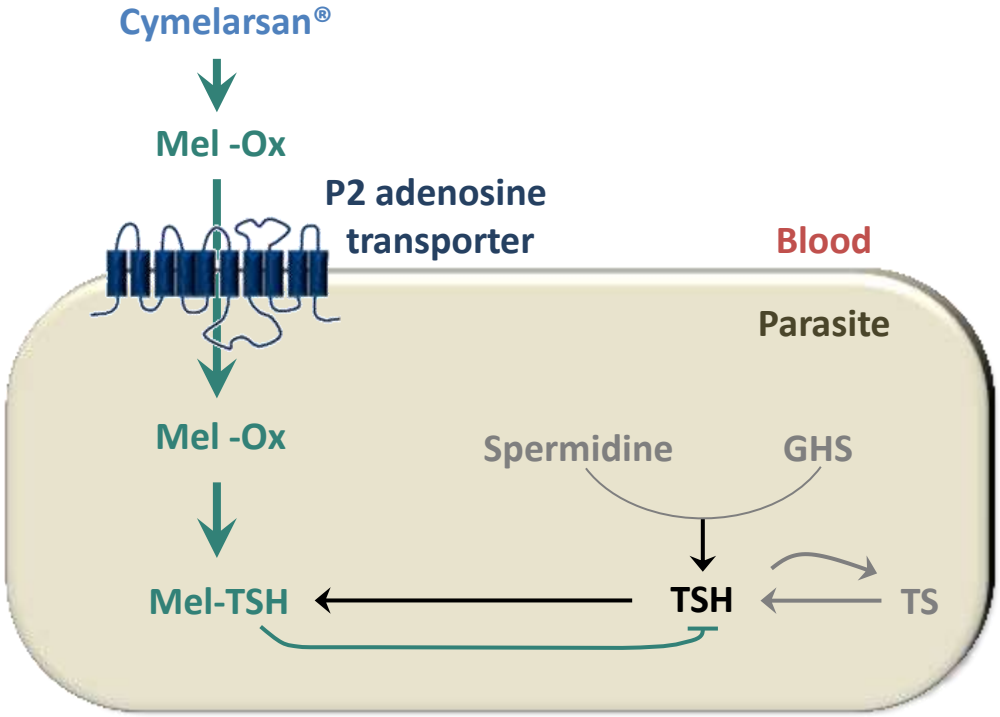
- Stage of infection during Cymelarsan® administration?

→ Late phase?

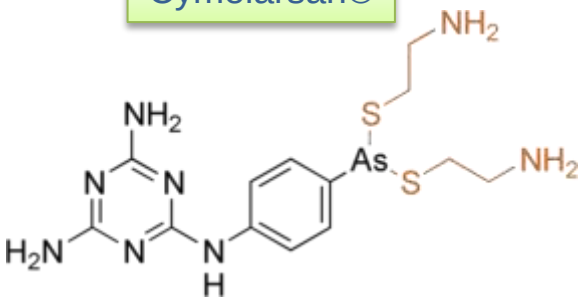
- Did *T. equiperdum* reach the central nervous system?

Cymelarsan® structure and main mechanism of action

An organo-arsenic drug analogue of purine



Cymélarsan®



Melarsen oxide + 2 Cysteamine

- Melarsen oxide is the active form
- Inhibits the trypanothione metabolism
- Alter the redox potential of the cell

Loss of mobility followed by cell lysis

This drug is believed to penetrate the blood–brain barrier

- 3–4 % of the maximum level reached in plasma

Adapted from Baker et al., 2013

Goals and objectives

Determine if the Cymelarsan® allows the elimination of *T. equipedum* from CSF of infected horses

✓ **Experimentally reproduce the disease** (in a time constrained context)

- Determine the best way of infection
- Determine the most suitable *T. equiperdum* infection dose

✓ **Ensure that the infection has reached the late phase**

- Research of *T. equiperdum* in CSF
- Eventually observed nervous clinical signs

✓ **Evaluate the Cymelarsan® efficacy**

- Determine the efficient dose of treatment
- Search for parasites in blood, tissues and CSF

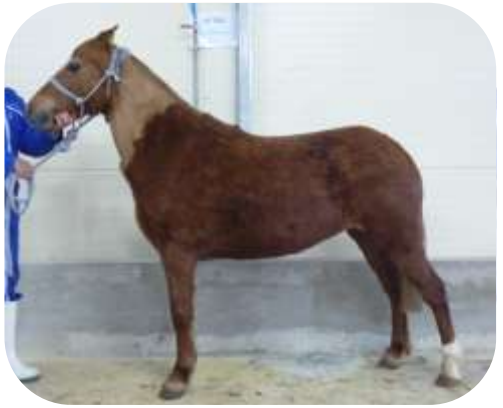
Animal experimentation

- **Experimental infectiology platform** (Tours, France)

- **Authorized by Ethic committee CEEA VdL N°19** (on February 27 2015)

Experimentations

- **4 Welsh ponies** (thereafter named A, B, C and D)
  - Females
  - About 11 years old



- **General monitoring**
  - Physical examination (partial: 3/day, full: 3/week)
  - Weight, temperature
  - Vaginal sampling

- **Blood sampling: 3/week**
  - Complete blood count and biochemical analysis
  - Serology (CFT dourine)

- **CSF sampling** (Pease et al., 2013)
  - Echography guided low risk protocol adapted to ataxic animals
  - Few blood contamination and good repeatability

