Histomonosis: the disease, diagnosis, life cycle & immunity

W.J.M. Landman

GD – Animal Health Service, Deventer, Netherlands
Antihistomonals no more!
The problem

- EU: exemption for nitarsonene denied (April 2005)

- No effective chemotherapeutics left!
Worldwide distribution
In vivo study herbal products

‘Standard’ inoculation dose of 200,000 histomonads/bird
Chickens reservoir & wild birds?
Diagnosis

• Clinical signs
• Postmortem
  • Liver & cecum lesions
• Fresh smears?
• Histopathology
  • Eosinophilic bodies
• Immunohistochemistry
• *In situ* hybridization

• Serology
  – ELISA’s
• Culture
• PCR
• Genotyping
Caecum tissue
Liver tissue
Blocking-ELISA

positive

negative
Culture medium

- 85% medium 199 with Hank’s salts
- 10% heat-inactivated horse serum
- 5% chicken embryo extract
- 10-12 mg organic white rice flour
Bürker-Türk haematocytometer

200 x
10% Dimethylsulfoxid (DMSO)
-10°C/min
qPCR

• Primer & probe design:
  – 31 *H. meleagridis* 5.8S rRNA DNA sequences (GenBank) from 4 research groups
  – Specificity: various trichomonad sequences (GenBank)
  – MGB probe
  – 81 bp product

• AM1840 extraction kit (Ambion)
• Quantifast pathogen PCR kit (Qiagen)
• ABI 7500 fast and LightCycler 480 cyclers
Results qPCR

- Successful detection reference isolate
- Detection limit: 330 histomonads/ml faeces
- 17 Dutch *H. meleagrisidis* (morphological identification) isolates from chickens or turkeys tested all positive
- A *Tetratrichomonas gallinarum* strain tested negative
Ribosomal gene cluster
C-profiling
H. meleagridis type III

Bar = 30 µm  Genbank submission nrs. DQ350439-47
Characteristics genotype III

- Distinct morphology
- Not culturable
- Not disease-associated
- ITS-1 sequences different (another species?)
- Different sequence 150 bp 3’ end SSU rRNA gene
- Phylogenetic analysis: closely related to *H. meleagridis* & *D. fragilis*
- *P. wenrichi*?
H. meleagris – life cycle

Turkey image by Terell Spencer, NCAT
Research Note—

Direct Lateral Transmission of Histomonas meleagridis in Turkeys

Jinghui Hu and L. R. McDougald
Inoculation

- Day 14
- Intracloacal
- 200,000 histomonads/bird
- 25% (10/40) poults
Survival of *H. meleagridis* inoculated and contact turkey poults

Days post inoculation

- Inoculated - females (n = 5)
- Inoculated - males (n = 5)
- Contact - females (n = 15)
- Contact - males (n = 15)
Transmission parameters

- Mean latent period: 1.8 days (95%: 0.9 - 3.2)
- Mean infectious period
  - until death: 13.6 days (7.1 - 20.7)
  - until recovery: 6.0 days (4.3 - 8.8)
- Proportion dying from infection: 82% (66 - 93)
- \( R_0 \) basic reproduction number: 5.0 (2.9 - 14.7)
- \( \beta \) transmission rate per day (estimated): 0.4
Simulations

Transmission experiment

Transmission model

Experiment simulation

Flock outbreak simulation
Five simulated outbreaks of *H. meleagridis* in a flock of 20,000 turkeys
Transmission

- Vector transmission
  - *Heterakis gallinarum*
  - Earthworm
- Direct transmission within flocks
  - ‘Cloacal drinking’?
Infection of Turkeys with *Histomonas meleagridis* by the Cloacal Drop Method

Jinghui Hu, L. Fuller, and L. R. McDougald

Department of Poultry Science, University of Georgia, Athens, GA 30602

Received 2 January 2004; Accepted 13 February 2004
Attemps to induce histomonosis with cyst stages failed (in our hands)

Oral & intracloacal inoculations
Immunity

• Active
  – Antibodies do not protect against reinfection

• Passive
  – Serum immunized birds does not protect against challenge

• Antibodies detected by ELISA
  – Indirect
  – Blocking
Antibody detection field

- Unexpected high seroprevalence layers
  - 87% ↔ 37% (Grafl et al., 2011)
- Confirmed in 5 flocks
  - Culture
  - PCR
- Importance of chickens as reservoir
Is there hope at all?

Yes
The effect of hanging

<table>
<thead>
<tr>
<th></th>
<th>Histomoniasis experiment 1 (%)</th>
<th>Histomoniasis experiment 2 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chickens</td>
<td>15-25</td>
<td>90</td>
</tr>
<tr>
<td>Turkeys</td>
<td>60-80</td>
<td>100</td>
</tr>
</tbody>
</table>
Thank you for your attention!