Histomoniasis

Diagnosis, Prophylaxis, Treatment - Research developments

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Agenda

- Diagnosis and monitoring
- Profylaxis and control
  - ‘General control measures’
  - Specific approaches
  - Search for products
- Conclusions
Diagnosis

- Clinical – necropsy:
  - High morbidity
  - Infection- ulcerations in caeca
  - Liver degeneration–necrosis
  - Peritonitis
  - High mortality (>50%) in affected farms in a short period
  - Pathognomonic (?)
  - Underdiagnosed? (chickens...)

Practical Use for Poultry Veterinarians 👍 👍 👍
Diagnosis & Monitoring

- Histopathology

Stained histological section of turkey liver

amoeboid *Histomonas meleagris* forms

100 μm
Diagnosis & Monitoring

In situ hybridisation

Immuno histochemical reaction


Singh., *Exp Parasitol.*, 2008
Diagnosis & Monitoring

- **Isolation**
  
  Last years increased interest and publications on culturing of *Histomonas meleagrisidis*
Diagnosis & Monitoring

- **Serology**
  - Indirect sandwich ELISA (Windisch and Hess, *Veterinary parasitology*, 2009) (SPF chickens and turkeys)
  - Blocking ELISA (van der Heijden et al., *Veterinary parasitology*, 2010) (chickens and turkeys)
  - Monitoring rather than diagnosis

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Diagnosis & Monitoring

- PCR
  - Based upon *Histomonas meleagridis* 18S rRNA gen
    - HIS5-PCR (Huber, et al., *Veterinary Parasitology*, 2005)
    - HM-PCR (Hafez, et al., *Avian Diseases*, 2005)
    - Hime-PCR (Bleyen, et al., *Veterinary Parasitology*, 2007)
    - PCR (Hess, et al., *Parasitology*, 2006)

Practical Use for Poultry Veterinarians ☻ ☻ ☻
Diagnosis & Monitoring

- PCR
  - Best matrix?

Different samples in 10 positive flocks
- 5 caeca \(\frac{10}{10}\) positive
- 30 faecal droppings \(\frac{8}{10}\) positive
- 2 wipes \(\frac{3}{10}\) positive
- 2 overshoes \(\frac{7}{10}\) positive
- 2 waterbottles \(\frac{6}{10}\) positive
- 1 sample of beetles \(\frac{3}{10}\) positive
- 2 samples of dust \(\frac{9}{10}\) positive

Souillard et al., JRA, 2009
Diagnosis & Monitoring

- PCR analysis of dust in farms **without** a recent history of *Histomonas meleagridis*
- Number of turkey flocks sampled: 53
- Number of positive samples: 3
- One farm connection with laying hens
- Prevalence: 5.6%
- In line with recent data from Dr. Landman (the Netherlands): 7.6% (39 farms)
  (*oral communication*)
- Study in France in 2008: 120 flocks: prevalence of 0.8%
  (Souillard et al., JRA, 2009)
Prophylaxis and Control

- General Control measures
- Specific Approaches
- Search for New Products
Prophylaxis and Control

General Control Measures: Worming?

Has failed to be a consistent control measure ‘in the field’, even when used ‘lege artis’ (taking into account (short) prepatent period of *Heterakis gallinarum* etc.)

Can be (partly) explained by possibility of direct transmission of the parasite
Prophylaxis and Control

General Control Measures: Litter treatment?

Treatment of the litter in the houses with
- Salt
- Desinfectants?
- Lime?
Prophylaxis and Control

General Control Measures: Microflora influencing?

- Study suggests that the level of NSP in diets influences the incidence of histomoniasis (Liebhart et al., 2011)

- Gut microflora is affected by *Histomonas meleagrisidis* in field conditions. (Réperant et al., 2012)
  - Shift of caecal bacterial flora
  - No identification of bacteria
Prophylaxis and Control

- History of control
  - Preventive
    - Arsenics (pentavalent organic)
      - Carbarsone (Carb-O-sep, Whitmoyer)
      - Nitarsonsone (Histostat-50, Solvay, Alpharma)
  - Curative and preventive
    - Nitro-imidazoles
      - Dimetridazole (Emtryl, Solvay)
      - Ipronidazole (Ipopran, Hoffman-La Roche)
    - Nitrofurans
      - Furazolidone (Furox, Smith-Kline)
      - Salfuride (Nifursol, Solvay)
      - Nifurtimox
Prophylaxis and Control