Selection of infection procedure

✓ **Selected strain: *Trypanosoma equiperdum* OVI**

- Rat blood stabilates
- Dilution of *T. equiperdum* in PSG buffer

Poney	1st infection
A	**Intraveinous** **5.10^4 Teq** → **D+4**
B	**Intravaginal** **5.10^4 Teq** → **D+10**
C	**Intravaginal** **$7.5.10^3$ Teq** → ✗
D	**Intravaginal** **1.10^3 Teq** → ✗

Selection of infection procedure

✓ **Selected strain: *Trypanosoma equiperdum* OVI**

- Rat blood stabilates
- Dilution of *T. equiperdum* in PSG buffer

Poney	1st infection	2nd infection	3rd infection	4th infection
A	**Intraveinous** **5.10^4 Teq** → D+4			
B	**Intravaginal** **5.10^4 Teq** → D+10			
C	**Intravaginal** **$7.5.10^3$ Teq** → ✗	**Intravaginal** **$7.5.10^3$** → ✗	**Intravaginal** **5.10^4** → ✗	**Intraveinous** **5.10^4** → D+7
D	**Intravaginal** **1.10^3 Teq** → ✗	**Intravaginal** **1.10^3 Teq** → ✗	**Intravaginal** **5.10^4** → ✗	**Intraveinous** **5.10^4** → D+7

Intravaginal: poorly reproducible (or an incubation time too long)

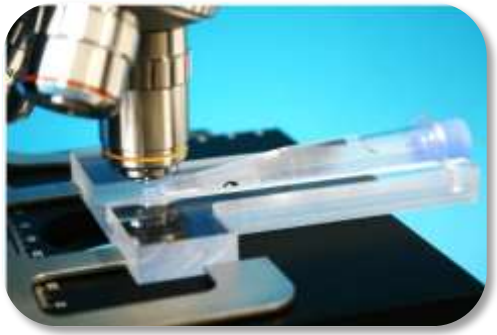
→ **Intraveinous:** fast and reproducible

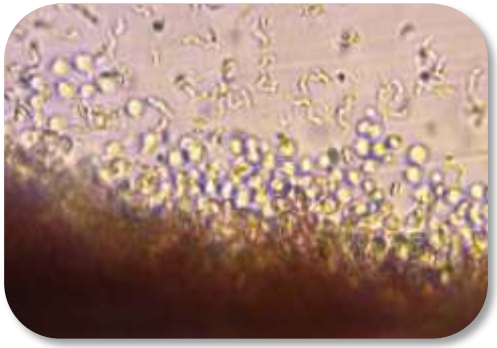
Identification of late infection phase

✓ Echography guided CSF sampling

- Pease et al. 2013

Poney	Time from blood to CSF
A	≤ 5 days
B	≤ 19 days
C	≤ 7 days
D	≤ 7 days





Centrigufation in
mAECT collector tubes

→ **Obtaining 4 animals with CSF infected by *T. equiperdum***

→ Late phase

Evaluation of Cymelarsan® efficacy

✓ Cymelarsan®

- Cymelarsan® dose for surra treatment in camels: 0.25 mg/kg

Poney	Time after Late phase	Cymelarsan® dose	Blood clearance	CSF clearance
A	13 days	0.5 mg/kg	≤ 4 hours	**No**

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Poney	Time after Late phase	Cymelarsan® dose	Blood clearance	CSF clearance
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B	6 days	0.5 mg/kg x1 0.5 mg/kg x5	≤ 4 hours	**No**

Evaluation of Cymelarsan® efficacy

Cymelarsan®

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Poney	Time after Late phase	Cymelarsan® dose	Blood clearance	CSF clearance
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B	6 days	0.5 mg/kg x1 0.5 mg/kg x5	≤ 4 hours	**No**
C	1 day	0.5 mg/kg x6	≤ 4 hours	**Yes**

Evaluation of Cymelarsan® efficacy

Cymelarsan®

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Poney	Time after Late phase	Cymelarsan® dose	Blood clearance	CSF clearance
A	13 days	0.5 mg/kg	≤ 4 hours	**No**
B	6 days	0.5 mg/kg x1 0.5 mg/kg x5	≤ 4 hours	**No**
C	1 day	0.5 mg/kg x6	≤ 4 hours	**Yes**
D	1 day	0.5 mg/kg x6	≤ 4 hours	**Yes**

These results need to be confirmed

Conclusion

✓ **Cymelarsan® is effective for parasite elimination from blood**

→ In less than 4 h

✓ **0.5 mg/kg of Cymelarsan® does not eliminate *T. equiperdum* from CSF**

→ At the origin of parasite relapse after treatment?

Is a single 0.5 mg/kg dose of Cymelarsan® adapted to dourine treatment?

✓ **An increased number of Cymelarsan® injections**

→ Observation of an elimination of *T. equiperdum* from CSF

→ These results need to be confirmed

Outlook

Evaluate the efficacy of multiple injections of Cymelarsan® for dourine treatment

- **A protocol including 8 Welsh ponies**
  - A conserved follow-up protocol
  - A single intravenous infection of 5.10^4 parasites
  - CSF sampling to ensure obtaining a late phase dourine
- **Cymelarsan® treatment**
  - One dose per day during 7 days
  - Evaluation of CSF clearance by echography guided sampling
- **In case of CSF clearance**
  - Induction of potential relapse by artificial immunosuppression of horses

A protocol currently in progress…

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