- History
  - Not possible to set Maximum Residue Levels (MRLs)
  - Potential risk for consumer

BANNED in EU
Prophylaxis and Control

- Nitarsone: 2004 negative EFSA opinion


Opinion of the Scientific Panel on Additives and Products or Substances used in Animal Feed on a request from the Commission related to the preliminary assessment of the safety of Nitarsone (4-nitrophenylarsonic acid), as a feed additive in accordance with Regulation (EC) N° 178/2002 and Regulation (EC) N° 1831/2003, article 15.

(Question N° EFSA-Q-2004-014)

Adopted on 28 October 2004
Prophylaxis and Control

- Prevention by vaccination
  - Passive immunisation
    Intra-peritoneal administration of pooled, concentrated, neutralizing antisera from immunized donor animals to naive turkeys: no protection

(Clarkson, Immunology, 1963; Bleyen et al., Avian Pathology, 2009)
Prophylaxis and Control

- Prevention by vaccination
  - Active immunisation
    - Lysed *Histomonas meleagris*is intramuscular injected (Hess et al., *Vaccine*, 2008; Bleyen et al., *Avian Pathology*, 2009)
    - In vitro passage (Tyzzer, 1934 & 1936; Dwyer & Honigberg, 1970; Hess, et al., 2008)
    - In vivo passage (Lund, 1959 & 1966)
    - In vitro passage (Hess et al. 2008, Liebhart et al. 2010)
Prophylaxis and Control

- Prevention by vaccination
  - Cloned *Histomonas meleagridis* passaged in vitro: protection (Hess et al., *Vaccine*, 2008)
  - Autogenous vaccination??
Search for products

- Since ban of preventive and curative products in Europe: increased search for “new” products

- Products with known activity against protozoa are of special interest

- Hurdles for screening:
  - In vitro vs in vivo results
  - Challenge studies: challenge model versus infections in the field
  - Spreading models (Hu and McDougald, Avian Diseases, 2003)
  - Field studies: low prevalence and severe impact of disease
- Artemisinin (anti-malaria):
  - Promising results in vitro
  - Limited effect in vivo

  Thøfner et al., *Avian Pathology*, 2012

- Phytoproducts
  - Protophyt®: based on garlic and cinnamon
  - Enteroguard®: volatile oils extracted from garlic, cinnamon, rosemary and lemon
  - Natustat ®: proprietary plant-derived product
  - Limited effect


  Van der Heijden and Landman, *Avian Pathology*, 2008
Paromomycin 2009 EFSA Opinion

Scientific Opinion of the Panel on Additives and Products or Substances used in Animal Feed

(Question No EFSA-Q-2009-00445)

Adopted on 13 May 2009
Search for products

- Paromomycin 2009 EFSA Opinion

**RECOMMENDATIONS**

Protective measures and restrictions in handling histobloc® should be considered.

A post-market monitoring plan should be implemented immediately after any authorisation.

A potential authorisation of the product should describe the composition of histobloc® as 80 g paromomycin sulphate kg⁻¹ additive, calcium carbonate and pre-gelatinised starch.

A validated analytical method for the determination of paromomycin sulphate in skin/fat should be developed.
Search for products

- Paromomycin 2009: In vivo studies and MoA elucidated at molecular level

Bleyen et al., *Veterinary Parasitol*, 2009
Search for products

- Nifurtimox 2010 (anti-trypanosomal activity)
  - Promising preventive action
  - Promising therapeutic action

- >> Nitrofuran: Possible consumer safety issue (no MRL)

  Hauck et al., Avian Diseases, 2010
Conclusions

- Histomoniasis bears many unknown factors in epidemiology, pathogenesis etc.
  - More research needed. Stalling at present after ‘golden decade’? This is truly an orphan disease.

- Available options
  - ‘alternatives’: Failed??
  - Biosecurity and hygienic measures may contribute but not control
  - Nitarsone: no MRL
  - Paromomycin: MRL available, in development
  - Vaccines: promising concept available, field validation needed, substantial and long term development work needed.